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Struktury klonalne populacji wytwarzających karbapenemazy szczepów *Klebsiella* spp. w Polsce: genotypy epidemiczne, ich filogeneza, mechanizmy lekooporności i czynniki zjadliwości

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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List of publications included in the dissertation

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2. **Biedrzycka M**, Urbanowicz P, Brisse S, Palma F, Żabicka D, Gniadkowski M, Izdebski R (2024) Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006–2019. Eur J Clin Microbiol Infect Dis. 44 (3):619-628, DOI: 10.1007/s10096-024-05016-1
3. **Biedrzycka M**, Urbanowicz P, Żabicka D, Hryniewicz W, Gniadkowski M, Izdebski R (2023) Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009–2019. Eur J Clin Microbiol Infect Dis. 42 (12):1449–1457, DOI: 10.1007/s10096-023-04682-x
4. **Biedrzycka M**, Izdebski R, Hryniewicz W, Gniadkowski M, Żabicka D. (2025) Carbapenemase-producing *Enterobacterales* from patients arriving from Ukraine in Poland, March 2022-February 2023. Infect Dis Ther. 14 (2):401-419, DOI: 10.1007/s40121-024-01097-9

Spis treści

1. List of abbreviations

AAHC – antibiotic-associated hemorrhagic colitis

AMR – antimicrobial resistance

ATLAS – Antimicrobial Testing Leadership and Surveillance

CAI – community-acquired infection

cKp – classical *Klebsiella pneumoniae*

CP – carbapenemase-producing, carbapenemase producer

CPE – carbapenemase-producing *Enterobacterales*

CR – carbapenem-resistant, carbapenem resistance

ECDC – European Centre for Disease Prevention and Control

ESBL – extended-spectrum-β-lactamase

ESKAPE – *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.

HAI – healthcare-associated infection

HGT – horizontal gene transfer

hv – hypervirulent, hypervirulence

ICE – integrative andconjugative element

IMP – imipenemase

In – integron

IPC – infection prevention and control

IS – insertion sequence

hvKp – hypervirulent *Klebsiella pneumoniae*

KPC – *Klebsiella pneumoniae* carbapenemase

KpSC – *Klebsiella pneumoniae* species complex

KoSC – *Klebsiella oxytoca* species complex

LPS – lipopolysaccharide

MBL – metallo-β-lactamase

Mbp – megabase pair

MDR – multidrug resistance, multidrug-resistant

MGE – mobile genetic element

MLST – multilocus sequence typing

NDM – New Delhi metallo-β-lactamase

PMQR – plasmid-mediated quinolone resistance

SLV – single locus variant

ST – sequence type

Tn – transposon

UTI – urinary tract infection

VIM – Verona integron-encoded metallo-β-lactamase

WHO – World Health Organisation

2. Streszczenie

Przybierające na sile zjawisko oporności na antybiotyki jest jednym z najpoważniejszych wyzwań współczesnej medycyny. Spośród wielu grup bakterii opornych na antybiotyki, pałeczki *Enterobacterales* wytwarzające karbapenemazy (CPE) stanowią jedno z głównych zagrożeń dla zdrowia publicznego na świecie. Kompleks gatunku *Klebsiella pneumoniae* (KpSC) należy do najważniejszych klinicznie CPE i jest obiektem szerokich badań nad czynnikami i mechanizmami swojej ekspansji. Drugą istotną grupą jest kompleks gatunku *Klebsiella oxytoca* (KoSC), który współdzieli z KpSC szeroką pulę genów wirulencji i oporności na antybiotyki. Gatunki należące do KoSC, choć przyćmiewane przez KpSC, także przyczyniają się do rozprzestrzeniania karbapenemaz na skalę globalną, jednak ich faktyczna rola w szerzeniu determinantów lekooporności nie została wystarczająco poznana. Od ponad dekady Polska jest krajem silnie dotkniętym problemem oporności na karbapenemy, Na całokształt sytuacji epidemiologicznej w kraju dominujący wpływ ma KpSC, jednak KoSC także odgrywa w niej istotną rolę.

Głównym celem niniejszej pracy było wyjaśnienie czynników i mechanizmów stojących za ekspansją *Klebsiella* spp. wytwarzających karbapenemazy (CP *Klebsiella* spp.) w Polsce, w okresie 2009-2019, oraz w czasie pierwszego roku pełnoskalowej wojny w Ukrainie (luty 2022-luty 2023). Szczególny nacisk położono na rozprzestrzenienie klonalne, horyzontalny transfer mobilnych elementów genetycznych (MGEs) i potencjalny import nowych genotypów. Niniejsza dysertacja składa się z czterech prac oryginalnych, przeprowadzonych na reprezentatywnej grupie izolatów klinicznych, zebranych z terenu całego kraju przez Krajowy Ośrodek ds. Lekowrażliwości Drobnoustrojów, z wykorzystaniem analiz bioinformatycznych sekwencji genomowych. Zastosowana metodologia opierała się przede wszystkim na technologii tzw. krótkich odczytów, wykorzystanej w analizach populacyjnych, która została uzupełniona o sekwencjonowanie w technologii tzw. długich odczytów, pozwalające na dokładne scharakteryzowanie konkretnych *locus* lub MGEs przyczyniających się do rozprzestrzeniania karbapenemaz.

Przedstawione analizy podkreśliły zróżnicowanie struktury klonalnej polskiej populacji KpSC wytwarzających karbapenemazy, zdominowanej w dużej mierze przez kilka tzw. światowych klonów wysokiego ryzyka. W początkowej fazie epidemii CPE w Polsce, klon *K. pneumoniae* ST147 uznawany był za jeden z mniej istotnych. Jego rosnącą rolę zaobserwowano w latach 2015-2019, kiedy to wytwarzający karbapenemazę typu NDM-1 genotyp ST147-Tn*125*F pochodzenia tunezyjskiego rozprzestrzenił się z jednego z warszawskich szpitali

powodując regionalne ognisko na terenie miasta i na Mazowszu. Ponadto, kilka spokrewnionych, ale nie zaliczonych do ogniska organizmów ST147-Tn*125*F zidentyfikowanych w tamtym czasie w innych miastach, reprezentowało ten sam genotyp pochodzący z endemicznych rejonów basenu Morza Śródziemnego, które zostały najprawdopodobniej wprowadzone do Polski na skutek niezależnych importów przez polskich turystów. W tym samym czasie, ST147 odegrał także istotną rolę w szerzeniu karbapenemaz typu VIM. Genotypy tego klonu z różnymi wariantami integronów zawierających geny *bla*$_{\text{VIM}}$ nazwane odpowiednio: ST147-In916, ST147-In2245 oraz ST147-In238 wywołały regionalne ogniska na terenach województw małopolskiego, śląskiego i lubelskiego. Później, po eskalacji wojny w Ukrainie, izolaty ST147 wytwarzające karbapenemazy typu NDM-1/-5 i/lub OXA-48 były jednymi z najczęściej izolowanych od pacjentów przybywających z tamtych terenów. Podczas początkowej fazy występowania klonu ST147 w Polsce, cała ówczesna populacja CPE była zdominowana przez endemiczny genotyp *K. pneumoniae* ST11-Tn*125*A wytwarzający NDM-1. Niniejsza dysertacja ukazała także szerszy kontekst epidemiologiczny występowania ST11-Tn*125*A w Europie, który był wykrywany także u pacjentów i migrantów przybywających z Ukrainy.

W ramach rozprawy poczyniono także kilka ważnych obserwacji epidemiologicznych dotyczących klonu *K. pneumoniae* ST395, będącego w Polsce istotnym producentem karbapenemaz typu OXA-48 już od 2013 roku. Na wzrost liczby przypadków *K. pneumoniae* ST395 w Polsce w ostatnich latach wpływ mogła mieć migracja pacjentów z Ukrainy, wśród których izolaty ST395 wytwarzające karbapenemazy typu NDM-1 i/lub OXA-48 stanowiły najliczniejszą grupę CPE i wykazały bliskie pokrewieństwo genetyczne z organizmami ST395 zidentyfikowanymi na terenie Rosji i izolowanymi od ukraińskich uchodźców wojennych w innych krajach europejskich. Innym scharakteryzowanym genotypem *K. pneumoniae,* wywodzącym się prawdopodobnie z Europy Wschodniej był ST307 wytwarzający karbapenemazy typu NDM-1 lub KPC-2/-3, który stanowił drugą pod względem liczebności grupę izolatów CPE od pacjentów przybywających z Ukrainy. Poza aspektem charakterystyki klonalnej izolatów *K. pneumoniae* wytwarzających karbapenenemazy wyizolowanych od pacjentów migrujących z terenu z Ukrainy, zwrócono także uwagę na stosunkowo częste występowanie genetycznych determinantów hiperwirulencji *K. pneumoniae* (hvKP) obecnych w klonach ST23, ST147, ST307 i ST395, wynikające prawdopodobnie z nabycia hybrydowych plazmidów typu IncFIB+HI1B pNDM-MAR, łączących geny AMR i wirulencji.

Spośród innych przedstawionych „klonów wysokiego ryzyka" *K. pneumoniae* ST437 i ST392 przyczyniły się w Polsce do ekspansji karbapenemaz typu VIM. Genotyp ST437-In238

był najliczniejszy wśród wszystkich izolatów *K. pneumoniae* wytwarzających VIM w latach 2009-2019 i obejmował łącznie ponad 25% wszystkich przypadków. Wywołał on rozległą epidemię regionalną w województwie lubelskim, która w ciągu zaledwie jednego roku rozprzestrzeniła się na 16 placówek tego regionu. ST392-In1667 natomiast spowodował regionalne ognisko epidemiczne w siedmiu szpitalach województwa kujawsko-pomorskiego. Co ciekawe, analizy filogenetyczne ST437 i ST392 w kontekście międzynarodowym wykazały, że oba wyżej wymienione genotypy były prawdopodobnie rodzimego pochodzenia.

KoSC odegrał szczególnie ważną rolę we wczesnej fazie rozprzestrzeniania się w Polsce karbapenemaz typu VIM. Trwająca dekadę (2009-2019) i obejmująca kilka regionów ekspansja genotypu *K. oxytoca* ST145-In237 była jednym z najbardziej niezwykłych zjawisk w całej epidemiologii VIM w kraju, bezprecedensowym również w skali międzynarodowej. Inną unikalną i niezaobserwowaną wcześniej cechą tego genotypu były częste delecje fragmentów chromosomu, obejmujące naturalny gen β-laktamazy bla_{OXY}, uważany za swoisty dla KoSC i historycznie wykorzystywany do identyfikacji gatunkowej. Mimo, że nie udało się jednoznacznie określić molekularnych mechanizmów tego zjawiska, postawiona w niniejszej pracy hipoteza delecji indukowanej przez fagi, wydaje się być prawdopodobną przyczyną.

W rozprawie podkreślono również rolę horyzontalnego transferu różnego rodzaju MGEs w rozprzestrzenianiu się karbapenemaz. Do najważniejszych z nich należą trzy pochodne transpozonu Tn*125* przenoszącego geny bla_{NDM}. Wariant Tn*125*A, charakterystyczny dla endemicznej w Polsce linii *K. pneumoniae* ST11, został wykryty także w organizmach reprezentujących ten klon pochodzenia ukraińskiego. Istotne powiązania z Europą Wschodnią zaobserwowano również dla wariantu Tn*125*K, dominującego w różnych klonach *K. pneumoniae* zebranych od pacjentów przybywających z Ukrainy. Tn*125*F natomiast został wykorzystany jako marker molekularny dla wspomnianego wcześniej genotypu ST147-Tn*125*F rozprzestrzeniającego się w Warszawie i na Mazowszu. Horyzontalny transfer genów kodujących VIM był uwarunkowany przede wszystkim przez plazmidy przenoszące integrony In238 i In916, oba o szerokim rozprzestrzenieniu międzygatunkowym. Praca ta przyczyniła się ponadto do scharakteryzowania kilku typów plazmidów przenoszących karbapenemazy. Były to przede wszystkim plazmidy typu $IncFII_K$+$IncFIB_K$, IncA, IncL i IncFIB+HI1B, o wysokim stopniu podobieństwa do cząsteczek tego typu obserwowanych wcześniej w Polsce i za granicą.

Przedstawione wyniki, wykazały jednoznacznie, że ekspansja wytwarzających karbapenemazy pałeczek *Klebsiella* spp. w Polsce była zjawiskiem wysoce dynamicznym i kształtowanym przede wszystkim przez: klonalne rozprzestrzenianie się genotypów epidemicznych, międzygatunkowy horyzontalny transfer MGEs zawierających geny

karbapenemaz, oraz import nowych linii epidemicznych wskutek podróży i migracji. Wszystkie cztery prace oryginalne tworzące rozprawę opisały złożoną epidemiologię CP *Klebsiella* spp. w Polsce na przestrzeni ostatnich 20 lat i znacząco przyczyniły się do pogłębienia wiedzy na temat sytuacji CPE w kraju.

3. Summary

Growing trends of antimicrobial resistance (AMR) are one of the greatest challenges in modern medicine. Currently, among numerous types of AMR bacteria, the carbapenemase-producing *Enterobacterales* (CPE) represent a major public health threat worldwide. The *Klebsiella pneumoniae* species complex (KpSC) is one of the most clinically significant CPE, being an object of extensive research on factors and mechanisms driving its expansion. Another important group is *Klebsiella oxytoca* species complex (KoSC), sharing a large common AMR and virulence gene pool with KpSC. KoSC members, overshadowed by KpSC, also contribute in the global dissemination of carbapenemases, but their actual role in the spread of AMR determinants remains unexplored. For more than a decade, Poland has been strongly affected by resistance to carbapenems, with the predominant position of KpSC, and a specific role of KoSC in the epidemiological situation.

The primary aim of this study was to elucidate the factors and mechanisms driving a part of the expansion of carbapenemase-producing (CP) *Klebsiella* spp. in Poland in the period 2006–2019, and during the first year of the full-scale war in Ukraine (February 2022–February 2023), with a particular emphasis on clonal dissemination, horizontal gene transfer of mobile genetic elements (MGEs), and potential importation of novel genotypes. The thesis comprises four original genomic-bioinformatic studies, utilizing representative groups of clinical isolates collected across Poland by the National Reference Centre for Susceptibility Testing. The methodology was predominantly based on the short-read whole genome sequencing (WGS) for large-scale analyses, supplemented by long-read sequencing to finely characterise the specific loci or MGEs contributing to the spread of carbapenemases.

The analyses highlighted the clonal diversity of the Polish CP KpSC population over the years, dominated largely by several well-known global "high-risk" clones. At the beginning of the CPE epidemic in Poland, *K. pneumoniae* ST147 was recognised as one of minor clones. Its growing role has been observed from 2015 to 2019, when an NDM-1 carbapenemase-producing ST147-Tn*125*F genotype of Tunisian origin has expanded from an index institution in Warsaw, causing a regional outbreak in the Warsaw area and Mazowsze. Moreover, several related but non-outbreak ST147-Tn*125*F organisms, detected at that time in different towns in the country, represented the same genotype from the endemic Mediterranean region, being likely results of independent imports by Polish tourists. In the similar period, ST147 has been involved in the spread of VIM-type carbapenemases, when its several genotypes with different VIM-encoding integrons, namely ST147-In916, ST147-In2245 and ST147-In238, caused

regional outbreaks in Małopolskie, Śląskie and Lubelskie, respectively. Later, during the first year of the war in Ukraine, NDM-1/-5- and/or OXA-48-producing ST147 isolates were one of the major *K. pneumoniae* groups linked with patients arriving from that country. The initial stage of dissemination of ST147 occurred when the entire Polish CPE population was immensely dominated by an endemic NDM-1-producing *K. pneumoniae* ST11-Tn*125*A genotype. This dissertation revealed also the broader epidemiological context of the ST11-Tn*125A* organisms in Europe which have been also recovered from patients arriving from Ukraine after the escalation of the war.

Several important epidemiological observations have been done for *K. pneumoniae* ST395, which has been a significant OXA-48-type spreader in Poland since 2013. The recent increase in its incidence might have been also influenced by the migration from Ukraine after February 2022, when NDM-1/ and/or OXA-48-producing ST395 isolates were the most numerous CPE linked with patients arriving from that country. These demonstrated close genetic relatedness. to such organisms identified in Russia and to the isolates obtained from Ukrainian war refugees in other European countries. Another *K. pneumoniae* genotypes originating likely from Eastern Europe and characterised in this thesis was ST307 with NDM-1 or KPC-2/-3. These were the second most common *K. pneumoniae* group gathered from patients arriving from Ukraine. Except for the clonal aspect of CP *K. pneumoniae* isolates linked with Ukrainian patients, another interesting feature emphasized in this dissertation was the relatively broad occurrence of hvKP (hypervirulence *K. pneumoniae*) genetic determinants present in ST23, ST147, ST307 and ST395 clones, resulting likely from acquisition and circulation of hybrid IncFIB+HI1B pNDM-MAR-type plasmids, combining AMR and virulence genes.

Other KpSC “high-risk” clones presented in this dissertation were *K. pneumoniae* ST437 and ST392, both contributing to the expansion of VIM-type enzymes in Poland. The ST437-In238 genotype has been recognized as a major Polish *K. pneumoniae* VIM producer from 2009 to 2019, comprising more than 25% of all KpSC VIM cases and being responsible for a large regional outbreak in Lubelskie, which in just one year expanded to 16 institutions of the region. ST392-In1667 has been detected in Kujawsko-Pomorskie and caused an outbreak in seven hospitals of the region. Interestingly, the phylogenetic analyses in the international context of ST437 and ST392 demonstrated, that both above mentioned genotypes likely emerged and disseminated on-site.

KoSC played a particularly important role in the early phase of VIM dissemination in Poland. A decade-long (2009-2019) multiregional expansion of a *K. oxytoca* ST145-In237-like

genotype was one of the most remarkable phenomena in entire VIM epidemiology in the country, being unprecedented also on the international scale. Another unique feature of the Polish *K. oxytoca* ST145-In237-like genotype, not reported previously, were frequent chromosomal deletions covering the natural β-lactamase *bla*$_{OXY}$ gene, usually considered as core for all KoSC members and historically used for species determination. Although the molecular mechanisms of this phenomenon have not been unambiguously explained, a phage-induced deletion hypothesized in this thesis seems to be a probable cause for some of the examined strains at least.

The dissertation emphasized also the role of the horizontal transfer of various MGEs in the dissemination of different carbapenemases. Among the most important were three individual *bla*$_{NDM}$-carrying Tn*125* transposon derivatives. Tn*125*A, characteristic for the endemic *K. pneumoniae* ST11 lineage in Poland, has been detected also in ST11 organisms obtained from patients migrating from Ukraine. Relevant associations with Eastern Europe have been observed also for the transposon variant Tn*125*K, prevailing in *K. pneumoniae* isolates of different STs gathered from patients arriving from Ukraine. Finally, Tn*125*F has been used as a molecular marker for the previously mentioned ST147-Tn*125*F genotype spreading in Warsaw and Mazowsze. The horizontal transfer of VIMs was facilitated primarily by In238 and In916 integrons-carrying plasmids, both of wide inter-species distribution. The study contributed also in characterisation of several types of carbapenemase-encoding plasmids. These were mainly IncFII$_K$+IncFIB$_K$, IncA, IncL and IncFIB+HI1B types, all demonstrating remarkable similarities to sets of international molecules of these types, and those identified previously in Polish CPE.

The overall findings of this thesis demonstrated that the expansion of CP-*Klebsiella* spp. in Poland has been a highly dynamic phenomenon, driven by the interplay of clonal spread of 'high-risk' genotypes, inter-species horizontal transfer of carbapenemase-encoding MGEs, and the importation of novel epidemic lineages by travelling and migration. All four original articles of the dissertation characterised the complex epidemiology of the CP *Klebsiella* spp. in Poland over the last 20 years and contributed significantly to the overall knowledge on the CPE situation in the country.

Title: Clonal structure of the population of carbapenemase-producing *Klebsiella* spp.in Poland: epidemic genotypes, their phylogeny, antimicrobial resistance mechanisms and virulence factors"

4. Introduction

4.1 Clinical significance of *Klebsiella* spp.

Klebsiella spp., a genus of the *Enterobacteriacae* family within the *Enterobacterales* order, comprises nineteen bacterial species (https://lpsn.dsmz.de/genus/klebsiella; as of 1st March, 2026) (1). These Gram-negative, non-motile, usually encapsulated and rod-shaped bacteria, can be found in association with diverse environmental niches; such as water, sewage, soil or plants, and mucosal surfaces of mammals, which they often colonize (1-5). *Klebsiella* spp. are frequently opportunistic pathogens, particularly dangerous to neonates, elderly and immunocompromised individuals, primarily in healthcare settings, but also in the community. From the clinical perspective, the most important groups are *Klebsiella pneumoniae* species complex (KpSC) and *Klebsiella oxytoca* species complex (KoSC) (2, 6-8), both of worldwide range (9, 10).

KpSC includes seven related species/subspecies: *K. pneumoniae*, *K. quasipneumoniae* subsp. *quasipneumoniae*, *K. quasipneumoniae* subsp. *similipneumoniae*, *K. quasivaricola*, *K. africana*, *K. variicola* subsp. *tropica* and *K. variicola* subsp. *variicola.* These differ from each other by 3-4% nucleotide divergence across core genes, but share common pool of antimicrobial resistance (AMR) and virulence genes (11). All KpSC members have been detected in the human gut and all with the exception of *K. variicola* subsp. *tropica* are causative agents of infections in humans (12). What is important, *K. pneumoniae* is the species of the highest clinical importance and the majority of the knowledge on *Klebsiella* spp. refers to it. Therefore, most of the KpSC characteristics presented in this dissertation focuses on *K. pneumoniae* as a representative reflecting feature common for other complex members. *K. pneumoniae* exhibits extensive plasticity, *i.e.* phenotypic and genetic diversity, enabling adaptation to a wide range of environmental and host-associated niches (9, 11). Interactions between *K. pneumoniae* and the host is a remarkably complex phenomenon; therefore, the bacterium can be either a commensal organism or a pathogen (13). As a component of the normal mammalian microbiota, *K. pneumoniae* colonizes mucous membranes in gut, it may also inhabit nasopharynx or respiratory tract (2, 6). The prevalence of the *K. pneumoniae* gut colonisation in humans in the community vary by country and demographic, e.g. data from Australia and USA indicated the level ~4-6% (14, 15), but recent health-care exposure might elevate this rate even up to ~25% (14, 16). Remarkably higher rates (up to even 87%) were observed in healthy adults populations in Korea, Japan, Singapore, Taiwan and Malaysia (17). *K. pneumoniae* demonstrates pathogenicity to wide range of hosts (13, 18), it has been described in medicine since the end

of XIX century. In 1882 Carl Friedländer, a German pathologist isolated an encapsulated bacillus from lungs of patient who had died of pneumonia (19). The organism was initially named "Friedländer's bacillus", then in 1886 it was changed into *Klebsiella* (20). In the pre-antibiotic era, *K. pneumoniae* was an important etiological factor of the community-acquired pneumonia (21), but since antibiotics have been commonly used in the treatment, its occurrence evolved into nosocomial environments (22). Current critical importance of *K. pneumoniae* in medicine arose from infections caused by two developed pathotypes: a classical one, associated with AMR, or a hypervirulent (11, 23, 24). In most cases, AMR and virulence genes are concentrated separately in distinct *K. pneumoniae* subpopulations (25, 26), nevertheless, their convergence, leading to the potentially risky co-existence of pathogenicity and resistance determinants in a single strain, has been increasingly reported (11, 27, 28). The first pathotype, classical *K. pneumoniae* (cKp), for many decades has been recognised as an opportunistic pathogen, being one of the most frequent etiological agents of healthcare-associated infections (HAIs). These are primarily: urinary tract infections (UTIs), pneumonia, and wound or soft-tissue infections, all having potential to progress into bacteraemia (2). At particular risk are patients in extreme age groups i.e. neonates and elderly, but also immunocompromised and those with inserted medical devices. Disruptions in immunological control of commensal strains leading to their overgrowth is a key risk factor of infections in these individuals (14, 16). Major factors limiting the treatment of HAIs is multidrug resistance (MDR), defined as resistance to at least three antimicrobial classes. MDR arises through accumulation of AMR genes, acquired primarily by horizontal gene transfer (HGT), and located mainly on larger conjugative plasmids (13). Currently, the biggest clinical challenge are the strains producing extended-spectrum-β-lactamases (ESBLs), *i.e.* enzymes conferring resistance to penicillins, cephalosporins, including the third- and fourth-generation compounds, and monobactams, and the carbapenemase producers (CPs), inactivating carbapenems, the last resort drugs, along with many other β-lactams (29, 30). Such strains are usually MDR due to accumulation of resistance mechanisms to other antimicrobial classes and were recognised by the World Health Organisation (WHO) as a critical public health threat (31). *K. pneumoniae* is a member of the so-called "ESKAPE" pathogens and stands for "K" in this acronym (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.), comprising six most significant species causing drug-resistant hospital infections (32). Additionally, *K. pneumoniae* has been prioritized as a target for new drugs and therapies (31). The precise estimation of the global *K. pneumoniae* disease burden is impeded by lack of precise data from many geographic regions. In Europe alone, more than

90,000 infections caused by CP and/or ESBL-producing *K. pneumoniae* strains lead upward of ~7,000 of deaths annually and constitute ~25% of the total disability-adjusted life years lost to MDR infections (33). AMR in *K. pneumoniae* is strongly correlated with high mortality, estimated at ~42% for carbapenem-resistant (CR) strains, compared with ~21% for non-CR organisms (34).

The substantial characteristics of the second pathotype, the hypervirulent *K. pneumoniae* (hvKp), are the ability to cause tissue-invasive, community-acquired infections (CAIs) in unusual sites in healthy individuals and their metastatic spread, and general sensitivity to antibiotics. Such syndromes, including pyogenic liver abscess in the absence of biliary tract disease, non-hepatic abscess, pneumonia, endophthalmitis, meningitis and necrotizing fasciitis have been describing from the mid-1980s. The infections were primarily observed in East and Southeast Asia (20, 35-37), but recently increasing number of cases have been reported worldwide (20, 38, 39). In contrast to cKp, possessing basic pathogenicity factors only, (2) the enhanced virulence of hvKp is driven by specific capsular serotypes and particular accessory genes located on mobile genetic elements (MGEs). Such elements are most typically plasmids and integrative and conjugative elements (ICEs), encoding additional siderophores systems of much rarer distribution in the overall *K. pneumoniae* population (25). The hvKp disease burden is high, with mortality rates up to several dozen percents (21), despite the general antibiotic susceptibility of hypervirulent (hv) strains, except for the natural resistance to ampicillin (described below), and the fact that patients are younger and usually with no additional co-morbidities (20, 36, 40, 41). Infections in critical body localisations are often associated with poor clinical prognosis, even survivors suffer serious morbidity, including neurologic sequelae, loss of vision or limb (20, 35, 36, 42, 43).

KoSC, the second most important *Klebsiella* group causing infections in humans, consists of nine closely related species: *Klebsiella oxytoca, Klebsiella michiganensis*, *Klebsiella grimontii*, *Klebsiella pasteurii*, *Klebsiella huaxiensis*, *Klebsiella spallanzanii*, and three unnamed ones (44), adopted to a variety of natural and host-associated niches (3, 45). First report on KoSC was made in 1886 by Carl Flügge, German bacteriologist, who cultivated "*Bacillus oxytocus pemiciosus*" from old milk, then the bacterium was renamed into "*Aerobacter oxytocum*" by David Bergey in 1923 (44) and further *Klebsiella oxytoca* by Hans Lautrop in 1956 (44, 46). All the mentioned above KoSC members are difficult to differentiate from each other by phenotypic characteristics, the precise species identification requires genome-based analysis. Such methodology has become widely used for the last dozen of years, therefore, organisms referred in the literature as *K. oxytoca* could actually represent any species

of this complex (44, 47). The current global distribution of each species among clinical KoSC isolates is largely unknown, but some reports indicate, that the most numerous species are *K. oxytoca* and *K. michiganensis* (47). KoSC is a human commensal detected on skin, oral cavity, or mucosal surfaces of intestinal and respiratory tracts (2, 48, 49). The intestinal carriage is relatively lower compared to KpSC and estimated at ~1.6% to ~9% in healthy individuals (50, 51). Increased incidence was observed in patients suffering from the inflammatory bowel disease (52), influenza (53), or infants and neonates hospitalised in intensive care units (54). As an opportunistic pathogen, KoSC is able to cause a variety of HAIs, including urinary tract, bloodstream, intra-abdominal, skin and soft tissue infections, pneumonia, and above all antibiotic-associated hemorrhagic colitis (AAHC), occurring after administration of antimicrobial agents (44, 47, 55). Infections caused by KoSC are much less notorious than KpSC, likely as a result of relatively lower colonisation frequency and are associated with lower mortality rate estimated at ~7% (56-58). Abilities to persist in a variety of wet environments, such as handwashing soaps and sinks, drainage systems, humidifiers, central venous catheters etc., predispose KoSC to cause nosocomial outbreaks, usually of environmental source (10, 44, 59-61). The growing threat of AMR, arising partially from a large pool of AMR genes shared with KpSC (59), is emerging clinical concern in KoSC. A particularly important role is played by ESBL- and/or CP strains, often involved in outbreaks in healthcare settings (44). In contrast to KpSC, the knowledge on KoSC virulence factors is highly limited. The best characterised is kleboxymycin, a toxin involved in AAHC (44), whilst information on the remaining ones, such as capsular antigens or siderophore systems are usually based on sequence homology, being extrapolations of the KpSC data, commonly non-including evidence from functional studies (59, 62).

4.2 General remarks on antimicrobial resistance in *Klebsiella* spp., acquired β-lactamases, major carbapenemases

Resistance to antibiotics, arising through the millennia of co-evolution of antimicrobial-producing organisms and environmental bacteria, is a natural phenomenon in microbiology (13, 63). The extraordinary capacity of microorganisms to accumulate AMR mechanisms to numerous antimicrobials in a relatively short time, has led to a specific arms race between bacteria and the academia and pharmaceutical industry; resulting in rapid emergence of resistance mechanisms to newly introducing drugs (64). AMR may be intrinsic/natural or acquired, and on the genetic level may emerge by random chromosomal point mutations or

horizontal gene transfer from a large pool of mobile AMR genes (29). A special emphasis in this section will focus on resistance mechanisms to penicillins, cephalosporins, monobactams and carbapenems; all representing β-lactam antibiotics. These constitute the major class of antimicrobial agents in human medicine, and the rapidly increasing resistance to them has been an urgent clinical and epidemiological challenge (65). The vast majority of individual AMR mechanisms and time of their occurrence will be discussed based on *K. pneumoniae.* Timelines showing the evolution of acquired resistance mechanisms to β-lactams in *K. pneumoniae* are presented in Figure 1.

KpSC and KoSC members are intrinsically resistant to ampicillin, one of aminopenicillins, due to the production of specific class A β-lactamases encoded in the core genome. The *bla*$_{SHV}$ gene, described from 1970s (Figure 1) is intrinsic to *K. pneumoniae* (66), while the remaining KpSC members possess conserved orthologues: *bla*$_{LEN}$ in *K. variicola* and *K. quasivariicola*, and *bla*$_{OKP}$ in the other species (12). Each of nine discerned *bla*$_{OXY}$ gene groups is typical to particular KoSC member and has been historically used in intra-complex species differentiation (44, 67). In 10-20% of KoSC clinical isolates, specific mutations in the *bla*$_{OXY}$ promoter region (68-71), leading to gene overexpression and resistance to third-generation cephalosporins (72, 73), have been observed. The chromosomal loci *fosA* and *oqxAB*, conferring reduced susceptibility to fosfomycin and quinolones, respectively, are considered to be natural for the both discussed species complexes (74-76). Increased resistance to important antimicrobials may be associated also with changes in expression level and/or activity and mutations in other core genes, particularly those regulating lipopolysaccharide (LPS) production, efflux and permeability (77, 78). Colistin resistance may be caused by inactivation of *mgrB* and point mutations in *phoPQ, pmrAB, ccrAB* (79). Regulation of activity of *ramA*, *soxS* and *rarA* genes, affecting AcrAB and/or OqxAB efflux pumps (75), is involved in resistance to carbapenems, fluoroquinolones, nitrofurantoin, tigecycline, and chloramphenicol (80-82). Specific mutations in genes *ompK35, ompK36*, coding for major porin channels, contribute significantly to resistance to various antiinfectives, including the carbapenems (83, 84).

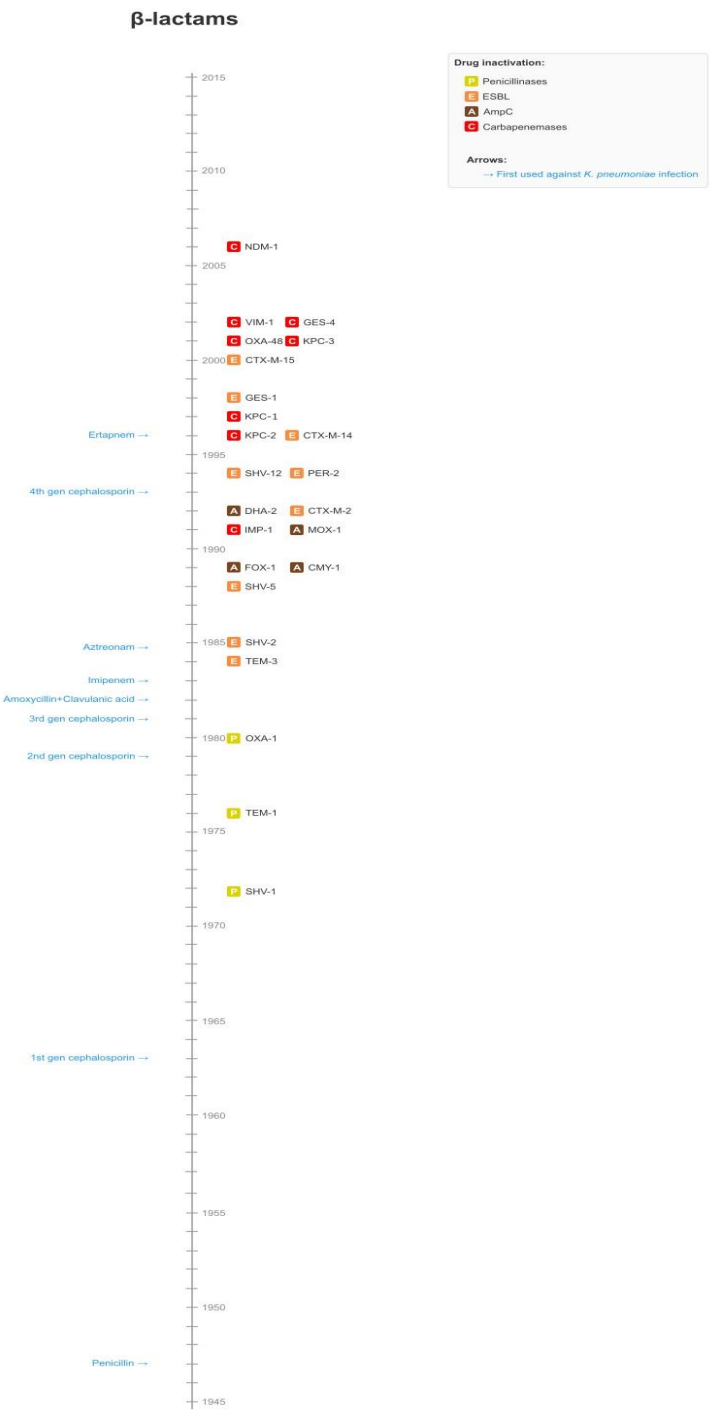

Figure 1. Timeline showing evolvement of selected *K. pneumoniae* resistance mechanisms against β-lactams. Resistance mechanisms are labelled with different symbols as indicated. Arrows on the left of the timeline indicate the first clinical use of the antibiotic against *K. pneumoniae* infection, adopted from Navon-Venezia et. al. (29).

Despite the considerable role of core genome most AMR determinants in *Klebsiella* spp. are located on large conjugative plasmids, assigned to accessory genome (29, 85, 86). *K. pneumoniae* is the species of particularly high plasmid permissiveness which makes it a reservoir of AMR genes circulating in the environment (13). Therefore, it was considered to play a role of "canary in the coalmine", the organism in which most of new mobile AMR genes were detected for the first time, before propagation into other Gram-negative pathogens (13, 25, 29). ESBL-encoding genes were reported since the early 1980s, shortly after introduction of oxyimino-cephalosporins (third-generation cephalosporins) into clinical practice. One of the earliest forms of such genes were mobile variants of *bla*$_{SHV}$ (87) and *bla*$_{TEM}$s (88) (Figure1). The successful spread of ESBL producers across *K. pneumoniae* populations in the 1990-2000s positioned this species as the major ESBL-positive pathogen, often associated with nosocomial outbreaks globally (29, 89). Unprecedented changes in ESBL epidemiology have been observed since 2000s, when *bla*$_{CTX-M}$ gene variants, originating from *Kluyvera* spp., became predominant ESBL (90) (Figure 1). Mobilisation of *bla*$_{CTX-M}$ genes was mediated by insertion sequences (IS), principally IS*Ecp1*, to plasmids of human opportunists (91, 92). Repeated mobilisation events resulted in independent circulation of different *bla*$_{CTX-M}$s, including *bla*$_{CTX-M-15}$, the predominant member of a CTX-M-1 subfamily that has been widespread in Europe, North America, the Middle East and India (93, 94). Genes coding for additional ESBL groups transferred horizontally to *Klebsiella* spp. were specific *bla*$_{OXA}$ variants (95) and much less common *bla*$_{GES}$s, *bla*$_{SFO}$s, *bla*$_{PER}$s or *bla*$_{VEB}$s (96). The current geographic range of ESBLs 40 years after their emergence, has reached high endemic rates in many parts of the world, and *K. pneumoniae* has been recognised as one of their major spreader (97). Dissemination of plasmid-borne AmpC-like cephalosporinase-encoding genes, such as *bla*$_{CMY-2}$, *bla*$_{DHA}$, *bla*$_{FOX}$ or *bla*$_{MOX}$ types, has been observed in parallel with the epidemiological success of ESBLs (Figure 1) (65, 98). Particularly concerning are ESBLs or AmpCs combined with porin loss and/or increased efflux, which enhances enzymatic β-lactam resistance. Moreover, the presence of multiple copies of β-lactamase genes and increased promoter strength lead to their overexpression, resulting even in lower-level carbapenem resistance (98).

Extensive use of carbapenems, as the last resort drugs in therapy of infections caused by ESBL producers in the 1990s, has engendered the evolution of acquired carbapenemases, enzymes hydrolysing almost all β-lactams and being resilient against β-lactamase inhibitors, such as clavulanic acid, sulbactam and tazobactam (99). The broad spread of such enzymes among *Enterobacterales* has given rise to the term carbapenem-producing *Enterobacterales* (CPE), among which *K. pneumoniae* has become a major worldwide representative due to its

strong association with nosocomial outbreaks (100). Resistance to the first clinically used carbapenem, namely imipenem, was reported in *K. pneumoniae* in 1985 (101), after barely two years of clinical use of the drug (102), whereas the earliest carbapenemase to be detected was IMP-1 (imipenemase), identified in 1988 in *Pseudomonas aeruginosa* and in 1991 in *Enterobacterales* in Japan (103).

The first group of carbapenemases of worldwide range in *Klebsiella* spp. were *Klebsiella pneumoniae* carbapenemases (KPCs), members of the class A of β-lactamases according to Ambler (104). These enzymes were detected originally in the USA in the mid-1990s (105) (Figure 1), and drove then extensive clonal expansion of the *K. pneumoniae* hyperepidemic sequence type (ST) 258 for around two decades (106). KPCs exhibit activity against all β-lactam antibiotics that were in use at that time ("classical" β-lactams) and are not sufficiently inactivated by the then commonly used β-lactamase inhibitors ("classical" β-lactamase inhibitors), posing a serious clinical challenge (107). Moreover, KPC producers are usually MDR, which limits available therapeutic options and leads to high attributed mortality rates, up to even ~50% (108). Apart from KPCs a number of other class A carbapenemases have been observed in *Enterobacterales* and *K. pneumoniae*, however, so far these have not disseminated on a scale comparable to KPCs (109).

The other globally dispersed acquired carbapenemases were Ambler class D OXA-type carbapenemases. Most of these highly diverse enzymes are divided into two groups: group I, characteristic to acinetobacters, and consisting of different subgroups of OXA-23-, -24- and -58-like enzymes, and group II, with OXA-48-related variants originating from *Shewanella* spp., mainly found in *Enterobacterales*. Enzymes representing the OXA-48-type are further assigned into three clusters: the OXA-48 cluster (A) with the most common OXA-48, -244 and -162 variants; the OXA-181 cluster (B) including OXA-181, -232, and -484; and the OXA-204 cluster (C) (110). The first OXA-48 producer was a *K. pneumoniae* strain identified in Turkey in 2001 (111) (Figure 1). During early 2000s, the OXA-48-type producers were distributed especially in the Middle-East and in North Africa. Their further spread to other geographic regions was mostly driven by travelling and migration; and the main molecular mechanism of the $bla_{\text{OXA-48}}$-like genes dissemination has been associated with specific plasmid backbones circulating in numerous *K. pneumoniae* clones and other *Enterobacterales* (110, 112). In general, the OXA-48-type enzymes present good penicillinase activity, weak carbapenem-hydrolyzing activity, no activity against oxyimino-cephalosporins and resistance to the classical β-lactamase inhibitors. Therefore, the production of ESBLs and/or porin deficiencies are required to provide extensive resistance to β-lactams, including high-level of resistance (113).

These characteristics contribute to diagnostic difficulties of OXA-48-type-producing organisms and their likely underdetection (110, 112).

Finally, the so-called metallo-β-lactamases (MBLs), constituting the structural-evolutionary class B of β-lactamases were discovered in the mid-1960s in species of lower clinical importance, such as *Bacillus cereus* (114), *Aeromonas* spp. (115) or *Stenotrophomonas maltophilia* (116), have elevated the AMR crisis to unprecedented scale (117, 118). MBLs demonstrate activity against all penicillins, cephalosporins, and carbapenems, sparing aztreonam, and are not inhibited by the clinically used β-lactamase inhibitors. Their hydrolytic properties are dependent on the interaction of the β-lactam molecule with zinc ions located in the active site of an enzyme (109). Globally, today the most frequently reported MBLs in *Enterobacterales* are New Delhi metallo-β-lactamases (NDMs), followed by Verona integron-encoded metallo-β-lactamases (VIMs), and imipenemases (IMPs) (119). Chronologically the first MBLs in *Enterobacterales* were IMPs, identified primarily in 1991 in *Serratia marcescens* in Japan (120), and shortly after transferred to *K. pneumoniae* (103). Since then, IMP producers have been mainly detected in Japan, China, other Far Eastern countries and Australia, and their distribution remains to be limited in the rest of the world (121). VIMs, discovered in *Pseudomonas* spp. in the mid-1990s in Italy and France (122), have been disseminating amongst *Enterobacterales* since the early 2000s (Figure 1), mostly in *K. pneumoniae,* circulating initially in Southern Europe. This initiated endemic situation in Greece, where a substantial role was played also by VIM-producing KoSC (117, 123, 124). Next, VIMs were introduced to the northern part of Europe, and to United States, where their frequency remains lower (117). NDMs, the most recently identified global type of MBLs, focused the worldwide attention around 2010, being known even to the lay public (109, 118). The NDM-1-producers, often called "superbugs", reached the intercontinental range over merely a couple of years (117, 118, 125). The first detection of NDM-1 was in 2008 in Sweden (Figure 1). A *K. pneumoniae* strain resistant to all antibiotics tested, except colistin, was obtained from a patient of Indian origin, who had been recently hospitalised in New Delhi (126). *bla*$_{\text{NDM}}$–like genes, residing in composite Tn*125* transposons, were primarily integrated into the chromosome of *A. baumannii* from an unknown environmental species. Then, their fragments were repeatedly and independently transferred onto plasmids of different incompatibility groups replicating in *Enterobacterales* (109, 127). Noticeably, NDM producers notoriously harbour also numerous other AMR genes, contributing to the MDR phenotypes which results in limited therapeutic options and high mortality rates (109).

Except the discussed above resistance to β-lactams, *Klebsiella* spp. acquired also mobile resistance mechanisms against other clinically important groups of drugs (13). Quinolones, antibiotics affecting bacterial DNA replication system, have been used since 1960s. Their consumption increased extensively from 1980s, and led to the emergence of quinolone resistance (128). *K. pneumoniae* has acquired all quinolone resistance mechanisms known in Gram-negatives; including increased efflux and target-site gene mutations, which are regulated by the core genome, as well as plasmid-mediated quinolone resistance (PMQR) mechanisms (29). The first plasmid-borne *qnrA* and *qnrB* genes, mobilised from *Shewanella* spp. and *Citrobacter* spp. respectively (129, 130) were identified in *K. pneumoniae* in 1994 in the United States (131) and in early 2000s in India (132) and soon after were successfully spread among KpSC and KoSC (133). Other PMQR genes, conferring simultaneously resistance to aminoglycosides, are *aac(6')-Ib-cr*s, located initially on plasmids, but recently found also in the chromosome (134). Noticeably, the expression of PMQR genes leads to low or moderate levels of quinolone resistance, but favours the appearance of chromosomal mutants in genes coding for DNA topoisomerases, *gyrA* and *parC* (135). Aminoglycosides are antibiotics that were used especially widely until the introduction of oxyimino-cephalosporins, fluoroquinolones and carbapenems in the 1980s (136). Within only 10 years, *K. pneumoniae* acquired a wide spectrum of plasmidic genes coding for aminoglycoside-modifying enzymes (AMEs), assigned to families such as *aac*, *ant*, *aad* or *aph*. While AMEs have narrow spectra of activity, limited to particular chemical modifications of these antibiotics, the 16S rRNA methylases, encoded by mobile *armA* gene family, observed since the early 2000s, confer resistance to all aminoglycosides (29, 137). Following *armA*, mobile genes specifying other 16S rRNA methylase families have been reported (136). The increased consumption of polymyxins, such as colistin, as the last line drugs has been observed since early 2000s, along with the dissemination of carbapenemase producers, especially CPE (138). Although major colistin resistance mechanisms are encoded chromosomally (described above), a plasmid-mediated *mcr-1* gene, encoding phosphoethanolamine transferase that modifies bacterial lipid A, was described in *K. pneumoniae* in China during the second decade of XXI century (139), and shortly after was expanded to other parts of the globe (140). Other *mcr* variants widespread in *K. pneumoniae* are *mcr-9* and *mcr-8* (141).

4.3 Major virulence factors in *Klebsiella* spp.

KpSC virulence factors are well examined, whilst, as it has been mentioned before, this knowledge in KoSC is highly limited and usually based on sequence homology. Basic pathogenicity factors enabling opportunistic infections in mammals are conserved in the chromosomal core genes of KpSC and KoSC members. This subset in KpSC includes type 1 and 3 fimbriae, a variety of surface K- and O-antigens, and siderophore systems (2, 11, 25, 44, 59, 62, 142, 143). Both types of fimbriae are involved in intestinal colonisation, formation of biofilm on catheters, and abilities to cause UTIs and pneumonia; however, the clinical impact of the allelic diversity of fimbriae-encoding loci *fim* and *mrk* remains unexplored (144, 145). The polysaccharide capsule and lipopolysaccharide (LPS) antigens are two major targets for novel control strategies against *K. pneumoniae*, most of all for effective vaccines. The polysaccharide capsule protects bacteria against desiccation, phage and protist predation. Capsule is recognised as a key virulence determinant, mediating suppression of the host immune response, preserving against antimicrobial immune-peptides, complement-mediated killing and phagocytosis. The chromosomal K- and O-loci, encoding enzymes involved in the biosynthesis of the polysaccharide capsule and LPS, respectively, are substantially diverse and contribute considerably to antigenic diversity of the bacterial population (146). To date, more than 185 of distinct K types and 13 O types (https://kaptive.readthedocs.io; as of 1st March, 2026) were identified. Except for several well-characterised K variants, little is known about the capsule-associated virulence. The K1 and K2 capsular types, being important characteristics of major hvKp clones, are related with enhanced pathogenicity and invasive disease (24, 26, 142). The K5 variant, occurring in various genetic backgrounds, is associated with liver abscesses (147), and K3 is linked with the rare rhinoscleromatis *K. pneumoniae* lineage (148). Additionally, such capsular types as K16, K20, K54, K57 or K64 have been recognised as virulence enhancers (20, 149). The LPS O1 and O2 O serotypes, the most widespread among clinical *K. pneumoniae* isolates, are considered as augmenting protection against phagocytosis (143, 146, 150). The major role of siderophores, the iron-chelating molecules, is to competitively capture iron ions from host proteins and other sources. Out of four such systems described in *K. pneumoniae*, only enterobactins, the most common in bacterial population are conserved in the chromosome (151). However, the role of enterobactins in virulence is limited because their activity is hampered by binding by human lipocalin-2 (Lcn-2), the element of innate immune system, which disrupts iron assimilation and induces the inflammatory response (152).

Other virulence factors involved in the infection enhancement or bacterial propensity to cause disease are encoded in accessory genome and spread by numerous MGEs (11). Acquired siderophore systems in KpSC, namely yersiniabactin (Ybt), aerobactin (Iuc) and salmochelin (Iro), encoded by the *ybt*, *iuc* and *iro* loci, respectively, are statistically associated with invasive CAIs in humans, and are not subjected to Lcn2 binding (25). These differ from each other in terms of iron-binding affinities and interaction with host immune system, including abilities to intra-macrophages survival. Iro is particularly important for the bacterial growth and correlated with enhanced virulence in murine sepsis model, whilst Iuc, demonstrates substantial role in increased iron acquisition, and was recognised as critical in virulence, both in *in vitro* and *in vivo* studies, and is a critical biomarker of hvKp isolates (11, 153, 154). The most widespread Ybt, present in almost 30-40% of *K. pneumoniae* human clinical isolates, enhances the bacterial growth and dissemination in spleen (11, 155). Colibactin (Clb), the genotoxic polyketide, infers damage of eukaryotic DNA, promotes colonization of gut and mucosal and dissemination to blood and other organs. It is also recognised as a causative agent of colorectal cancer (156, 157). Clb-producing *K. pneumoniae* strains constitute ~10% of the species population and are significantly associated with particular hvKp clones (11). A hypermucoviscous phenotype was historically considered as a characteristic feature of hvKp strains, and became probably the most well-known virulence marker in this species (20). Hypermucoid strains were initially defined by a positive "string test", resulting in production of a viscous filament ≥5mm in length by stretching a bacterial colony by inoculation loop (20). On a genetic level, hypermucoidy is caused by overproduction of the capsule, due to presence of at least one of the regulator loci, *rmpA* and *rmpA2* (158, 159), although more recent studies demonstrated hypermucoidy without the capsule overproduction (160). The confusing character of the string test has been supported by varying compatibility between clinical manifestation of infection and positive test rates, ranging from 51% (161) up to even 98% (162). What is more, some cKp isolates demonstrate also positive string test results (23), therefore its genetic basis and impact on a disease are not apparent (11). Mobile *iuc*, *iro*, *rmpA*/*rmpA2* loci are often co-carried by specific canonical *K. pneumoniae* virulence plasmids (described below) with strong linkage to individual hvKp clones (26). Furthermore, relatively high occurrence of Ybt in *Klebsiella* spp. population is driven by mobilisation of the *yb*t locus by ICE*Kp* elements, integrating typically into the chromosome. Several ICE*Kp* lineages were also involved in mobilisation of *clb* locus (155). A combination of core pathogenicity factors, first of all the K1/K2 capsular and O1/O2 LPS types, with particular plasmid-located loci: *iuc*, *iro* and *rmpA/rmpA2,* has been considered as an important predictor for clinical hypervirulence; therefore, these genetic determinants have been

commonly recognised as key virulence loci in *K. pneumoniae* (11, 23, 25). The genetic determinants of pathogenicity are a part of the gene pool that is commonly shared between KpSC and KoSC. Nevertheless, the K and O antigens in KoSC are much less studied, though some reports identified several capsular K types and O LPS variants in KoSC members (44, 62, 163). Of the remaining KpSC-specific virulence loci, the presence of *mrk*, *fim* (44) and *ybt* was also described in KoSC (62, 163). The best characterised KoSC-specific virulence factors are cytotoxins: kleboxymycin (also known as tilimycin) and tilivalline, produced as a result of the nucleophilic attack of free indole on kleboxymycin (164). Both toxins are involved in AAHC, a clinical syndrome causing pathological changes in the gut tissue due to damage of DNA in host cells. The syndrome occurs after administration of antimicrobial agents, especially penicillins, disturbing proliferation of normal microbiota and contributing favourable conditions for the overgrowth of KoSC (44, 50). The production of kleboxymycin is regulated by the kleboxymycin-biosynthetic gene cluster, likely acquired horizontally by KoSC from actinobacteria (165).

4.4 Mobile genetic elements associated with carbapenemase and virulence genes

AMR genes, including those encoding carbapenemases, are located mostly on MGEs. The most common primary genetic structures responsible for mobilisation and expansion of carbapenemase genes are ISs, transposons and integrons. These are further incorporated onto plasmids or chromosomes, and propagated via clonal spread and/or HGT (11, 29). *Klebsiella* spp. and *K. pneumoniae* in particular, acquired a huge pool of foreign DNA with accessory genes, including thousands of AMR-associated MGEs (11). This section focuses only on those harbouring the most relevant types of carbapenemase-encoding genes.

Numerous composite and simple transposons (Tn) are genetic platforms for NDM-, KPC- and OXA-48-type-encoding genes, whereas VIMs and IMPs are specified by gene cassettes inserted into integrons (In) (29, 166). DNA fragments (often with inserted AMR genes) bounded by two copies of the same or related IS, mobile as a single unit, are referred as composite transposons (166). Such structures have been identified as the direct genetic environment of bla_{NDMs} and several $bla_{\text{OXA-48}}$-type genes (29). The bla_{NDM}-carrying Tn*125* element, flanked by two copies of IS*Aba125*, was initially identified in the chromosome of *A. baumannii*. Tn*125* derivatives, differing by numerous random truncations on the 5' and/or 3' sides, have been widespread in *Klebsiella* and other *Enterobacterales* (127, 167). The primary Tn*1999* transposon, originating from *Shewanella xiamenensis*, harbours $bla_{\text{OXA-48}}$ and *lysR*

gene, bracketed by two copies of IS*1999* (168). Specific insertions located down- or upstream of the *bla*$_{\text{OXA-48}}$ gene allowed for distinction of five of Tn*1999* isoforms, of which two, namely Tn*1999.2* and Tn*1999.3,* are associated with stronger hybrid promoter, conferring 2-fold-higher OXA-48 enzymatic activity (169-172). Additionally, *bla*$_{\text{OXA48}}$-type genes have been incorporated into other transposonic structures, such as Tn*2013* or Tn*2016*, being transposable units formed by IS*Ecp1*-like elements (112). Simple transposons, bounded by terminal inverted repeats, contain usually the transposase and resolvase genes, and internal passenger gene(s) (166). *bla*$_{\text{KPC}}$ genes were identified as parts of unique composite Tn*3*-like transposons, namely Tn*4401* variants. This structure co-carry transposase and resolvase genes flanked by insertion sequences IS*Kpn6* and IS*Kpn7*. Internal deletions in a polymorphic region, located immediately upstream of *bla*$_{\text{KPC}}$, result in different levels of KPC expression and resistance, and allow to determine different isoforms of Tn*4401* (166, 173). While transposons are capable to move by themselves, most of integrons require site-specific recombination or location within an active transposon (166). AMR genes incorporated onto integrons are located on specific MGEs, referred to as integronic gene cassettes. Each cassette consists of a protein-encoding region and the *attC* recombination site, essential for its recruitment via site-specific recombination. The conserved segment at the 5' extremity (5'CS) of class 1 integrons contains the integrase (IntI) gene *intI*, the *aatI* recombination site and a promoter for the co-expression of all incorporated cassettes. The opposite 3'CS segment comprises truncated *qacE* (*qacEΔ1*) and *sul1* cassettes coding for resistance to quaternary ammonium salts and sulphonamides, respectively (166, 174). An individual integron may capture a number of gene cassettes providing an MDR phenotype. Specific combination of such cassettes has been used as a molecular marker to distinguish ~1400 of different class 1 integrons identified in Gram-negatives so far (175-177). The truncation of the *tniCQAB* module, located behind *qacEΔ1* and *sul1* cassettes, regulating the mobility of the progenitor Tn*402* transposon, has caused lack of the transposition function by the class 1 integrons; however, as mentioned above, these structures may be translocated by homologous recombination or other transposable structures (166, 175).

Plasmids have been recognised to be important platforms for other MGEs and acquired AMR genes in both Gram-negatives and Gram-positives (166). An individual plasmid is an extrachromosomal DNA molecule, replicating independently from bacterial chromosome and maintaining a fixed copy number per bacterial cell. Core housekeeping plasmid genes, coding for functions such as conjugation, mobilisation or vertical inheritance, form a backbone, which may be completed by niche-adaptive accessory genes. The transmission of plasmidic AMR genes in *K. pneumoniae* is usually driven by large conjugative (self-transmissible) or

mobilizable plasmids, assigned to major incompatibility groups (Inc), including IncF, IncN, IncA, IncC, IncH, IncR or IncX; however, small mobilizable molecules, such as Col-types, have also been involved (29). Carbapenemase-encoding genes are associated with diverse plasmid platforms, of which some have spread globally, across unrelated *K. pneumoniae* clones, remaining KpSC and KoSC members, and other *Enterobacterales* (11, 29, 44). The global expansion of KPCs in the early 2000s was driven by $IncFII_K$+/-$IncFIB_K$ pKpQIL-type plasmids, carried by the pandemic *K. pneumoniae* clonal group (CG) 258, arisen from ST258 (106). These molecules, with the typical resistance backbone containing *bla*$_{KPC-2}$ or *bla*$_{KPC-3}$ located in Tn*4401* isoforms and often *bla*$_{TEM-1}$- carrying Tn*3* transposon, have been variations of the firstly sequenced pKpQIL of ST258 from Israel (178). The other widespread *bla*$_{KPC}$-carrying plasmids are pKPN3 derivatives, formed as a result of IS*26*-mediated recombination between two co-existing IncFII-like plasmids: pKPN and pKpQIL (179). Apart from $IncFII_K$, *bla*$_{KPC}$ genes have also been associated with IncR-, IncN- or IncX3-type backbones, demonstrating broad host range and responsible for horizontal transmission of KPCs to other *Enterobacterales* (29, 30). The extensive intra- and inter-species expansion of OXA-48-type enzymes resulted mostly from the dissemination of pOXA-48a-like IncL-type plasmids (112). These usually do not contain any additional AMR genes, but are highly transmissible. As it has been demonstrated, insertion of the *bla*$_{OXA-48}$-carrying Tn*1999* transposon into a *tir* region has increased remarkably the mobility of these plasmids (180). The NDM-, VIM- and IMP-encoding genes have been incorporated into various plasmid backbones, of which some played an important role in regional or international dissemination of these carbapenemases (30). For example, *bla*$_{NDM-1}$ genes have been independently acquired by broad-host range IncA, IncC2, IncHI1, IncX3 or IncN2 molecules, which often co-carried additional AMR genes conferring MDR phenotypes (29, 127, 181). Multiple resistance determinants are located also on *bla*$_{VIM}$-encoding epidemic IncN-like plasmids, circulating in Greece in early 2000s (182). A significant role in the broad spread of *bla*$_{VIM-1}$ genes in Europe has been played by a specific lineage of IncA-type plasmids, identified first in Italy (183).

Plasmids are also major platforms aggregating key *K. pneumoniae* genetic virulence determinants. The loci *iuc*, *iro* and *rmpA/A2* are often co-localised on typically large, non-conjugative $IncFIB_K$ molecules, referred to as "*K. pneumoniae* virulence plasmids", KpVPs (154). The predominant structures, KpVP-1 (with an additional IncHI1B replicon) and KpVP-2, have been stably maintained within the *K. pneumoniae* hypervirulent lineages CG23, CG86, CG65, and CG380 and CG66 respectively (11). Apart from these two canonical molecules, mosaic structures combining fragments of KpVP-1 fused with conjugative AMR plasmids (27,

184), or virulence plasmids carrying individual conjugative machinery have been described (154). Despite the essential role of the plasmid-borne virulence loci, the most common virulence-associated MGEs in *K. pneumoniae* have been self-transmissible ICEs, namely ICE*Kp*s. These structures have mobilised *ybt*, and occasionally *clb* and *iro* loci, and are integrated at the *attO* site, present in four closely-located tRNA-*Asn* copies in the bacterial chromosome. The ICE*Kp*s are bounded by direct repeats at both extremities. Their 5' end is composed among others by the P4-like integrase gene *int, ybt* locus, and *mobBC* module, whereas the diverse clusters of cargo genes used in the ICEs classification are located at the 3' end (155).

4.5 Population structure and clonality of *Klebsiella* spp.

K. pneumoniae has a relatively large genome, of about 5-6 Mbps (megabase pairs) in size and comprising 5,000-6,000 genes, of which ~1,700 are core genes present in all the species members, whereas the remainder are variably dispersed in population as accessory genes. The resultant pangenome, comprising the sum of all core and accessory genes circulating in population, is highly diverse and estimated at more than 100,000 protein-coding sequences. The majority of the accessory genome is present in <10% of *K. pneumoniae* genomes, and is commonly shared with other bacterial species, mostly the remaining *Klebsiella* species and other *Enterobacterales* (25). An extensive phylogenetic analysis of *K. pneumoniae* revealed, that the population is composed of hundreds of deep-branching lineages , differing each from other by ~0.5% nucleotide divergence (25, 142), typically referred to as clones (11). The nomenclature of clones is based on seven-loci multilocus sequence typing (MLST) scheme, which allows to define STs. Subsets of related STs are grouped into CGs. Historically, CGs in different species were defined based on individual MLST schemes and included one central genotype with its single- and double-locus variants (SLVs and DLVs, respectively) (185). More recently, the CG definition for *K. pneumoniae* has been based on the core-genome MLST (cgMLST) scheme, allowing for the profound determination of individual phylogenetic lineages within existing STs (186). The majority of *K. pneumoniae* clones have been of local range and spread in a given time and place. However, a subset of lineages of wide geographical distribution, particularly those resistant to oxyimino-cephalosporins and carbapenems, contribute notably to the global *K. pneumoniae* disease burden and are considered as "global problem clones" or "high-risk clones" (25, 187) (Figure 2). In each locale, the predominant number of *K. pneumoniae* HAIs and outbreaks are caused by a relatively small subset of clones,

usually with AMR/MDR phenotypes (cKp). These are distinguished from hvKp clones causing the majority of severe CAIs on the basis of the accessory gene content, *i.e.* the presence of acquired AMR genes or key virulence loci, respectively. A genomic evolutionary analysis of the most widespread *K. pneumoniae* lineages highlighted crucial genetic features differentiating these two subsets of the population. Strong genetic diversity, frequent chromosomal recombination leading to *e.g.* extensive K antigen loci variety, frequent acquisition and loss of plasmids and phages has been observed in cKp AMR/MDR clones, in contrast to rare chromosomal recombination, significant depletion of pan-genome diversity, and reduced plasmid diversity in hvKp clones (26). Among the global high-risk MDR clones, an important group are: ST258, ST11, ST395, ST147, ST307, ST101 and ST15 (11, 29).

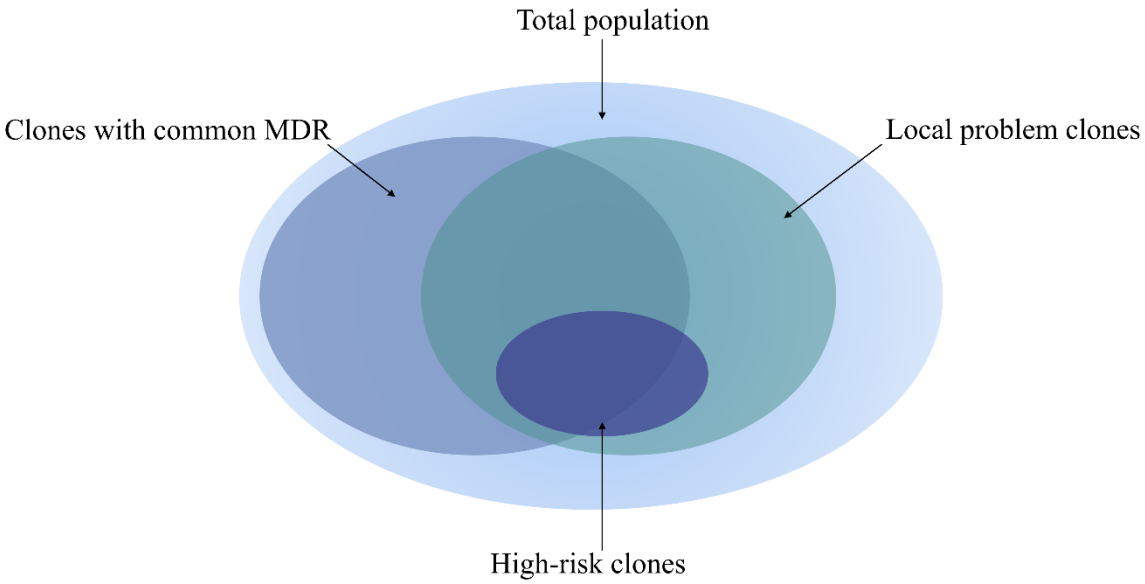

Figure 2. Population structure of *K. pneumoniae*, adopted from Wyres et al. (11)

The most recognizable and widespread member of CG258 is ST258, a hybrid clone emerged by recombination between ST11 and ST442, and inheriting 80% and 20% of genome from these two organisms, respectively (188). ST258 has been reported as a notorious clinical problem clone since the early 2000s, after acquisition of the *bla*$_{KPC}$–carrying pKpQIL plasmid backbone, facilitating successful dissemination across continents. Over time, ST258 has divided into two separate clades (189). The ST258 clade I evolved from clade II by recombination of a ~215 kb region, including *cps* genes, responsible for the biosynthesis of the capsular polysaccharide (K antigen) (188).

Although ST11 shares 6/7 MLST loci with ST258 and was historically recognised as a CG258 member, it should be considered separately as an older phylogenetic lineage, comprising isolates differentiated into three clonal groups: CG11, CG340 and CG3666, and often associated with ESBLs (186, 190). ST11 demonstrates a likewise potent association with KPCs, being the predominant KPC-carrying *K. pneumoniae* in China (191); however, in other parts of the world, such as Central or Western Europe, ST11 has been an important producer of NDMs or OXA-48 types, respectively (192, 193). Another genotype, which evolved from ST11 by a large-scale recombination and was historically assigned to CG258, is ST395 (194). First reports on this ST referred to an outbreak of OXA-48-producing isolates in a French hospital from 2010 (195). Since then, ST395 OXA-48-positive organisms have been observed in many countries in Europe, North Africa, Southeast Asia or Middle East. Additionally, ST395 has been reported also with other carbapenemases: KPCs in China and Italy, or NDMs in Russia and Germany (196).

ST147 is the best-known representative of CG147, comprising also such STs as ST392 (197). Recently ST147 emerged as a prominent global genotype, the spread of which had been driven primarily by the acquisition of plasmids with the *bla*$_{\text{CTX-M-15}}$ ESBL gene and fluoroquinolone resistance mutations (similar to other *K. pneumoniae* clones) in the early 2000s(198). The further spread of ST147 was accelerated by acquisition of carbapenemase-encoding plasmids later in the first decade of the 2000s, and resulted in a wide geographic distribution across all continents and with remarkable endemic rates in many countries (187, 199). The particularly affected regions have been North Africa and European Mediterranean countries, considered as endemic for ST147, including genotypes producing VIMs, NDMs, KPCs or OXA-48 types (199-201). An in-depth phylogenetic analysis of a global collection of CG147 revealed that ST147 has been divided into two separate clades, differing by capsular loci. The major ST147-K64 clade of global range, emerged likely around 1994 and has been associated with different carbapenemases, varying between geographic regions. The minor clade, ST147-K10, which emerged around 2002, has disseminated mainly in Asian countries and has been linked with NDMs and OXA-48 types. (197).

The major representative of CG307 is ST307, recognised as global spreader of ESBLs, due to strong association with *bla*$_{\text{CTX-M-15}}$-carrying plasmids (85), and carbapenemases, including KPCs, NDMs, OXA-48s and VIMs, with endemic rates in many countries (199). First clinical reports on ST307 isolates date back to around 2010 and soon after these organisms were observed almost all around the world (202) (203). KPC-producing ST307 genotypes have been disseminating in Italy (204), USA (205), Columbia (206) or South Korea (207). A spectacular

regional spread of CTX-M-15-producing organisms, of which a high proportion co-expressed also KPCs was described in the USA (Texas) (208). Apart from KPCs and ESBLs (CTX-M-15), ST307 has been reported also as an NDM- or OXA-48-type spreader (206-209). A comprehensive molecular dating and phylogenetic analysis of ST307 indicated emergence of this ST around 1994 and existence of its two deep-branching lineages. The first of them has been distributed globally, being encountered in Europe, Middle East, Asia, Australia and the Americas, whereas the second one has comprised organisms only from Texas so far. Samples from Texas were present also in the global branch, indicating USA as a potential place of origin of ST307 (203). Interestingly, ST307 has been recently displacing CG258 in the Americas and Southern Europe (85).

Of other "global problem" *K. pneumoniae* STs and/or CGs, ST101 has been found recently to be a significant cause of carbapenem-resistant (CR) *K. pneumoniae* infections in Europe (210), with a growing role of KPC-producing organisms *e.g.* in Italy (211) or OXA-48 producers in Czechia (172). In other parts of the world, *e.g.* South America (212, 213), Asia (214) and Africa (215), ST101 has been observed with diverse carbapenemases, especially NDMs or OXA-48 types. CG15, especially ST15, has been another important factor of *K. pneumoniae* infections of global presence (216). CG15 members have contributed also in spread of different ESBLs and carbapenemases around the world (11, 210). ST15 was involved in the intercontinental spread of OXA-48-type carbapenemases (193, 217), whereas ST14 has been described as a relevant NDM -producer in Europe and/or Asia (218-220).

In contrast to the MDR cKp clones, severe community-acquired hvKp infections, such as pyogenic liver abscess, pneumonia, meningitis or endophthalmitis, in all geographic regions have been usually dominated by the same, small subset of lineages, with the most common CG23, CG65 and CG86 (11). Extensive phylogenetic analysis of *K. pneumoniae* population revealed, that some virulent clones have been circulating among humans far longer than the well-known MDR clones. CG23, the most recognizable representative of hvKP, highly diffused in the Asian Pacific Rim, is associated with the serum-resistant K1 capsule. Another characteristic of the CG is strict connection with particular virulence factors, such as aerobactin (Iuc) and salmochellin (Iro) siderophores co-located on KpVP-1 plasmids, as well as with colibactin (Clb) encoded by the ICE*Kp* elements. Differentiation of individual CG23 sublineages was strictly associated with acquisition of the specific ICE*Kp*s. It has been estimated, that the first CG23 organisms emerged in the late 19th century, whereas CG23-I, the most widespread sublineage segregated around 1928, following the acquisition of colibactin-encoding ICE*Kp10*, decades before the liver abscess epidemic in the 1980s (221). Clb, an

attribute of CG23-I, has been recognised as an important factor enhancing epidemiological potential of the lineage, due to the promotion of gut colonisation and metastatic spread (156, 157). ST23, a major representative of CG23, is a well-known agent of severe infections in humans, with the geographic distribution primarily limited to Southeast Asia (153), but currently expanding also to other regions (222-224). Although the vast majority of CG23 isolates are in general susceptible to clinically relevant antimicrobials, a growing number of strains acquire various AMR genes, including those coding for ESBLs and carbapenemases (11, 153). Compared to CG23, CG65 and CG86 are much less frequent. Both CGs share common characteristics, including K2 capsular serotypes and KpVP-1 plasmid backbone, and the majority of infections are also reported from East and Southeast Asia. In contrast to CG86, CG65 is correlated with ICE*Kp10* element (153). On the other hand, CG86 demonstrates wider geographic distribution (225-227), and slight predisposition for animal infections (228). Similarly to CG23, a growing trend of AMR is being observed among CG65 and CG86, including CP isolates (229, 230).

The evolution of *K. pneumoniae* has proceeded via two separate pathways; a distinct subset of clones developed as the cKp pathotype, while another as hvKp. As it was described above, a number of MDR cKp lineages have successfully conquered all continents, whereas the less diverse hvKp clones have circulated mainly in Asia, though being repeatedly exported to other parts of the world. However, this epidemiological situation and the established population structure seems to have been recently disturbed. The growing number of scientific reports have been highlighting the on-going and worrisome phenomenon of "convergence" of the peculiar high-risk clones. On the one hand, the MDR cKp lineages, such as CG11, CG147, CG307 or CG101, have been acquiring virulence plasmids from the hvKp clones, and on the other, the canonical hvKp lineages CG23, CG65, or CG86 have been increasingly identified as AMR/MDR organisms, including CPE. A substantial role is being played also by hybrid virulence/AMR plasmids circulating more and more globally (11, 187).

KoSC has been notoriously overshadowed by the predominant KpSC, therefore, the knowledge on its population structures remains inadequate. Large discrepancies concern even such a fundamental issue as genome composition. According to a recent WGS-based large-scale analysis of clinical *K. oxytoca* isolates, the size of pan-genome of the species was estimated at more than 35,000 genes, among which 4,062 were recognised as core genes (~12%) (231). Another study, focused on a more diverse group containing also other KoSC species, distinguished pan-genome composed of almost 18,000 genes, with nearly 3,000 core genes (~17%) (59). Similarly to KpSC, the clonal structure of KoSC is composed of hundreds

of clones (44, 47), defined based on the 7-loci MLST scheme (232). The reference PubMLST database has discerned over 880 KoSC STs (as of the 1st of March 2026), but in contrast to KpSC, no "high-risk" clones, participating disproportionally in the global disease burden have been discerned so far. However, the international *K. oxytoca* clonal complex 2 (CC2) has been considered as a significant ESBL (233) and carbapenem host in many geographic regions (44). This finding seems to be consistent with the NCBI GenBank repository, in which three most numerous KoSC STs (ST2, ST199 and ST176), have been representatives of *K. oxytoca* CC2. Small hospital outbreaks caused by NDM-1-producing ST2 were recently reported in Denmark (234) or with different carbapenemases in Argentina (235). Predominance of ST2 carrying different carbapenemases has been observed also in a countrywide study from Spain (236). ST199 has been recorded among clinical samples in Denmark, Switzerland, the United Kingdom (231) and USA (237). ST176 has been noticed in Switzerland, Spain, Denmark, Sweden, Australia and USA (231, 238). Apart from CC2, a further relatively common *K. oxytoca* ST is ST145. It has been observed in a 4-years analysis in Spain as an important VIM-1 producer (239), whereas another multi-centre study from China indicated ST145 to be common in that country (240).

4.6 Molecular epidemiology of carbapenemase-producing *Klebsiella* spp. in Poland

Since 2005, changes in carbapenem resistance trends in invasive *K. pneumoniae* strains in Europe have been continuously monitored as a part of the Antimicrobial Testing Leadership and Surveillance (ATLAS) program, managed by the ECDC (European Centre for Disease Control and Prevention)-associated European Antimicrobial Resistance Surveillance Network (EARS-Net). Similar to other Central and Western European countries, the percentage of CR invasive *K. pneumoniae* strains in Poland was marginal at that time (<1%). A slightly growing trend was observed in the period 2011-2016, when this value was between 0.5-2.1%. A systematic, significant year-by-year increase was visible from 2017 to 2020, when the percentage of resistant strains was estimated at 6.4-8.2%. Then, a dramatic and rapid growth up to 19.5% of resistant strains occurred in 2021, and was most probably caused by the negative impact of the COVID-19 pandemic on the health-care system. Since that time to the end of 2024, the percentage of CR invasive *K. pneumoniae* strains remains at a constant level between ~16% and ~18% (https://atlas.ecdc.europa.eu/public/index.aspx). The routine CPE surveillance in Poland has been conducted by the National Reference Centre for Susceptibility Testing (NRCST) in Warsaw since 1997, and in general the NRCST data have been congruent with the

EARS-Net observations, even though the Centre has recorded a new increase tendency in 2024. According to the NRCST, for the entire CPE history in Poland, *Klebsiella* spp. have been predominant contributors to the entire CPE population (https://korld.nil.gov.pl).

The first CPE in Poland was a VIM-1-producing CG147 *K. pneumoniae,* identified in 2006 in Bydgoszcz (241). Till the end of 2008 the number of confirmed VIM cases in Poland was marginal (n=1-5), and then a gradual growth was observed from 2009 (n=22) to reach the level of 36 cases reported in 2012. The substantial role in VIMs' expansion at that time was played by *Enterobacter* spp., comprising 52.9% (n=64) of the 121 study isolates in the first national VIM/IMP CPE molecular analysis done by Izdebski et al. (242). The second most numerous taxon was *K. oxytoca,* (n=23; 19.0%), represented largely (n=19; ~82.6% of all KoSC) by the ST145 lineage which was observed in the central and western regions of the country since 2009. All ST145 isolates carried a $bla_{\text{VIM-1}}$-containing In237-like integron located chromosomally (242), identified originally in 2001 in *E. coli* from Greece, one of the earliest enterobacterial VIM producers ever detected (243). Another characteristic of this clone was the presence of the AmpC enzyme CMY-31, reported also in *K. oxytoca* circulating in Greece in the early 2000s (244). These two findings suggested mid-southern Europe to be a possible site of the original emergence of the VIM-producing *K. oxytoca* ST145, transmitted further to other parts of the continent (242). Interestingly, KpSC contributed only in 9.1% to the VIM CPE from 2006-2012, being surpassed even by *Serratia marcescens* (16.5%). However, this has changed in coming years. The following study of Polish VIM/IMP CPE by Izdebski et al. (245) was a genome-based epidemiology analysis, comprising all 934 isolates from 2006-2019. The predominance of enterobacters was maintained (n=365; 40.1%), but the second position was occupied by KpSC (n=214; 22.9%), followed by KoSC (n=106; 11.4%). This has indicated rapid spread of VIM-producing KpSC, mainly *K. pneumoniae* in 2013-2019.

The first KPC-producing *K. pneumoniae* isolate in Poland was identified in May 2008 in Warsaw. Subsequently, next 33 cases were identified by the NRCST till the end of 2008, followed by 86 isolates in 2009. Most of the isolates were from the metropolitan area of Warsaw, and all of these were included in the first molecular characteristics of KPC CPE done by Baraniak et al (246). The most numerous species in this study was *K. pneumoniae* (n=114; 95.8%), whereas *K. oxytoca* was reported sporadically (n=3; 2.5%). The *K. pneumoniae* population was dominated by ST258 (n=111; 97.4%), carrying mainly $bla_{\text{KPC-2}}$ located in the Tn*4401a* transposon variant. The remaining STs were ST11 (n=2; 1.8%) and ST23 (n=1; 0.9%). An important characteristic of all *K. pneumoniae* ST258 isolates were bla_{KPC}-carrying plasmids assigned to the pKpQIL-type. The epidemiological situation of KPCs in the country has

worsened in the following years. The second molecular analysis of KPC CPE done by Baraniak et al. (247), demonstrated further dissemination of ST258/ST512 genotypes in Poland from 2010 to 2014. The study focused on 169 KPC-producing *K. pneumoniae* isolates collected outside the Mazowsze region. The majority of these new cases (n=156; 90.2%) were collected from four regions: Świętokrzyskie, Lubelskie, Podlaskie and Śląskie. Several outbreaks reported in the main cities of Lubelskie (Lublin) and Świętokrzyskie (Kielce) were caused by the ST258-clade II organisms, carrying *bla*$_{\text{KPC-3}}$ in the Tn*4401b* transposon variant, located in ~80kb IncI2-like plasmids. Two different ST258 subclones spread in Podlaskie, including ST258-clade II with *bla*$_{\text{KPC-3}}$-carrying Tn*4401a* on IncFII-like plasmids, and ST258-clade II with *bla*$_{\text{KPC-3}}$-harbouring Tn*4401b* on various plasmids. The rapidly progressing outbreak in Śląskie was driven by *K. pneumoniae* ST512-clade II, carrying *bla*$_{\text{KPC-3}}$ in Tn*4401a*, residing in pKpQIL-type or IncX3 plasmids. Interestingly, the region Mazowsze with Warsaw reported a gradual decrease of KPC cases following the 2008-2009 outbreak (247). The remarkable changes in the KPC epidemiology observed in 2010-2014 in Poland demonstrated conversion of the localized regional spread of ST258-clade I organisms into the multiregional parallel spread of several individual *K. pneumoniae* ST258/ST512-clade I/II genotypes. Unfortunately, the knowledge on the molecular identity of KPC-producing *Klebsiella* spp. in Poland in following years is fragmentary. Data from the National Health Programme, supported by the Polish Ministry of Health, regarding AMR in *K. pneumoniae* isolates suggested the on-going clonal replacement of the CG258 organisms by ST147, ST15, ST392 and ST307 (248).

The epidemic of NDMs in Poland was initiated by an interregional outbreak of *K. pneumoniae,* with epicentres in Poznań and Warsaw (249). In November 2012, few cases of *K. pneumoniae* ST11 NDM-1 were reported in a hospital in Poznań, and after several months an outbreak was recorded in that city and its administrative region Wielkopolskie. The first identification of *K. pneumoniae* ST11 NDM-1 in Warsaw in July 2013 was preceded by admission of a patient previously hospitalised in Poznań. As it has been described by Baraniak et al. (249), *K. pneumoniae* ST11 NDM-1 was the absolutely predominant genotype (n=370; 98.9%) among all 374 NDM-producing *Enterobacterales* identified in Poland from 2012 to 2014. Its representative isolates revealed high clonal microdiversity confronted with uniformity in *bla*$_{\text{NDM-1}}$-carrying Tn*125* derivatives, located on IncFII(pKPX-1) and/or IncR plasmids. The same molecular markers were observed also in a *K. pneumoniae* ST11 isolate identified in Czechia in 2013 and may have suggested a possible spread of a specific ST11 NDM-1 subclone in Central Europe. A subsequent massive increase in NDM CPE, observed in 2015-2016, was driven mostly by the further expansion of the *K. pneumoniae* ST11-Tn*125*A (n=2094; ~98% of

all NDM CPE), with *bla*$_{NDM}$-like genes located on IncFII(pKPX-1)-type plasmids mainly (192). The cases occurred in 13/16 administrative regions of the country, mostly in Mazowieckie and Warsaw, with several hospitals reporting even more than 200 cases over that time. The second, though much less common NDM spreader was *K. pneumoniae* ST147-Tn*125F* (n=18; 0.8%), closely related to the genotype imported in March 2015 from Tunisia with victims of a terrorist attack (250). The cases were recorded in the same centre in Warsaw, which treated the patients from Tunisia; others were recorded in other institutions in that city or more distant locations (192). The first genomic analysis of representative *K. pneumoniae* ST11-Tn*125*A isolates identified from 2012 to 2018 all over Poland, done by Izdebski et al. (251), showed a strictly epidemic character of the dissemination, the isolates differed remarkably from other, epidemiologically non-related Polish *K. pneumoniae* ST11 strains. The SNP-based phylogeny analysis in the GenBank context, revealed the international character of this ST11 sublineage, with similarities to isolates identified in Greece, Bulgaria, Italy and other countries. The predominant role of *K. pneumoniae* ST11 in the further expansion of NDMs in Poland was visible in the period September 2021 – January 2022, when ST11 was still the major NDM carrier (n=48, ~94.1%) (248).

Over the years, the number of OXA-48-type CPE in Poland was far behind those with NDM- or KPC-like carbapenemases. Merely 54 OXA-48-type CPE were identified in Poland in the period 2013 - January 2017, all of which were included in the first countrywide molecular characteristics of such organisms, done by Izdebski et al. (252). The study demonstrated that the major role in the spread of OXA-48-type was played by *K. pneumoniae* (n=37), comprising 68.5% of the study isolates grouped into 11 STs. The most prevalent was ST395 (n=23; 62.2% of all *K. pneumoniae*), followed by ST15 (n=3; 8.1%), ST101 and ST152 (n=2 each; 5.4%). ST395 caused a multicentre outbreak, mainly in the Cracow area, including one hospital with 13 patients affected. *bla*$_{OXA-48}$ genes were located mostly in the Tn*1999.1* transposon variant, carried mainly by IncL-type plasmids. The genetic diversity of the ST395 and other *K. pneumoniae* isolates, revealed in the PFGE analysis, most probably arose from multiple foreign imports, as confirmed for 11 study isolates (252). The meaningful and systematic increase of OXA-48-type producers has been observed since the end of 2017 (Izdebski et al., unpublished), when the yearly number of cases (n=72) exceeded the total number from the period 2013 – January 2017 (n=54). Such trend has intensified in the following years, with an unprecedented growth in 2021 and 2022. Overall, after February 2017, till the end of 2022, the total number of OXA-48-type CPE achieved almost 1200 isolates, which was more than 20 times more (Izdebski et al., unpublished) than by January 2017(252). After February 2017 OXA-48-type

producers disseminated countrywide, but a large part of them was recovered from southern provinces: Śląskie and Małopolskie. An overwhelming number of isolates were assigned to *K. pneumoniae* (n=697; 86.9%). The *K. pneumoniae* isolates revealed diversified clonal structure, as reflected by 24 pulsotypes, with four major ones. Three of these were assigned to ST395 (n=374; 53.7% of all *K. pneumoniae*), the predominant ST from the early period, and all had been observed at that time (252). Following February 2017, the ST395 pulsotypes occurred with different frequencies in Cracow, and caused additionally regional outbreaks in Podkarpacie, Pomorze and Wielkopolskie. The latter one was especially spectacular by its size, and it originated from a major regional COVID-19 hospital in there. The last of the four major pulsotypes represented ST147 (n=189; 27.1%), and was detected for the first time from an outpatient in Cracow. The remarkable spread of ST147 isolates was observed from 2019 to 2020 in Śląskie, mainly in a single centre. Starting from March 2022, the beginning of the full-scale war in Ukraine, the OXA-48-type CPE isolates have been frequently identified from Ukrainian refugees (Izdebski et al., unpublished).

This doctoral dissertation includes four original articles addressing in details individual aspects of molecular epidemiology of the major carbapenemase-producing KpSC and KoSC clones observed in Poland in recent years.

The first paper (#1) (253) attempts to estimate the scale of the spread of NDM-1-producing *K. pneumoniae* ST147 of Tunisian (Mediterranean) origin in Poland, since its introduction in March 2015 (250). In order to identify potential representatives of that, polymorphism of the Tn*125*-like elements, characteristic for different NDM-producing *K. pneumoniae* clones and genotypes, was used as a screening marker. PCR mapping was performed for all 8925 NDM-producing *K. pneumoniae* isolates, collected by the NRCST from April 2015 to the end of 2019. As a result, the presence of a Tn*125*F variant identified in two index Tunisian isolates, was revealed in 126 cases. The PFGE analysis demonstrated that all the 126 isolates shared the same pulsotype as the Tunisian index strain, therefore, all were assigned to a single ST147 branch. Two additional ST147 NDM-1 isolates from 2016 of the same pulsotype but with different Tn*125* variants (O and J, respectively), were also included in the study. Short-read WGS was performed for 47 representative isolates of ST147-Tn*125*F from almost all hospitals and the entire period, and for the two ST147-Tn*125*O/J strains. Furthermore, six isolates were sequenced with long-read sequencing. As it has been shown, the ST147-Tn*125*F organisms spread through the country since March 2015, affecting 6/16 administrative regions. The cases were recorded in 33 hospitals in 20 cities, mainly in Warsaw (n=94; 74.6%) and predominantly in the index centre (Warsaw IV; n=75; 59.5%). The

collection of 126 isolates constituted 1.4% of all 8925 NDM-producing *K. pneumoniae* identified in Poland from April 2015 – 2019, and were "masked" by the predominant ST11 Tn*125*A lineage. Moreover, the actual incidence rate of ST147-Tn*125*F might had been significantly underestimated due to necessary restrictions in the NRCST surveillance programme, discussed in details in the paper. The genomic analysis showed that the entire group of 49 isolates shared O2v1 LPS and KL64 capsular antigens. The in-sample clonality analysis demonstrated the general relatedness of all isolates, but also revealed a cluster of higher homogeneity. The cluster (n=41; ~83.7%) comprised two index isolates of the Tunisian origin, and those originated from 24 medical centres located mainly in Warsaw, including the Warsaw IV centre, where the index patients had been hospitalised. Based on the above, the cluster was interpreted as a result of an on-going outbreak, being a direct result of the terrorist attack in Tunisia in March 2015. The remaining seven Tn*125*F isolates, plus one with Tn*125*J, were located apart of the outbreak cluster as related outliers. The phylogenetic analysis in the international context, positioned the Polish study sample within the KL64 phylogenetic lineage, together with 183 international KL64 ST147 genomes, of which 35 were NDM-1 producers, including 11 with the Tn*125*F structure. The closest relatives of the sample were Tn*125*F-carrying isolates from Tunisia, Egypt and France, indicating the Mediterranean origin of the genotype. The high level of genetic homogeneity of the study sample was visible also in plasmid replicon profiles, consisting mostly $IncFII_K$-, FIB/pKPHS1- and IncR-like replicons. Interestingly, identical plasmid profiles were observed also in the relatives from Tunisia, Egypt and France. Long-read sequencing of six representatives of different plasmid profiles, revealed the structure of bla_{NDM-1}-carrying plasmids. The outbreak cluster isolates, two isolates from Tunisia and a single non-outbreak case carried Tn*125*F with bla_{NDM-1} on $IncFII_{K2}$+$IncFIB_K$ plasmids of different sizes. The second non-outbreak isolate had Tn*125*F on a ~176.5-kb plasmid of the $IncFII_{K2}$+FIB_K+R multireplicon type, whereas a ~148.7 kb IncC-like molecule with bla_{NDM-1} in Tn*125J* was found in the other non-outbreak isolate with the most different plasmid profile. The study was one of the largest genome-based analyses of *K. pneumoniae* ST147 genotype at that time, demonstrating continuous spread of the organism in Poland since 2015, being a consequence of its import from Tunisia (250) but also probable repeated introductions from the Mediterranean basin The study sample presented high level of genetic similarity observed on SNP level, which proved a recent start of its expansion in the country, and in general the relatively recent emergence of the ST147 KL64 Tn*125*F genotype in its endemic reservoir in North African countries.

The main objective of the second (#2) article (254) was to exhibit the genomic characteristics of VIM-producing KpSC population in Poland from 2006 to 2019. The study comprised 214 isolates, collected by the NRCST from 86 medical centres located in 49 cities of 14/16 regions of the country. All isolates were subjected to short-read WGS, and eight of them were selected to long-read sequencing. Among 40 identified STs, five were the most numerous: ST437 (n=57; 26.6% of all KpSC), ST147 (n=33; 15.4%), ST15 (n=25; 11.7%), ST277 (n=18; 8.4%) and ST392 (n=13; 6.1%). The most common *bla*$_{VIM}$-carrying integrons were In238 (n=104; 48.6%) and In916 (n=64; 29.9%), both distributed widely across the STs. The in-sample SNP-based clonality analysis demonstrated that several genotypes of *K. pneumoniae* of the all major STs contributed in eight regional outbreaks in Lubelskie, Małopolskie, Ślaskie, Wielkopolskie, Świętokrzyskie, Warmińsko-Mazurskie, Pomorskie and Kujawsko-Pomorskie voivodeships, described in details in the paper. Wider phylogenetic analysis of the main *K. pneumoniae* STs showed several substantial similarities between Polish and international genomes of the same ST deposited in the RefSeq repository. The analysis of isolates subjected to long-read sequencing showed that many of acquired AMR genes resided on plasmids of various types. Detailed structure of six *bla*$_{VIM}$-harbouring plasmids revealed a number of similarities to previously described molecules in Poland and other countries. Particularly interesting were IncA plasmids with In916, and double-replicon IncFIB+HI1B pNDM-MAR-type plasmids with In238. Isolates of the ST147 outbreak II and ST277 outbreak VII carried the IncA-In916 molecules with high homology to such plasmids from Italian *Enterobacterales* in which they had been primarily identified (183), but also to those observed in Polish *Enterobacter* spp. (245) or *Klebsiella* (163, 255). The In238-carrying IncFIB+HI1B pNDM-MAR-type plasmids, detected in the ST437 outbreak I and ST147 outbreak IV isolates, were hybrids combining fragments of the canonical KpVP-1 *K. pneumoniae* virulence plasmid (256) with a prototypic resistance plasmid pNDM-MAR (257). In fact, such molecules have been reported in Poland, but so far only with *bla*$_{NDM-1}$ genes (255). Another important *bla*$_{VIM}$-carrying MGE was a genomic island (GI), being a *clc*-type integrative and conjugative element (ICE) harbouring In1654, identified in the ST15 outbreak VI isolates. The only virulence loci observed in the study sample were *ybt*, detected in 24.3% of isolates and located in six ICE*Kp* variants in various STs, but largely in ST147. The study, together with the described below the original paper #3 regarding VIM-producing KoSC, provided extensive epidemiological analysis of VIM-producing *Klebsiella* spp. in Poland. With the exception of IncA-In916 plasmids, most of the characterised genotypes and detected *bla*$_{VIM}$-harbouring genetic elements

originated likely on-site, indicating Polish hospitals as the environment of emergence, differentiation and dynamic dissemination of VIM CPE.

The main objective of the third (#3) original article (163) was to expose the role of KoSC in expansion of VIM-type carbapenemases in Poland. The detailed WGS-based analysis was performed for 106 KoSC isolates collected by the NRCST from 2009 to 2019, of which four, representing predominant *bla*$_{VIM}$-carrying integron types were selected to long-read sequencing. The isolates were gained from 60 centres located in 35 cities of all 16 administrative regions. The highest number of cases was observed in Mazowieckie (n=56; 52.8%), with more than 32% of all isolates identified in Warsaw. The taxonomic distribution of the study sample was dominated by *K. oxytoca* (n=92; 86.6%) and followed by *K. michiganensis* (n=11, 10.4%), and *K. grimontii*, *K. pasteurii* and *K. spallanzanii* (n=1; 0.9% each). The study was principally focused on the most numerous *K. oxytoca* ST145 (n=83; 90.2% of the species and 78.3% of all KoSC isolates), highly correlated with the *bla*$_{VIM-1}$-harbouring In237-like integron. The in-sample SNP analysis of all ST145 isolates discerned a tight cluster of closely related organisms, formed by 78 In237-like carriers, indicating clonal character of their dissemination. The remaining ST145 representatives with In916 or In238 were located apart the cluster. In the international context, the Polish ST145-In237-like isolates formed an individual phylogenetic lineage positioned separately from the Polish ST145 with In916 and In238, and from the few international ST145 genomes from China, the United Kingdom, United States and Spain. Interestingly, a significant fraction of the ST145-In237-like outbreak organisms (n=24; 30.8% of all ST145-In237-like) lacked the natural β-lactamase *bla*$_{OXY}$ gene, being considered as core for all KoSC members and historically used for species determination (44, 67). The comparison performed for all *bla*$_{OXY}$-negative genomes against the oldest *bla*$_{OXY}$-positive representative subjected to long-read sequencing showed that loss of the *bla*$_{OXY}$ gene was a result of a series of various independent chromosomal deletions. Interestingly, a single ST145 isolate from China was also *bla*$_{OXY}$-negative. The study demonstrated also genetic variability of the chromosomal loci comprising the In237-like integron in the outbreak ST145 organisms. In a *bla*$_{OXY}$-positive isolate the In237-like element was a part of a unique mosaic region (MR). The region contained numerous additional AMR genes, followed directly by a phage-like segment (‘Phage 1’), inserted downstream of the *bla*$_{OXY}$ gene. A similar scheme of the ‘Phage 1’-MR was observed in the *bla*$_{OXY}$-negative representative, but another phage-like structure was located directly behind the MR, replacing the fragment of the chromosome containing originally the *bla*$_{OXY}$ gene. The detailed structure of *bla*$_{VIM}$-carrying plasmids was revealed for two non-outbreak *K. oxytoca* ST145 isolates with

In916 or In238 integron. Similarly as in paper #2, In916 was located on a ~134-kb IncA-type plasmid of high homology with the previously identified IncA-In916 molecules from Polish *Enterobacter* spp. (245) and *K. pneumoniae* (254, 255), and a set of plasmids from Italian *Enterobacterales* (183) and *Aeromonas* from the Netherlands (258). Major differences between these resulted from multiple rearrangements within the AMR mosaic region containing an IS*26*-*bla*$_{\text{SHV-12}}$-In916-IS*26* module. In238 was assigned to a ~23-kb plasmid of unknown replicon, comprising regions specifying plasmid replication, stability, conjugal transfer, and type I and II toxin-antitoxin systems of generally low similarity to few international molecules only. The presence of pathogenicity- and virulence-encoding loci characteristic to KpSC varied among the study sample. The LPS O-antigen and the yersiniabactin locus were observed broadly (in 98.1% and 88.7% isolates, respectively). The kleboxymycin biosynthesis cluster was detected with lower frequency (37.7%), whereas the CPS K-antigen locus was identified only occasionally (4.7%). The study contributed largely to epidemiology and genetics of KoSC, recognised as a significant VIM producer in Poland, positioned as the third most common taxon among all VIM CPE in 2006-2019. The substantial part of the work concerned the ST145-In237-like organisms, emphasizing their specific characteristics, usually not observed among the remaining VIM CPE, neither in Poland, nor abroad. The long-term clonal expansion of the ST145-In237-like genotype was assumed to be one of the most extraordinary phenomena observed in the entire VIMs epidemiology in the country so far.

The profound genomic analysis of CPE isolates collected during the first year of the full-scale war in Ukraine from patients arriving from this country was the aim of the fourth (#4) original work (259). The study material included 65 CPE isolates obtained from 57 Ukrainian patients hospitalised all around Poland, of which all underwent short-read WGS and 10 were selected to long-read sequencing. Among six identified CPE species, the special emphasis was placed on *K. pneumoniae* represented mainly by ST395, ST307, ST11, ST147 and ST23. The study exhibited different aspects of the genetic diversification in a CP *K. pneumoniae* population. The distribution of carbapenemases varied across STs, however, the most numerous were NDMs, followed by OXA-48-type enzymes. High variety was observed also in *K. pneumoniae* K and O serotype variants. The in-sample clonality analysis of five predominant STs demonstrated well the correlation between the SNP distribution with K serotypes, and less so with carbapenemases. The lowest level of genetic diversity was revealed in ST307 isolates, all assigned to the KL102 serotype. More heterogeneous were ST395 and ST11, whereas the highest variety was observed in ST147. The phylogeny in the international context exposed close relatedness of the study ST395, ST307-NDM-1 and ST23 K57 to isolates from Russia.

What is more, the most of the study ST395, ST307, ST147 and ST23 isolates demonstrated close genetic relationship with genomes of *K. pneumoniae* recovered from Ukrainian war refugees reported in Germany and The Netherlands (260-263). Another interesting feature exposed in the study were elevated virulence scores, observed in the ST307-NDM-1 group, individual ST395 genotypes and the entire ST23 K57 group. Such characteristic was not previously observed in Polish CP *K. pneumoniae.* Similar organisms linked with Ukrainian patients were reported also in other European studies (261, 262), and were identified previously in Russia (227, 264-266). Among diverse carbapenemase-encoding plasmids, a significant fraction were IncFIB+HI1B hybrid molecules of the pNDM-MAR-type, combing homologous segments of the MDR pNDM-MAR plasmid (257) and the typical *K. pneumoniae* virulence plasmid KpVP-1 (256). Their presence explains the high prevalence of virulence loci in the *K. pneumoniae* ST395, ST307 and ST23 K57 isolates. Despite several limitations, concerning principally epidemiological randomness of the study sample and gaps in essential epidemiological information, the study characterised a set of the actual CP *K. pneumoniae* genotypes circulating in Ukraine. The results obtained were consistent with those from previous European analyses, demonstrating broad circulation of several epidemic NDM-1 and/or OXA-48-producing *K. pneumoniae* ST395, ST307, ST147 and ST23 lineages, and spread of the pNDM-MAR-like plasmids, indicating their substantial epidemic potential and risk for public health in a broad geographic perspective.

4.7 Discussion

The four original works constituting this doctoral dissertation have a significant impact on the knowledge on the CPE epidemiology in Poland in recent years. Each of these is an element of a complex and long-term series of interconnected epidemiologic reports, describing comprehensively consecutive phases of the CPE dissemination in the country. The aim of this section of the dissertation is to provide a discussion of the results of all the four articles, in the context of the international knowledge on the subject. However, it must be emphasized that the study material was recovered over a relatively long period, and that most of the analyses (#1-#3) terminated at the end of 2019. Therefore, these do not refer to the very current epidemiological situation, which has been strongly influenced by the COVID-19 pandemic in 2020-2021 and massive migration from Ukraine after February 2022. On the other hand, numerous epidemiological observations highlighted in the publications *e.g.* clonal spread of particular *K. pneumoniae* genotypes could have significantly affected the present situation.

Therefore, the unpublished data on the Poland's CPE epidemiology in 2019-2024, collected with the participation of the author of this thesis, will be referenced partially in this section as well.

All works (#1-#4) provided extensive genomic characteristic of individual KpSC and KoSC epidemic genotypes involved in the dissemination of different carbapenemases on regional, interregional or countrywide levels. Their common aim was to comprehensively describe the epidemiological phenomena associated with their spread in given periods. The critical part of each paper regarding KpSC (#1, #2, #4) was focused on the "global problem" *K. pneumoniae* clones, such as ST147, ST392, ST11, ST437, ST395, or ST307.

ST147 was described in articles #1, #2 and #4 as an important NDM, VIM and OXA-48-type spreader in Poland and Ukraine. It is considered to have emerged in the early 2000s (198, 267) and since that time its current global distribution has been documented by a large number of reports, including those on spectacular CP *K. pneumoniae* countrywide or regional outbreaks, *e. g.* in Greece or Italy (268, 269). Some regions of the world, such as North Africa, east Mediterranean basin or India, have been recognised as endemic for ST147, and constitute the reservoir for imports of the organisms to other areas (199). The example of such event was described by Izdebski et al. in the report on the ST147 NDM-1 colonization of Polish tourists – victims of the terrorist attack in Tunisia in March 2015 (250). Consequences of this transfer were analysed further in the paper #1. Molecular screening of ~9000 NDM-producing *K. pneumoniae* non-duplicate isolates, collected in Poland from April 2015 till the end of 2019, enabled elucidation of the incidence and scale of spread of the specific *K. pneumoniae* ST147-Tn*125*F genotype. In less than four years the genotype has spread in the Warsaw IV centre, where index patients had been hospitalised (n=75; 59.5%), and expanded to 33 hospitals in 20 cities, mainly in Warsaw (n=94; 74.6%), affecting 6 administrative regions of the country. Therefore, it demonstrated epidemic potential and gaps in infection prevention and control (IPC) systems of the centres; however, when compared to ST11-Tn*125*A the scale of the outbreak must have been recognised as limited. The North African (Mediterranean) origin of these organisms was confirmed by their phylogenetic analysis in the international context, which exposed the closest genetic similarity to Tn*125*F-carrying isolates from Tunisia, Egypt and France. It should also be underlined that several non-outbreak ST147-Tn*125*F isolates most probably represented repeated, independent imports of basically the same organisms from the same endemic region, *i. e.* North-African countries, being common touristic destinations. The article (#1) was one of the larger genomic studies on *K. pneumoniae* ST147 at that time. The epidemic potential of ST147 was revealed also in the publication #2, in which ST147 was

second most common among all VIM-producing KpSC in Poland (15.4%), and each of the three identified ST147 genotypes was responsible for a regional outbreak in another part of the country. Interestingly, the outbreak genotypes showed closer relatedness to ST147 OXA-48 producers from Germany, Greece or India, whereas the ST147-Tn*125*F organisms from Poland, described in #1, were more distant. The broad geographic distribution of ST147 was supported also by the paper #4, in which *K. pneumoniae* ST147 was found to be one of the major CP *K. pneumoniae* clones linked with patients arriving from Ukraine. As it has been revealed by phylogenetic analysis in the international context, some of the study ST147 organisms had the closest relatives in Russia, indicating circulation of the specific ST147 lineages over a large territory in Eastern Europe, whereas some other representatives of the study ST147 NDM-1/-5 revealed similarity to ST147 isolates from endemic regions, such as Egypt or India. Considering the entire CPE and CP *K. pneumoniae* epidemiology in Poland from 2006 to 2024, the role of ST147 should be recognised as minor until very recently, despite all the phenomena identified already in papers #1 and #2. Between 2013 and 2021, the clonal structure of CP *K. pneumoniae* in Poland was vastly dominated by the *K. pneumoniae* ST11-Tn*125*A genotype (192, 251), which *e. g.* in 2019-2021 constituted ~70% of all CP *K. pneumoniae* (Izdebski et al., manuscript in preparation). The first relevant changing trends started to occur around that time, when a remarkable spread of an OXA-48-type-producing ST147 genotype commenced especially in Śląskie. This was paralleled or followed by the dissemination of other ST147 lineages with KPC-3, NDM-1, NDM-5 or NDM-1+OXA-48. Interestingly, none of these was related to the "Tunisian" ST147-Tn*125*F genotype described in the paper #1 or to VIM producers from the study #2 but, some were clearly related to the ST147 NDM-1 or NDM-5 isolates from Ukrainian patients in 2022, reported in the paper #4. Overall, the total contribution of ST147 in Polish CP *K. pneumoniae* grew recently from 11.8% in 2019 up to 45.2% in 2024 (Izdebski et al., manuscript in preparation). This has largely affected the contemporary clonal structure of the entire CP *K. pneumoniae* population in Poland, causing rapid replacement of the former leader, ST11 (14.9% in 2024). Although, the origin of this epidemiological phenomenon has been complex, one of its driving factors could have been the migration of patients arriving from Ukraine after the escalation of war in there in 2022, and gaps in the infection control systems in Polish hospitals. Another epidemic clone, ST392, a member of CG147, was described in details in the publication #2. ST392 with the *bla*$_{\text{VIM-4}}$-carrying In1667 integron was recognised as a causative agent of an outbreak in Kujawsko-Pomorskie, recorded in 2014-2019 in seven hospitals of this region. Interestingly, no essential similarities between the study ST392-In1667 outbreak genotype and international genomes of the ST were found, indicating probably that

this individual genotype might have emerged and disseminated on site. The intercontinental spread of ST147, ST392 and other members of CG147 has been strongly influenced by acquisition of diverse carbapenemases by different lineages of these clones (270). Papers #1, #2 and #4, as well as a series of reports from other countries, and the recent, though not-published data from Poland, have highlighted this essential correlation (110, 268, 271-274). These also exposed the in-depth clonal-genomic characteristic of a number of CG147 genotypes involved in local, regional, countrywide or international spreads with NDM-, OXA-48- or VIM-type enzymes.

ST11 has been reported as highly relevant CP organism of global range, originally being associated with ESBLs (198, 275, 276) and more recently with diverse carbapenemases, including *e.g.* KPCs in China (277-279), OXA-48 types in Spain (280, 281), or NDMs in different geographic regions (193). As it was mentioned above, its crucial role in Poland was observed from 2012/2013, when in only two years, the ST11 NDM-1-producing organisms caused the largest interregional CPE outbreak in a non-endemic country (249). The further spread of the *K. pneumoniae* ST11-Tn*125*A genotype (192), representing the international lineage circulating in South-Eastern and Middle Europe (251), created a critical epidemiological problem in Poland and contributed largely to all NDM CPE and CPE overall in the country (192, 251). The predominant role of ST11 was still observed by the end of 2021 (248) (Izdebski et al., manuscript in preparation). The wider epidemiological context of the ST11 circulation, was exposed in the publication #4, in which several ST11-Tn*125*A isolates were recovered from patients arriving from Ukraine. These organisms represented the same South-Eastern European, Poland-endemic lineage, (251), therefore, the origin of the ST11-Tn*125*A isolates in the study sample could not have been unambiguously determined. Such organisms could have spread independently in Ukraine, but the possibility of their acquisition in Poland could not be excluded either, especially that most of these were recovered in Poland >48 h from admission of patients. This has highlighted the critical role of the patient screening on admission in IPC strategies, given the intensity of travelling and migration in our times. However, it should be underlined, that K. *pneumoniae* ST11 organisms have been identified from Ukrainian patients after February 2022 also in Germany (262).

Another globally distributed *K. pneumoniae* genotype was ST437, representing CG10268 based on the cgMLST scheme, which accounted for more than 25% of all VIM-producing KpSC described in the article #2. The individual ST437-In238 genotype was responsible for the regional outbreak in Lubelskie, which in only two years expanded from a single hospital to 16 other institutions of the area. The emerging character of ST437 has been

widely supported by scientific reports from around the world. *K. pneumoniae* ST437 recognised as the "global problem", having been reported in the Americas (282, 283), Europe (284, 285) or Asia (286, 287). Its strong association with KPC-type carbapenemases has been observed in Brazil (288) or in Canada (289), whereas several studies from Europe showed its important role in the spread of NDMs and/or OXA-48 types (285, 290-295). The relatively small genetic distance between all the study ST437 isolates (1-14 SNPs) over the 2-years period indicated a strictly regional dissemination of these organisms. This was further supported by the phylogenetic analysis in the international context, showing that the ST437-In238 genotype from Lubelskie, Poland, formed a separate, local phylolineage. In general, such spectacular expansions of CPE in individual regions should prompt and contribute local IPC specialists to verify existing protocols and identify gaps, that might be responsible for outbreaks. In summary, the publication #2 has demonstrated the "successful" regional spread of the VIM-4-producing ST437 genotype which has strongly influenced the overall epidemiology of VIM CPE in Poland.

ST395 has been recognised as a major OXA-48-type spreader in Poland since the beginning of the OXA-48 CPE occurrence in the country (252). Out of five OXA-48-producing ST395 genotypes identified from 2013 to January 2017, four affected the Cracow area, and one was detected in Poznań. As it has been emphasized, documented imports from Russia, Ukraine and Georgia probably initiated the occurrence of some of these genotypes (252). The especially high contribution of ST395 to all CP *K. pneumoniae* was observed in 2021-2024, which might have been additionally associated with the migration from Ukraine after February 2022 (Izdebski et al., manuscript in preparation). NDM- and/or OXA-48-type-producing ST395 was the most prevalent *K. pneumoniae* ST linked with patients arriving from Ukraine described in the article #4. The study ST395 isolates demonstrated close genetic relatedness to such organisms identified in Russia and to other ST395 representatives recovered from Ukrainian war refugees in Germany and The Netherlands (260-263). All these findings indicated broad circulation of specific ST395 lineages in Eastern Europe. The study ST395 organisms were characterised also by elevated virulence scores resulting from the presence of the hvKp loci. This finding was supported by an extensive genomic study demonstrating stronger association of ST395 isolates originating from Russia, Belarus and Italy with specific virulence loci, such as *iucABCD* and/or *rmpA/rmpA2* (196). The overall results of the genomic analysis of ST395 described in the publication #4 are consistent with other reports, and emphasize an important role of this genotype in dissemination of carbapenemases, especially NDMs and OXA-48-types over a large territory of Eastern and Middle-Eastern Europe. As it was mentioned at the

beginning of this paragraph, in 2021-2024 a number of *K. pneumoniae* ST395 isolates with different carbapenemases have been identified in Poland. Among them, five separate phylolineages, corresponding to isolates with different capsular serotypes, K2, K39, K64 and KL108, and producing NDM-1/-5 alone or with combination with OXA-48-types, revealed close similarity to the isolates previously identified in patients arriving from Ukraine, described in paper #4.

The incidence of ST307 in Poland was marginal over the years (254). The first in-depth molecular characteristic of ST307 in the country was performed in the publication #4, and regarded the strains from the patients arriving from Ukraine. The ST307 NDM-1- or KPC-2/-3-producing organisms were the second most numerous CP *K. pneumoniae* group (n=11; ~22.0%) in the study sample. The study ST307-NDM-1 cluster was the most genetically related with isolates identified in Russia, as it has been demonstrated by the phylogenetic analysis in the international context. Additionally, close genetic relatedness was observed between the study ST307 organisms and the isolates recovered from Ukrainian patients in Germany and The Netherlands, which further supported the Eastern-European origin of this lineage (262, 263). Similarly to the previously mentioned ST395 linked with Ukrainian patients (#4), the ST307-NDM-1 genotype demonstrated enhanced virulence scores. Moreover, as in case of ST147 and ST395, the increasing contribution of ST307 in Poland has been observed since 2021-2022, when it was the second most numerous genotype among the invasive *K. pneumoniae* ESBL- and/or carbapenemase-producing isolates (248). All these isolates demonstrated strong association with ESBL-encoding genes and carried *bla*$_{\text{CTX-M}}$ types, predominantly the *bla*$_{\text{CTX-M-15}}$ variant, whereas ~8.6% of them were carbapenemase producers. The particularly relevant growing trend was observed in 2024 when the total contribution of ST307 among all CP *K. pneumoniae* was estimated at more than 8% (Izdebski et al., manuscript in preparation). NDM-1- or KPC-3-producing ST307 KL102 isolates identified at that time were related to the similar isolates obtained from patients arriving from Ukraine in 2022, presented in paper #4, indicating their probable origins. *K. pneumoniae* ST307 has been recognised as the prominent global AMR spreader, strongly associated with ESBLs, especially CTX-M-15, and with various carbapenemases (199). Its emerging character has been observed *e.g.* in the USA (208) or Italy (296), where ST307 KPC producers have recently displaced CG258. The broad geographic distribution of ST307 organisms, highlighted in the publication #4, supported their high epidemic potential and indicated the necessity of further epidemiological studies, especially in the face of the potential convergence of AMR and virulence determinants

Apart from the global epidemiological phenomena, such as the spread of the "high-risk" clones of KpSC, the publication #3 focused on the more localized problem, associated with the notoriously overshadowed group, the KoSC. Members of this complex, and especially *K. oxytoca*, were recognised as significant VIM producers in Poland. In the period 2006-2019 these were placed at the third position among all VIM CPE, behind *Enterobacter* spp. and the above-mentioned *K. pneumoniae* (245). The 10-years long (2009-2019) country-wide expansion of the epidemic *K. oxytoca* ST145-In237-like genotype was recognised as one of the most spectacular phenomena in the entire VIM CPE epidemiology in Poland. Such massive monoclonal dissemination of KoSC, seems to be unique also at the international level. To the best of my knowledge, the country-wide genomic-based studies available so far, *e.g.* from Spain, Ireland or the United Kingdom have demonstrated high diversity of KoSC, with larger number of clones spreading over smaller territories (59, 236, 297). The publication #3 highlighted also the wider geographic circulation of the ST145-In237-like genotype. The genomic characteristics of the lineage, *i.e.* the *bla*$_{\text{VIM-1}}$-carrying In237-like integron, the presence of the acquired CMY-31 AmpC-like cephalosporinase, and the identical *bla*$_{\text{OXY}}$ allele indicated its likely Greek origin (244), as it was discussed in the paper. However, the lack of genomes of the VIM-1+CMY-31-producing *K. oxytoca* isolates from Greece has not allowed to demonstrate this hypothesis unambiguously, and the phylogenetic analysis in the international context described in the paper #3 had no sufficient comparative material. The limited number of the ST145 genomes in the NCBI repositories might suggest the marginal role of the clone in the expansion of AMR determinants worldwide, and the lower interest of researchers in KoSC in general. On the other hand, single reports from Spain (239) and China (240) emphasized the broad occurrence of ST145 and its contribution in local CPE populations. The further exceptional feature of the ST145-In237-like genotype emphasized in the publication #3, was the frequent chromosomal deletions containing *bla*$_{\text{OXY}}$ genes. To my knowledge, such phenomenon has not been reported so far in KoSC. Unfortunately, the hypothesis of the phage-induced deletion was based on a limited number of representatives subjected to long-read sequencing, therefore, it cannot be considered as a universal explanation. The comprehensive genomic characteristic of VIM-producing KoSC in Poland, reported in publication #3 contributed remarkably to the overall knowledge on these organisms and indicated the possible and overlooked role of ST145 and its specific outbreak ST145-In237-like genotype.

Another element linking the four articles each with other was the HGT of various MGEs demonstrated to be the major factor of the spread of carbapenemases and other AMR genes

across the *Klebsiella* species, clones and lineages. The massive spread of NDMs was a matter of great concern in Poland since 2012/2013 (192, 249, 251). As it has been mentioned, numerous derivatives of the *bla*$_{NDM}$-carrying Tn*125* transposon, differing in truncations at the 5' and 3' extremities have been widespread in *Enterobacterales* (167, 168), therefore, the polymorphism of this particular structure was extensively used as the molecular marker in articles #1 and #4. The Tn*125*F variant, characteristic to the *K. pneumoniae* ST147 genotype imported to Poland from Tunisia, was used in the screening of 8925 NDM-producing *K. pneumoniae* isolates and allowed for selection of the 126 representatives. On the other hand, the Tn*125*A element specific for the major Polish ST11 NDM producer (192, 251), was detected in several NDM-1-producing ST11 isolates from Ukrainian patients analysed the in paper #4. As it was discussed above, the presence of this peculiar Tn*125* polymorph in the study sample might suggest the parallel spread of the ST11-Tn*125*A organisms in Ukraine, or their possible acquisition on-site in Polish hospitals. Yet another Tn*125* polymorph, Tn*125*K [originally named mistakenly as Tn*125*V (255)] prevailed among the NDM-1-producing *K. pneumoniae* of different STs from patients arriving from Ukraine described in the paper #4 . Relevant associations of Tn*125*K with isolates originating from Eastern Europe have been reported in Poland already before. In 2016 it was primarily identified in a *K. pneumoniae* isolate obtained from a patient from Belarus (192). Subsequently, the same structure was determined in 2018 in *K. pneumoniae* ST23 K57 outbreak isolates from Silesia, demonstrating close similarity to isolates from Russia and Germany (255).

A particularly broad inter-species circulation was observed in case of two major *bla*$_{VIM}$ genetic platforms identified in this thesis, the In238-like and In916 integrons. In238 was identified first in a Polish *P. aeruginosa* from 1998 (298), and then it has become common in VIM-producing *Pseudomonas* spp. (299, 300) and *Enterobacter* spp. (245) in the country. The first In238-carrying CPE isolate was a sporadic *K. pneumoniae* ST11 isolate from 2008 included in the publication #2. The same report demonstrated also the role of In238-harbouring *K. pneumoniae* genotypes, ST437-In238 and ST147-In238, in the epidemic expansion of VIM-like carbapenemases in two regional KpSC populations. The second integron, In916, observed exclusively in European *Enterobacterales* from 2010s, was propagated principally on IncA-type plasmids described for the first time in Italy (183, 284, 301). The spread of such molecules was reported in Polish KpSC and KoSC in the articles #2 and #3, respectively, but also in *K. pneumoniae* ST23 (255), *Enterobacter* spp. (245), and *Citrobacter* spp. (302).

Another important group of plasmids characterised widely in this dissertation were the pNDM-MAR types. These hybrid molecules combining AMR regions (257) with segments

containing several virulence loci from the typical *K. pneumoniae* virulence plasmid KpVP-1 (154), were genetic platforms for various carbapenemase genes: *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-48}}$ (publication #4), but also for *bla*$_{\text{VIM-4}}$ (publication #2). The chronologically first were the *bla*$_{\text{NDM-1}}$-carrying pNDM-MAR-like molecules, observed for the first time in Poland in 2018 (255), which have been further relatively often recovered from *K. pneumoniae* isolates from patients arriving from Ukraine in 2022 (publication #4). The pNDM-MAR-like structures with *bla*$_{\text{OXA-48}}$ or *bla*$_{\text{VIM-4}}$ were reported for the first time in Poland in publications #4 and #2, respectively. Apart from the AMR and/or virulence loci load, these articles highlighted also the high level of similarity of the pNDM-MAR-like plasmids characterised in Poland, to such molecules found in Russia or in Germany in isolates of the Ukrainian origin, which indicates the broad spread of such structures in CPE in Eastern Europe. Finally, the *bla*$_{\text{NDM-1}}$-carrying IncFII(pKPX-1) plasmids, predominant in the endemic ST11-Tn*125*A lineage, circulating in Poland since 2012, were identified in a set of *K. pneumoniae* NDM-1-producing ST11 isolates originating from patients from Ukraine (paper #4).

The problem of virulence in *K. pneumoniae* was discussed in #4 publication. In contrast to the vast majority of the previously analysed CP *K. pneumoniae* in Poland, carrying no typical hvKp loci (251, 253), the substantial overrepresentation of the *iuc* and *rmpA/A2* loci was observed in ST23, ST307-NDM-1, and several ST395 genotypes from the patients arriving from Ukraine. Association of these STs with the hvKp genetic determinants was observed recently in Germany in isolates of the Ukrainian origin (261, 262), relatively often in Russia (227, 264-266), but also in Western Europe (303, 304). Although the occurrence of the hvKp genes in CP *K. pneumoniae* is highly worrisome, the actual virulence potential of such isolates as those reported in the article #4 requires confirmation in functional studies. Interestingly, the ST23 organisms were of the K57 capsule serotype and belonged to a distinct phylogenetic lineage than the canonical hvKp ST23 K1 clone (305). ST23 K57 isolates of high similarity to those from Russia or Germany have been sporadically reported in Poland from 2015 (255), and since 2022 this genotype has been frequently reported in Ukrainian war refugees (261-263). From 2021 ECDC recognised such organisms carrying carbapenemases genes as potential threat for public health in the EU/EEA countries (306, 307).

The genetic determinants of several putative KoSC virulence factors were analysed in the publication #3. Of the virulence-encoding loci characteristic for KpSC, the LPS O-antigen and yersinibactin loci were observed with the highest frequency in KoSC isolates described in paper #3, whereas the CPS K-antigen locus was identified sporadically. These were consistent with observations made by other researchers (47, 62), which might indicate that the above-

mentioned virulence loci, along with numerous AMR genes, constitute the common gene pool shared between KpSC and KoSC. Moreover, the frequent occurrence of the kleboxymycin-encoding gene cluster, the most well-known KoSC-specific virulence factor, has been also observed in other studies (47, 62). Despite the relatively high level of genetic similarity between the LPS O-antigen and yersinibactin virulence loci between KpSC and KoSC, the actual virulence potential of particular allelic variants detected in KoSC requires functional studies (47, 62).

This thesis has several limitations. The major one regards the retrospective character of the all papers included (#1-#4). The laboratory and bioinformatic analyses were performed done on bacterial isolates collected over longer periods, and the MDR organisms had been already diffused over remarkable parts of the country. However, the publication #4 was somehow exceptional because the study was carried out on CPE isolates obtained during the first year of the full-scale war in Ukraine. The study material reflected mostly the *K. pneumoniae* genotypes of Ukrainian origin, that might have penetrated then the local population of CP *K. pneumoniae* in Poland (as it happened, indeed). Although all the papers represented the current methodological state-of-the-art in epidemiological studies on CPE, the findings provided have not been used in molecular investigations of on-going outbreaks or local IPC strategies, which would have essentially affected their clinical impact. The real-time application of such extensive analyses, requiring wide collaboration between specialist from different areas and centres located around the Poland, would have resulted in strengthening the partnership between hospitals and the NRCST, benefiting potentially in more country-wide epidemiological studies in the future.

The second important limitation of this dissertation refers to the representativeness of the study samples, which concerns specifically the publication #4. The collection of 65 CPE isolates gathered from patients arriving from Ukraine was surprisingly small, compared to the overall large number of war refugees to whom medical services in Poland were provided (308, 309), and to the analogous collections of CPE isolates in Germany or The Netherlands (260-263). The reason for that was the low intensity of screening at admission of the Ukrainian patients in Polish hospitals. This might have resulted in the overall lower genetic diversity of the study sample, but to the best of my knowledge, the genotypes identified were broadly observed in other European studies on the CPE from Ukrainian war refugees (260-263). The value of the works may have been diminished also by the relatively low number of isolates selected to long-read sequencing, which affected especially the paper #3. An increased number of isolates for which long-reads would be obtained, could have helped to better explain of the

genetic basis of chromosomal deletions comprising KoSC-specific *bla*$_{OXY}$ genes in the *K. oxytoca* ST145-In237-like organisms. On the other hand, the range and level of the analyses performed and the methodology used were cost-effective and affordable.

In summary, each of the articles presented detailed properties of the epidemic *K. pneumoniae* and *K. oxytoca* genotypes, participating in the spread of major carbapenemases in Poland over a period of last twenty years. The implemented WGS technologies allowed for the determination of the detailed genetic characteristics and phylogenetic positions of the lineages discerned, both in the in-sample and in the international contexts. Apart from the population clonal structures, all the works provided also the detailed analysis of MGEs associated with AMR and/or virulence, especially the carbapenemase- and/or hypervirulence-encoding plasmids. The studies described visualised multiple hospital outbreaks caused by the dangerous organisms, expanding on the regional and interregional scales, which altogether revealed severe shortcomings and gaps in the IPC systems on the hospital, regional and country levels, providing a serious threat for public health in Poland.

5. Objectives of the study

The main objective of this thesis was to understand the factors and mechanisms driving a part of the expansion of CP *Klebsiella* spp. in Poland over a period 2006-2019, and during the first year of the full-scale war in Ukraine, from February 2022 to February 2023. In order to achieve this purpose, the role of such phenomena as clonal expansion of individual organisms, and HGT of MGEs carrying carbapenemase and virulence genes were to be addressed. Epidemic genotypes were to be identified and characterized in molecular and epidemiological terms, and their possible origins, including imports, were to be elucidated. Extensive molecular and genomic/bioinformatic studies of CP KpSC and KoSC isolates from Polish medical institutions were to be performed with several detailed aims as below:

- to determine the scale of the spread of the *K. pneumoniae* ST147 genotype of the Tunisian origin among the NDM-1 producing KpSC in Poland, 2015-2019; to assess the phylogenetic position of this genotype in the global *K. pneumoniae* ST147 population;
- to reveal the clonal structure of VIM-type carbapenemase-producing *K. pneumoniae* in Poland, 2006-2019; to identify and comprehensively characterise the epidemic genotypes, contributing essentially in the expansion of VIM CPE in the country;
- to re-evaluate the role of KoSC, and especially the *K. oxytoca* ST145 clone, in the epidemiology of VIM CPE in Poland in 2006-2019; to infer the phylogeny and clonality markers of the ST145-In237 genotype;
- to examine the collection of the CP *K. pneumoniae* isolates identified in Poland from patients arriving from Ukraine during the 1st year of the full-scale war; to identify and characterise the genotypes that might constitute a threat for public health in Poland upon importation.

6. Publications included in the dissertation

6.1 Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19

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Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015–19

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Objectives: To assess the spread of New Delhi metallo-β-lactamase-1 (NDM-1)-producing *Klebsiella pneumoniae* ST147 organisms in Poland since an introduction from Tunisia in March 2015, including their phylogenetic position in the global population of the high-risk clone.

Methods: Out of 8925 unique NDM-positive *K. pneumoniae* isolates identified in Poland from April 2015 till December 2019, 126 isolates, including the Tunisian imports, were related by PFGE and bla_{NDM} gene-carrying Tn*125* transposon derivatives. Forty-seven representative isolates were sequenced by Illumina MiSeq. The phylogeny, resistome, virulome and plasmid replicons were analysed and compared with the international ST147 strains. Plasmids of six isolates were studied by the MinION sequencing.

Results: A high homogeneity of the 47 isolates was observed, with minor variations in their resistomes and plasmid replicon profiles. However, the detailed SNP comparison discerned a strict outbreak cluster of 40 isolates. All of the organisms were grouped within the ST147 phylogenetic international lineage, and four NDM-1 producers from Tunisia, Egypt and France were the closest relatives of the Polish isolates. Yersiniabactin genes (YbST280 type) were located within the ICE*Kpn12*-like element in most of the outbreak isolates, characterized by O2v1 and KL64 antigen loci. The $bla_{NDM\ 1}$ genes were located in double-replicon $IncFII_{K2}$+$IncFIB_K$ plasmids.

Conclusions: The continuous spread of *K. pneumoniae* ST147 NDM-1 in Poland since 2015, largely in the Warsaw area, is demonstrated by this genomic analysis. The isolates showed a high degree of homogeneity, and close relatedness to organisms spreading in the Mediterranean region.

Introduction

New Delhi metallo-β-lactamase (NDM)-producing Enterobacterales, including *Klebsiella pneumoniae*, are one of the leading threats in infectious diseases, challenging not only South Asia and North Africa, their major endemic reservoirs, but also many other regions with continuous importation and subsequent outbreaks.[1–4] It seems most likely that the bla_{NDM} gene emerged originally in *Acinetobacter* sp. within the Tn*125* transposon, which in Enterobacterales is randomly truncated, yielding a multiplicity of derivatives.[1,5] A wide variety of plasmids harbour bla_{NDM} genes, with the most frequent replicon types being FII, C, X or R,[1,2] and these are found in a large taxonomic and clonal diversity of organisms, indicating efficient horizontal transfer of bla_{NDM}s. However, clonal spread of NDM producers has been significant in some areas too.[2,4] The *K. pneumoniae* population is dominated by a number of pandemic clones, some of which broadly disseminate resistance genes, including those encoding carbapenemases. High-risk clones that were recognized early on, such as ST11 or ST15, are genetically diverse, whereas the more recently emerged ones, such as ST258, ST307 and ST147, have been less differentiated so far.[6]

Since late 2012 a nation-wide outbreak of NDM-1-producing *K. pneumoniae* ST11 has been observed in Poland, converting into endemicity in consecutive regions.[4,7] With time, new imports of NDM producers occurred, including in March 2015, when Polish nationals who suffered a terrorist attack in Tunisia and were treated locally, were then transported to Warsaw. They were colonized by *K. pneumoniae* of the newly emerging ST147, with $bla_{NDM\ 1}$ localized in a different Tn*125* derivative from that of the epidemic *K. pneumoniae* ST11.[8] From April 2015 to December 2016, 19 clonally related, non-duplicate ST147 NDM-1 isolates were recovered in the index hospital but also in several other centres in Warsaw and other regions.[7] This indicated a new NDM outbreak, though limited when compared with that of ST11 at the end of 2016. Here we investigated the history of spread of the *K. pneumoniae* ST147 NDM-1 lineage till the end of 2019, also

providing the whole-genome sequencing (WGS) data for selected organisms representing the entire time span 2015–19.

Materials and methods

Study material and initial molecular typing

Between April 2015 and December 2016, the National Reference Centre for Susceptibility Testing (NRCST) in Warsaw confirmed 2136 unique *K. pneumoniae* NDM isolates,[7] followed by 6789 in 2017–19 ($n=8925$ in total). NDMs were detected by the CarbaNP test and PCR.[9,10] Polymorphism of the Tn*125*-like elements, used as a screening marker for different NDM-producing *K. pneumoniae* clones in Poland, was assessed by PCR-mapping.[7] The variant Tn*125*F of the two index Tunisian ST147 isolates,[8] found later in one more victim isolate and in 18 other ST147 isolates from 2015–16,[7] was then identified in 105 isolates from 2017–19. All these were typed by PFGE,[11] using the criteria of Tenover *et al.* for pulsotype definition,[12] and like those from 2015–16,[7] they represented a single pulsotype. Therefore, the group of *K. pneumoniae* NDM Tn*125*F isolates from 2015–19, including the index Tunisian ones, accounted for 126 isolates in total (9, 12, 86, 11 and 8 from 2015 to 2019, respectively). Two additional Polish ST147 NDM-1 isolates from 2016 were included; these represented the same pulsotype as the above but had different Tn*125* variants, Tn*125*J and Tn*125*O.[7]

WGS

Genomes of 47 representative *K. pneumoniae* ST147 Tn*125*F isolates and the two additional strains were sequenced with the Illumina MiSeq platform (Illumina, San Diego, CA, USA); reads were assembled with SPAdes 3.10.1.[13] Six isolates were selected also for long-read sequencing by MinION (Oxford Nanopore Technologies, Oxford, UK). The MiSeq and MinION hybrid read assemblies were done with Unicycler.[14]

Clonality, Tn*125*, K/O-serotype and virulome analyses

STs were determined as described previously.[15] The SNP analysis of the sample was done using BioNumerics Version 7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium), using one of the index Tunisian isolates, 1203/15, as a reference. The SNP-based phylogenetic analysis against all *K. pneumoniae* ST147 genomes available in GenBank was performed with Parsnp as described previously.[4] The phylogenetic tree was created by iTOL (https://itol.embl.de). Tn*125*-like structures were analysed using Lasergene (DNASTAR, Madison, WI, USA). O and K antigen types were determined in the study isolates and all *K. pneumoniae* genomes in GenBank by Kaptive (https://github.com/katholt/Kaptive). Virulomes were identified using BIGSdb with an identity threshold of 90% and the Institut Pasteur MLST databases (https://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html).

Plasmid and resistome analyses, susceptibility testing

Plasmid replicon types were identified with PlasmidFinder 2.1.[16] Plasmids were compared using BLASTn, and visualized with BRIG (http://brig.sourceforge.net/). The IncF and IncC plasmid replicon sequence typing (RST)/MLST was performed using PubMLST (https://pubmlst.org/organisms/plasmid-mlst).[17] The S1 nuclease analysis was performed as reported previously.[18] Antimicrobial resistance genes were detected by ResFinder 3.1, with 100% sequence identity criterion.[19] Susceptibility testing was carried out by broth microdilution or agar dilution (fosfomycin), according to the EUCAST guidelines for performance and interpretation of results (http://eucast.org).

Nucleotide sequence accession numbers

Genomic sequences have been deposited in the NCBI under the Bio-Project and Bio-Samples numbers PRJNA666795 and SAMN16327524-72, respectively. Sequences of IncFII$_K$+IncFIB$_K$-type plasmids are available under the following GenBank accession numbers: MW363911, isolate 650/19 (plasmid p650F; 118 156 bp); MW363914, 1203/15 (p1203F; 107 192 bp); MW363916; 1298/15 (p1298F; 124 439 bp); MW363917, 1433/15 (p1433F; 113 614 bp); IncR-type plasmids: MW363912, isolate 650/19 (plasmid p650R; 70 614 bp); MW363915, 1203/15 (p1203R; 56 351 bp); the IncFII$_K$+IncFIB$_K$+IncR plasmid: MW363913, isolate 1183/20 (plasmid p1183F/R; 176 549 bp); the IncC-type plasmid: MW363918, isolate 2273/16 (plasmid p2273C; 148 692 bp).

Results

Basic epidemiological data

Since March 2015, the *K. pneumoniae* ST147 NDM-1 Tn*125*F isolates have been observed in Poland. Their highest number was recorded in 2017 ($n=86$), with ~20% of isolates recovered from infections and ~80% from carriage. In total, 126 isolates were identified during the entire period 2015–19 (1.4% of all 8927 NDM-producing *K. pneumoniae*) in 33 hospitals in 20 cities, mainly Warsaw ($n=94$), and largely in the index centre (Warsaw IV; $n=75$). The cases were recorded in 6/16 administrative regions of the country (Figure S1, available as Supplementary data at *JAC* Online).

Genomic analyses

The WGS analysis was performed on 47 ST147 NDM-1 Tn*125*F isolates, representing all years of recovery (2015–19), and almost all hospitals (29/33) and towns (19/20), in which these occurred. The more-affected hospitals were represented proportionally (e.g. Warsaw IV, $n=14$). The sample contained also two additional ST147 isolates with Tn*125*O (5918/16) and Tn*125*J (2273/16) variants.[7] The Tn*125*F elements were of 5353 bp, covering a 253 bp part of the upstream IS*Aba125 tnpA* gene to a 1260 bp fragment of *groEL*.[5] Tn*125*O was of 2019 bp and was likely a Tn*125*F derivative, having the same 5′-end but being truncated by an IS*6*-like element 480 bp from the 3′-end of the *iso* gene. The 6372 bp Tn*125*J was unique, starting with 123 bp of the 5′ IS*Aba125 tnpA* and ending with 581 bp of IS*CR21*.[7]

Like the 2015–16 isolates,[7,8] all of the newer ones were classified into ST147. The SNP analysis of the 47 isolates and the two additional strains revealed 307 polymorphic positions in total within ~5 Mb (88%) of the reference Tunisian isolate 1203/15 genome, with 3 to 91 SNPs between any individual genome and the reference (Table S1 and Figure 1). The mean SNP value was 16 and the median was 13. The additional ST147 strains with Tn*125*O (5918/16) and Tn*125*J (2273/16) exhibited 6 and 49 SNPs from the reference, respectively. The majority of the Tn*125*F isolates ($n=40$) plus the additional one with Tn*125*O grouped into a tight cluster of organisms with 0–13 SNPs between each other and with up to 22 SNPs from the reference. The seven remaining Tn*125*F isolates and the additional one with Tn*125*J were located apart. Three and two of these formed clusters with 23–27 and 25–28 SNPs, whereas the last three isolates were individual outliers with 49–91 SNPs from the reference isolate.

The 49 isolates were uniform in O- and K-antigens, O2 variant 1 (O2v1) and KL64, respectively (Table S2). Of the virulence genes, the majority of the isolates, including all but two of the main cluster, had the *fyuA*, *irp1/2* and *ybtAEPQSTUX* genes, responsible for yersiniabactin synthesis and secretion.[20,21] These were of the

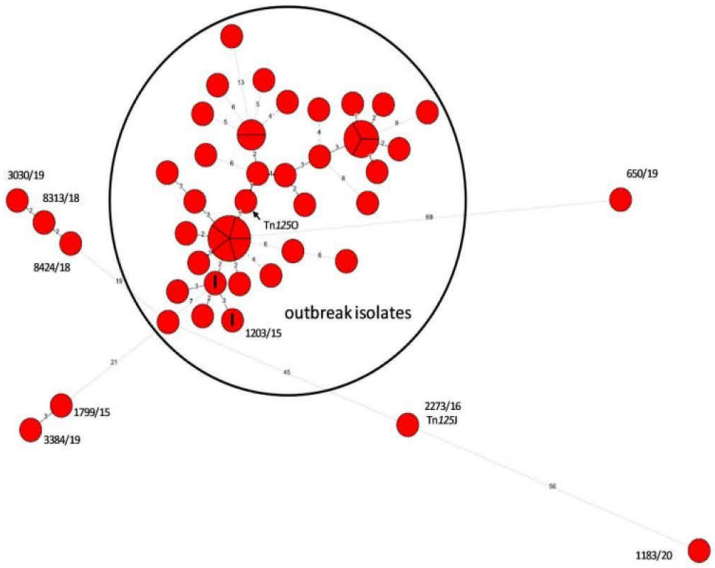

Figure 1. SNP-based minimum spanning tree of all study Polish *K. pneumoniae* ST147 isolates, constructed using BioNumerics (Applied Maths). Lengths of branches are related to numbers of SNPs in linked pairs of isolates (as shown on the branches). I—index isolates from the terrorism victims in Tunisia, one of which, 1203/15, was used as a reference in this analysis.[8] Tn*125*-like elements different than Tn*125*F are indicated at the circles of the corresponding isolates. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

YbST280-type, and resided within a 98 927 bp ICE*Kp12*-like element (33 SNPs from ICE*Kp12*, truncated by an IS*630*-like element).[22]

Comparison with international isolates

The study isolates were subjected to the phylogenetic analysis together with 255 ST147 strains identified among 9261 *K. pneumoniae* genomes available in GenBank (as of 1 June 2020). The comparison involved ~3.3 Mb (57%) of the reference isolate 1203/15 genome. The entire ST147 population was split into two main clades or branches, one of which was remarkably stable and correlated largely with the K-antigen KL10 (Figure S2). The second clade with a majority of the genomes, was more differentiated in general; however, it was vastly dominated by a lineage associated with KL64. Considering the entire *K. pneumoniae* GenBank WGS collection, KL64 was detected mainly in fractions of ST147 (58.4% of all ST147) and ST11 (35.6%) genomes. Consistent with the K-antigen data, the whole Polish study sample clustered into the KL64 phylogenetic lineage together with 183 international isolates, 35 of which had NDM-1, and 11 of which had Tn*125*F (Table S2, Figure S2). The closest relatives of all but two Polish isolates were four isolates from Tunisia, Egypt and France (11–27 SNPs from the reference isolate; mean value, 16; median, 13), all having Tn*125*F. The two Polish isolates that fell outside the sample were the most distant Tn*125*F isolate (1183/20), and the additional one with Tn*125*J (2273/16). Interestingly, 1183/20 was related more closely to two non-NDM *K. pneumoniae* ST147 isolates from Poland, identified in 2013–14 during the EuSCAPE survey (26 and 36 SNPs with 1183/20).[23]

Plasmid analysis

Plasmid replicon profiles showed remarkable stability, consisting of FII_K, FIB_K, FIB/pKPHS1-like and R replicons in almost every study isolate, and in their relatives from Tunisia, Egypt and France (Table 1). Few isolates had various additional replicons, and a totally different pattern was found in the additional isolate 2273/16 with Tn*125*J, containing C, FIB/pKPHS1, Col440I and ColBS512 replicons. Of all the IncF-type replicons identified, only $IncFII_K$ was typeable by the IncF RST scheme representing allele 2 ($IncFII_{K2}$).[17] The IncC-type replicon in the isolate 2273/16 was classified by pMLST to ST1.

Six isolates were selected for the MinION sequencing, representing the predominant four-replicon profile (index Tunisian isolates 1203/15 and 1298/15), three profiles with the additional types FII/pBK30683 (isolate 1433/15), N (650/19) or HI1B (1183/20), and the basically different pattern (2273/16) (Table 1). The complete plasmid assemblies revealed that in four isolates (1203/15, 1298/15, 1433/15 and 650/19) the $bla_{NDM\ 1}$ gene in Tn*125*F resided on double-replicon plasmids $IncFII_{K2}$+$IncFIB_K$ of varying size (~107.2 to ~124.4 kb) (Figure S3). These showed ≥50% coverage and 98%–99% identity with 67 plasmids with the FII_{K2}+FIB_K replicons (at least) from GenBank, of which 52 had bla_{KPC} carbapenemase genes (pKpQIL-like plasmids)[24] and two carried $bla_{NDM\ 1}$, though with 74%–75% coverage only and different Tn*125*-like elements (pKP15-T2 and FDAARGOS_442_plasmid_unnamed1, respectively). Apart from the FII_{K2}+FIB_K, the four isolates had also plasmids of the IncFIB/pKPHS1 type (~111.5 kb, no resistance genes) and the IncR type with the ESBL $bla_{CTX\ M\ 15}$ gene (~56.3 kb

Table 1. *K. pneumoniae* ST147 isolates included in the genomic study with closest international relatives: basic epidemiological data and resistomes[a]

City with the hospital symbol (number of WGS isolates/total number of isolates)	Isolate or assembly no. (country of isolation)[b]	Type of Tn*125*	Plasmid content	Acquired antimicrobial resistance genes						
				β-lactams	aminoglycosides[c]	fluoroquinolones[c]	sulphonamide	tetracycline	trimethoprim	phenicol
Outbreak isolates										
Warsaw I (3/3)	**1433/15**[d] 6008/17	Tn*125*F	FII_K+FIB_K, FIB, R, FII	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
	9074/19	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}	*aph(3″)-Ib*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw II (1/1)	7224/16	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw III (1/1)	5007/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw IV (13/75)	**1203/15**[e] **1298/15**[f] 3697/15, 3820/15, 4150/15, 4919/15, 7435/16, 5338/17, 2581/17, 1180/20	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
	6378/16	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(3′)-IIa*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
	583/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}	*aph(3″)-Ib*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
	10000/19	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw IV (add. str.)	5918/16	Tn*125*O	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}	*aph(3″)-Ib*, *aac(3′)-IIa*, *aph(6)-Id*	–	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw V (1/2)	2528/18	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw VI (1/2)	6757/16	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw VII (1/1)	4199/18	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw VIII (2/2)	3177/15, 5420/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Warsaw IX (1/1)	5164/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}	*aph(3″)-Ib*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Wołomin I (1/2)	3787/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Wołomin II (1/1)	3789/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Wieliszew (1/1)	3312/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aac(6′)-Ib-cr*, *aac(3′)-IIa*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Majdan (2/6)	7132/16, 3654/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
N.D.Maz. (1/1)	5242/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Sochaczew (1/1)	3855/16	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}	*aph(3″)-Ib*, *aac(3′)-IIa*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Sokołów Podl. (1/1)	5459/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aac(6′)-Ib-cr*, *aac(3′)-IIa*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Rudka (1/1)	8861/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Ząbki (1/1)	7632/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–

Olsztyn (1/1)	6791/16	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Gižycko (1/2)	6365/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Olecko (1/1)	4366/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Łomża (1/1)	7190/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Katowice (1/2)	9220/18	Tn*125*F	FII_K+FIB_K, R, M1	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}	*aph(3″)-Ib*, *aph(6)-Id*	*qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Bielsko-Biała (1/1)	4146/17	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Non-outbreak isolates										
Warsaw IV (1/75)	**1183/20**[g]	Tn*125*F	FII_K+FIB_K+R, FIB+HI1B, ColE440I	bla_{NDM-1} bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	*catA1*
Warsaw X (1/4)	**650/19**[h]	Tn*125*F	FII_K+FIB_K, FIB, R, N	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*, *aadA5*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*, *dfrA17*	–
Toruń (add. str.)	**2273/16**[i]	Tn*125*J	C, ColBS512, FIB, ColE440I	bla_{NDM-1}, bla_{OXY-6}	*aph(3′)-IV*, *aac(3)-IId*, *aac(6′)-Ib3*, *rmtC*	*aac(6′)-Ib-cr*	*sul1*	–	–	–
Toruń (2/2)	1799/15	Tn*125*F	FII_K+FIB_K, FIB, R, ColE440I	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*, *qnrS1*	*sul1*	*tet(A)*	*dfrA1*	–
	3384/19	Tn*125*F	FII_K+FIB_K, FIB, R, Q2, ColE440I/II	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}, bla_{SES-7}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aph(6)-Id*, *aacA4′-4i*	*aac(6′)-Ib-cr*, *qnrB1*, *qnrS1*	*sul1*	*tet(A)*	*dfrA1*, *dfrB3*	–
Lublin I (1/1)	8424/18	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Lublin II (1/1)	8313/18	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
Inowrocław (1/1)	3030/19	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
International isolates										
	GCF_002140135 (TN)	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*	*sul1*	*tet(A)*	*dfrA1*	–
	GCF_004803035 (EG)	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	–	–	–	–
	GCF_009856925 (FR)	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–
	GCF_009856915 (FR)	Tn*125*F	FII_K+FIB_K, FIB, R	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	*aph(3″)-Ib*, *aac(6′)-Ib-cr*, *aac(3′)-IIa*, *aph(6)-Id*	*aac(6′)-Ib-cr*, *qnrB1*	*sul1*	*tet(A)*	*dfrA1*	–

[a]Only acquired resistance genes are shown, as identified by ResFinder 3.2.[19]
[b]Abbreviations: TN, Tunisia; EG, Egypt; FR, France.
[c]The *aac(6′)-Ib-cr* gene shown in the 'aminoglycosides' and 'fluoroquinolones' columns is the same gene, conferring resistance to both classes of antimicrobials.
[d](1433/15) the following resistance genes were located on the individual plasmids in this isolate: $IncFII_K$+$IncFIB_K$ (p1433F; 113 614 bp): bla_{NDM-1}, bla_{OXA-1}, *aac(6′)-Ib-cr*, *aac(3′)-IIa* and *qnrB1*; IncR (56 351 bp): $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(3″)-Ib*, *aph(6)-Id*, *dfrA14*, *sul1* and *tet(A)*; IncFIB (111 565 bp); none; IncFII (68 996 bp): none.
[e](1203/15) the following resistance genes were located on the individual plasmids in this isolate: $IncFII_K$+$IncFIB_K$ (p1203F; 107 192 bp): bla_{NDM-1} and *qnrB1*; IncR (p1203R; 56 351 bp): $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(3″)-Ib*, *aph(6)-Id*, *dfrA14*, *sul1 and tet(A)*; IncFI (111 565 bp): none.
[f](1298/15) the following resistance genes were located on the individual plasmids in this isolate: $IncFII_K$+$IncFIB_K$ (p1298F; 124 439 bp): bla_{NDM-1}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, *aac(6′)-Ib-cr* and *qnrB1*; IncR (56 351 bp): $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(3″)-Ib*, *aph(6)-Id*, *dfrA14*, *sul1* and *tet(A)*; IncFI (111 565 bp): none.
[g](1183/20) the following resistance genes were located on the individual plasmid in this isolate: $IncFII_K$+$IncFIB_K$+IncR (p1183F/R; 176 549 bp): bla_{NDM-1}, bla_{OXA-1}, bla_{TEM-1}, *aac(6′)-Ib-cr*, *qnrB1*, *aph(3″)-Ib*, *aph(6)-Id*, *dfrA14*, *sul1* and *tet(A)*; IncFIB+IncHI1B (218 862 bp): none; IncCol440I (3089 bp): none.
[h](650/19) the following resistance genes were located on the individual plasmids in this isolate: $IncFII_K$+$IncFIB_K$ (p650F; 118 156 bp): bla_{NDM-1}, bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{TEM-1}; *aac(6′)-Ib-cr*, *aac(3′)-IIa* and *qnrB1*; IncR (p650R; 70 614 bp): $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(3″)-Ib*, *aph(6)-Id*, *dfrA14*, *sul1* and *tet(A)*; IncFIB (111 565 bp): none; IncN (34 224 bp): *aadA5*, *sul1* and *dfrA17*.
[i](2273/16) the following resistance genes were located on the individual plasmid in this isolate: IncC (p2273C; 148 692 bp): bla_{NDM-1}, bla_{OXY-6}, *rmtC*, *aac(6′)-Ib-cr*, *sul1*, *aph(3′)-IV*, *aac(3)-IId*, *aac(6′)-Ib3*; IncFIB (111 909 bp): none; IncColBS512 (2089 bp): none; ColE440I (4163 bp): none.

in 1203/15, 1298/15 and 1433/15; ~70.6 kb in 650/19) (Figure S4). The last of the MinION-sequenced isolates with the FII_{K2} and FIB_K replicons (1183/20) had $bla_{NDM\ 1}$ located on a triple-replicon hybrid plasmid FII_{K2}+FIB_K+R (~176.5 kb) (Figure S5), confirmed *in vivo* by S1 nuclease analysis (data not shown). Its IncR part (~46 kb) matched well the corresponding fragments of the autonomous IncRs in the other study isolates and of those present in GenBank (e.g. acc. no. CP061055), and did not carry $bla_{CTX\ M\ 15}$. The subsequent BLASTn analysis, done with three plasmids of the reference isolate 1203/15 and short reads of the remaining isolates has confirmed the replicon profiling data and stability of the major plasmid pattern, identifying reads with >89% coverage/>99% identity for the IncFII_{K2}+FIB_K plasmids, >99% coverage/>99% identity for the IncFIB/pKPHS1-like plasmids, and >79% coverage/>99% identity for the IncRs in most of the isolates.

The last isolate analysed by MinION, characterized by the unique replicon profile (2273/16), had $bla_{NDM\ 1}$ in Tn*125*J located on an IncC (ST1)-type plasmid (~148.7 kb), also carrying the cephalosporinase $bla_{CMY\ 6}$ gene (Figure S6).

Resistomes and susceptibility

The resistome analysis revealed 13 different gene profiles, with the predominant combination of genes detected in most of the main cluster isolates (n = 32/40; Table 1). The diversity resulted more from absence of some genes of the major profile than from acquisition of new ones (e.g. $bla_{GES\ 7}$). A totally different resistome characterized the additional strain 2273/16 with Tn*125*J. Twenty-two acquired resistance genes have been identified altogether, forming profiles of 8–16 genes per organism. Based on the MinION data, all these genes were plasmid-located (Table 1). Apart from $bla_{NDM\ 1}$, the FII_{K2}+FIB_K-like plasmids also carried the *qnrB1* fluoroquinolone resistance gene, and some molecules additionally had others (e.g. in isolate 1298/15). The $bla_{CTX\ M\ 15}$ and $bla_{TEM\ 1}$ β-lactamase genes plus *tet*(A), *dfrA1* and *sul1*, were found on all IncR-like plasmids. The IncC-type plasmid, besides $bla_{NDM\ 1}$ and $bla_{CMY\ 6}$, also carried a number of aminoglycoside resistance genes, including *rmtC*.

Twenty isolates (including 14 of the main cluster), representing all resistomes, were used in susceptibility testing. All these showed MDR phenotypes with variations for individual drugs, in general correlating well with resistomes (Table S3). The cluster isolates were apparently more uniform than the outsiders. β-Lactam resistance resulted mainly from the NDM-1 plus CTX-M-15 production in most of the isolates, with aztreonam resistance due to CTX-M-15, efficiently reversed by avibactam. Aminoglycosides, tigecycline and fosfomycin were active *in vitro* against the majority of the isolates, and only one of these was resistant to colistin.

Discussion

Since March 2015, the *K. pneumoniae* ST147 NDM-1 Tn*125*F genotype has been spreading in Poland, being masked by the predominant *K. pneumoniae* ST11 NDM-1 Tn*125*A lineage.[4,7] Its actual incidence rate, approximated in this study by 126 cases by the end of 2019, has been significantly underestimated. Because of the huge general NDM increase, in March 2018 the NRCST stopped confirming *K. pneumoniae* NDM carriage cases in the endemic Warsaw region, and in July 2019 it did so in the entire country. This has caused lower ST147 NDM-1 Tn*125*F rates recorded in 2018–19 through the significant reduction of the surveillance cultures. Another factor might be enhanced control measures in the most affected index hospital, Warsaw IV, in which numbers of NDM infections decreased remarkably over that period.

The comparison of the isolates within the sample revealed relatedness of all these in general but the SNP analysis revealed a cluster of higher clonal homogeneity. This formed the majority of the isolates with the Tn*125*F element plus one with Tn*125*O, likely being a truncated Tn*125*F derivative. Other differences shown by a few individual isolates included the lack or presence of a single plasmid or the lack of the yersiniabactin-encoding ICE*Kp12*-like element, all which might be assigned to single genetic events. The cluster included the Tunisian isolates, and originated from 24 centres, mainly in Warsaw (including the Warsaw IV hospital and some others) among which the index patients had circulated. All these data prompted us to interpret the cluster as resulting from an on-going outbreak in Poland, as an indirect consequence of the terrorist attack in Tunisia in March 2015.[8]

The analysis of the isolates in the international context has helped understanding of the status of the related organisms, though classified outside of the outbreak. The level of their relatedness to the cluster was similar to that of the ST147 NDM-1 Tn*125*F isolates from other, mainly Mediterranean countries. This indicated that the entire Polish population of these organisms might arise concurrently by two means, *i.e.* the clonal outbreak discussed just above, and repeated transfers of related organisms by mass tourism. Mediterranean and especially North African countries are the area of spread or endemicity of ST147, including variants with NDM-1.[6,8,25,26] It has been defined as an emerging high-risk clone linked with resistance.[6,27–29] Its global distribution and frequent association with carbapenemases (NDM, VIM, KPC, OXA-48 types) and/or ESBLs (CTX-M-15) has been confirmed in this study as well.

The stability of the ST147 NDM-1 Tn*125*F genotype was observed in plasmid profiles too, with $bla_{NDM\ 1}$-carrying IncFII_{K2}+IncFIB_K plasmids plus IncR and IncFIB/pKPHS1 types. The FII_{K2}+FIB_K structures with the pKPN4-like backbone have been extensively studied in pKpQIL-like plasmids, reported worldwide with bla_{KPC}s in *K. pneumoniae* ST258/ST512.[30–34] Like the study by Messaoudi *et al.*,[26] this study indicated that the FII_{K2}+FIB_K may be common $bla_{NDM\ 1}$ plasmids in the Mediterranean region. The FII_{K2}+FIB_K+R hybrid observed here resulted from fusion of two primarily separate plasmids pre-existing in the disseminating genotype. Similar arrangements of IncRs with other types have been reported on multiple occasions.[27,35] The IncC-like plasmid harbouring $bla_{NDM\ 1}$ and $bla_{CMY\ 6}$ genes in the outlier ST147 Tn*125*J strain, represented a common type associated with spread of resistance genes in various hosts worldwide, including bla_{NDM}s.[36–38]

This has been one of the largest genomic studies on the *K. pneumoniae* ST147 clone, associated with various resistance mechanisms, including ESBLs and major carbapenemase types. Several reports demonstrated its dissemination in the Mediterranean basin,[6,39] being the source of imports to other regions.[6,8,27,40] The relative genetic stability of the KL64 lineage observed on the SNP level probably reflects recent start of its large-scale expansion.[6] This should be considered when typing groups of ST147 isolates, e.g. in outbreak investigations, even when performed with WGS technologies.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S3 and Figures S1 to S6 are available as Supplementary data at *JAC* Online.

References

1 Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int* 2014; **2014**: 249856.

2 Wu W, Feng Y, Tang G *et al.* NDM metallo-β-lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev* 2019; **32**: e00115-18.

3 Touati A, Mairi A. Epidemiology of carbapenemase-producing *Enterobacterales* in the Middle East: a systematic review. *Expert Rev Anti Infect Ther* 2020; **18**: 241–50.

4 Izdebski R, Sitkiewicz M, Urbanowicz P *et al.* Genomic background of the *Klebsiella pneumoniae* NDM-1 outbreak in Poland, 2012-18. *J Antimicrob Chemother* 2020; **75**: 3156–62.

5 Poirel L, Bonnin RA, Boulanger A *et al.* Tn*125*-related acquisition of bla_{NDM}-like genes in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2012; **56**: 1087–9.

6 Peirano G, Chen L, Kreiswirth BN *et al.* Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. *Antimicrob Agents Chemother* 2020; **64**: e01148-20.

7 Baraniak A, Machulska M, Zabicka D *et al.* Towards endemicity: large-scale expansion of the NDM-1-producing *Klebsiella pneumoniae* ST11 lineage in Poland, 2015-16. *J Antimicrob Chemother* 2019; **74**: 3199–204.

8 Izdebski R, Bojarska K, Baraniak A *et al.* NDM-1- or OXA-48-producing Enterobacteriaceae colonising Polish tourists following a terrorist attack in Tunis. *Euro Surveill 2015* 2015; **20**: pii=21150.

9 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; **18**: 1503–7.

10 Lee K, Lim YS, Yong D *et al.* Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623–9.

11 Seifert H, Dolzani L, Bressan R *et al.* Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; **43**: 4328–35.

12 Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

13 Bankevich A, Nurk S, Antipov D *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; **19**: 455–77.

14 Wick RR, Judd LM, Gorrie CL *et al.* Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595.

15 Diancourt L, Passet V, Verhoef J *et al.* Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82.

16 Carattoli A, Zankari E, Garcia-Fernandez A *et al.* In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.

17 Villa L, Garcia-Fernandez A, Fortini D *et al.* Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. *J Antimicrob Chemother* 2010; **65**: 2518–29.

18 Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995; **226**: 235–40.

19 Zankari E, Hasman H, Cosentino S *et al.* Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640–4.

20 Paczosa MK, Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol Mol Biol Rev* 2016; **80**: 629–61.

21 Lin CT, Lin TH, Wu CC *et al.* CRP-cyclic AMP regulates the expression of type 3 fimbriae via cyclic di-GMP in *Klebsiella pneumoniae*. *PLoS One* 2016; **11**: e0162884.

22 Lam MMC, Wick RR, Wyres KL *et al.* Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICE*Kp* in *Klebsiella pneumoniae* populations. *Microb Genom* 2018; **4**: e000196.

23 David S, Reuter S, Harris SR *et al.* Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol* 2019; **4**: 1919–29.

24 Leavitt A, Chmelnitsky I, Ofek I *et al.* Plasmid pKpQIL encoding KPC-3 and TEM-1 confers carbapenem resistance in an extremely drug-resistant epidemic *Klebsiella pneumoniae* strain. *J Antimicrob Chemother* 2010; **65**: 243–8.

25 Gamal D, Egea P, Elias C *et al.* High-risk clones and novel sequence type ST4497 of *Klebsiella pneumoniae* clinical isolates producing different alleles of NDM-type and other carbapenemases from a single tertiary care center in Egypt. *Int J Antimicrob Agents* 2020; **56**: 106164.

26 Messaoudi A, Haenni M, Mansour W *et al.* ST147 NDM-1-producing *Klebsiella pneumoniae* spread in two Tunisian hospitals. *J Antimicrob Chemother* 2017; **72**: 315–6.

27 Papagiannitsis CC, Izdebski R, Baraniak A *et al.* Survey of metallo-β-lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008-11. *J Antimicrob Chemother* 2015; **70**: 1981–8.

28 Baraniak A, Izdebski R, Fiett J *et al.* Comparative population analysis of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases colonizing patients in rehabilitation centers in four countries. *Antimicrob Agents Chemother* 2013; **57**: 1992–7.

29 Hasan CM, Turlej-Rogacka A, Vatopoulos AC *et al.* Dissemination of bla_{VIM} in Greece at the peak of the epidemic of 2005-2006: clonal expansion of *Klebsiella pneumoniae* clonal complex 147. *Clin Microbiol Infect* 2014; **20**: 34–7.

30 Leavitt A, Chmelnitsky I, Carmeli Y *et al.* Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. *Antimicrob Agents Chemother* 2010; **54**: 4493–6.

31 Baraniak A, Izdebski R, Zabicka D *et al.* Multiregional dissemination of KPC-producing *Klebsiella pneumoniae* ST258/ST512 genotypes in Poland, 2010-14. *J Antimicrob Chemother* 2017; **72**: 1610–6.

32 Garcia-Fernandez A, Villa L, Carta C *et al. Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother* 2012; **56**: 2143–5.

33 Chen L, Chavda KD, Melano RG *et al.* Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York Hospitals. *Antimicrob Agents Chemother* 2014; **58**: 2871–7.

34 Findlay J, Hopkins KL, Alvarez-Buylla A *et al.* Characterization of carbapenemase-producing *Enterobacteriaceae* in the West Midlands region of England: 2007-14. *J Antimicrob Chemother* 2017; **72**: 1054–62.

35 Compain F, Frangeul L, Drieux L *et al.* Complete nucleotide sequence of two multidrug-resistant IncR plasmids from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2014; **58**: 4207–10.

36 Hancock SJ, Phan MD, Peters KM *et al.* Identification of IncA/C plasmid replication and maintenance genes and development of a plasmid multilocus sequence typing scheme. *Antimicrob Agents Chemother* 2017; **61**: e01740-16.

37 Carattoli A, Villa L, Poirel L *et al.* Evolution of IncA/C bla_{CMY-2}-carrying plasmids by acquisition of the bla_{NDM-1} carbapenemase gene. *Antimicrob Agents Chemother* 2012; **56**: 783–6.

38 Giske CG, Froding I, Hasan CM *et al.* Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of bla_{NDM-1} in India, Sweden, and the United Kingdom. *Antimicrob Agents Chemother* 2012; **56**: 2735–8.

39 Tavoschi L, Forni S, Porretta A *et al.* Prolonged outbreak of New Delhi metallo-β-lactamase-producing carbapenem-resistant *Enterobacterales* (NDM-CRE), Tuscany, Italy, 2018 to 2019. *Euro Surveill* 2020; **25**: pii=2000085.

40 Samuelsen O, Toleman MA, Hasseltvedt V *et al.* Molecular characterization of VIM-producing *Klebsiella pneumoniae* from Scandinavia reveals genetic relatedness with international clonal complexes encoding transferable multidrug resistance. *Clin Microbiol Infect* 2011; **17**: 1811–6.

Supplementary data

Isolate	Year	Number of SNPs	Country	Remarks	References
NMI1203_15	2015	0	Tunisia/Poland	ST147 NDM-1 Tn*125*F outbreak isolate (index and reference)	8
NMI1298_15	2015	3	Tunisia/Poland	ST147 NDM-1 Tn*125*F outbreak isolate (index)	8
NMI1433_15	2015	10	Tunisia/Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI3177_15	2015	6	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI3697_15	2015	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI3820_15	2015	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI4150_15	2015	7	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI4919_15	2015	6	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI3855_16	2016	9	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI5918_16	2016	6	Poland	ST147 NDM-1 Tn*125*O additional strain/outbreak isolate	7
NMI6378_16	2016	7	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI6757_16	2016	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI6791_16	2016	7	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI7132_16	2016	9	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI7224_16	2016	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI7435_16	2016	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	7
NMI2581_17	2017	8	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI3312_17	2017	10	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI3654_17	2017	17	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI3787_17	2017	8	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI3789_17	2017	9	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI4146_17	2017	9	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI4366_17	2017	11	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5007_17	2017	16	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5164_17	2017	15	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5242_17	2017	22	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5338_17	2017	13	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5420_17	2017	15	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI5459_17	2017	14	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI583_17	2017	5	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI6008_17	2017	14	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI6365_17	2017	16	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI7190_17	2017	14	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI7632_17	2017	13	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI8861_17	2017	14	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI2528_18	2018	14	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI4199_18	2018	13	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI9220_18	2018	15	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI10000_19	2019	11	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI9074_19	2019	22	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI1180_20	2020	15	Poland	ST147 NDM-1 Tn*125*F outbreak isolate	this study
NMI1799_15	2015	25	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	7
NMI3384_19	2019	28	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI8313_18	2018	25	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI8424_18	2018	23	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI3030_19	2019	27	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI650_19	2019	74	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI1183_20	2020	91	Poland	ST147 NDM-1 Tn*125*F non-outbreak isolate	this study
NMI2273_16	2016	49	Poland	ST147 NDM-1 Tn*125*J additional strain/non-outbreak isolate	7
GCF_900511505.1	2013	58	Poland	ST147, EuSCAPE survey	23
GCF_900511365.1	2014	54	Poland	ST147, EuSCAPE survey	23
GCF_003123945.1	2017	11	France	ST147 NDM-1 Tn*125*F	-
GCF_004803035.1	-	27	Egypt	ST147 NDM-1 Tn*125*F	-

Table S1. Numbers of SNPs between the study Polish and related international *K. pneumoniae* ST147 isolates, and the reference index isolate (1203/15).

Table S2. Allelic profiles of virulence-associated genes and yersiniabactin sequence types of the study Polish *K. pneumoniae* ST147 isolates.

	type 3 fimbriae encoding genes								yersiniabactin encoding genes											
Isolates	*mrkA*	*mrkB*	*mrkC*	*mrkD*	*mrkF*	*mrkH*	*mrkI*	*mrkJ*	*fyuA*	*irp1*	*irp2*	*ybtA*	*ybtE*	*ybtP*	*ybtQ*	*ybtS*	*ybtT*	*ybtU*	*ybtX*	YbST
n=41[a]	6	3	2	12	8	7	15	12	2[b]	129	125	11	51	4	5	6	11[d]	2	15	280/280-like
n=1	6	3		12	8	7	15	12	2	129	125	11	51[c]	4	5	6	11	2	15	280
n=4	6	3	2	12	8	7	15	12												
n=2			2	12	8	7	15	12												
n=1	6	3	2	12	8	7	15													

[a] – including control strains 2273/16 and 5918/16

[b] – two isolates differed by single mutations in the *fyuA* 2 allele

[c] – one isolate differed by a single mutation in the *ybtE* 51 allele

[d] – eight isolates differed by single mutations in the *ybtT* 11 allele

Table S3. MICs of antimicrobials for the *K. pneumoniae* ST147 isolates

	Isolates	MIC (mg/L)[a,b]																	
		AMX	AMC	PIP	TZP	CTX	CAZ	FEP	ATM	AZA	ERT	IPM	MEM	AMK	GEN	CIP	TGC	SXT	FOF	CST
outbreak isolates	1203/15[c]	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**>32**	*4*	**>32**	4	**64**	**>32**	2	**>32**	4	0.25
	1298/15[c]	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**64**	0.25	**>32**	*4*	**>32**	8	**64**	**>32**	2	**>32**	4	0.25
	1433/15	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**16**	2	**>32**	8	**64**	**>32**	2	**>32**	8	0.25
	3855/16	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**64**	≤0.125	**>32**	2	**>32**	2	2	**>32**	2	**>32**	8	0.5
	5918/16	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**32**	**64**	0.25	**>32**	2	**>32**	2	**8**	**4**	2	**>32**	4	0.25
	6378/16	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	0.25	**>32**	*4*	**>32**	1	**32**	**16**	2	**>32**	4	0.25
	6791/16	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**>32**	*4*	**>32**	**16**	2	**>32**	2	**>32**	4	0.5
	583/17	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**>32**	*4*	**>32**	1	0.25	**>32**	2	**>32**	4	0.25
	3312/17	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**32**	**128**	0.25	**>32**	*4*	**>32**	8	2	**>32**	2	**>32**	8	**16**
	4146/17	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**>32**	*4*	**>32**	8	2	**>32**	1	**>32**	4	0.5
	4366/17	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	≤0.125	**>32**	*4*	**>32**	8	2	**>32**	2	**>32**	4	1
	5164/17	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**64**	≤0.125	**>32**	*4*	**>32**	4	2	**>32**	2	**>32**	4	0.25
	4199/18	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**128**	≤0.125	**>32**	*4*	**>32**	8	0.25	**>32**	2	**>32**	16	0.25
	10000/19	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	≤0.125	≤0.125	**>32**	*4*	**>32**	8	2	**>32**	2	**>32**	8	0.25
non-outbreak isolates	1799/15	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	0.25	**>32**	*4*	*8*	4	0.5	**>32**	**8**	**>32**	**>128**	0.25
	3384/19	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	0.25	**>32**	2	**>32**	8	2	**>32**	**8**	**>32**	**>128**	0.5
	8313/18	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**32**	**128**	0.25	**>32**	2	**>32**	8	**32**	**>32**	**8**	**>32**	4	1
	1183/20	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	1	0.5	**>32**	**>32**	**>32**	**16**	**64**	**>32**	2	**>32**	**64**	1
	650/19	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**64**	**128**	0.25	**>32**	2	*8*	8	**64**	**>32**	**4**	**>32**	4	0.25
	2273/16	**>256**	**>256**	**>256**	**>256**	**>256**	**>256**	**32**	**8**	0.25	**>32**	**8**	**>32**	**>256**	**>256**	**4**	0.5	0.5	16	0.25

[a] – abbreviations: AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; PIP, piperacillin; TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; AZA, aztreonam-avibactam ; ERT, ertapenem; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; FOF, fosfomycin ; CST, colistin.

[b] – bold style refers to resistance, italics (in case of carbapenems only) – intermediate resistance, according to EUCAST (http://eucast.org). For TGC ECOFF values were used.

[c] – MICs for 1203/15 and 1298/15 isolates were reported previously.[8]

Figure S1. Geographic distribution of the *K. pneumoniae* ST147 NDM-1 isolates in Poland, March 2015 – December 2019. Dots represent medical centres where the isolates were collected. Sizes of the dots are roughly proportional to numbers of cases. The dot corresponding to the Warsaw index hospital HW11 is grey in order not to mask other centres in the city and the Mazowieckie region.

Figure S2. SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST147 isolates (n=49) compared with all international ST147 genomes available in GenBank (n=255). Numbers on the inner circle are the original numbers of the study isolates or GenBank assembly numbers. The presence of carbapenemase genes is indicated by colours: the *bla*$_{\text{NDM-1}}$ gene in the Tn*125*F element is indicated in red; *bla*$_{\text{NDM-1}}$ in other Tn*125* derivatives are in black; *bla*$_{\text{NDM-4,-5,-7,-9}}$ genes are in gray; *bla*$_{\text{KPC-2,-3}}$ are in blue; *bla*$_{\text{OXA-48,-181,-204,-232}}$ are in purple; *bla*$_{\text{VIM-1,-27}}$ and *bla*$_{\text{IMP-14}}$ are in green. The presence of *bla*$_{\text{CTX-M-15}}$ is indicated in yellow. The abbreviations: AE, United Arab Emirates; AU, Australia; BD, Bangladesh; BE, Belgium; CA, Canada; CH, Switzerland; CN, China; CO, Columbia; CZ, Czechia; DE, Germany; DK, Denmark; EG, Egypt; ES, Spain; FR, France; GB, United Kingdom; GR, Greece; HU, Hungary; IL, Israel; IN, India; IT, Italy; JP, Japan; KR, South Korea; LB, Lebanon; LK, Sri Lanka; LU, Luxemburg; MY, Malaysia; NG, Nigeria; NO, Norway; NP, Nepal; PE, Peru; PH, Philippines; PK, Pakistan; PL, Poland; PT, Portugal; RO, Romania; RS, Serbia; RU, Russia; SE, Sweden; SG, Singapore; TH, Thailand, TN, Tunisia; TR, Turkey; US, USA. K-serotypes are indicated on outer circle. The tree was constructed using Parsnp and visualized with iTOL.

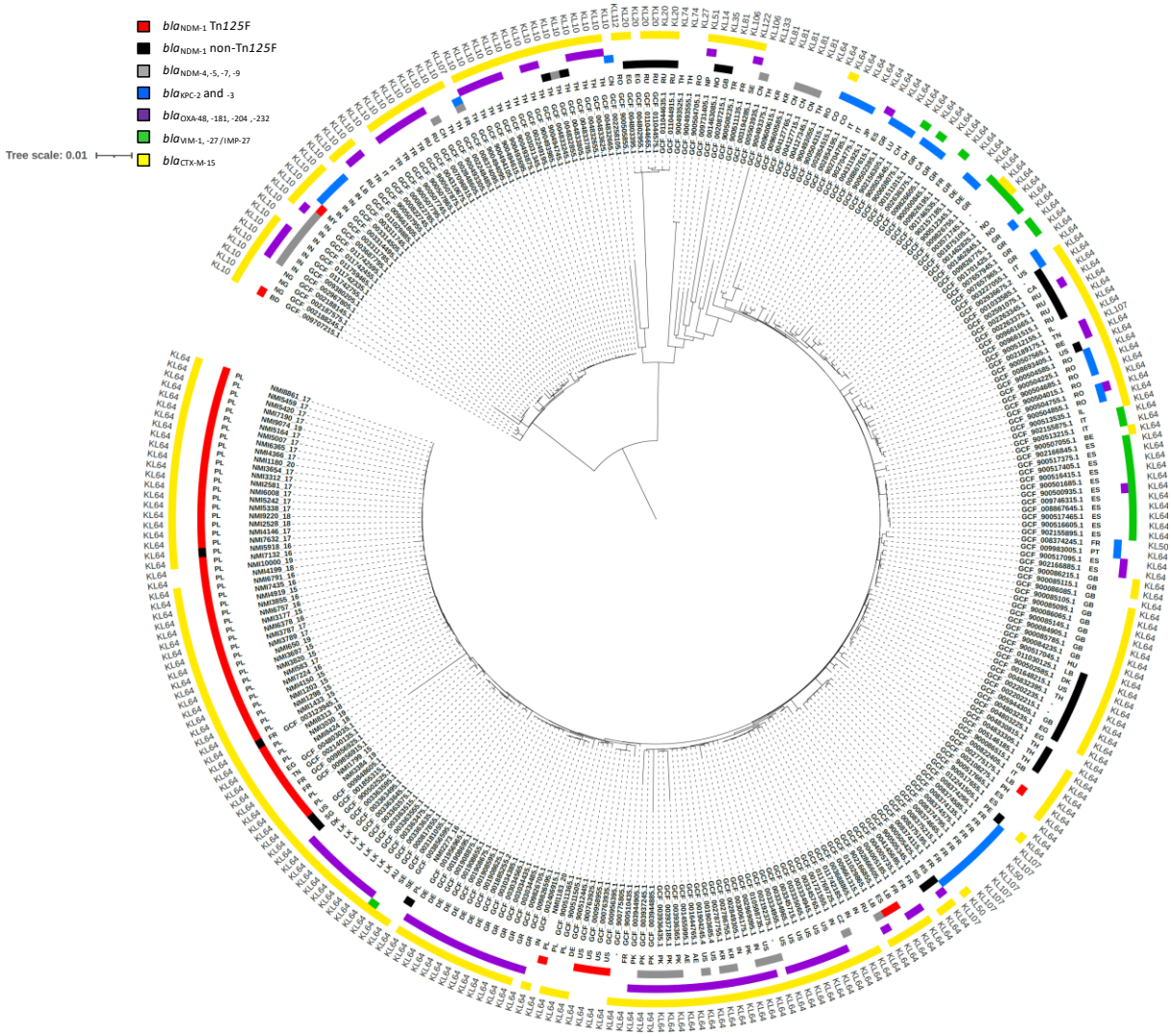


Figure S3. Comparison of the NDM-1-encoding IncFII$_K$+IncFIB$_K$-type plasmid p1203F (inner, thin black circle) to p1298F, p1433F, p650F from the study isolates, and selected plasmids from GenBank: pKPN4 (CP000649), pKpQIL-IT (JN233705), FDAARGOS_442_plasmid_unnamed1 (CP023928), pKP15-T2 (MN657248). The outer thick gray ring refers to the annotation of p1203F, with the selected genes indicated. The percentage of sequence identity is reflected by colour intensity. The picture was created using the BRIG software (http://brig.sourceforge.net/).

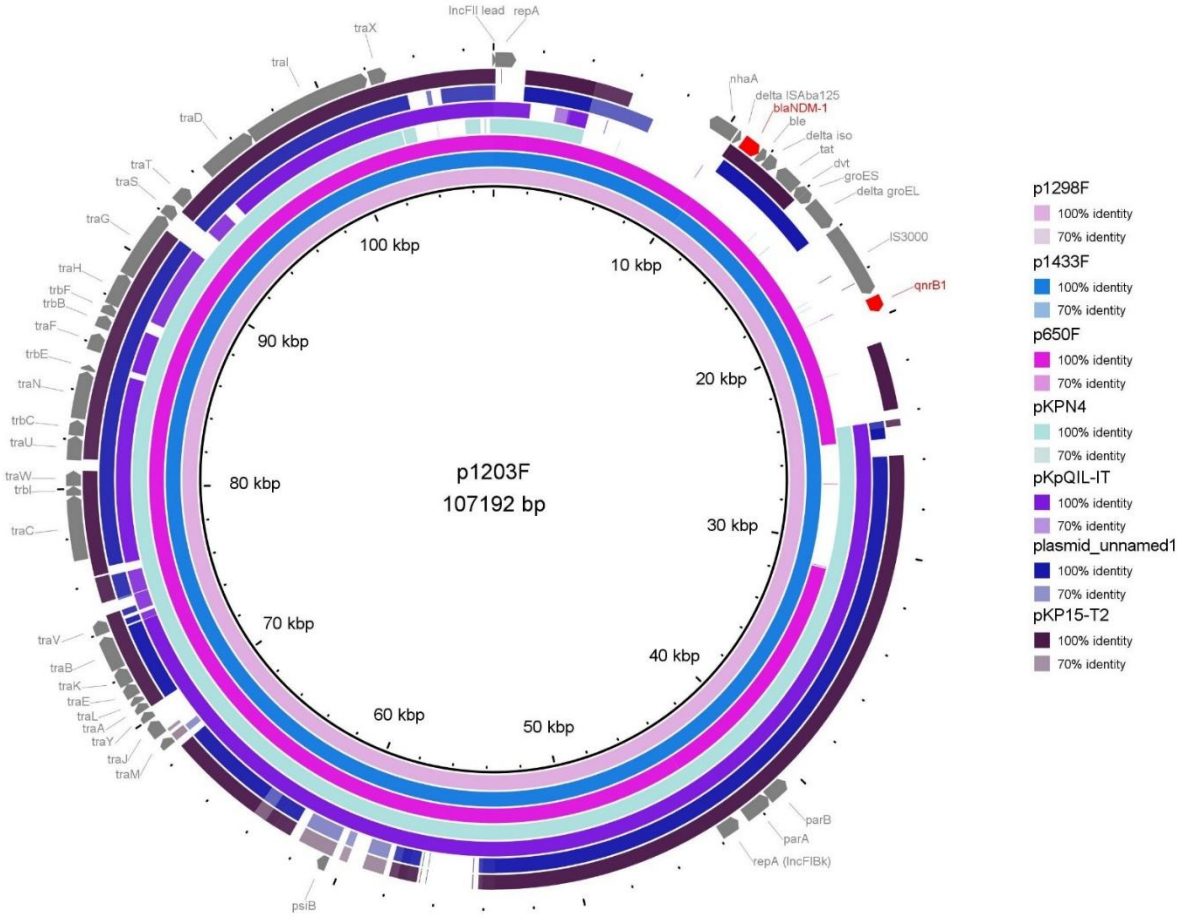


Figure S4. Comparison of the NDM-1-encoding hybrid arrangement IncFII$_K$+IncFIBK+IncR plasmid p1183F/R (inner, thin black circle) to p1203R, p1298R, p1433R, p650R, p1203F, p1298F, p1433F and p650F from the study isolates. The outer thick gray ring refers to the annotation of p1183F/R, with the selected genes indicated. The percentage of sequence identity is reflected by colour intensity. The picture was created using the BRIG software (http://brig.sourceforge.net/).

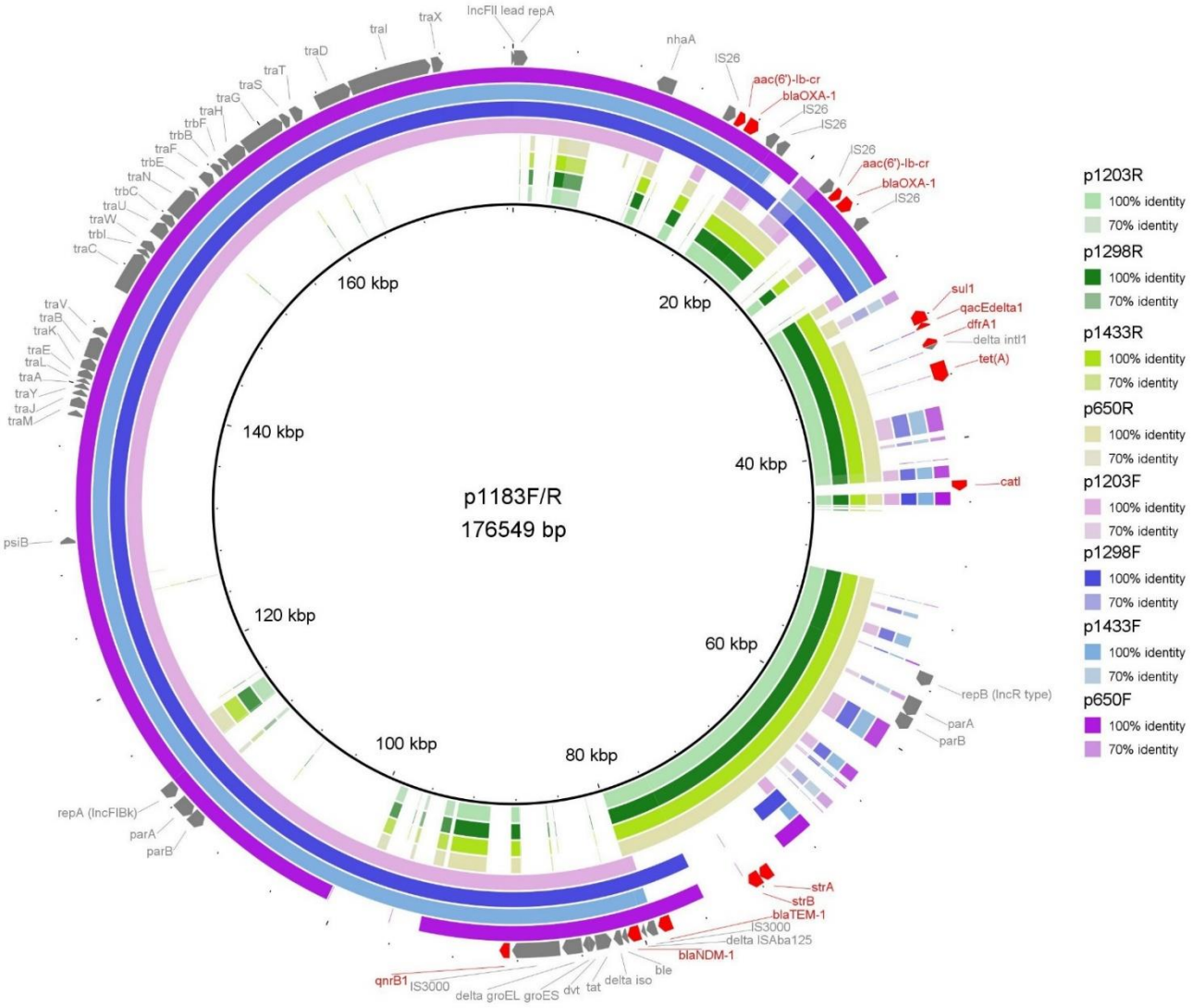


Figure S5. Comparison of the IncR-type plasmid p1203R (inner, thin black circle) to p1298R, p1433R, p650R from the study isolates, and selected plasmids from GenBank: pHKP0067.2 (CP061055), plasmid_unnamed4 (CP017989), pKp_Goe_579-4 (CP018316), pHKP0018.2 (CP061064), pSg1-NDM (CP011839), pD16KP0109-1 (CP052366), p4_1_2.2 (CP023841), pDA33140-96 (CP029586), plasmid_tig00000004 (CP021761). The outer thick gray ring refers to the annotation of p1203R, with the selected genes indicated. The percentage of sequence identity is reflected by colour intensity. The picture was created using the BRIG software (http://brig.sourceforge.net/).

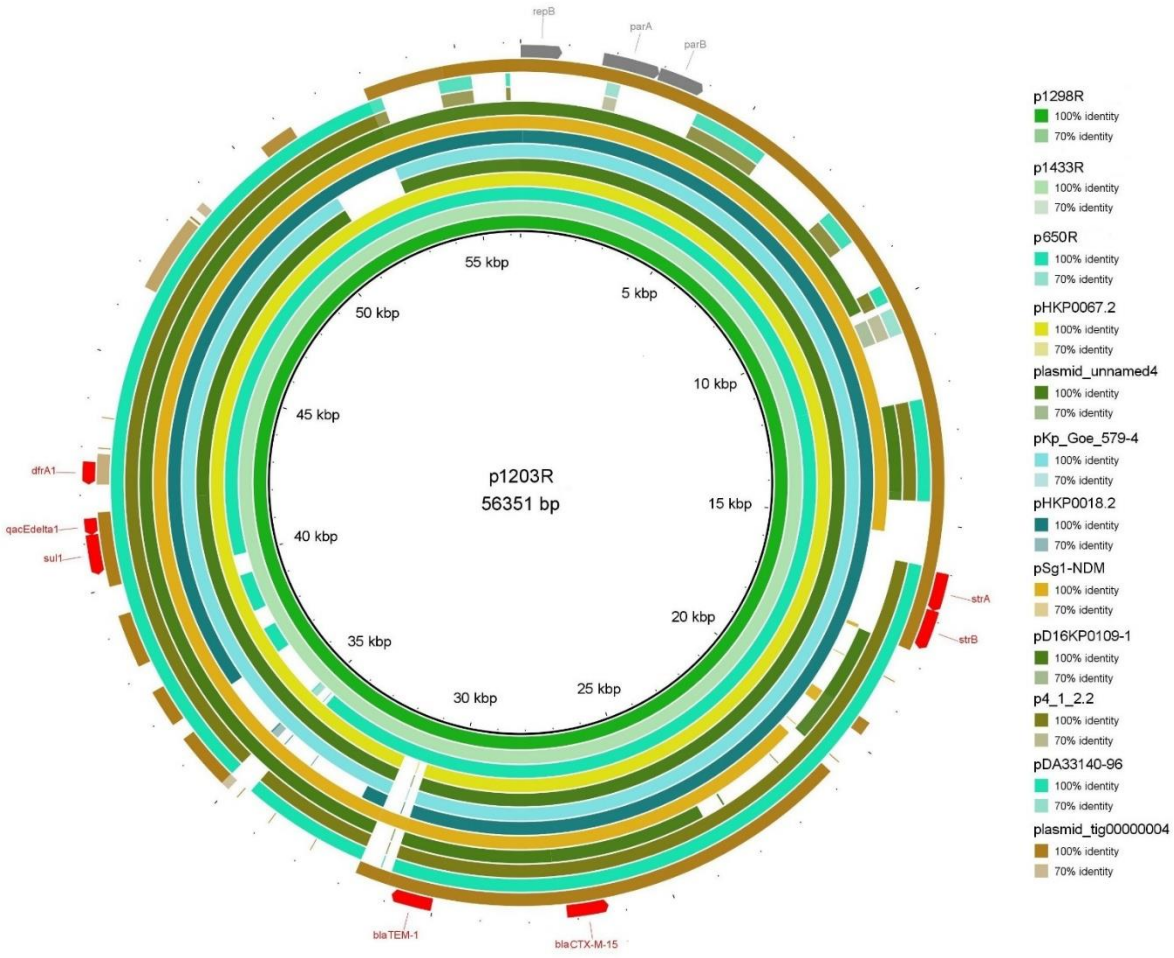
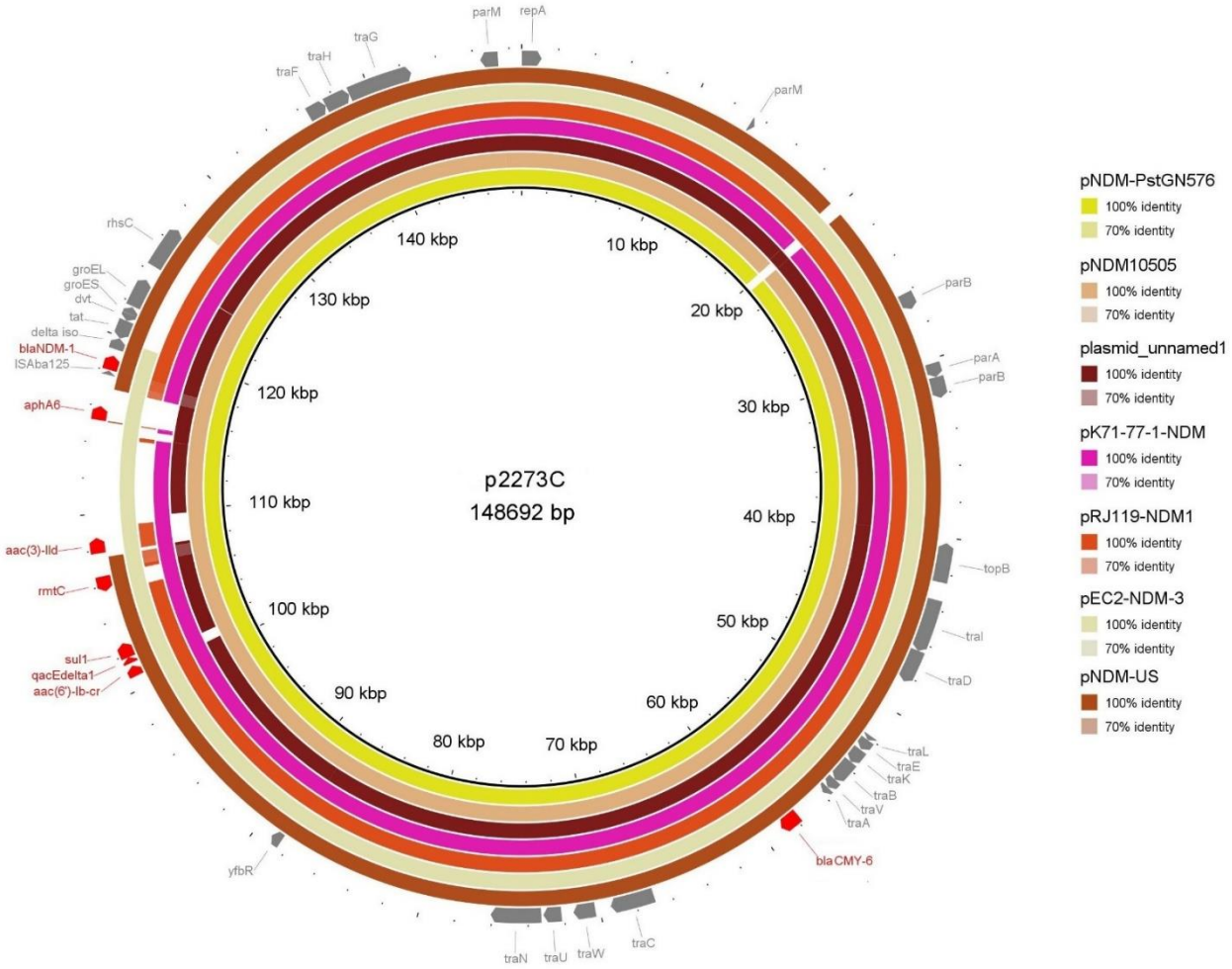


Figure S6. Comparison of the NDM-1-encoding IncC-type p2273C plasmid (inner, thin black circle) to selected plasmids from GenBank: pNDM-PstGN576 (KJ802405), pNDM10505 (JF503991), plasmid_unnamed1 (CP020524), pK71-77-1-NDM (CP040884), pRJ119-NDM1 (KX636095), pEC2-NDM-3 (KC999035), pNDM-US (CP006661). The outer thick gray ring refers to the annotation of p2273C, with the selected genes indicated. The percentage of sequence identity is reflected by colour intensity. The picture was created using the BRIG software (http://brig.sourceforge.net/).



6.2 Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006–2019

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RESEARCH

Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006–2019

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Abstract

Purpose This study was aimed at comprehensive genomic analysis of VIM-type carbapenemase-producing *Klebsiella pneumoniae* species complex (KpSC) in Poland.

Methods All non-duplicate 214 VIM-producing KpSC isolates reported in Poland in 2006–2019 were short-read sequenced and re-identified by the average nucleotide identity scoring. Their clonality/phylogeny was assessed by cgMLST and SNP in comparison with genomes from international databases. Serotypes, VIM-encoding integrons, resistomes, virulomes and plasmid replicons were identified by various bioinformatic tools. Structures of plasmids and genomic islands with VIM integrons were analysed for representative long-read sequenced isolates.

Results The KpSC isolates were the second most prevalent VIM-positive *Enterobacterales* (23.1%) in Poland in 2006–2019, following *Enterobacter* spp. (40.1%). Their significance emerged in 2014 and then grew consequently, owing to eight regional outbreaks of *K. pneumoniae* sequence types (STs) ST437, ST147, ST15, ST277 and ST392. These carried different VIM integrons, mainly In238 and In916 types, located on IncFIB + IncHI2 (pNDM-MAR)-, IncA- or IncM-like plasmids, or *clc*-type integrative and conjugative elements. Despite relatedness of the outbreak clusters to isolates from other countries, e.g. Greece, Spain, Slovakia or Germany, most of them have apparently emerged on site by horizontal acquisition of resistance determinants from other species, including *Enterobacter* spp. and *Pseudomonas* spp.

Conclusions This work shows dynamic epidemiology of VIM-producing organisms, driven by a mix of circulation of different VIM-encoding elements, and parallel clonal spread of multiple organisms.

Keywords VIM · KpSC · *Klebsiella pneumoniae* · ST437 · ST147 · Poland

Introduction

Global spread of antimicrobial resistance (AMR) in clinically relevant bacteria is a severe challenge for public health [1], and Enterobacterales with the *Klebsiella pneumoniae* species complex (KpSC) belong to the key AMR pathogens [2]. A particular concern refers to carbapenemase-producing Enterobacterales (CPE) which compromise carbapenems, the last-resort antibiotics until recently [3]. The VIM family of metallo-β-lactamases is one of the five major carbapenemase types [4]. It emerged in CPE around 2000 in Greece, and soon VIM CPE caused a country-wide outbreak, followed by high-level endemicity [5]. Subsequently, these spread broader in Europe [6–10]. Usually *K. pneumoniae* has been an important contributor to VIM CPE populations, split into clones or sequence types (STs), with some having expanded in certain countries, such as ST147 in Greece or ST15 in Hungary [5, 11]. bla_{VIM} gene cassettes, usually of bla_{VIM-1} type in CPE, reside in mainly class 1 integrons, (e.g. In-e541 or In916), spreading horizontally with conjugative plasmids of various types (e.g. IncA, IncFII, IncHI2) or, rarely, genomic islands (GIs) [5–7, 10, 12–14].

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The first VIM CPE isolate reported in Poland was a *K. pneumoniae* in 2006, confirmed by the National Reference Centre for Susceptibility Testing (NRCST) [12]. Subsequently, annual numbers of VIM CPE grew, exceeding 100 isolates in 2017, 200 in 2019, and reaching a total of 927 isolates during 2006–2019 [7]. By the end of 2013 *Enterobacter* spp. prevailed, followed by *Klebsiella oxytoca* species complex (KoSC) and *Serratia marcescens*, whereas KpSC played a minor role [7, 12]. In 2014–2019, the number of KpSC grew significantly, contributing importantly to the overall VIM CPE increase. The first molecular analysis of VIM CPE from 2006 to 2012 [12] was followed by an extensive genomic study of all 927 isolates from 2006 to 2019. After reports on *Enterobacter* spp [7]. and KoSC [15], here we describe the KpSC isolates from that period, aiming at dissecting the genomic basis of their remarkable increase since 2014.

Materials and methods

Bacterial isolates

The study comprised 214 non-duplicate VIM-producing KpSC isolates, collected in 2006–2019 by the NRCST national CPE surveillance program from 86 medical centres in 49 cities of 14/16 main administrative regions of Poland (Table S1) [7]. The isolates were from various infections ($n=128$; 59.8%), mainly of the urinary ($n=60$; 28%) or the respiratory tracts ($n=29$; 13.5%), or from carriage ($n=86$; 40.2%). The KpSC isolates were initially identified as *K. pneumoniae* with VITEK 2 (bioMérieux, Marcy l'Etoile, France) and tested for carbapenemases by CarbaNP [16], phenotypic and PCR tests [17]. One isolate (isolate 9546/19), being of sequence type (ST) 23 and serotype K1, was included also in a separate analysis of carbapenemase-producing *K. pneumoniae* ST23 isolates in Poland [18].

Whole-genome sequencing and molecular species identification

All of the isolates were sequenced by MiSeq (Illumina, San Diego, USA), with reads assembled de novo with SPAdes 3.15.5 [19]. Additionally, eight selected isolates were sequenced by MinION (Oxford Nanopore Technologies, Oxford, UK) [20], followed by hybrid assemblies performed with Unicycler 0.4.8 [21]. The species/subspecies identification was based on average nucleotide identities, using FastANI 1.32 with a > 95% cutoff [22].

Clonal and phylogenetic analyses

Multilocus sequence typing (MLST) [23] of the KpSC isolates, and their STs were determined in silico by the mlst tool (https://github.com/tseemann/mlst). The clonality of the isolates was analyzed by core-genome MLST (cgMLST) [24] and sublineages (SLs), clonal groups (CGs) and cgMLST-based Life Identification Numbers (LINs) codes [25] were identified based on the Klebsiella BIGSdb Pasteur nomenclature (https://bigsdb.pasteur.fr/klebsiella/). The clonality by single-nucleotide polymorphism (SNP) was assessed by BioNumerics 7.6.3 (Applied Maths NV, Sint-Martens-Latem, Belgium) with a strict SNP filtering template. The study was performed for individual STs, using their chronologically first isolates as references. All KpSC genomes from RefSeq (16,227 as of 1st February, 2023) (https://www.ncbi.nlm.nih.gov/refseq) were used in the phylogenetic analysis of the Polish isolates. The analysis was carried out by SNP-based comparisons within STs, using Parsnp v.1.5.4 (https://github.com/marbl/parsnp). Phylogenetic trees were visualized by iTOL (https://itol.embl.de).

Integrons, plasmids and genomic islands contaning bla_{VIM} genes

Structures of integrons, plasmids and GIs with bla_{VIM} genes were defined using Geneious Prime 2022.0.1 (Biomatters, Auckland, New Zealand) and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmid replicon types were identified with ABRicate and the PlasmidFinder database [26]. The plasmid and GI structures were visualized using BRIG (http://brig.sourceforge.net) and Easyfig 2.2.5. (http://mjsull.github.io/Easyfig).

Acquired AMR genes, serotypes and virulence genes

The composition of acquired AMR genes (resistomes) in the study isolates was determined by ABRicate and the ResFinder database with a 99.5% identity criterion [27]. The capsular (K) and LPS (O) serotypes, and virulence genes (virulomes) were identified by Kaptive [28], Kleborate [29], BIGSdb [24], Geneious Prime and BLASTn [30].

Results

Epidemiology of VIM KpSC– general remarks

VIM KpSC has been increasing in Poland from 2014, ranking the second type of VIM CPE in 2006–2019 overall (*Enterobacter* spp., 40.1%; KpSC, 23.1%) (Table S1) [7].

VIM-producing KpSC occurred almost in all regions of the country, but their regional distribution was highly uneven in absolute numbers and incidence. The most affected area was Lubelskie with 69 isolates (32.7% of all country VIM KpSC); in other regions these numbers were 1–26 (0.5–12.1%). In Lubelskie KpSC was dominant among all VIM CPE (77.5%), whereas in three other regions (Kujawsko-Pomorskie, Warmińsko-Mazurskie and Świętokrzyskie) KpSC was the most numerous group (31.4-42.3%).

Taxonomy and clonality

Three phylogroups of the KpSC isolates, corresponding to different species: *K. pneumoniae* (Kp1, $n=207$; 96.7%), *K. variicola* subsp. *variicola* (Kp3, $n=4$; 1.9%) and *K. quasipneumoniae* subsp. *similipneumoniae* (Kp4, $n=3$; 1.4%) were identified (Table S1). The isolates were classified into 40 STs, with five *K. pneumoniae* STs being collectively predominant ($n=146$; 68.2%) and having > 10 isolates each (Fig. 1, Table S2). These were: ST437 ($n=57$; 26.6% of all KpSC), ST147 ($n=33$; 15.4%), ST15 ($n=25$; 11.7%), ST277 ($n=18$; 8.4%) and ST392 ($n=13$; 6.1%). In order to define clonal relationships more precisely, STs were grouped into LIN codes and their corresponding sublineages (SLs) and clonal groups (CGs). The 35 STs belonged to 25 SLs, and comprised 1–2 CGs each. Furthermore, isolates of each CG had 1–11 distinct LIN codes. The single ST23 isolate (9546/19) was presented in details in a previous report [18].

bla$_{VIM}$ genes and integron types

Five *bla*$_{VIM}$ variants were detected, predominantly of the *bla*$_{VIM-1}$ group ($n=202$; 94.4%), including *bla*$_{VIM-4}$ ($n=130$; 60.7%), *bla*$_{VIM-1}$ ($n=71$; 33.2%) and *bla*$_{VIM-28}$ ($n=1$; 0.5%) (Tables S2 and S3). Representatives of the *bla*$_{VIM-2}$ group ($n=12$; 5.6%) contained *bla*$_{VIM-2}$ ($n=3$; 1.4%) and *bla*$_{VIM-20}$ ($n=9$; 4.2%).

Altogether 12 integrons with the *bla*$_{VIM}$ genes were detected, including one new element, In2245 (5'CS_*bla*$_{VIM-1}$_*bla*$_{OXA-10}$_3'CS). The predominant types were In238 (5'CS_*aacA4*_*bla*$_{VIM-4}$_3'CS) with or without a *bla*$_{VIM-4}$ 3'-terminal repeat ($n=104$; 48.6%), and In916 (5'CS_*bla*$_{VIM-1}$_*aacA4*_*aphA15*_*aadA1*_*catB2*_3'CS) with sequence variation in *aphA15* ($n=64$; 29.9%). Both In238 (found in 20 STs) and In916 (23 STs) were distributed widely across the clones. ST437 and ST277 were uniform in the integron content (In238 or In916) but ST147, ST392 and ST15 varied widely in that respect.

Major *K. pneumoniae* genotypes and their epidemiology

The most prevalent ST437 with In238 was observed in 2018–2019 in 17 hospitals of 10 cities, largely in Lubelskie ($n=54/57$; 94.7% of ST437 isolates), and mainly in one centre ($n=36$; 63.1%) (Tables S2 and S4; Figure S1). All these isolates had K36 and O4 serotypes, and together with sporadic ST11 and ST258 isolates were classified into SL258. The ST437 isolates belonged to CG10268 and eight LIN codes, differing by two allelic mismatches at most in cgMLST. The SNP analysis confirmed this result, showing a cluster with 0–11 SNPs between closest relatives, and documenting so a regional outbreak I.

ST147 was identified in 2015–2019 in 17 centres in nine towns mostly of three regions (Tables S2 and S5; Figure S2). The 33 isolates were of K64 and O2v1 serotypes, and together with ST392 (described below) all these belonged to SL147 and CG147. Three groups of ST147 cgMLST LIN codes with 29 isolates altogether, corresponding to SNP clusters with different integrons, revealed three regional outbreaks: outbreak II in Małopolskie ($n=11$; 1–28 SNPs between closest relatives; In916), outbreak III in Śląskie ($n=6$; 2–4 SNPs; In2245) and outbreak IV in Lubelskie ($n=12$; 0–4 SNPs; In238).

ST15 was recorded in 2014–2019 in 13 hospitals in 11 cities of two regions mainly (Tables S2 and S6; Figure S3). Its 25 isolates belonged to SL15 and CG15, and two groups of related LIN codes ($n=20$ altogether), correlating with SNP clusters, serotypes, integrons and geographic origins. These corresponded to regional outbreaks V in Wielkopolskie ($n=5$; 5–21 SNPs between closest relatives; K24 and O1v1; In1444) and VI in Świętokrzyskie ($n=25$; 0–17 SNPs; O1v1 and KL48; In1654).

An interregional outbreak VII comprised all the 18 ST277 isolates, identified in 2017–2019 in seven hospitals in six cities of two neighboring regions, Warmińsko-Mazurskie and Pomorskie (Tables S2 and S7; Figure S4). These all were of SL277 and CG10463, and represented six closely related LIN codes, reflecting an SNP cluster (0–17 SNPs between closest relatives). The ST277 isolates had serotypes K46 and O3b, and integron type In916.

As mentioned above, ST392 belonged to SL147 and contained CG147, and was recorded in 2014–2019 in seven hospitals of seven cities, spread in the country (Tables S2 and S8; Figure S5). Differing from ST147 by K27 and O4 serotypes, 8/13 ST392 isolates with In1667 had closely related LIN codes, corresponding to a SNP cluster (5–15 SNPs between closest relatives) from a hospital outbreak VIII in Kujawsko-Pomorskie.

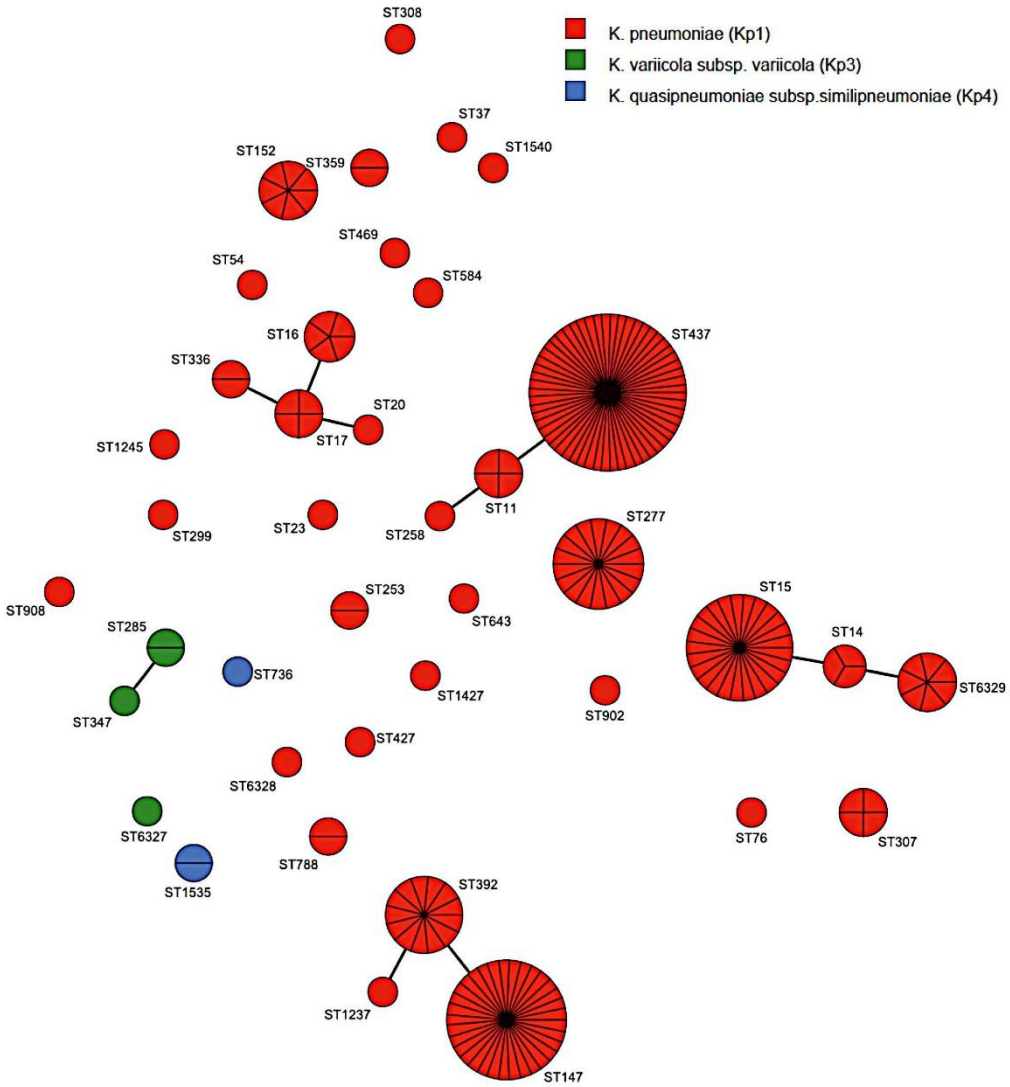

Fig. 1 Population structure of *K. pneumoniae* species complex (KpSC) isolates identified in a genome-based epidemiologic analysis of VIM carbapenemase-producing KpSC, Poland, 2006–2019. The minimum-spanning tree was constructed on the basis of 7-loci multilocus sequence type data. Each circle represents 1 ST, and each fragment of a pie chart corresponds to 1 isolate. The size of a circle is proportional to the number of isolates of that ST. Connecting lines link single-locus variants; the other links are not shown due to uncertainty in them representing phylogenetic relationships. ST, sequence type

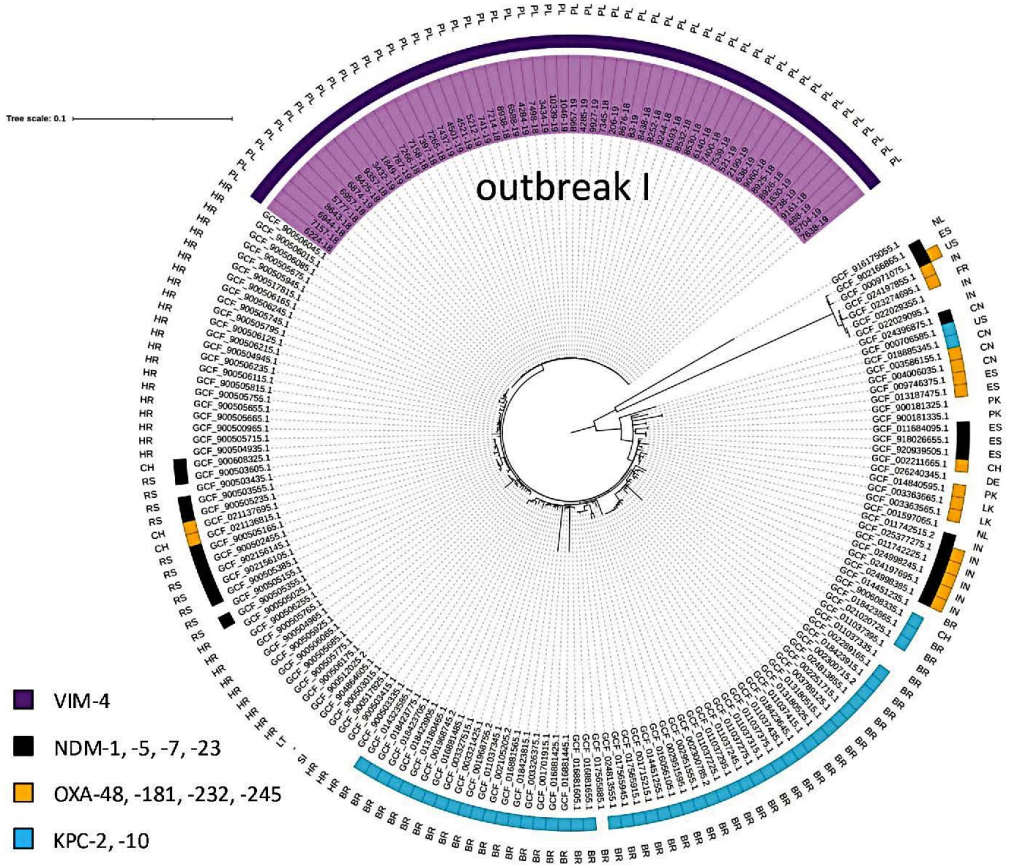

Fig. 2 SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST437 isolates compared with the international ST437 genomes available in RefSeq. Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: BR, Brazil; CH, Switzerland; CN, China; DE, Germany; ES, Spain; FR, France; HR, Croatia; IN, India; LK, Sri Lanka; LT, Lithuania; NL, Netherlands; PL, Poland; PK, Pakistan; RS, Serbia; SI, Slovenia; US, United States of America. The tree was constructed using Parsnp and visualized with iTOL

Phylogeny of the major *K. pneumoniae* Genotypes

The phylogenetic analysis of 135 international and 57 Polish ST437 genomes revealed that the entire ST437 population was dominated vastly by one relatively homogeneous clade (< 200 SNPs between any isolate and the Polish reference) (Fig. 2). The clade consisted largely of three clusters: Polish VIM ST437 outbreak I; isolates from Croatia, Serbia and Switzerland, some with NDM or OXA-48 types; and KPC producers from Brazil.

The ST147 phylotree with 556 RefSeq and 33 Polish genomes had also a prevailing clade, grouping serotype K64 isolates from all over the world, often with carbapenemases (Figure S6). Polish VIM ST147 outbreaks' II-IV clusters had closer relatives in OXA-48 producers from Germany (34–37 SNPs with the Polish reference), Greece (49–65 SNPs) and India (40 SNPs). The previously studied NDM-1-producing ST147 K64 isolates imported to Poland from Tunisia [31] were more distant.

The analysis of 907 ST15 international and 25 Polish isolates revealed complex structure of a large phylotree (Figure

S7); therefore, positions of the study isolates were defined within their branch shared with 205 international isolates (Figure S8). The VIM ST15 outbreaks' V-VI clusters were separated into two subbranches. Outbreak V neighbored isolates from Africa, Slovakia and Spain (VIM-/OXA-48-like producers; 55–63 SNPs from the Polish reference). Outbreak VI grouped with isolates from worldwide (some with KPC, NDM or OXA-48), including Italy or Switzerland (26–86 SNPs).

A small number of 16 international ST277 genomes available made defining the phylogenetic position of outbreak VII non-conclusive (Figure S9). Otherwise, ST392 had 82 genomes from all around the world, which, with the 13 study isolates, composed a tree with relatively low diversity level overall (81–242 SNPs from the Polish reference) (Figure S10). Polish isolates with outbreak VIII formed a distinct branch.

Acquired AMR genes

Apart from the bla_{VIM} genes, the KpSC isolates had many other acquired AMR genes (1–18 genes per isolate; 9.3 in average) (Table S9). The resistomes varied, even within individual STs and outbreak clusters; some of these had more AMR genes than the average, e.g. ST437 outbreak I, ST147 outbreak II or ST277 outbreak VII (14.1 AMR genes per isolate each). Representative isolates of most of the outbreaks were long-read sequenced, so that we could define their resistomes, considering also multiple copies of some AMR genes (Table S10). We identified $bla_{CTX\text{-}M}$-type genes of extended-spectrum β-lactamases (ESBLs), commonly $bla_{CTX\text{-}M\text{-}15}$ (n = 109; 50.9% of KpSC isolates). All isolates had multiple genes of aminoglycoside-modifying enzymes, and one-fourth of isolates (n = 53; 24.8%) carried *armA*, encoding a 16S rRNA methylase inactivating all aminoglycosides. Genes of resistance to fluoroquinolones (*aac(6')-Ib-cr*, *n* = 46; 21.5%; *qnrB1/B6/S1*, *n* = 94; 43.9%), trimethoprim (*dfrA1/12/14/27/30*, *n* = 117; 54.7%) and sulphonamides (*sul1/2*, *n* = 149; 69.6%) were abundant too.

Plasmids and GIs

Twelve plasmid replicon types were identified in the KpSC isolates (1–9 replicons per isolate; mean 4.9), most frequently IncFI (*n* = 319; 30.6% of all replicons), Col (*n* = 308; 29.6%) and IncFII (*n* = 182; 17.5%) (Table S11). Replicon patterns varied both across and within STs. The actual content of plasmids and their AMR genes was revealed in seven long-read sequenced isolates, including outbreak representatives (Table S10), in which detailed structure of bla_{VIM}-harboring plasmids was analyzed; an IncA plasmid with In916 from the sporadic ST23 isolate (9546/19) was published previously [18]. Outbreak VI isolates had a GI with bla_{VIM} that was long-read sequenced in a representative isolate as well.

The outbreak I ST437 and outbreak IV ST147 isolates had double-replicon IncFIB + IncHI1B pNDM-MAR-type plasmids [32] with In238, namely p5777FH of 246,869 bp (ST437) and p7160FH of 272,076 bp (ST147) (Figure S11). The pNDM-MAR types contain fragments of the virulence plasmid pK2044/KpVP-1 [33], which in the prototypic pNDM-MAR itself (AMR plasmid from Moroccan *K. pneumoniae* ST15) provide the HI1B replicon and tellurium resistance locus *ter* only [32] (Figure S12). The pNDM-MAR was the best match of p5777FH (coverage, 81%; identity, 99.5%) and p7160FH (76%; 99.5%), revealing multiple backbone rearrangements though. Moreover, pNDM-MAR had a totally different bla_{NDM-1}-carrying AMR region and in another location than the study plasmids, which differed from each other by several AMR genes besides In238.

The outbreak II ST147 and outbreak VII ST277 isolates had In916 located on IncA (ST12)-like plasmids, so as the single ST23 isolate (9546/19) described previously [18] (Figure S13). The p2254A (ST147) of 162,628 bp, p7089A of 203,105 bp (ST277) and p9546/19_2 of 158,944 bp (ST23) were most similar to In916-carrying IncAs from Polish *Enterobacter hormaechei* [7] and *K. oxytoca* [15] (coverage, 67–100%; identity, 99.9–100%) but also to such molecules from e.g. Italy or France [6, 10, 13]. A remarkable part of these were AMR regions with multiple genes and mobile elements (~37.8-~53.2 kb), including an IS*26*–bla_{SHV-12}–In916–IS*26* module, described first in pGB_VIM in Italy [7, 10]. Their structural variation, observed also in the study (Figure S14), was the main source of these plasmids' diversity. Some of these IncA-type plasmids, including the study p7089A and p7536 from France [34], had an additional mosaic region (~39.2 kb) of unknown function (Figure S13).

A part of the outbreak V ST15 and all outbreak VIII ST392 isolates carried IncM2-type plasmids, being p381M of 89,194 bp with In1444 (ST15) and p1359M of 90,265 bp with In1667 (ST392) (Figure S15). A series of molecules with conserved scaffolds and various AMR genes (*e.g.* $bla_{CTX-M-3/-15}$, *aac(3)-IIa*, *aac(6')-Ib*, and/or *armA*) were related to the study plasmids (coverage, 95–98%; identity, ~99.7–100%), originating from a large geographic span. The two alternative integrons in the study plasmids, In1444 (bla_{VIM-20}) and In1667 (bla_{VIM-4}), were inserted in the same position between IS*26* and IS*Ec28*.

The outbreak VI ST15 isolates contained GIs harboring In1654 (bla_{VIM-4}), being *clc*-type integrative and conjugative elements (ICEs) [35], inserted into the $tRNA^{Gly}$ gene (locus KPHS_t00610). Like previously reported Polish bla_{VIM}-carrying GIs/ICEs *Eh*GI3 from *E. hormaechei* [7],

and the ICE*6441*-PL1-PL21 series from *Pseudomonas* spp. [36], the ICE*6441*-PL22 element (107,565 bp) represented the main lineage GI2 of carbapenemase-encoding ICEs [36–38]. Consistently, In1654 was inserted into the Tn*4380* transposon, identical to ICE*6441*-PL12 in a *Pseudomonas alloputida* isolate; these two elements differed from each other only by a 3585 bp-long fragment associated with IS*CR3* [36].

Virulomes

Besides the single ST23 K1 isolate (9546/19), reported elsewhere [18], none of the isolates contained the increased virulence loci *iuc*, *iro*, *rmpA/A2* or *clb* [39]. Fifty-two isolates (24.3%) carried different loci of the *ybt* cluster coding for the synthesis of yersiniabactin [29], located on six ICE variants (ICE*Kp3*, *4*, *5*, *10*, *11*, *12*) in 11 STs, largely ST147 ($n=33$) (Table S12).

Discussion

As elsewhere in Europe [5, 6, 9, 11, 40], VIM producers are amongst the four most frequently observed CPE types in Poland [7]. After the period of incidental occurrence in 2006–2013, they started to grow steadily, and one of the main factors of that has been the KpSC spread. From 2014 to 2017, KpSC, and *K. pneumoniae* mainly, achieved the important role among VIM CPE; however, with *Enterobacter* spp. being the most prevalent [7]. This study was meant to reveal the genetic structure and characteristics of VIM KpSC populations, disseminating rapidly in Poland.

The genome sequence analysis of all 214 VIM KpSC isolates from 2006 to 2019, inferring taxonomy, clonality, phylogeny, and VIM genetic determinants and platforms identified eight clonal outbreaks in different regions. Each of these commenced in 2014–2018, and all were still ongoing in 2019, correlating with the VIM KpSC increase rates. The outbreaks were caused by various organisms, defined by classifications at several phylogenetic levels (SL, ST, CG and LIN code), and their corresponding resistomes, bla_{VIM} variants, integrons, plasmids or GIs.

Especially outbreak I contributed to the overall situation. It started only in 2018 but in 2-year time has become the largest one, constituting more than one-fourth ($n=57$; 26.6%) of all KpSC. Outbreak I expanded from one to seven hospitals in Lublin, the main city of Lubelskie, six local centres, and three institutions in other regions. In 2019 outpatients appeared in Lublin, indicating efflux of the epidemic organism to the community. It was *K. pneumoniae* ST437 with In238 (bla_{VIM-4}) on IncFIB + IncHI1B pNDM-MAR-type plasmids. ST437, a single-locus variant of ST258, described in 2011, was known the main KPC-type carbapenemase host in Brazil [41], unlike other countries with ST258 predominant [3]. More recently ST437 was reported with NDM- and/or OXA-48-type carbapenemases e.g. in China [42], India [43], USA [44], and Southern Europe [45–48]. All these, plus the ST437 phylotree (Fig. 2) showed the emerging and high-risk character of this clone.

ST15 and ST147 were peculiar in having segregated 2–3 outbreak genotypes each, discerned by cgMLST/SNP, bla_{VIM} integrons and carriers. Outbreak VI caused by ST15 with In1654 (bla_{VIM-4}) on *clc*-like ICEs was remarkable ($n=25$; 11.7%). ST15 belongs to early *K. pneumoniae* STs [23], from 2 to 3 decades ago being a major global ESBL producer [49, 50], and remaining the predominant VIM host in Hungary [11]. The more recent ST147 has been one of the most expansive multidrug-resistant *K. pneumoniae* clones, with a reservoir in the Mediterranean basin [51, 52]. ST147 was the main factor of the nationwide VIM outbreak in Greece, the first large-scale CPE manifestation in Europe [5]. Multiple ST147 lineages with diverse carbapenemases have been spreading since then, even within single countries [51, 52], including Poland, where besides the three VIM outbreak genotypes, we have identified epidemic NDM-1-producing variants from North Africa [31].

Phylogenetic comparisons of the outbreak isolates with RefSeq genomes showed some relationships, like e.g. ST147 outbreaks II-IV with isolates from Germany or Greece, or ST15 outbreak V with isolates from Spain or Slovakia. These illustrated broad circulation of these organisms. However, the relationships were not close enough to indicate a direct import of the individual genotypes to Poland from abroad (the international isolates also had different or no carbapenemases). The only presumable import was the first Polish isolate, *K. pneumoniae* ST1237 from 2006, an ST147 variant with In-e541, typical for the epidemic organism in Greece [5, 6, 12]. However, it has not dispersed in Poland.

Of bla_{VIM} integrons, two types, In238 and In916, prevailed and spread to multiple clones, including four outbreak clusters. The In238 type has comprised multiple elements, varying in individual bla_{VIM-1}-like cassettes by mutations and/or 3'-terminal duplications [7, 53]. Recorded first in Polish *Pseudomonas aeruginosa* in 1998 [54], it has been common in *Pseudomonas* spp. [36, 53] and *Enterobacter* spp. in Poland [7]. However, a sporadic *K. pneumoniae* ST11 isolate from 2008 in this study was the first Enterobacterales with In238. Interestingly, In238 variants have been observed broader in Mid-Southern Europe [8, 55, 56]. These have transmitted with various platforms, in Polish KpSC often the IncFIB + IncHI1B pNDM-MAR-type plasmids [32] and GI2-like *clc*-type ICEs [36, 37]. Strikingly, origins of these two genetic configurations have been

unclear, given their lack in *Enterobacter* spp. and other VIM CPE in Poland [7]. Otherwise, In916 has been observed from early 2010s, exclusively in European Enterobacterales [6, 10, 13, 57], and usually on IncA-type plasmids [6, 10, 13]. In Poland these spread in *Enterobacter* spp. [7], *K. oxytoca* [15], and *K. pneumoniae* (first in ST152 from 2014), being closely related to Italian variants [10]. Their rapid proliferation has been an important factor of the VIM CPE spread in Poland.

This study revealed genomic determinants of the increase of VIM-producing KpSC in 2014–2019 in Poland, and together with those on *Enterobacter* spp. [7] and KoSC [15], provide comprehensive country data on epidemiology of VIM CPE. These all demonstrated the major role of multiple parallel clonal outbreaks in different regions and of the horizontal expansion of VIM genetic determinants/platforms. Their common elements were the two essential integron types, In238 of pseudomonadal origin and the enterobacterial In916, and the IncA-type plasmids spreading In916. The KpSC-specific element were the pNDM-MAR-type plasmids with In238 in the key outbreak organism ST437. The observations indicated that apart from the IncA-like plasmids, most of the organisms and genetic elements emerged on site or were of unknown origin. The study demonstrates Polish hospitals to be an environment favoring the emergence, and dynamic dissemination and differentiation of CPE, indicating shortcomings in the infection control system.

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Author contributions All authors contributed to the study concept and design. Survey and microbiological analysis were performed by MB, PU, DZ and RI. Bioinformatic analyses was done by MB, PU, SB, FP and RI. Draft of the manuscript was written by RI and its final version was prepared by SB and MG. The manuscript was approved by all the authors.

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Data availability Genomic sequences have been deposited in the NCBI under the BioProject number PRJNA1005055, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1005055, and BioSample numbers SAMN36972333- 36972546. Plasmid and genomic islands sequences have been assigned the following accession numbers: PQ441977, p381M; PQ441978, p1359M; PQ441979, p7089A; PQ441980, p2254A; PQ441981, p5777A; PQ441982, p7160FH; PQ463789, ICE6441-PL22.

Declarations

Ethical approval The isolates used in the current study were obtained during a routine national surveillance activity of the National Reference Centre for Susceptibility Testing, under the mandate of the Ministry of Health. The study was performed in a retrospective manner with an anonymization of patients' data; thus, ethical approval and informed consent were not required.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399(10325):629–655
2. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48(1):1–12
3. Bush K, Bradford PA (2020) Epidemiology of β-lactamase-producing pathogens. Clin Microbiol Rev 33(2):e00047–e00019
4. Boyd SE, Livermore DM, Hooper DC, Hope WW (2020) Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrob Agents Chemother 64(10):e00397–e00320
5. Hasan CM, Turlej-Rogacka A, Vatopoulos AC, Giakkoupi P, Maatallah M, Giske CG (2014) Dissemination of bla_{VIM} in Greece at the peak of the epidemic of 2005–2006: clonal expansion of *Klebsiella pneumoniae* clonal complex 147. Clin Microbiol Infect 20(1):34–37
6. Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabak J, Derde LP, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M (2015) Survey of metallo-β-lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008-11. J Antimicrob Chemother 70(7):1981–1988
7. Izdebski R, Biedrzycka M, Urbanowicz P, Zabicka D, Gniadkowski M (2023) Genome-based epidemiologic analysis of VIM/IMP carbapenemase-producing *Enterobacter* spp., Poland. Emerg Infect Dis 29(8):1618–1626
8. Kristof K, Toth A, Damjanova I, Janvari L, Konkoly-Thege M, Kocsis B, Koncan R, Cornaglia G, Szego E, Nagy K, Szabo D (2010) Identification of a bla_{VIM-4} gene in the internationally successful *Klebsiella pneumoniae* ST11 clone and in a *Klebsiella oxytoca* strain in Hungary. J Antimicrob Chemother 65(6):1303–1305
9. Oteo J, Ortega A, Bartolome R, Bou G, Conejo C, Fernandez-Martinez M, Gonzalez-Lopez JJ, Martinez-Garcia L, Martinez-Martinez L, Merino M, Miro E, Mora M, Navarro F, Oliver A, Pascual A, Rodriguez-Bano J, Ruiz-Carrascoso G, Ruiz-Garbajosa P, Zamorano L, Bautista V, Perez-Vazquez M, Campos J (2015) Prospective multicenter study of carbapenemase-producing *Enterobacteriaceae* from 83 hospitals in Spain reveals high

in vitro susceptibility to colistin and meropenem. Antimicrob Agents Chemother 59(6):3406–3412
10. Arcari G, Di Lella FM, Bibbolino G, Mengoni F, Beccaccioli M, Antonelli G, Faino L, Carattoli A (2020) A multispecies cluster of VIM-1 carbapenemase-producing Enterobacterales linked by a novel, highly conjugative, and broad-host-range IncA plasmid forebodes the reemergence of VIM-1. Antimicrob Agents Chemother 64(4):e02435–e02419
11. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, Abudahab K, Goater R, Giani T, Errico G, Aspbury M, Sjunnebo S, Eu SWG, Group ES, Feil EJ, Rossolini GM, Aanensen DM, Grundmann H (2019) Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. Nat Microbiol 4(11):1919–1929
12. Izdebski R, Baraniak A, Żabicka D, Sękowska A, Gospodarek E, Hryniewicz W, Gniadkowski M (2018) VIM/IMP carbapenemase-producing Enterobacteriaceae in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother 73(10):2675–2681
13. Emeraud C, Petit C, Gauthier L, Bonnin RA, Naas T, Dortet L (2022) Emergence of VIM-producing *Enterobacter cloacae* complex in France between 2015 and 2018. J Antimicrob Chemother 77(4):944–951
14. Tato M, Coque TM, Baquero F, Canton R (2010) Dispersal of carbapenemase bla_{VIM-1} gene associated with different Tn402 variants, mercury transposons, and conjugative plasmids in *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 54(1):320–327
15. Biedrzycka M, Urbanowicz P, Zabicka D, Hryniewicz W, Gniadkowski M, Izdebski R (2023) Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009–2019. Eur J Clin Microbiol Infect Dis 42(12):1449–1457
16. Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 18(9):1503–1507
17. Baraniak A, Machulska M, Zabicka D, Literacka E, Izdebski R, Urbanowicz P, Bojarska K, Herda M, Kozinska A, Hryniewicz W, Gniadkowski M, Group N-PS (2019) Towards endemicity: large-scale expansion of the NDM-1-producing *Klebsiella pneumoniae* ST11 lineage in Poland, 2015-16. J Antimicrob Chemother 74(11):3199–3204
18. Biedrzycka M, Izdebski R, Urbanowicz P, Polanska M, Hryniewicz W, Gniadkowski M, Literacka E (2022) MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009-19. J Antimicrob Chemother 77(12):3367–3375
19. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19(5):455–477
20. Izdebski R, Sitkiewicz M, Urbanowicz P, Krawczyk M, Brisse S, Gniadkowski M (2020) Genomic background of the *Klebsiella pneumoniae* NDM-1 outbreak in Poland, 2012-18. J Antimicrob Chemother 75(11):3156–3162
21. Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13(6):e1005595
22. Jain C, Rodriguez RL, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9(1):5114
23. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S (2005) Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol 43(8):4178–4182
24. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decre D, Brisse S (2014) Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis 20(11):1812–1820
25. Hennart M, Guglielmini J, Bridel S, Maiden MCJ, Jolley KA, Criscuolo A, Brisse S (2022) A dual barcoding approach to bacterial strain nomenclature: genomic taxonomy of *Klebsiella pneumoniae* strains. Mol Biol Evol 39(7):msac135
26. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H (2014) In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58(7):3895–3903
27. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67(11):2640–2644
28. Wick RR, Heinz E, Holt KE, Wyres KL (2018) Kaptive web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. J Clin Microbiol 56(6):e00197–e00118
29. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE (2021) A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. Nat Commun 12(1):4188
30. Stewart J, Judd LM, Jenney A, Holt KE, Wyres KL, Hawkey J (2022) Epidemiology and genomic analysis of *Klebsiella oxytoca* from a single hospital network in Australia. BMC Infect Dis 22(1):704
31. Biedrzycka M, Urbanowicz P, Guzek A, Brisse S, Gniadkowski M, Izdebski R (2021) Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19. J Antimicrob Chemother 76(10):2538–2545
32. Villa L, Poirel L, Nordmann P, Carta C, Carattoli A (2012) Complete sequencing of an IncH plasmid carrying the bla_{NDM-1}, $bla_{CTX-M-15}$ and *qnrB1* genes. J Antimicrob Chemother 67(7):1645–1650
33. Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, Fung CP, Chen HJ, Liu YM, Wang JT, Fang CT, Chang SC, Shu HY, Liu TT, Chen YT, Shiau YR, Lauderdale TL, Su IJ, Kirby R, Tsai SF (2009) Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. J Bacteriol 191(14):4492–4501
34. Hadjadj L, Cassir N, Saidani N, Hoffman C, Brouqui P, Astoul P, Rolain JM, Baron SA (2022) Outbreak of carbapenem-resistant enterobacteria in a thoracic-oncology unit through clonal and plasmid-mediated transmission of the bla_{OXA-48} gene in Southern France. Front Cell Infect Microbiol 12:1048516
35. Johnson CM, Grossman AD (2015) Integrative and conjugative elements (ICEs): what they do and how they work. Annu Rev Genet 49:577–601
36. Urbanowicz P, Izdebski R, Biedrzycka M, Gniadkowski M (2024) VIM-type metallo-β-lactamase (MBL)-encoding genomic islands in *Pseudomonas* spp. in Poland: predominance of clc-like integrative and conjugative elements (ICEs). J Antimicrob Chemother 79(5):1030–1037
37. Botelho J, Mourao J, Roberts AP, Peixe L (2020) Comprehensive genome data analysis establishes a triple whammy of carbapenemases, ICEs and multiple clinically relevant bacteria. Microb Genom 6(10):mgen000424
38. Roy Chowdhury P, Scott M, Worden P, Huntington P, Hudson B, Karagiannis T, Charles IG, Djordjevic SP (2016) Genomic islands 1 and 2 play key roles in the evolution of extensively drug-resistant ST235 isolates of *Pseudomonas aeruginosa*. Open Biology 6(3):150175

39. Russo TA, Marr CM (2019) Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev 32(3):e00001–00019
40. Matsumura Y, Peirano G, Devinney R, Bradford PA, Motyl MR, Adams MD, Chen L, Kreiswirth B, Pitout JDD (2017) Genomic epidemiology of global VIM-producing *Enterobacteriaceae*. J Antimicrob Chemother 72(8):2249–2258
41. Andrade LN, Curiao T, Ferreira JC, Longo JM, Climaco EC, Martinez R, Bellissimo-Rodrigues F, Basile-Filho A, Evaristo MA, Del Peloso PF, Ribeiro VB, Barth AL, Paula MC, Baquero F, Canton R, Darini AL, Coque TM (2011) Dissemination of bla_{KPC-2} by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* species in Brazil. Antimicrob Agents Chemother 55(7):3579–3583
42. Weng X, Shi Q, Wang S, Shi Y, Sun D, Yu Y (2020) The characterization of OXA-232 carbapenemase-producing ST437 *Klebsiella pneumoniae* in China. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale 2020:5626503
43. Sahoo S, Sahoo RK, Dixit S, Behera DU, Subudhi E (2023) NDM-5-carrying *Klebsiella pneumoniae* ST437 belonging to high-risk clonal complex (CC11) from an urban river in eastern India. 3 Biotech 13(5):139
44. Ceron S, Salem-Bango Z, Contreras DA, Ranson EL, Yang S (2023) Clinical and genomic characterization of carbapenem-resistant *Klebsiella pneumoniae* with concurrent production of NDM and OXA-48-like carbapenemases in Southern California, 2016–2022. Microorganisms 11(7):1717
45. Seara N, Oteo J, Carrillo R, Perez-Blanco V, Mingorance J, Gomez-Gil R, Herruzo R, Perez-Vazquez M, Astray J, Garcia-Rodriguez J, Ruiz-Velasco LM, Campos J, de Burgos C, Ruiz-Carrascoso G (2015) Interhospital spread of NDM-7-producing *Klebsiella pneumoniae* belonging to ST437 in Spain. Int J Antimicrob Agents 46(2):169–173
46. Di Marcantonio S, Perilli M, Alloggia G, Segatore B, Miconi G, Bruno G, Frascaria P, Piccirilli A (2024) Coexistence of bla_{NDM-5}, $bla_{CTX-M-15}$, $bla_{OXA-232}$, $bla_{SHV-182}$ genes in multidrug-resistant *K. pneumoniae* ST437-carrying OmpK36 and OmpK37 porin mutations: first report in Italy. J Glob Antimicrob Resist 37:24–27
47. Novovic K, Trudic A, Brkic S, Vasiljevic Z, Kojic M, Medic D, Cirkovic I, Jovcic B (2017) Molecular epidemiology of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in Serbia from 2013 to 2016. Antimicrob Agents Chemother 61(5):e02550–e02516
48. Pirs M, Cerar Kisek T, Krizan Hergouth V, Seme K, Mueller Premru M, Jeverica S, Logar M, Mrvic T, Znidarsic B, Jordan Markocic O, Lejko Zupanc T (2019) Successful control of the first OXA-48 and/or NDM carbapenemase-producing *Klebsiella pneumoniae* outbreak in Slovenia 2014–2016. J Hosp Infect 101(2):142–149
49. Breurec S, Guessennd N, Timinouni M, Le TA, Cao V, Ngandjio A, Randrianirina F, Thiberge JM, Kinana A, Dufougeray A, Perrier-Gros-Claude JD, Boisier P, Garin B, Brisse S (2013) *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. Clin Microbiol Infect 19(4):349–355
50. Baraniak A, Izdebski R, Fiett J, Sadowy E, Adler A, Kazma M, Salomon J, Lawrence C, Rossini A, Salvia A, Vidal Samso J, Fierro J, Paul M, Lerman Y, Malhotra-Kumar S, Lammens C, Goossens H, Hryniewicz W, Brun-Buisson C, Carmeli Y, Gniadkowski M (2013) Comparative population analysis of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases colonizing patients in rehabilitation centers in four countries. Antimicrob Agents Chemother 57(4):1992–1997
51. Rodrigues C, Desai S, Passet V, Gajjar D, Brisse S (2022) Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. Microb Genom 8(1):000737
52. Peirano G, Chen L, Kreiswirth BN, Pitout JDD (2020) Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. Antimicrob Agents Chemother 64(10):e01148–e01120
53. Urbanowicz P, Izdebski R, Baraniak A, Zabicka D, Hryniewicz W, Gniadkowski M (2021) Molecular and genomic epidemiology of VIM/IMP-like metallo-β-lactamase-producing *Pseudomonas aeruginosa* genotypes in Poland. J Antimicrob Chemother 76(9):2273–2284
54. Patzer J, Toleman MA, Deshpande LM, Kaminska W, Dzierzanowska D, Bennett PM, Jones RN, Walsh TR (2004) *Pseudomonas aeruginosa* strains harbouring an unusual bla_{VIM-4} gene cassette isolated from hospitalized children in Poland (1998–2001). J Antimicrob Chemother 53(3):451–456
55. Scoulica EV, Neonakis IK, Gikas AI, Tselentis YJ (2004) Spread of bla_{VIM-1}-producing *E. Coli* in a university hospital in Greece. Genetic analysis of the integron carrying the bla_{VIM-1} metallo-β-lactamase gene. Diagn Microbiol Infect Dis 48(3):167–172
56. Bitar I, Papagiannitsis CC, Kraftova L, Chudejova K, Mattioni Marchetti V, Hrabak J (2020) Detection of five *mcr-9*-carrying *Enterobacterales* isolates in four Czech hospitals. mSphere 5(6):e01008–01020
57. Porres-Osante N, Azcona-Gutierrez JM, Rojo-Bezares B, Undabeitia E, Torres C, Saenz Y (2014) Emergence of a multiresistant KPC-3 and VIM-1 carbapenemase-producing *Escherichia coli* strain in Spain. J Antimicrob Chemother 69(7):1792–1795

Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019

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Table S1. Annual and geographic distribution of VIM-producing *K. pneumoniae* species complex in Poland in 2006-2019

K. pneumoniae species complex	Year														Total	% of all VIM CPE
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019		
K. pneumoniae (Kp1)	1	-	1	3	-	3	3	2	20	8	16	28	59	63	**207**	**22.3**
K. variicola subsp. *variicola* (Kp3)	-	-	-	-	-	-	-	-	-	-	-	1	2	1	**4**	**0.4**
K. quasipneumoniae subsp. *similipneumoniae* (Kp4)	-	-	-	-	-	-	-	-	-	-	-	2	-	1	**3**	**0.3**
Total	**1**	-	**1**	**3**	-	**3**	**3**	**2**	**20**	**8**	**16**	**31**	**61**	**65**	**214**	**23.1**
Administrative regions																
Dolnośląskie	-	-	-	-	-	-	-	-	-	1	2	-	1	4	**8**	**9.9**
Kujawsko-Pomorskie	1	-	-	-	-	-	-	-	6	2	-	-	-	-	**9**	**37.5**
Lubelskie	-	-	-	-	-	-	1	-	-	-	-	-	37	31	**69**	**77.5**
Lubuskie	-	-	-	-	-	-	-	-	-	-	-	1	-	-	**1**	**5.9**
Łódzkie	-	-	-	-	-	-	-	-	1	-	4	6	5	3	**19**	**14.6**
Małopolskie	-	-	-	-	-	-	-	-	-	-	-	-	1	11	**12**	**22.2**
Mazowieckie	-	-	1	3	-	-	1	1	8	3	4	3	-	2	**26**	**12.9**
Opolskie	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**	**14.3**
Podkarpackie	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**	**2.4**
Podlaskie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pomorskie	-	-	-	-	-	-	-	-	2	-	3	4	3	4	**16**	**18.8**
Śląskie	-	-	-	-	-	-	-	-	-	2	-	6	4	-	**12**	**25.0**
Świętokrzyskie	-	-	-	-	-	-	-	-	-	-	1	9	5	1	**16**	**31.4**
Warmińsko-Mazurskie	-	-	-	-	-	-	-	-	-	-	-	-	3	8	**11**	**42.3**
Wielkopolskie	-	-	-	-	-	3	1	1	3	-	2	2	-	1	**13**	**21.0**
Zachodniopomorskie	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
															214	**23.1**

Table S2. Taxonomy, clonality, serotypes, integrons and *bla*$_{VIM}$ gene variants in the VIM-producing KpSC study isolates

phylogroup	sublineage	ST[b]	Clonal group	Serotype	*bla*$_{VIM}$ gene	Integron[c]	LINcode	Remarks
Kp1 (n=207)	SL258 (n=62)	**ST437** (n=57)	CG10268	O4; KL36	*bla*$_{VIM-4}$	In238	0_0_105_1_1_1_9_0_0_0 (n=45)	
							0_0_105_1_1_1_9_0_2_0 (n=5)	
							0_0_105_1_1_1_9_0_3_0 (n=2)	
							0_0_105_1_1_1_9_0_4_0 (n=1)	regional outbreak I
							0_0_105_1_1_1_9_0_5_0 (n=1)	
							0_0_105_1_1_1_9_0_6_0 (n=1)	
							0_0_105_1_1_1_9_0_7_0 (n=1)	
							0_0_105_1_1_1_9_1_0_0 (n=1)	
		ST11 (n=4)	CG340 (n=3)	O3b; KL125	*bla*$_{VIM-4}$	In238	0_0_105_0_3_0_1_47_0_0	
			CG3666 (n=1)	O2v1; KL24	*bla*$_{VIM-4}$	In238	0_0_105_11_0_0_0_48_0_0	
		ST258 (n=1)	CG258	O2v2; KL106	*bla*$_{VIM-28}$	In1517	0_0_105_6_0_0_0_128_3_0	
	SL147 (n=47)	**ST147** (n=33)	CG147	O2v1; KL64	*bla*$_{VIM-1}$ (n=19)	In916 (n=13)	0_0_197_0_4_0_2_24_1_0 (n=5)	
							0_0_197_0_4_0_2_22_0_0 (n=2)	
							0_0_197_0_4_0_2_24_0_0 (n=2)	regional outbreak II
							0_0_197_0_4_0_2_25_0_0 (n=1)	
							0_0_197_0_4_0_2_26_0_0 (n=1)	
							0_0_197_0_4_0_2_21_0_0 (n=1)	
							0_0_197_0_4_0_2_0_3_0 (n=1)	
						In2245 (n=6)	0_0_197_0_4_0_2_23_1_0 (n=5)	regional outbreak III
							0_0_197_0_4_0_2_23_0_0 (n=1)	
					bla$_{VIM-4}$ (n=12)	In238	0_0_197_0_4_0_43_0_0_0	regional outbreak IV
					bla$_{VIM-2}$ (n=2)	In1008	0_0_197_0_4_0_2_23_2_0	
		ST392 (n=13)	CG147	O4; KL27	*bla*$_{VIM-4}$ (n=12)	In1667 (n=8)	0_0_197_0_25_6_0_0_1_0 (n=2)	
							0_0_197_0_25_6_0_1_0_0 (n=2)	
							0_0_197_0_25_6_0_0_0_0 (n=1)	regional outbreak VIII
							0_0_197_0_25_6_0_2_0_0 (n=1)	
							0_0_197_0_25_6_0_3_0_0 (n=1)	
							0_0_197_0_25_6_0_4_0_0 (n=1)	
						In238 (n=3)	0_0_197_0_25_0_0_25_0_0	
						In1654 (n=1)	0_0_197_0_25_6_0_0_1_0	
					bla$_{VIM-1}$ (n=1)	In916	0_0_197_0_25_0_0_1_2_0	
		ST1237 (n=1)	CG147	O2v1; KL64	*bla*$_{VIM-1}$	In-e541	0_0_197_0_4_10_0_2_1_0	
	SL15 (n=25)	**ST15** (n=25)	CG15	O1v1; KL48 (n=16)	*bla*$_{VIM-4}$ (n=15)	In1654	0_0_0_0_2_0_54_1_3_0 (n=12)	
							0_0_0_0_2_0_54_1_3_1 (n=1)	regional outbreak VI
							0_0_0_0_2_0_54_2_0_0 (n=1)	
							0_0_0_0_2_0_54_3_0_0 (n=1)	
					bla$_{VIM-1}$ (n=1)	In916	0_0_0_0_2_0_54_1_3_0	
				O1v1; KL112 (n=4)	*bla*$_{VIM-4}$	In238	0_0_0_0_2_0_77_0_0_0 (n=2)	
							0_0_0_0_2_0_78_0_0_0 (n=1)	

						0_0_0_0_2_0_0_120_0_0 (n=1)	
			O1v1; KL24 (n=5)	*bla*$_{VIM-20}$	In1444	0_0_0_0_2_25_0_0_0_0 (n=4)	regional outbreak V
						0_0_0_0_2_25_0_1_0_0 (n=1)	
SL277 (n=18)	**ST277** (n=18)	CG10463	O3b; KL46	*bla*$_{VIM-1}$	In916	0_0_193_3_0_0_0_0_0_0 (n=12)	
						0_0_193_3_0_0_0_0_4_0 (n=2)	
						0_0_193_3_0_0_0_0_1_0 (n=1)	interregional outbreak VII
						0_0_193_3_0_0_0_0_2_0 (n=1)	
						0_0_193_3_0_0_0_0_3_0 (n=1)	
						0_0_193_3_0_0_0_2_0_0 (n=1)	
SL17 (n=12)	**ST16** (n=5)	CG16	O3b; KL51	*bla*$_{VIM-20}$ (n=4)	In1444	0_0_22_27_0_0_5_0_0_0	
				bla$_{VIM-4}$ (n=1)	In1443-like	0_0_22_27_0_0_0_29_0_0	
	ST17 (n=4)	CG17 (n=3)	O5; KL25	*bla*$_{VIM-1}$ (n=2)	In916	0_0_22_24_1_6_1_0_0_0 (n=1)	
						0_0_22_24_48_0_0_0_0_0 (n=1)	
				bla$_{VIM-4}$ (n=1)	In1667	0_0_22_24_1_6_0_0_0_0	
		CG1123 (n=1)	O2v2; KL2	*bla*$_{VIM-1}$	In916	0_0_22_12_0_1_2_0_0_0	
	ST336 (n=2)	CG17	O5; KL25	*bla*$_{VIM-1}$ (n=1)	In916	0_0_22_24_3_0_9_0_0_0	
				bla$_{VIM-4}$ (n=1)	In238a	0_0_22_24_3_3_0_0_0_0	
	ST20 (n=1)	CG20	O1v2; KL28	*bla*$_{VIM-4}$	In238	0_0_22_2_0_0_3_0_0_0	
SL14 (n=10)	**ST6329** (n=7)	CG14	O1v1; KL2	*bla*$_{VIM-4}$	In238	0_0_1_1_60_0_0_1_0_0	
	ST14 (n=3)	CG14	O1v1; KL2	*bla*$_{VIM-1}$ (n=2)	In2240	0_0_1_1_61_0_0_0_0_0	
				bla$_{VIM-4}$ (n=1)	In238	0_0_1_1_60_0_0_0_0_0	
SL152 (n=7)	**ST152**	CG152	O4; KL149	*bla*$_{VIM-1}$ (n=4)	In916	0_0_220_0_0_6_0_0_0_0 (n=2)	
						0_0_220_0_0_0_7_0_0_0 (n=2)	
				bla$_{VIM-4}$ (n=3)	In238	0_0_220_0_0_0_2_8_0_0 (n=1)	
						0_0_220_0_0_0_2_8_1_0 (n=1)	
						0_0_220_0_0_7_0_0_0_0 (n=1)	
SL307 (n=4)	**ST307**	CG307	O2v2; KL102	*bla*$_{VIM-1}$ (n=3)	In916	0_0_369_0_0_0_0_0_1_1 (n=2)	
						0_0_369_0_0_0_0_0_1_75 (n=1)	
				bla$_{VIM-4}$ (n=1)	In238	0_0_369_0_0_0_43_0_0_0	
SL1626 (n=2)	**ST788**	CG12252	O3b; KL46	*bla*$_{VIM-1}$	In916	0_0_227_7_0_1_0_0_0_0	
SL3010 (n=2)	**ST253**	CG10539	O2v1; KL110	*bla*$_{VIM-1}$	In916	0_0_42_24_2_0_0_0_0_0	
SL359 (n=2)	**ST359**	CG359	O1v2; KL10	*bla*$_{VIM-1}$	In916	0_0_91_0_0_0_8_0_0_0 (n=1)	
						0_0_91_0_0_0_8_0_1_0 (n=1)	
SL6 (n=2)	**ST299** (n=1)	CG10246	O1v1; KL7	*bla*$_{VIM-4}$	In238	0_0_127_2_1_0_0_2_0_0	
	ST908 (n=1)	CG6	O1v1; KL48	*bla*$_{VIM-4}$	In238a	0_0_127_8_14_1_2_0_0_0	
SL1245 (n=1)	**ST1245**	CG10145	O2v2; KL3	*bla*$_{VIM-4}$	In238	0_0_15_1_1_0_0_0_0_0	
SL1427 (n=1)	**ST1427**	CG10060	OL101; KL155	*bla*$_{VIM-1}$	In916	0_0_346_1_2_0_0_0_0_0	
SL1878 (n=1)	**ST902**	CG1878	O5; KL125	*bla*$_{VIM-4}$	In238	0_0_113_1_0_0_1_0_2_0	
SL2004 (n=1)	**ST584**	CG584	O3b; KL38	*bla*$_{VIM-1}$	In916-like	0_0_52_4_20_0_0_0_0_0	
SL23 (n=1)	**ST23**	CG23	O1v2; KL1	*bla*$_{VIM-1}$	In916	0_0_429_0_42_0_1_1_0_0	
SL292 (n=1)	**ST469**	CG10359	O3b; KL139	*bla*$_{VIM-1}$	In916	0_0_250_2_0_0_1_0_0_0	
SL308 (n=1)	**ST308**	CG308	O1v2; KL28	*bla*$_{VIM-2}$	In1008	0_0_93_1_0_0_0_0_0_0	
SL34 (n=1)	**ST643**	CG10062	O1v1; KL37	*bla*$_{VIM-4}$	In238a	0_0_13_8_6_0_0_0_0_0	

	SL3598 (n=1)	**ST1540**	CG3598	O2v1; KL24	*bla*$_{VIM-1}$	In916	0_0_177_7_0_0_0_0_0_0
	SL37 (n=1)	**ST37**	CG3648	O4; KL15	*bla*$_{VIM-4}$	In238	0_0_109_23_4_1_1_0_0_0
	SL427 (n=1)	**ST427**	CG427	O3b; KL31	*bla*$_{VIM-1}$	In916	0_0_226_4_0_0_0_0_0_0
	SL54 (n=1)	**ST54**	CG54	O3b; KL14	*bla*$_{VIM-4}$	In238	0_0_166_0_0_0_3_0_1_0
	SL76 (n=1)	**ST76**	CG10052	O3/O3a; KL10	*bla*$_{VIM-1}$	In916	0_0_46_5_0_1_0_1_0_0
	-	**ST6328** (n=1)	-	O1v1; KL63	*bla*$_{VIM-1}$	In916	0_0_1197_0_0_0_0_0_0_0
Kp3 (n=4)	-	**ST285** (n=2)	-	OL103; KL67	*bla*$_{VIM-1}$	In916	-
	-	**ST347** (n=1)	-	O3/O3a; KL6	*bla*$_{VIM-4}$	In238	-
	SL3982 (n=1)	**ST6327** (n=1)	CG3982	OL103; KL47	*bla*$_{VIM-1}$	In916	1_0_119_1_0_0_0_0_0_0
Kp4 (n=3)	-	**ST736** (n=1)	-	O3/O3a; KL53	*bla*$_{VIM-1}$	In916	-
	SL1535 (n=2)	**ST1535**	CG1535	O3/O3a; KL114	*bla*$_{VIM-4}$	In238	2_1_21_0_5_0_0_0_0_0

Table S3. VIM-encoding class 1 integrons identified in the *K. pneumoniae* group study isolates.

Integron type	Integron variant[a]	Gene cassette array	Number of STs	Country, year and species of the first identification[b, c]	GenBank entry
with bla_{VIM-1}-like genes					
In238 (n=104)	In238 (n=101)	5'CS_*aacA4*_$bla_{VIM-4rpt}$_3'CS	17	Poland, 1998, *P. aeruginosa* Poland, 2008, *K. pneumoniae*	AJ585042/AY702100[d]
	In238a (n=3)	5'CS_*aacA4*_bla_{VIM-4}_3'CS	3	Poland, 2009, *E. hormaechei* Poland, 2015, *K. pneumoniae*	JQ003906 (Hungary 2010)
In1654 (n=16)	In1654	5'CS_$bla_{VIM-4rpt}$_3'CS	2	Poland, 2010, *P. aeruginosa* Poland, 2014, *K. pneumoniae*	MW595328
In1667 (n=9)	In1667	5'CS_$bla_{VIM-4rpt}$_*aacA4*_3'CS	2	Poland, 2009, *P. aeruginosa* Poland, 2014, *K. pneumoniae*	MW595333
In916 (n=64)	In916 (n=61)	5'CS_bla_{VIM-1}_*aacA4_aphA15_aadA1_catB2*_3'CS	21	Spain, before 2014, *E. coli* Poland, 2013, *E. coli* & *C. freundii* Poland, 2014, *K. pneumoniae*	KF856617
	In916-like (n=1)	5'CS_bla_{VIM-1}_*aacA4_ΔaphA15*	1	Poland, 2018, *K. pneumoniae*	-
	In2240 (n=2)	5'CS_bla_{VIM-1}_*aacA4_aphA15f_aadA1_catB2*_3'CS	1	Poland, 2015, *E. hormaechei* Poland, 2019, *K. pneumoniae*	OQ116829
In2016 (n=6)	**In2245**	5'CS_bla_{VIM-1}_bla_{OXA-10}_3'CS	1	Poland, 2017, *K. pneumoniae*	OQ116834
In590 (n=1)	In-e541	5'CS_bla_{VIM-1}_*aacA7_dhfr1_aadA1*_3'CS	1	Greece, 2001, *E. coli* Poland, 2006, *K. pneumoniae*	AY339625
In1443 (n=1)	In1443-like	5'CS_bla_{VIM-4}_*aacA7_aacA4*_bla_{OXA-1}_*ΔcatB3*	1	Poland, 2019, *K. pneumoniae*	-
In1517 (n=1)	In1517	5'CS_$bla_{VIM-28rpt}$_*orf1_orf2*_3'CS	1	Poland, 2009, *K. pneumoniae*	MH071811
with bla_{VIM-2}-like genes					
In1008 (n=12)	In1008 (n=3)	5'CS_bla_{VIM-2}_*aacA4*_3'CS	2	Poland, 2001, *P. aeruginosa* Poland, 2007, *S. marcescens* Poland, 2015, *K. pneumoniae*	AM087408
	In1444 (n=9)	5'CS_bla_{VIM-20}_*aacA4*_3'CS	2	Poland, 2006, *E. hormaechei* Poland, 2011, *K. pneumoniae*	MF678584

[a] – new integron is indicated in bold style.

[b] – when the first report was from another country, then it is followed by the first Polish case(s); if the first Polish record was from non-Enterobacterales and/or non-*Klebsiella* Enterobacterales, it is then followed by the first Polish Enterobacterales and *Klebsiella*, respectively.

[c] – date of isolation of the first Polish organism with a given integron may be earlier than that of the first isolate reported ever in another country.

[d] – the original In238 record (AJ585042) contains a 2 nt error in the bla_{VIM-4} coding sequence; the subsequent *P. aeruginosa* In238 entry from Hungary from 2003 has been provided.

Table S4. SNP scores between *K. pneumoniae* ST437 isolates[a,b]

Isolate	Year	Voivodeship	City	Hospital	Number of SNPs	VIM variant	Remarks
5777-18[a]	2018	Lubelskie	Lublin	HL5	0	VIM-4	ST437-In238-VIM-4; regional outbreak I
6944-18	2018	Lubelskie	Lublin	HL5	1	VIM-4	ST437-In238-VIM-4; regional outbreak I
8643-18	2018	Podkarpackie	Mielec	HR1	1	VIM-4	ST437-In238-VIM-4; regional outbreak I
7345-18	2018	Lubelskie	Lublin	HL18	2	VIM-4	ST437-In238-VIM-4; regional outbreak I
7157-18	2018	Lubelskie	Lublin	HL5	3	VIM-4	ST437-In238-VIM-4; regional outbreak I
7214-18	2018	Lubelskie	Lublin	HL5	3	VIM-4	ST437-In238-VIM-4; regional outbreak I
4501-19	2019	Lubelskie	Lublin	HL5	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
4521-19	2019	Lubelskie	Radzyń Podlaski	HL7	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
5212-19	2019	Lubelskie	Puławy	HL6	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
6224-18	2018	Śląskie	Zabrze	HS17	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
6957-18	2018	Lubelskie	Radzyń Podlaski	HL7	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
7158-18	2018	Lubelskie	Lublin	HL5	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
741-19	2019	Lubelskie	Lublin	HL5	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
7498-18	2018	Lubelskie	Lublin	HL5	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
8938-18	2018	Lubelskie	Lublin	HL12	4	VIM-4	ST437-In238-VIM-4; regional outbreak I
7400-18	2018	Lubelskie	Lublin	HL5	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
1046-19	2019	Lubelskie	Łuków	HL8	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
206-19	2019	Lubelskie	Poniatowa	HL9	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
6140-18	2018	Lubelskie	Lublin	HL5	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
7266-18	2018	Lubelskie	Lublin	HL5	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
7437-19	2019	Lubelskie	Lublin	AL4	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
7539-18	2018	Lubelskie	Lublin	HL5	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
8957-19	2019	Lubelskie	Lublin	HL5	5	VIM-4	ST437-In238-VIM-4; regional outbreak I
4284-19	2019	Lubelskie	Lublin	HL5	6	VIM-4	ST437-In238-VIM-4; regional outbreak I
4285-19	2019	Lubelskie	Lublin	HL5	6	VIM-4	ST437-In238-VIM-4; regional outbreak I
1849-19	2019	Lubelskie	Lublin	HL5	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
7397-18	2018	Lubelskie	Lublin	HL5	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
8252-18	2018	Lubelskie	Lublin	HL5	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
8438-18	2018	Świętokrzyskie	Kielce	HT6	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
8530-18	2018	Lubelskie	Lublin	HL5	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
8532-18	2018	Lubelskie	Lublin	HL5	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
8676-18	2018	Lubelskie	Lublin	HL11	7	VIM-4	ST437-In238-VIM-4; regional outbreak I
10339-19	2019	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
3434-19	2019	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
7265-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
738-19	2019	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
787-19	2019	Lubelskie	Lublin	HL20	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
83-19	2018	Lubelskie	Biała Podlaska	HL3	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
8425-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
8925-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
8926-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
9060-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
9161-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
9357-18	2018	Lubelskie	Lublin	HL5	8	VIM-4	ST437-In238-VIM-4; regional outbreak I
1630-19	2019	Lubelskie	Lublin	AL5	9	VIM-4	ST437-In238-VIM-4; regional outbreak I
488-19	2019	Lubelskie	Lublin	HL20	9	VIM-4	ST437-In238-VIM-4; regional outbreak I
521-19	2019	Lubelskie	Lublin	HL5	9	VIM-4	ST437-In238-VIM-4; regional outbreak I
636-19	2019	Lubelskie	Lublin	HL5	9	VIM-4	ST437-In238-VIM-4; regional outbreak I
8593-18	2018	Lubelskie	Krasnystaw	HL21	9	VIM-4	ST437-In238-VIM-4; regional outbreak I
9927-19	2019	Lubelskie	Lublin	HL5	10	VIM-4	ST437-In238-VIM-4; regional outbreak I
2199-19	2019	Lubelskie	Lublin	HL1	10	VIM-4	ST437-In238-VIM-4; regional outbreak I
6588-19	2019	Lubelskie	Lublin	AL4	10	VIM-4	ST437-In238-VIM-4; regional outbreak I
9244-18	2018	Lubelskie	Lublin	HL5	10	VIM-4	ST437-In238-VIM-4; regional outbreak I
6874-19	2019	Lubelskie	Lublin	HL5	11	VIM-4	ST437-In238-VIM-4; regional outbreak I
5704-19	2019	Lubelskie	Lublin	HL11	12	VIM-4	ST437-In238-VIM-4; regional outbreak I
7638-19	2019	Lubelskie	Lublin	HL11	13	VIM-4	ST437-In238-VIM-4; regional outbreak I
3432-19	2019	Lubelskie	Lublin	HL5	14	VIM-4	ST437-In238-VIM-4; regional outbreak I

[a] – reference isolate, *i.e.* the Poland's index isolate of ST437 as confirmed by the National Reference Centre for Susceptibility Testing

[b] – the SNP analysis of the ST437 isolates revealed 127 polymorphic positions within ~4.8 Mb (91%) of the index isolate reference genome.

Table S5. SNP scores between *K. pneumoniae* ST147 isolates[a,b]

Isolate	Year	Voivodeship	City	Hospital	Number of SNPs	VIM variant	Remarks
2274/15[a]	2015	Śląskie	Katowice	HS8	0	VIM-2	ST147-In1008-VIM-2; hospital dissemination
2279/15	2015	Śląskie	Katowice	HS8	6	VIM-2	ST147-In1008-VIM-2; hospital dissemination
6349/17	2017	Śląskie	Katowice	HS18	31	VIM-1	ST147-In2245-VIM-1; regional outbreak III
7094/17	2017	Śląskie	Sosnowiec	AS5	31	VIM-1	ST147-In2245-VIM-1; regional outbreak III
6410/17	2017	Śląskie	Katowice	HS18	32	VIM-1	ST147-In2245-VIM-1; regional outbreak III
7909/17	2017	Śląskie	Katowice	HS18	32	VIM-1	ST147-In2245-VIM-1; regional outbreak III
7605/17	2017	Śląskie	Katowice	HS18	33	VIM-1	ST147-In2245-VIM-1; regional outbreak III
8057/17	2017	Śląskie	Siemianowice Śląskie	HS1	34	VIM-1	ST147-In2245-VIM-1; regional outbreak III
4323/18	2018	Śląskie	Katowice	HS781	34	VIM-1	ST147-In916-VIM-1; regional outbreak II
4321/18	2018	Śląskie	Katowice	HS780	35	VIM-1	ST147-In916-VIM-1; regional outbreak II
2615/19	2019	Małopolskie	Miechów	HK23	38	VIM-1	ST147-In916-VIM-1; regional outbreak II
2348/19	2019	Małopolskie	Kraków	HK5	39	VIM-1	ST147-In916-VIM-1; regional outbreak II
2639/19	2019	Małopolskie	Kraków	HK5	39	VIM-1	ST147-In916-VIM-1; regional outbreak II
2254/19	2019	Małopolskie	Kraków	HK2	44	VIM-1	ST147-In916-VIM-1; regional outbreak II
3674/19	2019	Małopolskie	Kraków	HK13	44	VIM-1	ST147-In916-VIM-1; regional outbreak II
2410/19	2019	Małopolskie	Kraków	HK2	47	VIM-1	ST147-In916-VIM-1; regional outbreak II
2537/19	2019	Małopolskie	Kraków	HK5	48	VIM-1	ST147-In916-VIM-1; regional outbreak II
2783/19	2019	Małopolskie	Kraków	HK5	48	VIM-1	ST147-In916-VIM-1; regional outbreak II
3345/19	2019	Małopolskie	Kraków	HK20	49	VIM-1	ST147-In916-VIM-1; regional outbreak II
7160/18	2018	Lubelskie	Lublin	HL5	48	VIM-4	ST147-In238-VIM-4; regional outbreak IV
7215/18	2018	Lubelskie	Lublin	HL5	48	VIM-4	ST147-In238-VIM-4; regional outbreak IV
7401/18	2018	Lubelskie	Lublin	HL5	48	VIM-4	ST147-In238-VIM-4; regional outbreak IV
8336/18	2018	Lubelskie	Lublin	HL5	50	VIM-4	ST147-In238-VIM-4; regional outbreak IV
8385/18	2018	Lubelskie	Łęczna	HL10	50	VIM-4	ST147-In238-VIM-4; regional outbreak IV
8582/18	2018	Lubelskie	Lublin	HL5	50	VIM-4	ST147-In238-VIM-4; regional outbreak IV
7942/18	2018	Lubelskie	Lublin	HL5	51	VIM-4	ST147-In238-VIM-4; regional outbreak IV
8253/18	2018	Lubelskie	Lublin	HL5	52	VIM-4	ST147-In238-VIM-4; regional outbreak IV
3412/19	2019	Lubelskie	Lublin	HL4	53	VIM-4	ST147-In238-VIM-4; regional outbreak IV
742/19	2019	Lubelskie	Lublin	HL5	53	VIM-4	ST147-In238-VIM-4; regional outbreak IV
614/19	2019	Świętokrzyskie	Chęciny	HT14	54	VIM-4	ST147-In238-VIM-4; regional outbreak IV
819/19	2019	Lubelskie	Lublin	HL4	54	VIM-4	ST147-In238-VIM-4; regional outbreak IV
7315/19	2019	Lubelskie	Lublin	HL11	66	VIM-1	ST147-In916-VIM-1; single case
1603/18	2018	Łódzkie	Łódź	HE9	115	VIM-1	ST147-In916-VIM-1; single case

[a] – reference isolate, *i.e.* the Poland's index isolate of ST147 as confirmed by the National Reference Centre for Susceptibility Testing
[b] – the SNP analysis of the ST437 isolates revealed 283 polymorphic positions within ~5.1 Mb (92%) of the index isolate reference genome.

Table S6. SNP scores between *K. pneumoniae* ST15 isolates[a,b]

Isolate	Year	Voivodeship	City	Hospital	Number of SNPs	VIM variant	Remarks
381-14 [a]	2014	Wielkopolskie	Poznań	HP2	0	VIM-20	ST15-In1444-VIM-20; regional outbreak V
4779-19	2019	Wielkopolskie	Poznań	HP1	5	VIM-20	ST15-In1444-VIM-20; regional outbreak V
2866-14	2014	Wielkopolskie	Poznań	HP2	10	VIM-20	ST15-In1444-VIM-20; regional outbreak V
1981-14	2014	Wielkopolskie	Poznań	HP2	11	VIM-20	ST15-In1444-VIM-20; regional outbreak V
3980-16	2016	Wielkopolskie	Poznań	HP1	29	VIM-20	ST15-In1444-VIM-20; regional outbreak V
4369-15	2015	Mazowieckie	Warszawa	HW13	202	VIM-4	ST15-In238-VIM-4; single case
7167-17	2017	Lubuskie	Gorzów Wielkopolski	HF5	296	VIM-4	ST15-In238-VIM-4; single case
4565-16	2016	Mazowieckie	Radom	HM1	295	VIM-4	ST15-In238-VIM-4; hospital transmission
5002-16	2016	Mazowieckie	Radom	HM1	300	VIM-4	ST15-In238-VIM-4; hospital transmission
5975-16	2016	Świętokrzyskie	Kielce	HT8	290	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
5403-17	2017	Świętokrzyskie	Starachowice	HT9	292	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
4096-17	2017	Świętokrzyskie	Włoszczowa	HT12	295	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
5496-17	2017	Świętokrzyskie	Kielce	HT6	296	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
5719-17	2017	Świętokrzyskie	Kielce	HT8	296	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
3994-17	2017	Świętokrzyskie	Końskie	HT7	297	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
6411-17	2017	Świętokrzyskie	Kielce	HT6	297	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
7365-17	2017	Świętokrzyskie	Kielce	HT6	298	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
8048-17	2017	Łódzkie	Radomsko	HE20	298	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
7637-17	2017	Świętokrzyskie	Kielce	HT6	300	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
6908-17	2017	Świętokrzyskie	Kielce	HT6	300	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
1256-18	2018	Świętokrzyskie	Kielce	HT6	301	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
8379-18	2018	Łódzkie	Łódź	HE7	306	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
906-18	2018	Świętokrzyskie	Końskie	HT7	306	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
8256-19	2019	Łódzkie	Radomsko	HE20	312	VIM-4	ST15-In1654-VIM-4; regional outbreak VI
9065-17	2017	Łódzkie	Piotrków Trybunalski	HE19	310	VIM-1	ST15-In916-VIM-1; single case

[a] – reference isolate, *i.e.* the Poland's index isolate of ST15 as confirmed by the National Reference Centre for Susceptibility Testing

[b] – the SNP analysis of the ST15 isolates revealed 714 polymorphic positions within ~4.6 Mb (84%) of the index isolate reference genome.

Table S7. SNP scores between *K. pneumoniae* ST277 isolates[a,b]

Isolate	Year	Voivodeship	City	Hospital	Number of SNPs	VIM variant	Remarks
7089-17[a]	2017	Pomorskie	Gdańsk	HG1	0	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
1009-18	2018	Pomorskie	Gdańsk	HG1	9	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
3360-19	2019	Pomorskie	Wejherowo	HG7	9	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
9204-17	2017	Pomorskie	Kartuzy	HG10	9	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
1444-18	2018	Warmińsko-Mazurskie	Elbląg	HN19	11	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
2725-18	2018	Pomorskie	Kartuzy	HG10	11	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
1157-18	2018	Warmińsko-Mazurskie	Elbląg	HN19	11	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
5701-18	2018	Warmińsko-Mazurskie	Elbląg	HN19	12	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
8336-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	15	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
9107-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	15	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
7104-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	15	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
8939-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	16	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
9299-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	16	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
9542-19	2019	Warmińsko-Mazurskie	Elbląg	HN19	16	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
743-19	2019	Warmińsko-Mazurskie	Elbląg	HN20	20	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
8012-19	2019	Pomorskie	Gdynia	HG336	20	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
8475-19	2019	Pomorskie	Sztum	HG22	22	VIM-1	ST277-In916-VIM-1; interregional outbreak VII
7213-18	2018	Pomorskie	Gdańsk	HG1	28	VIM-1	ST277-In916-VIM-1; interregional outbreak VII

[a] – reference isolate, *i.e.* the Poland's index isolate of ST277 as confirmed by the National Reference Centre for Susceptibility Testing

[b] – the SNP analysis of the ST277 isolates revealed 88 polymorphic positions within ~4.9 Mb (93%) of the index isolate reference genome.

Table S8. SNP scores between *K. pneumoniae* ST392 isolates[a,b]

Isolate	Year	Voivodeship	City	Hospital	Number of SNPs	VIM variant	Remarks
1359-14 [a]	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	0	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
1535-14	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	6	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
3826-14	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	9	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
2925-14	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	10	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
3827-14	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	12	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
2259-14	2014	Kujawsko-Pomorskie	Bydgoszcz	HC1	15	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
83-15	2015	Kujawsko-Pomorskie	Bydgoszcz	HC1	19	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
3347-14	2014	Pomorskie	Gdynia	HG3	21	VIM-4	ST392-In1667-VIM-4; regional outbreak VIII
2522-14	2014	Pomorskie	Gdańsk	HG1	11	VIM-4	ST392-In1654-VIM-4; single case
1257-15	2015	Mazowieckie	Warszawa	HW9	56	VIM-4	ST392-In238-VIM-4; single case
4366-18	2018	Świętokrzyskie	Końskie	HT7	63	VIM-1	ST392-In916-VIM-1; single case
3926-16	2016	Mazowieckie	Wólka Ostrożeńska	HWA15	68	VIM-4	ST392-In238-VIM-4; single case
9513-19	2019	Lubelskie	Puławy	HL6	69	VIM-4	ST392-In238-VIM-4; single case

[a] – reference isolate, *i.e.* the Poland's index isolate of ST392 as confirmed by the National Reference Centre for Susceptibility Testing

[b] – the SNP analysis of the ST392 isolates revealed 158 polymorphic positions within ~4.6 Mb (86%) of the index isolate reference genome.

Table S9. Resistomes of the *K. pneumoniae* species complex isolates

ST	clonal group	integron	n isolates	outbreak	acquired AMR genes[a]			AMR genes/ isolate
					resistance to β-lactams	resistance to aminoglycosides	resistance to other groups	
ST437	CG10268	In238	57	I	*bla*$_{VIM-4}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	*aac(3)-IId*, *aac(6')-Ib*, (*aac(6')-Ib-cr*), (*aph(3'')-Ia*), (*armA*)	(*dfrA12*), (*dfrA30*), (*sul1*), (*sul2*), (*qnrS1*), (*catA1*), (*catA2*), (*tet(D)*), (*msrE*)	14.1
ST11	CG340	In238	3		*bla*$_{VIM-4}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), *aac(6')-Ib*, *aph(3')-Ia*, (*aph(3'')-Ib*), *aph(6)-Id*	(*dfrA12*), (*dfrA14*), *sul1*, (*sul2*), *tet(D)*	8.7
	CG3666	In238	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*aac(6')-Ib*	*dfrA14*, *sul1*,	6.0
ST258	CG258	In1517	1		*bla*$_{VIM-28}$	*aac(6')-Ib*, *aph(3')-XVa*, *ant(2'')-Ia*	*floR*, *tet(G)*	6.0
ST147	CG147	In916	11	II	*bla*$_{VIM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	(*aac(3)-IIa*), *aac(6')-Ib*, (*aph(6)-Id*), *aph(3')-XV*, *aadA1*	(*dfrA1*), (*dfrA14*), *sul1*, (*sul2*), (*qnrS1*), (*catA1*), *catB2*	14.1
			2		*bla*$_{VIM-1}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	*aac(6')-Ib*, (*aph(3'')-Ib*), *aph(6)-Id*, *aph(3')-XV*, *aadA1*	(*dfrA1*), (*dfrA14*), *sul1*, *sul2*, *qnrS1*, (*catA1*), *catB2*	13.0
		In2245	6	III	*bla*$_{VIM-1}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), *bla*$_{OXA-10}$, (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), (*aac(6')-Ib-cr*), (*aph(3')-Ia*), *aph(6)-Id*, (*aph(3')-VI*)	(*dfrA1*), *sul1*, (*qnrS1*), (*catA1*)	9.5
		In238	12	IV	*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), (*aac(3)-IId*), *aac(6')-Ib*, (*aac(6')-Ib-cr*), *aph(3')-Ia*, (*armA*)	*dfrA12*, *sul1*, (*sul2*), (*qnrS1*), *catA1*, (*catA2*), (*msrE*)	12.5
		In1008	2		*bla*$_{VIM-2}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(3)-IIa*, *aac(6')-Ib*, (*aph(6)-Id*), *aph(3')-VI*	*dfrA1*, *sul1*, *qnrS1*, *catA1*	11.5
ST392	CG147	In1667	8	VIII	*bla*$_{VIM-4}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	(*aac(3)-IId*), *aac(6')-Ib*, (*aph(3'')-Ib*), (*aph(6)-Id*), (*aadA16*), (*armA*)	(*dfrA12*), (*dfrA14*), (*dfrA27*), (*sul1*), (*sul2*), (*qnrB6*), *msrE*, (*mphE*)	13.7
		In238	3		*bla*$_{VIM-4}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	*aac(6')-Ib*, (*aph(3')-Ia*), (*aph(3'')-Ib*), (*aph(6)-Id*)	(*dfrA14*), *sul1*, (*sul2*), (*qnrB1*)	6.7
		In1654	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$	*aac(3)-IId*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA16*, *armA*	*dfrA14*, *dfrA27*, *sul2*, *msrE*, *mphE*	13.0
		In916	1		*bla*$_{VIM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(6')-Ib*, *aph(3')-XV*, *aadA1*	*dfrA14*, *sul1*, *sul2*, *qnrB1*, *qnrS1*, *catB2*	13.0
ST1237	CG147	In-e541	1		*bla*$_{VIM-1}$	*aac(6')-I*, *aac(6')-II*, *aph(3')-Ia*, *aph(6)-Id*, *aadA1*	*dfrA1*, *sul1*	8.0
ST15	CG15	In1654	15	VI	*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, (*bla*$_{OXA-1}$), *bla*$_{TEM-1}$	(*aac(3)-IIa*), (*aac(6')-Ib-cr*), (*aph(3')-Ia*), (*aph(3'')-Ib*), (*aph(6)-Id*), (*aph(3')-VI*)	(*dfrA14*), (*sul1*), (*sul2*), (*catB1*)	8.8
		In916	1		*bla*$_{VIM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(6')-Ib*, *aac(6')-Ib-cr*, *aph(3')-XV*, *aadA1*	*dfrA12*, *sul1*, *sul2*, *catB2*	12.0
		In238	4		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, (*bla*$_{OXA-9}$), (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), *aac(6')-Ib*, (*aac(6')-Ib-cr*), *aph(3')-Ia*, (*aph(3'')-Ib*), (*aph(6)-Id*), (*armA*)	(*dfrA12*), (*dfrA14*), *sul1*, (*sul2*), (*catB2*), (*msrE*), (*mphE*)	11.3
		In1444	5	V	*bla*$_{VIM-29}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	(*aac(3)-IId*), *aac(6')-Ib*, (*aph(3')-Ia*), *armA*	*sul1*, *msrE*, (mphA), mphE	9.0
ST277	CG10463	In916	18	VII	*bla*$_{VIM-1}$, (*bla*$_{CTX-M-15}$), (*bla*$_{CTX-M-210}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), (*aac(3)-IId*), *aac(6')-Ib*, (*aph(3'')-Ib*), (*aph(6)-Id*), *aph(3')-XV*, *aadA1*, (*aadA2*), (*armA*)	(*dfrA12*), *dfrA14*, (*sul1*), (*sul2*), (*qnrB1*), (*qnrS1*), *catB2*, (*tet(A)*), (*msrE*), (*mphA*), (*mphE*)	14.1
ST16	CG16	In1444	4		*bla*$_{VIM-26}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(3)-IId*, *aac(6')-Ib*, *aph(3')-Ia*, *armA*	*sul1*, (*msrE*), (*mphE*)	10.5
		In1443-like	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(6')-I*, *aph(3')-Ia*, *ant(2'')-Ia*	*dfrA12*, *sul1*	9.0
ST17	CG17	In916	2		*bla*$_{VIM-1}$, (*bla*$_{CTX-M-15}$), (*bla*$_{TEM-1}$)	*aac(6')-Ib*, (*aph(3'')-Ib*), (*aph(6)-Id*), *aph(3')-XV*, *aadA1*	*sul1*, (*sul2*), (*qnrS1*), *catB2*	9.0
		In1667	1		*bla*$_{VIM-4}$	*aac(3)-IId*, *aac(6')-Ib*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA16*, *armA*	*dfrA27*, *sul1*, *sul2*, *qnrB6*, *msrE*, *mphE*	14.0
	CG1123	In916	1		*bla*$_{VIM-1}$	*aac(6')-Ib*, *aph(3')-XV*, *aadA1*	*dfrA14*, *sul1*, *qnrS1*, *catB2*	8.0
ST336	CG17	In916	1		*bla*$_{VIM-1}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(6')-Ib*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *aph(3')-XV*, *aadA1*	*dfrA14*, *sul1*, *sul2*, *qnrS1*, *catB2*	14.0
		In238a	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-238}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(6')-Ib*, *aac(6')-Ib-cr*, *aph(3'')-Ib*, *aph(6)-Id*	*dfrA14*, *sul1*, *sul2*, *qnrB1*	12.0
ST20	CG20	In238	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(3)-IIa*, *aac(6')-Ib*, *aph(3'')-Ib*, *aph(6)-Id*, *armA*	*dfrA14*, *sul1*, *sul2*, *msrE*	13.0
ST6329	CG14	In238	7		*bla*$_{VIM-4}$, (*bla*$_{CTX-M-15}$), (*bla*$_{OXA-1}$), (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), *aac(6')-Ib*, *aac(6')-Ib-cr*, *aph(3'')-Ib*, *aph(6)-Id*	*dfrA14*, *sul1*, (*sul2*), (*qnrB1*)	11.1
ST14	CG14	In2240	2		*bla*$_{VIM-1}$, *bla*$_{CTX-M-27}$, *bla*$_{LAP-2}$, (*bla*$_{TEM-1}$)	*aac(6')-Ib*, *aph(3'')-Ib*, *aph(6)-Id*, *aph(3')-XV*, *aadA1*	*dfrA14*, *sul1*, *sul2*, *qnrS1*, *catB2*	13.5
		In238	1		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	*aac(3)-IIa*, *aac(6')-Ib*, *aph(3'')-Ib*, *aph(6)-Id*	*dfrA14*, *sul1*, *sul2*, *qnrB1*	12.0
ST152	CG152	In916	4		*bla*$_{VIM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	(*aac(3)-IIa*), *aac(6')-Ib*, (*aph(3')-Ia*), (*aph(3'')-Ib*), (*aph(6)-Id*), *aph(3')-XV*, *aadA1*, *aadA16*	(*dfrA1*), (*dfrA14*), (*dfrA27*), *sul1*, (*sul2*), (*qnrS1*), (*catA1*), *catB2*, (*tet(D)*)	17.0
		In238	3		*bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$	(*aac(3)-IIa*), *aac(6')-Ib*, (*aac(6')-Ib-cr*), *aph(3'')-Ib*, *aph(6)-Id*, *aadA16*, *armA*	(*dfrA1*), (*dfrA16*), (*dfrA27*), *sul1*, *sul2*, (*tet(C)*), *msrE*	14.0
ST307	CG307	In916	3		*bla*$_{VIM-1}$, (*bla*$_{CTX-M-15}$), *bla*$_{OXA-1}$, (*bla*$_{TEM-1}$)	(*aac(3)-IIa*), *aac(6')-Ib*, (*aph(3'')-Ib*), (*aph(6)-Id*), *aph(3')-XV*,	*dfrA14*, *sul1*, *sul2*, *qnrS1*, *qnrB1*, (*catA1*), *catB2*, (*msrE*), (*mphE*)	15.0

					aadA1, (armA)		
		In238	1	bla_{VIM-4}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}	*aac(3)-IId, aac(6')-Ib, aph(3')-Ia*	*dfrA30, sul1, qnrS1, catA1, catA2, tet(D)*	13.0
ST788	CG12252	In916	2	bla_{VIM-1}	*aac(6')-Ib, (aph(3'')-Ib), aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14,(sul1), (sul2), (qnrS1), catB2*	9.0
ST253	CG10539	In916	2	bla_{VIM-1}, $bla_{CTX-M-3}$	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1, armA*	*dfrA12, dfrA14, sul1, sul2, qnrS1, catB2, msrE, mphE*	16.0
ST359	CG359	In916	2	bla_{VIM-1}	*aac(6')-Ib, (aph(3'')-Ib), (aph(6)-Id), aph(3')-XV, aadA1*	*dfrA14, sul1, (sul2), (qnrS1), catB2*	9.0
ST299	CG10246	In238	1	bla_{VIM-4}	*aac(6')-Ib*	*dfrA1, sul1*	4.0
ST908	CG6	In238a	1	bla_{VIM-4}	*aac(6')-Ib, aac(6')-Ib-cr*	*sul1*	4.0
ST1245	CG10145	In238	1	bla_{VIM-4}	*aac(6')-Ib, aac(6')-Im, aph(2'')-Ib*	*sul1, catA1, tet(B)*	7.0
ST1427	CG10060	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14, sul1, sul2, qnrS1, catB2*	11.0
ST902	CG1878	In238	1	bla_{VIM-4}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}	*aac(3)-IIa, aac(6')-Ib, aph(3'')-Ib, aph(6)-Id*	*dfrA14, sul1, sul2, qnrB1*	12.0
ST584	CG584	In916-like	1	bla_{VIM-1}	*aac(6')-Ib, aph(3')-XV, aadA1*	*dfrA14, qnrS1, catB2*	7.0
ST23	CG23	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, (aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14, sul1, sul2, qnrS1, catB2*	11.0
ST469	CG10359	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1, aadA16*	*dfrA12, dfrA14, dfrA27, sul1, sul2, qnrS1, qnrB6, catB2*	15.0
ST308	CG308	In1008	1	bla_{VIM-2}	*aac(6')-Ib, aph(6)-Id*	*sul1*	4.0
ST643	CG10062	In238a	1	bla_{VIM-4}	*aac(6')-Ib, aac(6')-Ib-cr,*	*sul1*	4.0
ST1540	CG3598	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14, sul1, sul2, qnrS1, catB2*	11.0
ST37	CG3648	In238	1	bla_{VIM-4}, $bla_{CTX-M-3}$, $bla_{CTX-M-14}$, bla_{TEM-1}	*aac(3)-IId, aac(6')-Ib, armA*	*sul1, msrE, mphE*	10.0
ST427	CG427	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14, sul1, sul2, qnrS1, catB2*	11.0
ST54	CG54	In238	1	bla_{VIM-4}, $bla_{CTX-M-15}$, bla_{OXA-10}, bla_{TEM-1}	*aac(3)-IIa, aac(6')-Ib, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id*	*dfrA14, sul1, sul2*	12.0
ST76	CG10052	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, aph(3')-XV, aadA1, aadA16*	*dfrA14, dfrA27, sul1, sul2, qnrS1, qnrB6, catB2*	14.0
ST6328	-	In916	1	bla_{VIM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{DHA-1}	*aac(3)-IIa, aac(6')-Ib, aph(6)-Id, aph(3')-XV, aadA1*	*dfrA14, sul1, qnrB2, qnrB4, catB2*	14.0
ST285	-	In916	2	bla_{VIM-1}, bla_{LEN-13}	*aac(6')-Ib, aph(3'')-Ib, (aph(6)-Id, aph(3')-XV, aadA1*	*(dfrA14), sul1, sul2, qnrS1, catB2*	11.5
ST347	-	In238	1	bla_{VIM-4}, bla_{CMY-4}, bla_{LEN-57}	*aac(6')-Ib*	*dfrA14, sul1, sul2, qnrS1, tet(A)*	9.0
ST6327	CG3982	In916	1	bla_{VIM-1}	*aac(6')-Ib, aph(3')-XV, aadA1*	*dfrA14, catB2*	6.0
ST736	-	In916	1	bla_{VIM-1}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}	*aac(3)-IIa, aac(6')-Ib, aph(3')-Ia, aph(3')-XV, aadA1*	*dfrA14, sul1, sul2, qnrB1, catB2, tet(C)*	15.0
ST1535	CG1535	In238	2	bla_{VIM-4}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}	*(aac(3)-IIa), aac(6')-Ib, aph(3'')-Ib, aph(6)-Id, armA*	*(dfrA14), sul1, sul2, qnrB1, msrE*	13.0

[α] – symbols in parentheses refer to the genes that occurred not in all isolates of the corresponding genotypes.

Table S10. Resistomes of the isolates subjected to the long-read WGS analysis

Isolate (ST)	Plasmids with *bla*$_{VIM}$ gene	Other plasmids	AMR genes located on contigs not assigned to individual plasmids or chromosome	Chromosome
5777/18	**IncFIB+HI1B (pNDM-MAR):** *bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *aac(3)-IId, aac(6')-Ib, aph(3')-Ia* (x2), *mph(A), sul1, qnrS1*	**IncFIB$_K$+FII$_K$:** *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *aac(3)-IId, aac(6')-Ib-cr, catA1, tet(D)* **IncFIB (pQil):** *aph(3')-Ia, dfrA30, sul1*	*aadA2, armA, dfrA12, mph(E), msr(E), sul2*	*bla*$_{CTX-M-15}$
7160/18	**IncFIB+HI1B (pNDM-MAR):** *bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *aac(3)-IId, aac(6')-Ib, aadA2, aph(3')-Ia* (x2), *armA, dfrA12, mph(A), mphE, msr(E), sul1* (x2), *sul2, qnrS1*	**IncFIB$_K$:** *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *aac(3)-IIa, aac(6')-Ib-cr, aadA2, catA1, dfrA12, sul1*	-	-
2254/19	**IncA:** *bla*$_{VIM-1}$, *bla*$_{SHV-11}$, *aac(6')-Ib3, aadA1, aph(3')-XV, catB2, dfrA14, qnrS1, sul2*	**IncR:** *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *aac(3)-IIa, aac(6')-Ib-cr, aph(6)-Id, catA1, dfrA1, qnrS1, sul1, tet(A)*	-	-
9546/19	**IncA:** *bla*$_{VIM-1}$, *bla*$_{SHV-12}$, *aac(6')-Ib3, aadA1, aph(3'')-Ib, aph(3')-XV, aph(6)-Id, catB2, dfrA14, qnrS1, sul1, sul2*	-	-	-
7089/17	**IncA:** *bla*$_{VIM-1}$, *bla*$_{SHV-12}$, *aac(6')-Ib3, aadA1, aph(3'')-Ib, aph(3')-XV, aph(6)-Id, catB2, dfrA14, qnrS1, sul1, sul2*	-	*bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *aac(3)-IIa* (x4), *aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, dfrA12, qnrB1, sul2, tet(A)*	-
381/14	**IncM2:** *bla*$_{VIM-20}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *aac(3)-IId, aac(6')-Ib3, armA, mph(E), msr(E), sul1*	**IncFIB$_K$:** *bla*$_{OXA-1}$, *aac(6')-Ib-cr, aph(3'')-Ia, mphA*	-	*bla*$_{CTX-M-15}$
1359/14	**IncM2:** *bla*$_{VIM-4}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *aac(3)-IId, armA, mph(E), msr(E), sul1*	**IncFIB$_K$:** *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, sul2, tet(A)* **IncFII$_K$+Q1:** *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, *arr3, aac(3)-IId, aac(6')-Ib-cr, aadA16, aph(3'')-Ib, aph(6)-Id, dfrA27, qnrB6, sul1* (x2), *sul2*	*dfrA14*	-
5975/16	-	**IncFIB$_K$:** *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *aac(6')-Ib-cr, aph(3')-Ia, tet(A)*	-	**ICE6441-PL22:** *bla*$_{VIM-4}$, *aph(3')-VIa, sul1* (x2)

Table S11. Replicon types of the *K. pneumoniae* species complex isolates

ST	Clonal group	Integron	n isolates	Outbreak	Replicon types[a]	n replicon types	n replicon types /isolate
ST437	CG10268	In238	57	I	(FIB_K), FIB, (FIB), (FII), (HI1B), Col440I, Col440II, ColRNAI	6	7.7
ST11	CG340	In238	3		(C), FIA, FIB_K, FII, (FII), R, Col440I	2	6
	CG3666	In238	1		C, FII	1	2
ST258	CG258	In1517	1		X3	1	1
ST147	CG147	In916	11	II	A, (FIB), (FIB), (HI1B), R, Col440I	3	4.7
			2		A, (FIA), FIB, (FII), (R), Col440I, (ColRNAI), ColMG828	2	6
		In2245	6	III	FII, R, Col440I	1	3
		In238	12	IV	FIB_K, FIB, FII, HI1B, Col440I, Col440II	1	6
		In1008	2		FII, R, Col440I	1	3
ST392	CG147	In1667	8	VIII	(FIB_K), (FIB), FII, (FII), (HI1B), M2, Col440I	3	4.9
		In238	3		(C), (FIB_K), (FII), Col440I	3	3
		In1654	1		FIB_K, FII, FII, M2, Col440I	1	5
		In916	1		A, FIB, FII, Col440I	1	4
ST1237	CG147	In-e541	1		FIB, HI1B, R, Col440I	1	4
ST15	CG15	In1654	15	VI	FIB_K, FII, (FII), (M1)	4	2.3
		In916	1		A, FIB_K, FIC, FII, FII	1	5
		In238	4		C, (FIA), FIB_K, (FIB), FII, (N3), (R), (Col440I), (Col440II), (ColRNAI), (ColpVC)	3	5.5
		In1444	5	V	(FIB_K), (FIB), FII, (FII), (M2), Col440I, Col440II, (ColRNAI), ColpVC	5	7.4
ST277	CG10463	In916	18	VII	A, (FIB), FII	2	2.9
ST16	CG16	In1444	4		FIB_K, FII, M2, Col440I, Col440II	1	5
		In1443-like	1		FIB_K, FII	1	2
ST17	CG17	In916	3		A, (FIA), (FIB_K), (FII), (Col440I)	2	3
		In1667	1		FIA, FIB_K, FII, M2	1	4
	CG1123	In916	1		A, FIB_K, FII, R, Col440I, Col440II, ColRNAI	1	7
ST336	CG17	In916	1		A, FIB_K, FII, N, Col440I	1	5
		In238a	1		FIA, FIB_K, FII, FII, N3, Col440I	1	6
ST20	CG20	In238	1		C, FIB_K, FII	1	3
ST6329	CG14	In238	7		N3	1	1
ST14	CG14	In2240	2		A, FIB_K, FII	1	3
		In238	1		M1	1	1
ST152	CG152	In916	4		A, (FIA), FIB_K, FII, (FII), (Col440I), (ColRNAI)	3	5
		In238	3		C, (FIA), (FII), ColRNAI	2	2.7
ST307	CG307	In916	3		A, (FIB_K), (FIB), FII	2	3
		In238	1		FIB_K, FIB, FIB, FII, HI1B, ColRNAI	1	6
ST788	CG12252	In916	2		(A), (FIB_K), (FII)	2	2
ST253	CG10539	In916	2		A, FII, M2, Col440I, Col440II, ColRNAI	1	6
ST359	CG359	In916	2		A, FIB_K, I1, ColRNAI	1	4
ST299	CG10246	In238	1		FIB, FII, M1, Col440I	1	4
ST908	CG6	In238a	1		FIA, FIB_K, FII, N3	1	4
ST1245	CG10145	In238	1		A, FIB_K, FII, Col440I, ColRNAI	1	5
ST1427	CG10060	In916	1		A, FIB_K, FII	1	3
ST902	CG1878	In238	1		FIB_K, FII, N3	1	3
ST584	CG584	In916-like	1		L, N	1	2
ST23	CG23	In916	1		A, FIB_K	1	2
ST469	CG10359	In916	1		A, FIB_K, FII, N, Col440I	1	5
ST308	CG308	In1008	1		FIB_K, FII, M1, R, Col440I	1	5
ST643	CG10062	In238a	1		FIB_K, FII, N3, R, Col440I	1	5
ST1540	CG3598	In916	1		A	1	1
ST37	CG3648	In238	1		M2, Col440II	1	2
ST427	CG427	In916	1		A, FIB_K	1	2
ST54	CG54	In238	1		C, FIB_K, FII, Col440I, Col440II, ColRNAI	1	6
ST76	CG10052	In916	1		A, FIA, FIB_K, N	1	4
ST6328	-	In916	1		FIA, FIB_K, HI1B, HI2, HI2A	1	5
ST285	-	In916	2		A, FIA, Col440I	1	3
ST347	-	In238	1		C, N	1	2
ST6327	CG3982	In916	1		A, FIA, FII	1	3
ST736	-	In916	1		A, FIB_K, FII, HI2, HI2A, ColRNAI	1	6
ST1535	CG1535	In238	2		C, FIB_K, Col440I	1	3

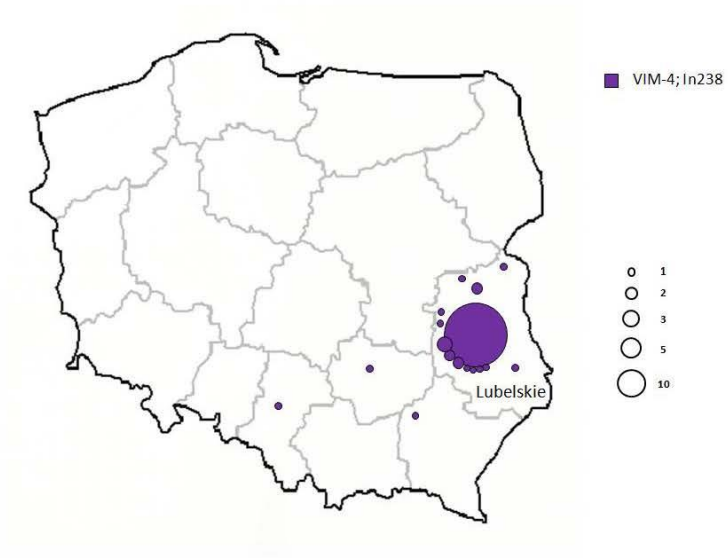
[a] – symbols in parentheses refer to the replicon types that occurred not in all isolates of the corresponding genotypes.

Table S12. Yersiniabactin phylolineages, YbSTs and mobile elements carrying *ybt* genes in the *K. pneumoniae* species complex isolates

ST	K serotype	n isolates	Yersiniabactin, *ybt*		
			Phylo lineage	YbST	Mobile element
ST437	KL36	57	-	-	-
ST11	KL125	3	-	-	-
	KL24	1	15	230	ICE*Kp11*
ST258	KL106	1	-	-	-
ST147	KL64	33	16	280/280-like	ICE*Kp12*
ST392	KL27	13	-	-	-
ST1237	KL64	1	-	-	-
ST15	KL48	16	-	-	-
	KL24	5	-	-	-
	KL112	4	-	-	-
ST277	KL46	18	-	-	-
ST16	KL51	5	-	-	-
ST17	KL25	2	15	231	ICE*Kp11*
		1	-	-	-
	KL2	1	-	-	-
ST336	KL25	2	15	230/230-like	ICE*Kp11*
ST20	KL28	1	14	151-like	ICE*Kp5*
ST6329	KL2	4	-	-	-
		3	16	530/530-like	ICE*Kp12*
ST14	KL2	2	-	-	-
		1	16	530-like	ICE*Kp12*
ST152	KL149	4	10	325/325-like	ICE*Kp4*
		3	-	-	-
ST307	KL102	2	9	157-like	ICE*Kp3*
		1	10	17	ICE*Kp4*
		1	-	-	-
ST788	KL46	2	-	-	-
ST253	KL110	2	-	-	-
ST359	KL10	2	-	-	-
ST299	KL7	1	-	-	-
ST908	KL48	1	-	-	-
ST1245	KL3	1	-	-	-
ST1427	KL155	1	-	-	-
ST902	KL125	1	-	-	-
ST584	KL38	1	-	-	-
ST23	KL1	1	1	47	ICE*Kp10*
ST469	KL139	1	-	-	-
ST308	KL28	1	-	-	-
ST643	KL37	1	-	-	-
ST1540	KL24	1	-	-	-
ST37	KL15	1	14	151-like	ICE*Kp5*
ST427	KL31	1	-	-	-
ST54	KL14	1	-	-	-
ST76	KL10	1	-	-	-
ST6328	KL63	1	-	-	-
ST285	KL67	2	-	-	-
ST347	KL6	1	-	-	-
ST6327	KL47	1	-	-	-
ST736	KL53	1	-	-	-
ST1535	KL114	2	-	-	-

Figure S1. Geographic distribution and clonal analysis of *K. pneumoniae* ST437 KL36 in Poland. A) Geographic distribution of the isolates shown on the map of the country with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. B) SNP-based minimum spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots

A



B

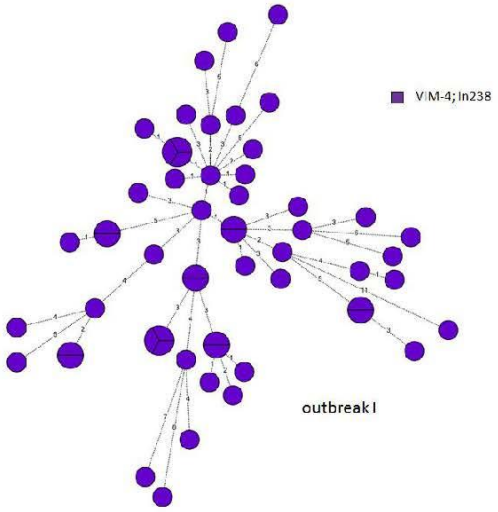
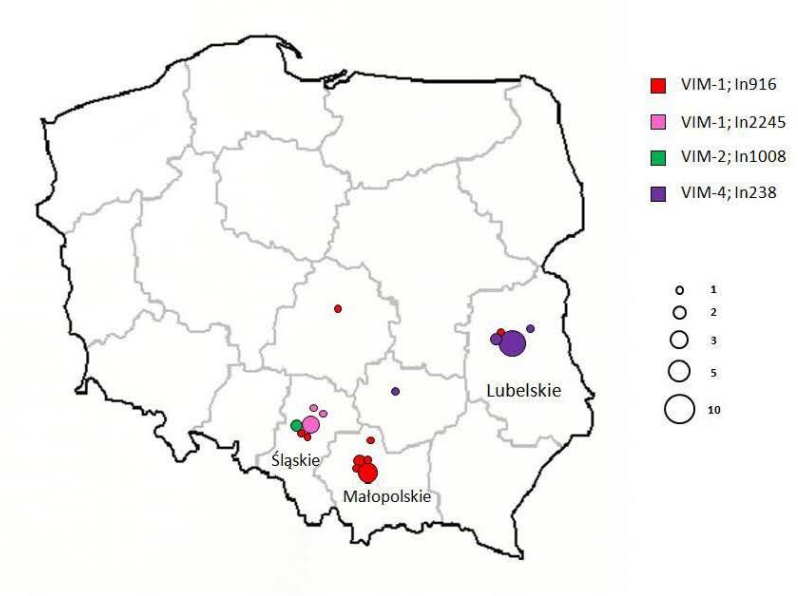


Figure S2. Geographic distribution and clonal analysis of *K. pneumoniae* ST147 KL64 in Poland. A) Geographic distribution of the isolates shown on the map of the country with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. B) SNP-based minimum spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots

A



B

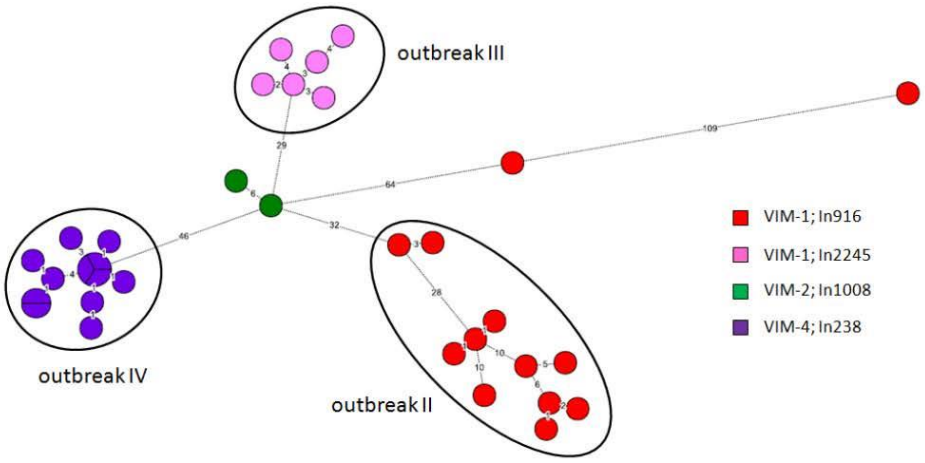
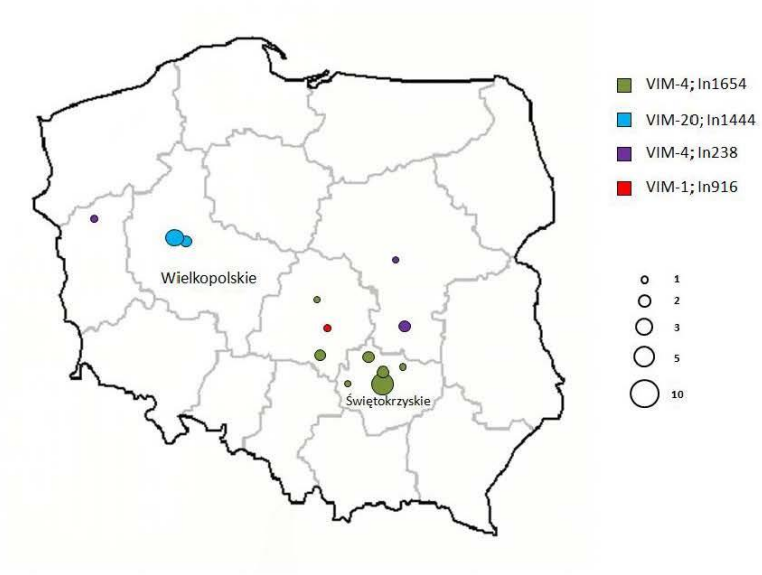


Figure S3. Geographic distribution and clonal analysis *of K. pneumoniae* ST15 in Poland. A) Geographic distribution of the isolates shown on the map of the country with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. B) SNP-based minimum spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots

A



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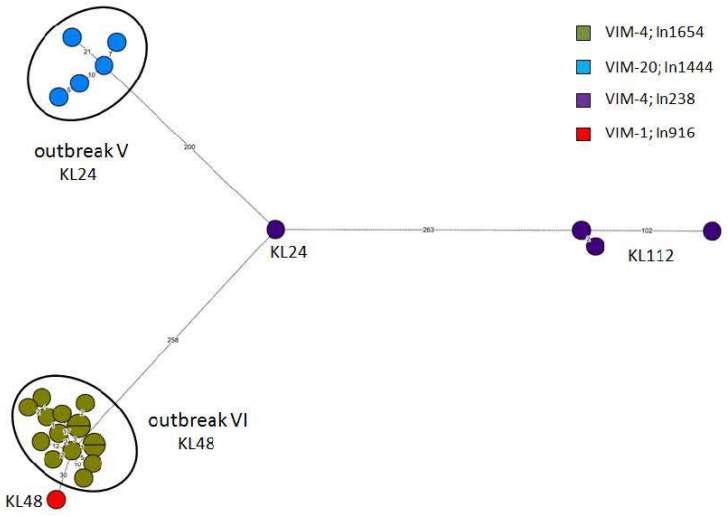
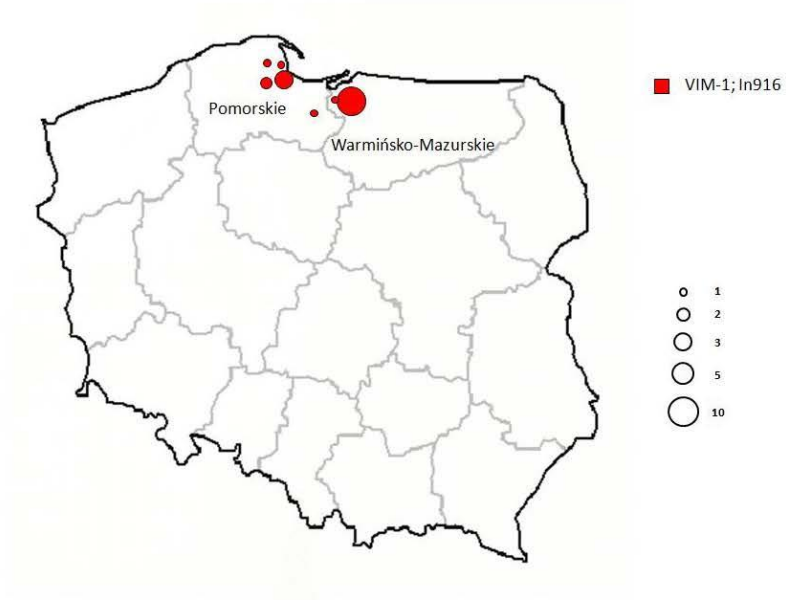


Figure S4. Geographic distribution and clonal analysis *of K. pneumoniae* ST277 KL46 in Poland. A) Geographic distribution of the isolates shown on the map of the country with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. B) SNP-based minimum spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots

A



B

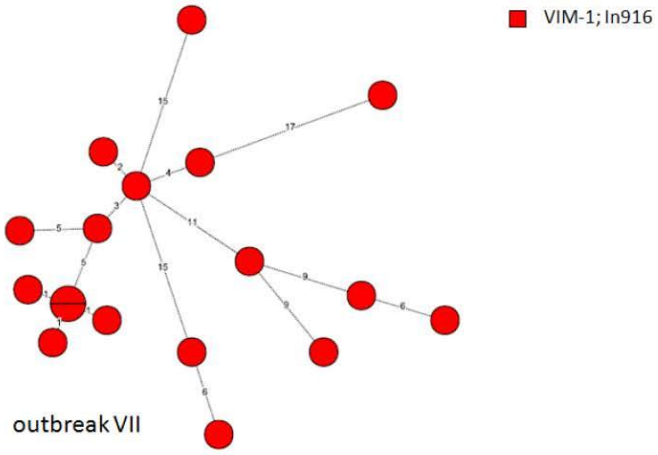
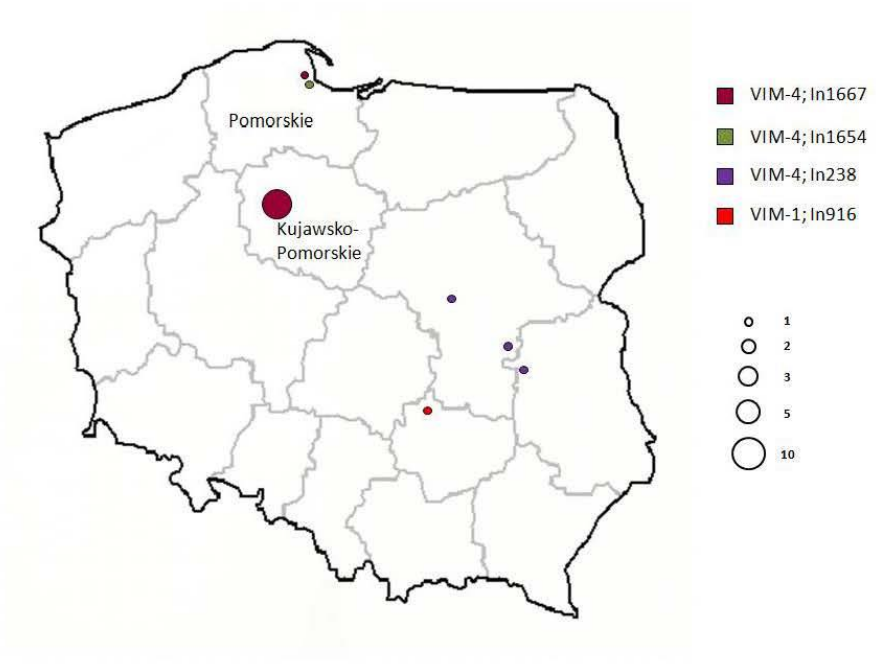


Figure S5. Geographic distribution and clonal analysis *of K. pneumoniae* ST392 KL27 in Poland. A) Geographic distribution of the isolates shown on the map of the country with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. B) SNP-based minimum spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots

A



B

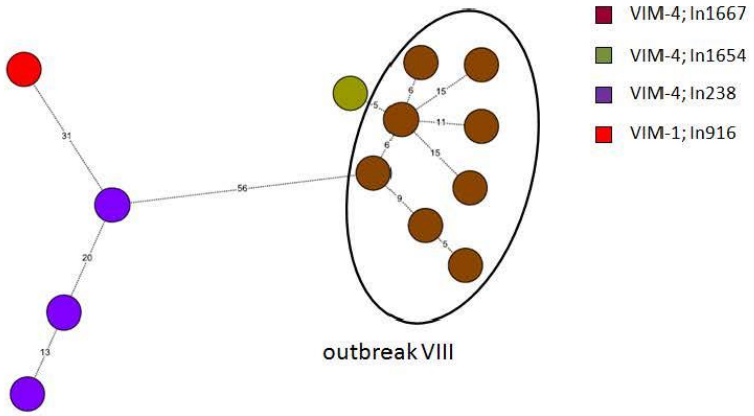


Figure S6. SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST147 isolates compared with the international ST147 genomes available in RefSeq. Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: AE, United Arab Emirates; AU, Australia; BD, Bangladesh; BE, Belgium; BR, Brazil; BY, Belarus; CA, Canada; CH, Switzerland; CN, China; CO, Columbia; CZ, Czechia; DE, Germany; DK, Denmark; EG, Egypt; ES, Spain; FR, France; GB, United Kingdom; GH, Ghana; GR, Greece; HU, Hungary; IL, Israel; IN, India; IR, Iran; IT, Italy; JO, Jordan; JP, Japan; KE, Kenya; KR, South Korea; LB, Lebanon; LK, Sri Lanka; LU, Luxemburg; MY, Malaysia; NG, Nigeria; NL, Netherlands; NO, Norway; NP, Nepal; PE, Peru; PH, Philippines; PK, Pakistan; PL, Poland; PT, Portugal; QA, Qatar; RO, Romania; RS, Serbia; RU, Russia; SE, Sweden; SG, Singapore; SN, Senegal; TH, Thailand, TN, Tunisia; TR, Turkey; US, United States of America. The tree was constructed using Parsnp and visualized with iTOL.

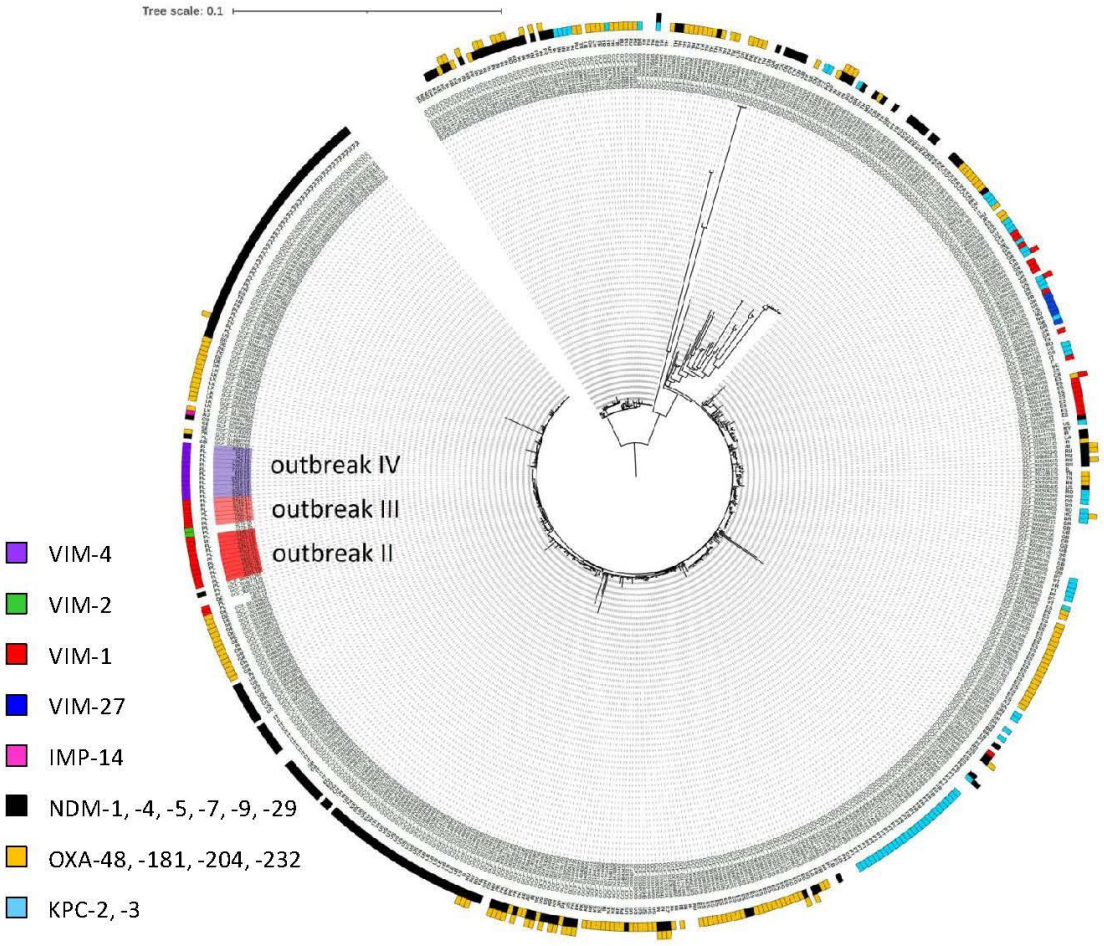


Figure S7. A) SNP-based phylogenetic tree of the study Polish *K. pneumoniae* ST15 isolates and all the 907 international ST15 genomes available in RefSeq. The red circle indicates a clade with all Polish isolates and 698 international genomes. B) SNP-based phylogenetic tree of the ST15 clade, containing all study Polish *K. pneumoniae* isolates and 698 selected international ST15 genomes, indicated by the red circle in panel A. The blue circle indicates a branch with all Polish and 205 international genomes. The trees were constructed using Parsnp and visualized with iTOL.

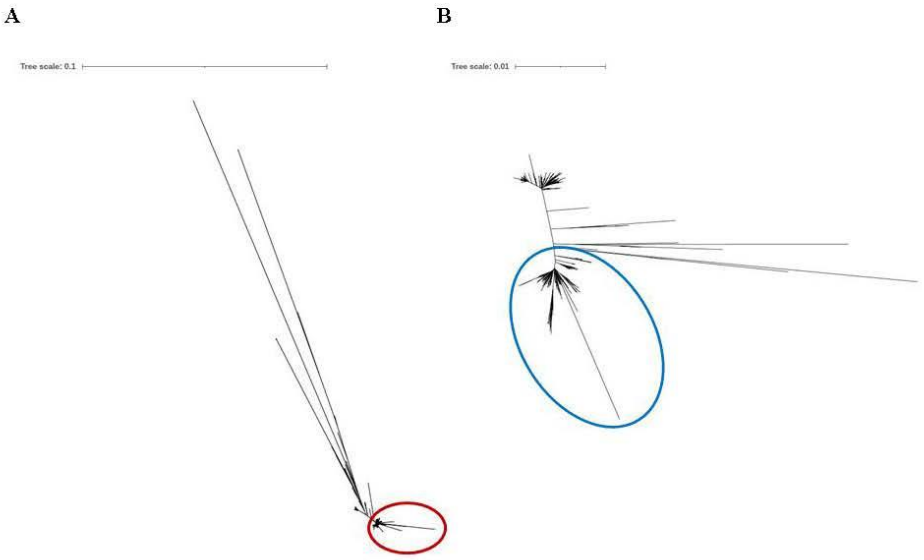


Figure S8. SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST15 isolates and the most related 205 international ST15 genomes available in RefSeq (indicated with blue circle in Appendix Figure S7, Panel B). Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: AT, Austria; BD, Bangladesh; BE, Belgium; BR, Brazil; CH, Switzerland; CN, China; CO, Columbia; CZ, Czechia; DE, Germany; ES, Spain; FR, France; GB, United Kingdom; GH, Ghana; HK, Hong Kong; HR, Croatia; HU, Hungary; IN, India; IT, Italy; KE, Kenya; LB, Lebanon; ME, Montenegro; MG, Madagascar; MY, Malaysia; NG, Nigeria; NL, Netherlands; PK, Pakistan; PL, Poland; PT, Portugal; RO, Romania; RS, Serbia; SK, Slovakia; TH, Thailand, TN, Tunisia; TR, Turkey; TT, Trinidad and Tobago; US, United States of America; VN, Viet Nam; ZA, South Africa. The tree was constructed using Parsnp and visualized with iTOL.

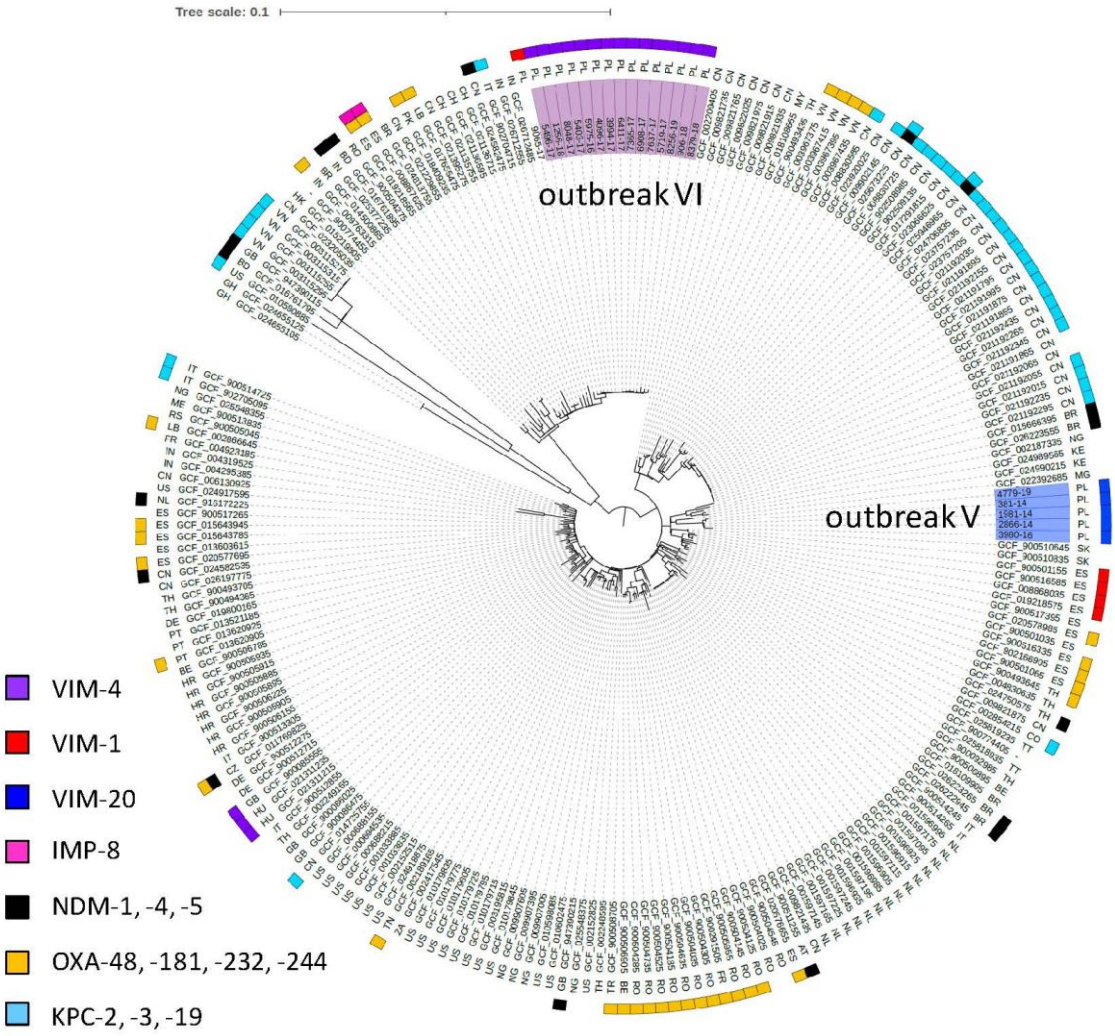


Figure S9. SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST277 isolates compared with the international ST277 genomes available in RefSeq. Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: BR, Brazil; CN, China; GB, United Kingdom; IT, Italy; NL, Netherlands; PL, Poland; PT, Portugal; TH, Thailand. The tree was constructed using Parsnp and visualized with iTOL.

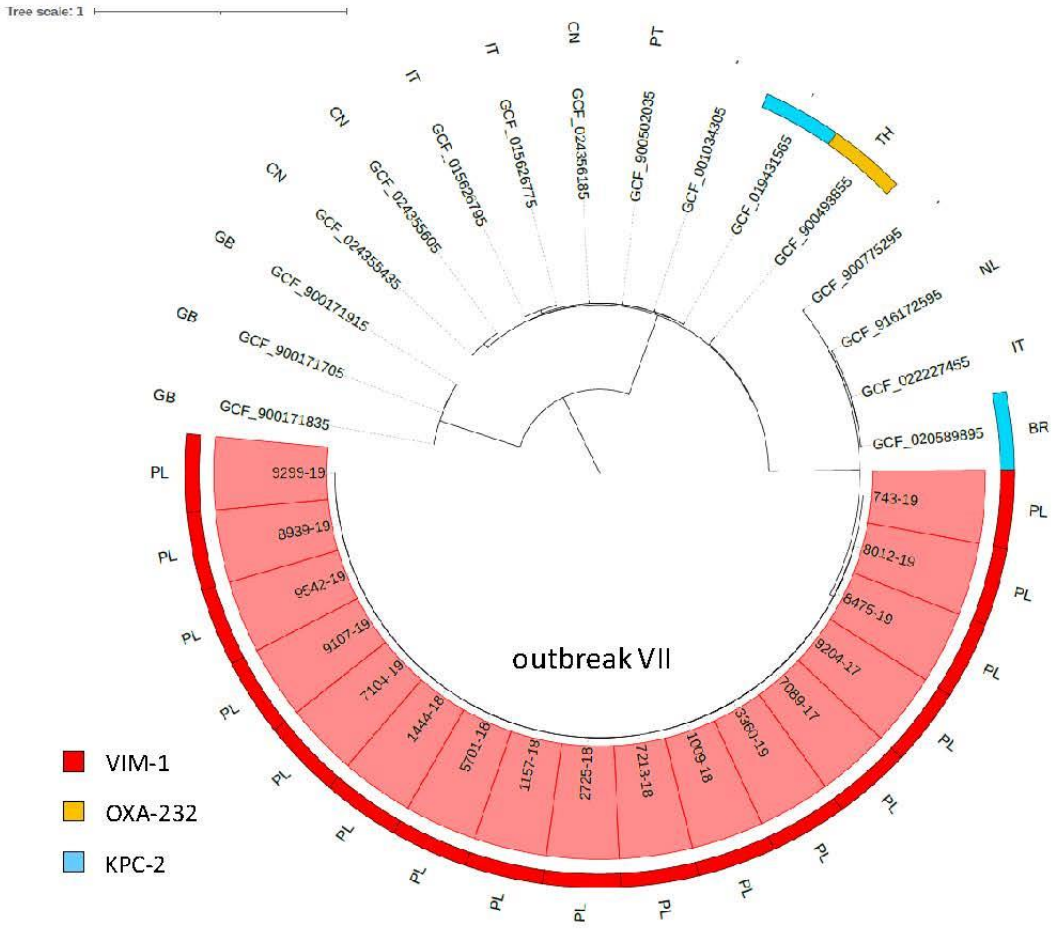


Figure S10. SNP-based phylogenetic tree of all study Polish *K. pneumoniae* ST392 isolates compared with the international ST392 genomes available in RefSeq. Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: AT, Austria; AU, Australia; BE, Belgium; BR, Brazil; CH, Switzerland; CN, China; CO, Columbia; DE, Germany; ES, Spain; FR, France; GB, United Kingdom; IT, Italy; KR, South Korea; LB, Lebanon; LU, Luxemburg; MX, Mexico; MY, Malaysia; NG, Nigeria; PH, Philippines; PL, Poland; PT, Portugal; RU, Russia; TN, Tunisia; TR, Turkey; TZ, Tanzania; UA, Ukraine; US, United States of America, VN, Viet Nam. The tree was constructed using Parsnp and visualized with iTOL.

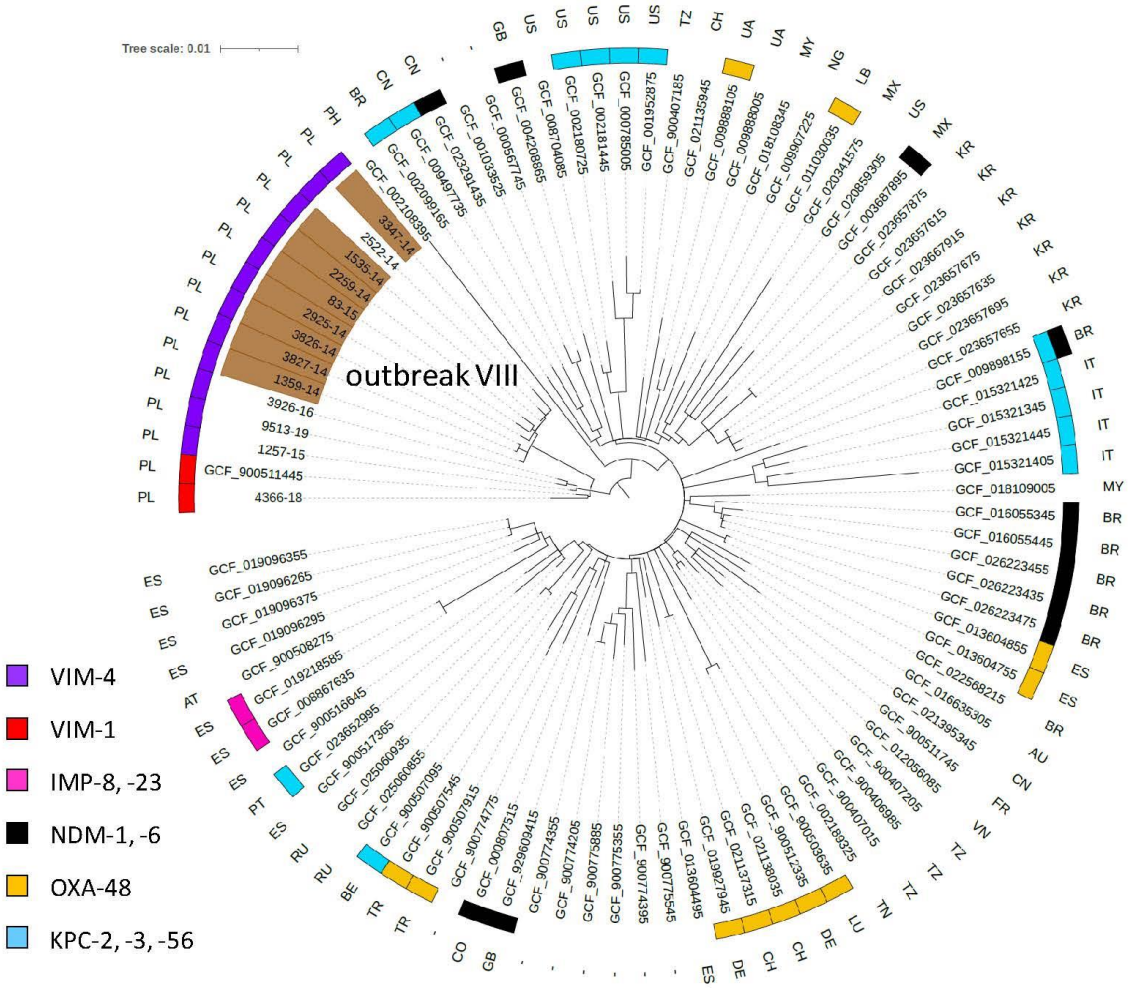


Figure S11. Comparison of the VIM-4-encoding (integron In238) IncFIB+IncHI1B p7160FH plasmid (inner, thin black circle) to IncFIB+IncHI1B p5777FH plasmid and previously reported plasmids of the highest homology: pNDM-MAR (Morocco; JN420336), p4313/18_1 (Poland, ON081621), p7902/18_1 Poland, ON081624), pKpvST383L (United Kingdom, CP034201), pKP-1PI_HIB_FIB (Italy, CP071028), phvKpST147_NDM-1_1659 (Russia, CP072810), pFQ61_ST383_NDM-5 (Qatar, CP091814), pCDI231-348.3 (China, CP077784), p51015_NDM-1 (Czechia, CP050380) and unnamed 1 (USA, CP117746). The outer thick black ring refers to the annotation of p7160FH, with the selected genes indicated. The percentage of sequence identity is reflected by color intensity. The picture was created using BRIG software (http://brig.sourceforge.net/).

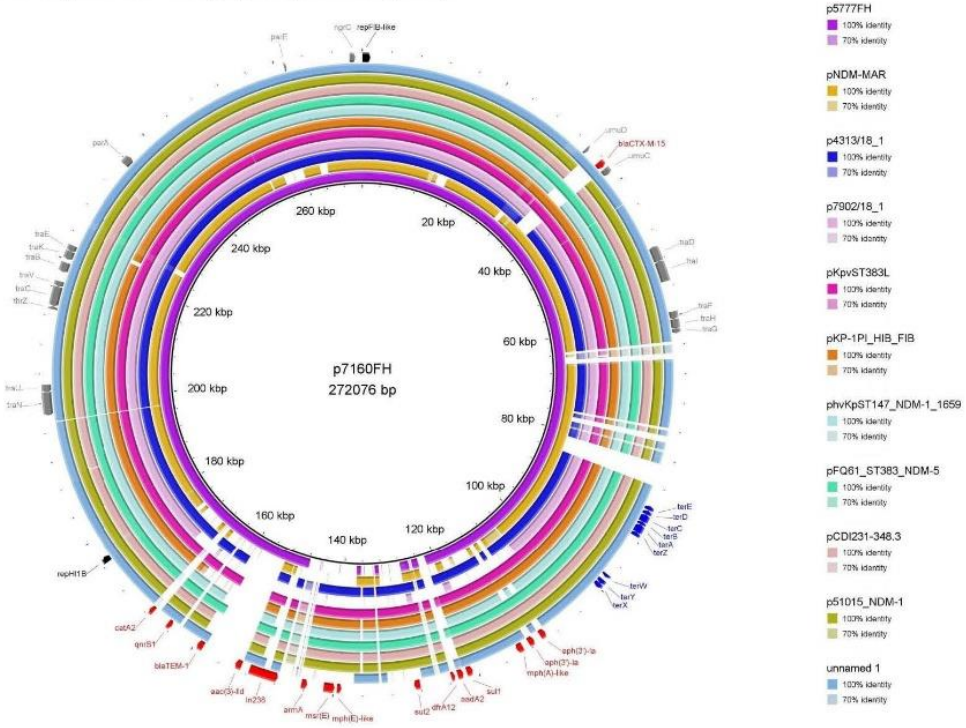


Figure S12. Comparison of the study FIB+HI1B pNDM-MAR .like plasmids: p7160FH and p5777FH with the reference plasmid pNDM-MAR (Morocco, JN420336) and the previously reported p4313/18_1 (Poland, ON081621), containing a larger fragment of pK2044/KpVP-1 with virulence loci *iuc* and *rmpA/A2*. The picture was created using the Easyfig 2.2.5 software.

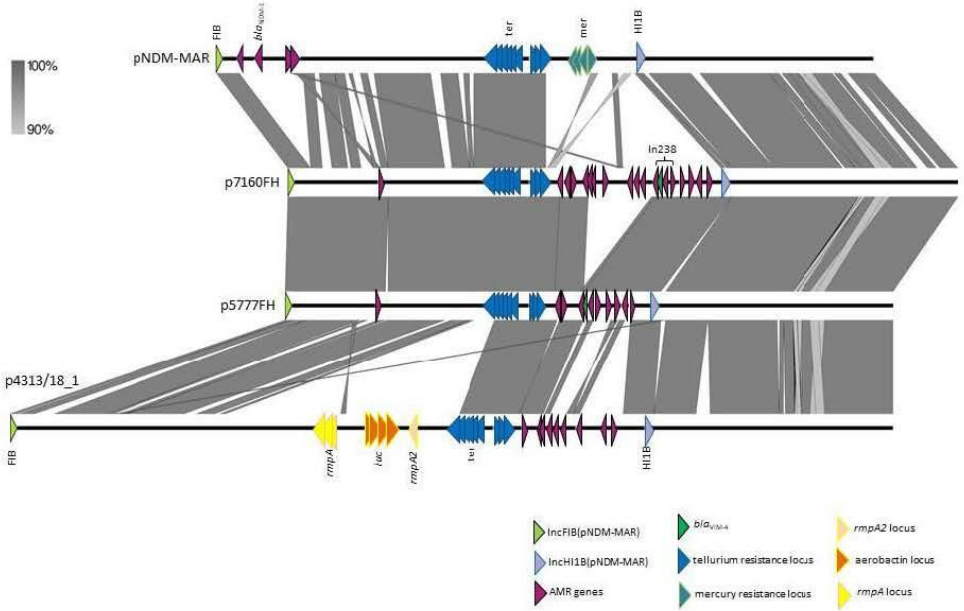


Figure S13. Comparison of the VIM-1-encoding (integron In196) IncA p7089A plasmid (inner, thin black circle) to IncA p2254A plasmid and previously reported plasmids of the highest homology: p9546/19_2 (Poland; ONO081626), p743A (Poland, OQ111274), p5955A (Poland, OQ111275), p7753A (Poland, OQ111276), p253A (Poland, OR232699), plasmid unnamed (USA, CP132210), plasmid unnamed 1 (USA, CP132322), pRIVM0001 (The Netherlands, MH220284), pKC-BO-N1-VIM (Italy, MG228427), pGA_VIM (Italy, MN783743), pFDL-VIM (Italy, MN783744) and p7536 (France, CP071789). The outer thick black ring refers to the annotation of p7089A, with the selected genes indicated. The percentage of sequence identity is reflected by color intensity. The picture was created using BRIG software (http://brig.sourceforge.net/).

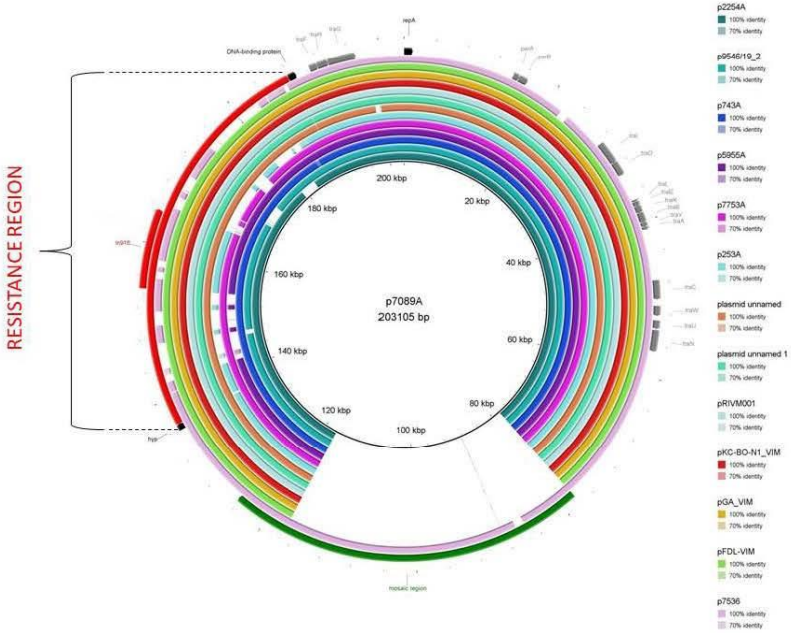


Figure S14. Comparison of the AMR region of the In916-carrying IncA-like plasmid p7089A with the corresponding part of p9546/19_2 (Poland, *K. pneumoniae*, ON081626), p743A (Poland, *E. hormaechei*, OQ111274) and p2254A (this study). The percentage of sequence identity is reflected by the gray color intensity. Individual loci (antibiotic AMR genes, mobile genetic elements and integration sites) are marked by colored arrows or triangles as explained below. The picture was created using the Easyfig 2.2.5 software

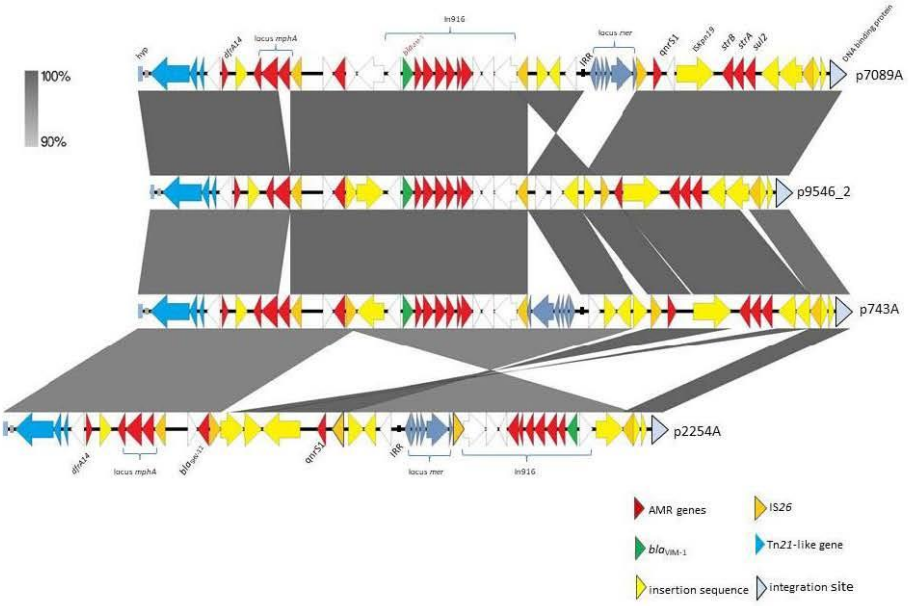
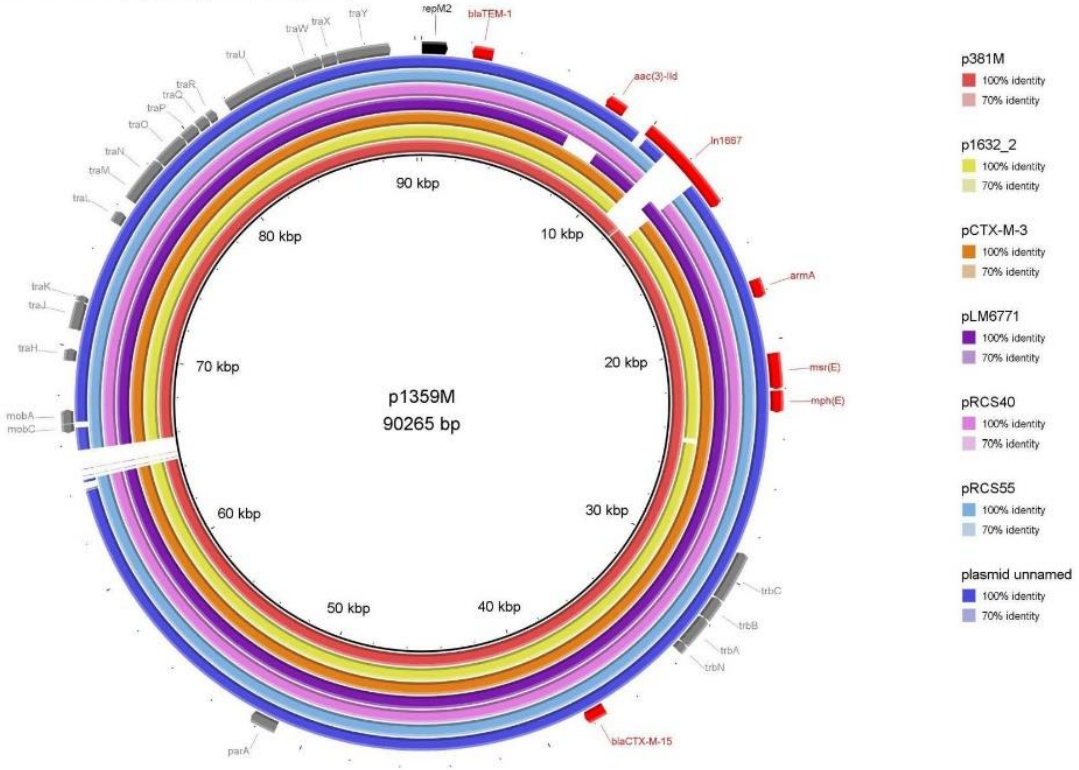


Figure S15. Comparison of the VIM-4-encoding (integron In1667) IncM2 p1359M plasmid (inner, thin black circle) toVIM-20-encoding (integron In1444) IncM2 p381M plasmid and previously reported plasmids of the highest homology: p1632_2 (China, CP084499), pCTX-M-3 (Poland, AF550415), pLM6771 (South Korea, KX009507), pRCS40 (France, LT985241), pRCS55 (France, LT985387) and plasmid unnamed (Taiwan, CP044337). The outer thick black ring refers to the annotation of p1359M, with the selected genes indicated. The percentage of sequence identity is reflected by color intensity. The picture was created using BRIG software (http://brig.sourceforge.net/).



6.3 Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009–2019

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ORIGINAL ARTICLE

Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009–2019

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Abstract

Purpose To elucidate the role of the *Klebsiella oxytoca* species complex (KoSC) in epidemiology of VIM-type MBL-producing Enterobacterales in Poland.

Methods The study comprised all 106 VIM-positive KoSC isolates collected by the Polish National Reference Centre for Susceptibility Testing during 2009–2019 from 60 institutions in 35 towns. All isolates were sequenced by Illumina MiSeq, followed by MinION sequencing of selected organisms. Genomes were subjected to bioinformatic analysis, addressing taxonomy, clonality, phylogeny and structural characterisation of key resistance determinants within their chromosomal and plasmidic loci.

Results Among five species identified, *K. oxytoca* was predominant ($n=92$), followed by *Klebsiella michiganensis* ($n=11$). MLST distinguished 18 STs, with the most prevalent *Klebsiella oxytoca* ST145 ($n=83$). The clone segregated a lineage with the In237-like integron [$bla_{\text{VIM-1}}$–*aacA4* genes; $n=78$], recorded in 28 cities almost all over the country. The integron was located in a ~ 49–50 kb chromosomal mosaic region with multiple other resistance genes, linked to a ~ 51 kb phage-like element. The organism might have originated from Greece, and its evolution in Poland included several events of chromosomal ~ 54–258 kb deletions, comprising the natural β-lactamase bla_{OXY} gene. A group of other isolates of various species and clones ($n=12$) carried the integron In916 on self-transmissible IncA-type plasmids, effectively spreading in Italy, France and Poland.

Conclusion KoSC has been one of the major VIM producers in Poland, owing largely to clonal expansion of the specific *K. oxytoca*–In237-like lineage. Its apparently enhanced epidemic potential may create a danger on international scale.

Keywords VIM · Enterobacterales · *Klebsiella oxytoca* species complex · KoSC · ST145 · Poland

Introduction

Carbapenemase-producing Enterobacterales (CPE) are considered to be largely responsible for the current antimicrobial resistance (AMR) crisis around the world [1]. One of their critically important groups is metallo-β-lactamase (MBL) producers, including those with VIM-type enzymes. Initially, these were described in *Pseudomonas* spp. in the mid-1990s, followed soon by Enterobacterales [2, 3]. In general, the VIM-type carbapenemases are encoded by gene cassettes usually of class 1 integrons that in enterobacteria are located on a variety of plasmids and only occasionally in the chromosome. The first successful VIM CPE country-wide spread in Europe was observed in Greece in the early 2000s, leading to endemic situation with numerous host species, VIM variants and their genetic determinants [4–6]. Soon, regional or interregional dissemination of such organisms has been reported in other European countries, including Spain, Italy or Hungary [6–9].

The first VIM-producing Enterobacterales isolate in Poland, *Klebsiella pneumoniae*, was confirmed in 2006 by the National Reference Centre for Susceptibility Testing (NRCST) in Warsaw [10]. Till the end of 2012, 118 VIM CPE isolates were reported, with predominance of *Enterobacter* spp. ($n=64$; 54.2%), followed by *Klebsiella oxytoca* ($n=23$; 19.5%) [11]. Since then, annual numbers

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of VIM CPE multiplied, resulting in a total of 927 isolates from 2006 to 2019 [12]. Although surpassed by *K. pneumoniae* in recent years, the *K. oxytoca* species complex (KoSC) has remained to be a highly relevant fraction of VIM CPE, ranking third among the taxonomic groups ($n=106$; 11.4%). Here, we report the genomic analysis of all VIM-producing KoSC isolates, collected from the first identification in 2009 till the end of 2019, being part of a comprehensive WGS study of all Polish VIM CPE confirmed by the NRCST in 2006–2019.

Materials and methods

Bacterial isolates, WGS and species identification

The study comprised 106 non-duplicate VIM-producing KoSC isolates from 105 patients, collected by the NRCST during the CPE surveillance in Poland from 2009 to 2019 (Table S1) [12]. The isolates were from 60 centres in 35 cities of all 16 main administrative regions. The majority of the isolates were detected in the region Mazowieckie ($n=56$; 52.8%) and often in Warsaw ($n=34$; 32.1%). Around a half of the isolates were recovered from infections ($n=52$; 49.1%), mainly of the urinary tract ($n=27$; 25.5%) and wounds ($n=15$; 14.1%), and most of the remaining ones were from carriage ($n=53$; 50.0%). The KoSC isolates were tested for the carbapenemase presence by CARBA NP [13], phenotypic and PCR tests [14] and sequenced by MiSeq (Illumina, San Diego, CA, USA), with reads assembled with SPAdes 3.15.5 [15]. Four isolates, representing predominant bla_{VIM}-carrying integron types, were subjected to long-read sequencing by MinION (Oxford Nanopore Technologies, Oxford, UK). The MiSeq and MinION hybrid assemblies were done with Unicycler v.0.4.8 [16]. Species identification was based on average nucleotide identity (ANI) scores, using FastANI v.1.32 with ≥ 95% cutoff [17] and RefSeq reference genomes.

MLST, clonal and phylogenetic analyses, characterisation of bla_{OXY} genes

MLST was performed by the mlst tool (https://github.com/tseemann/mlst). The in-sample clonality SNP analysis was done by BioNumerics v.7.6.3 (Applied Maths NV, Sint-Martens-Latem, Belgium). The SNP-based phylogenetic analysis in the international context was performed with all 271 KoSC genomes available in RefSeq as of the 1st of February 2023, using Parsnp v.1.5.4 (https://github.com/marbl/parsnp). Phylotrees were visualised by iTOL (https://itol.embl.de). Variants of the intrinsic KoSC β-lactamase bla_{OXY} genes were identified using the BIGSdb database (https://bigsdb.pasteur.fr/klebsiella/). Broader bla_{OXY}-containing genomic regions were studied using the progressive Mauve algorithm [18].

Integrons with bla_{VIM} genes and their chromosomal and plasmid context; resistomes andantimicrobial susceptibility

The composition of bla_{VIM}-carrying integrons was characterised manually using Geneious Prime v.2022.0.1 (Biomatters, Auckland, New Zealand) and BLASTn. Chromosomal loci containing bla_{VIM}-carrying integrons and bla_{OXY} deletions were analysed using Mauve [18]; PHASTER [19] was applied to identify phage-like elements. Plasmid replicon types were identified by ABRicate using PlasmidFinder database [20]. The structural analysis of plasmids was executed using BLASTn and visualised by BRIG (http://brig.sourceforge.net/). Easyfig v.2.2.5 (http://mjsull.github.io/Easyfig/) was used to illustrate specific structures of the chromosome or plasmids with AMR genes. Acquired AMR genes (resistomes) were detected using ResFinder database, with the 99.5% identity criterion [21]. Susceptibility to 19 antimicrobials was tested for 23 representative KoSC isolates by broth microdilution, using Sensititre™ Gram Negative EUMDRXXF AST (Thermo Fisher Scientific, Waltham, MA, USA), MICRONAUT-S Pseudomonas MIC (Bruker Daltonics, Bremen, Germany), ComASP® cefiderocol (Liofilchem, Roseto degli Abruzzi, Italy) and aztreonam-avibactam in-house plates. EUCAST breakpoints (http://eucast.org) were used for interpretation of the results.

Serotypes and virulence genes

Identification of putative virulence determinants: capsule (CPS, K) and lipopolysaccharide (LPS, O) loci and yersiniabactin and kleboxymycin biosynthesis gene clusters was performed using Kaptive [22], Kleborate [23], Geneious Prime v.2022.0.1 and BLASTn as described previously [24].

Results

Taxonomy, clonality and bla_{OXY} genes

Five different KoSC species were detected among the 106 isolates: *K. oxytoca* ($n=92$; 86.8%), *K. michiganensis* ($n=11$, 10.4%) and *K. grimontii*, *K. pasteurii* and *K. spallanzanii* ($n=1$; 0.9% each) (Table S1). The isolates were classified into 18 distinct STs, including five novel ones, with ten and six STs assigned to *K. oxytoca* and *K. michiganensis*, respectively (*K. spallanzanii* is not included in the MLST scheme) (Table S1). The *K. oxytoca* population was dominated vastly by ST145 ($n=83$; 90.2% and 78.3% of *K. oxytoca* and all KoSC isolates, respectively). The occurrence

of the remaining STs was marginal, including 13 STs with single isolates only.

Six phylogroups of the bla_{OXY} gene were congruent with the taxonomic distribution, with lineage 2 characteristic for *K. oxytoca* and lineages 1 and 5 for *K. michiganensis* (Table S1) [25, 26]. A total of 17 gene alleles were distinguished, which in general correlated well with STs. The most abundant was the $bla_{OXY\text{-}2\text{–}22}$ variant ($n=59$; 55.7% of all KoSC), observed only in the *K. oxytoca* ST145 isolates; however, a remarkable fraction of this clone ($n=24$; 22.6% of all KoSC) were bla_{OXY} negative (confirmed by PCR [25]; addressed below).

bla_{VIM} variants and bla_{VIM}-carrying integrons

Four bla_{VIM} gene variants were detected, three of which represented the $bla_{VIM\text{-}1}$ lineage: $bla_{VIM\text{-}1}$ (n=96; 90.6%), $bla_{VIM\text{-}4}$ (n=7; 6.6%) and the novel $bla_{VIM\text{-}79}$ (n=1; 0.9%) (Table S1). The remaining $bla_{VIM\text{-}2}$ genes were sporadic (n=2; 1.9%). Altogether, nine bla_{VIM}-carrying integrons were found, with predominance of In237-like elements ($bla_{VIM\text{-}1}$; n=81, 76.4% in total), followed by In916 ($bla_{VIM\text{-}1}$; n=13; 12.3%), and In238, In238a and In238-79 together ($bla_{VIM\text{-}4/\text{-}79}$; n=8; 7.5% in total) (Tables S1 and S2). The In237-like integron differs from In237 by two SNPs at positions 60 and 68 in the $bla_{VIM\text{-}1}$ *attC* site [11]. The In238a element differs from In238 by not having a specific 169-bp duplication at the 3′-end of the bla_{VIM} cassette (present also in In238-79, In237 and In237-like elements) [11, 27, 28], whereas In238-79 differs from In238 by one point mutation converting $bla_{VIM\text{-}4}$ to $bla_{VIM\text{-}79}$.

Epidemiology, clonality and phylogeny of the *K. oxytoca* ST145 clone

In the study period, the predominant *K. oxytoca* ST145 clone ($n=83$) was recorded in 46 hospitals in 28 towns of 14/16 Polish administrative regions, mainly Mazowieckie ($n=49$; 59.0% of ST145) with Warsaw ($n=28$) (Figure S1A). The vast majority of the ST145 isolates carried the In237-like integron ($n=78$ in total; 93.9%); few isolates had In916 or In238-like elements ($n=4$; 4.8%, and $n=1$; 1.2%, respectively).

All of the ST145 isolates were subjected to the SNP-based clonal comparative analysis that revealed 1446 polymorphic positions within 4.9 Mb (78%) of the reference genome, characterising the original VIM-producing ST145 isolate from 2009 (isolate NMI776/09 with the In237-like integron). SNP numbers between any individual isolate and the reference ranged from 14 to 126 SNPs (Table S3). However, the 78 isolates with the In237-like element formed a distinct cluster with 0–69 SNPs between each other, indicating clonal outbreak, separated clearly from the remaining five isolates with In916 or In238 integrons (Figure S1B). The further in-depth investigation of the outbreak isolates has split them into those with the natural β-lactamase bla_{OXY} gene (allele $bla_{OXY\text{-}2\text{–}22}$; $n=59$) and those lacking the gene ($n=24$). The comparison of all bla_{OXY}-negative genomes with the 'oldest' ST145-In237-like bla_{OXY}-positive isolate (isolate NMI2092/09) revealed that bla_{OXY} negatives have arisen from a series of chromosomal deletions, ranging from ~ 54 to ~ 258 kb (Table S4). The majority of the deletions characterised single isolates, and the phylogenetic ST145 analysis revealed these to be distributed across the phylotree, indicating mainly independent and unique character of the deletions (Fig. 1). However, some identical or similar in size deletions (~ 152 kb, ~ 159–161 kb and ~ 216 kb) were observed in multiple isolates each, and the analysis showed these to form clusters of closely related isolates, demonstrating spread of some of the bla_{OXY}-negative sublineages, combined with further modifications of the original deletions.

The phylogenetic analysis of all 83 Polish and eight international ST145 genomes identified in RefSeq has separated the outbreak ST145-In237-like isolates from those with the In916 or In238 integrons and the isolates from other countries (China, $n=5$; and the UK, USA and Spain, $n=1$ each) (Fig. 1). Of note, a single isolate from China (GCF_002265085) was the only other bla_{OXY}-negative ST145 record found in RefSeq; however, the lack of bla_{OXY} was observed also in isolates of ST2 from the UK (GCA_022432685) and undefined ST from South Africa (GCF_015694225).

Phylogeny of minor STs

Only seven of the remaining STs identified in Polish KoSC were represented in the RefSeq database. A single VIM-1-positive *K. oxytoca* ST2 isolate was related to a Spanish VIM-1 producer (229 SNPs; another bla_{VIM} integron), and both were located on the main branch of the ST2 phylotree, along with multiple other isolates from European countries mainly (Figure S2). The other STs had only few RefSeq genomes each (results not shown).

Acquired AMR genes and susceptibility patterns

Seventy-one acquired AMR gene profiles were defined in the study isolates, with 4–19 genes per genome and a mean of 11.1 (Table S1). The AMR gene content varied even within the ST145-In237-like outbreak cluster, presenting 46 gene profiles, each with up to 17 isolates. The only AMR genes common for all of the outbreak isolates were $bla_{VIM\text{-}1}$ and *aacA4* of the In237-like integrons. Apart from other aminoglycoside resistance genes, all or most of the outbreak isolates carried the AmpC-like cephalosporinase gene $bla_{CMY\text{-}31}$

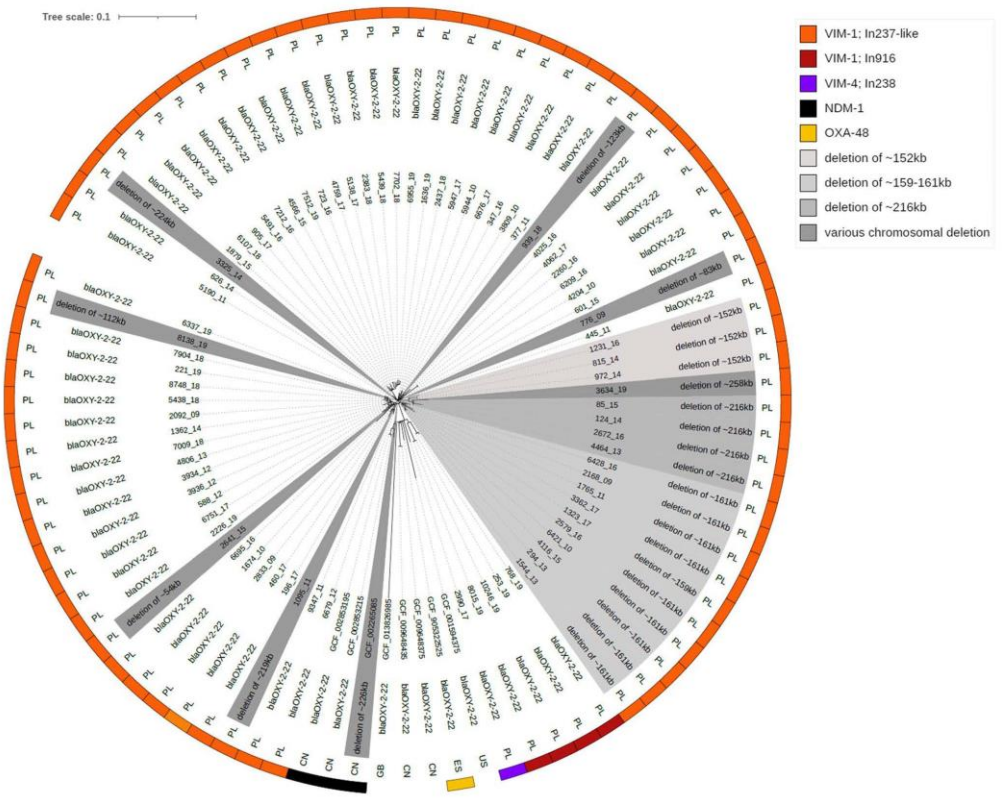

Fig. 1 SNP-based phylogenetic tree of Polish *K. oxytoca* ST145 isolates compared with all international ST145 genomes available in RefSeq. Numbers on the inner circle are the original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colours. Country symbols: CN, China; ES, Spain; GB, Great Britain; PL, Poland; US, USA. The tree was constructed using Parsnp and visualised with iTOL

(91.0%) and genes of resistance to sulphonamides (100%), trimethoprim (91.0%) and phenicols (69.2%).

Twenty-three isolates (including four ST145-In237-like outbreak organisms), representing all species, STs and various resistomes, were subjected to susceptibility testing. All these showed AMR phenotypes correlating well with the resistomes, and in general, the isolates were not extensively drug resistant (Table S5). Levels of resistance to carbapenems varied, and the majority of the isolates were susceptible or susceptible at increased exposure to meropenem (and, consistently, its combination with vaborbactam). All of the isolates had low MICs of aztreonam with avibactam, and all but one were susceptible to cefiderocol. Aminoglycosides (amikacin and gentamicin) and quinolones (especially levofloxacin) were active in vitro against the majority of the organisms, and all isolates were fully susceptible to colistin.

Chromosomal AMR locus with the In237-like integron in the outbreak ST145 clone

The previous, standard molecular biology study has assigned the In237-like integron to the chromosome of the early *K. oxytoca* ST145 outbreak isolates, 2009–2012 [11]. The present analysis has confirmed that observation and revealed details of the In237-like chromosomal loci. Two outbreak isolates, namely the first bla_{OXY}-positive and bla_{OXY}-negative isolates NMI2092/09 and NMI776/09, respectively, used as references in the clonal analyses described above, were long-read sequenced. The examination of the NMI2092/09 isolate has shown the In237-like integron to reside in a Tn*21*-like mercury resistance transposon, truncated by IS*26* and located within a unique ~ 52 kb mosaic region (MR) with multiple other mobile elements (Fig. 2). The

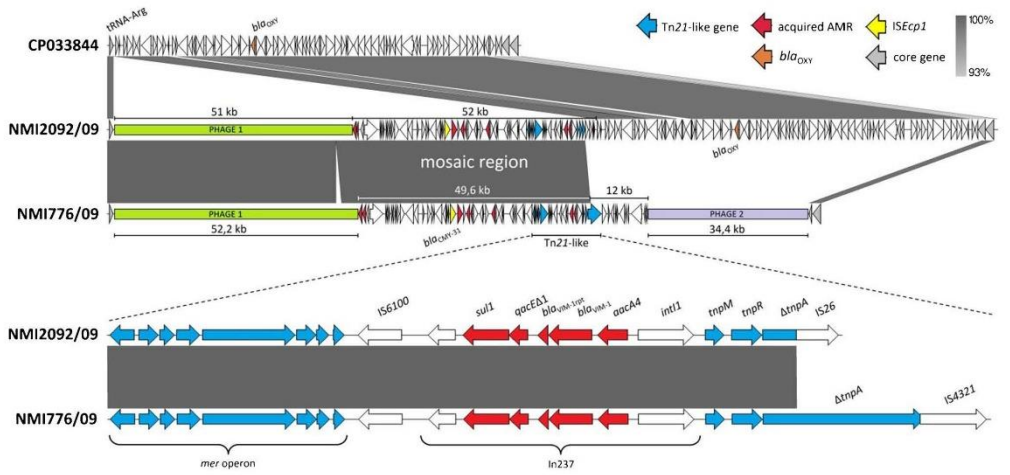

Fig. 2 Genetic context of the chromosomal AMR islands and adjacent regions in the bla_{OXY}-positive isolate NMI2092/09 and bla_{OXY}-negative isolate NMI776/09, compared to the corresponding loci with $tRNA^{Arg}$ and bla_{OXY} genes loci in the *K. oxytoca* RefSeq reference strain FDAARGOS 500 (GenBank accession number CP033844). The entire fragment compared is flanked by the $tRNA^{Arg}$ gene (*K. oxytoca* FDAARGOS 500 locus tag EGY21_24185) and fimbrial protein genes (locus tags EGY21_24550 and EGY21_24555). Arrows indicate all CDSs proportionally to their sizes and orientation; the only selected genes or mobile elements are marked with colours and/or names. The shaded areas between linear structures indicate homologous regions and the level of their sequence identity. The Tn*21*-like structures with the In237-like integron are enlarged below the chromosomal comparisons using dotted lines

MR contained also eight additional AMR genes (Table S1), including bla_{CMY-31} within an IS*Ecp1* transposition module, and it followed directly a ~ 51 kb phage-like segment, 'Phage 1', identified by PHASTER to be intact but of no extensive identity to any phage known. The entire 'Phage 1'–MR structure was inserted into a $tRNA^{Arg}$ gene with a partial, 45 bp duplication, placed ~ 29.5 kb downstream of the $bla_{OXY-2-22}$ gene. The bla_{OXY}-negative NMI776/09 isolate also contained a 'Phage 1'–MR combo in the $tRNA^{Arg}$ gene, sharing ~ 49.6 kb of MR with NMI2092/09, with the In237-like-Tn*21* truncated by IS*4321*. Interestingly, another phage-like structure of ~ 34 kb, 'Phage 2', plus additional ~ 12 kb of unknown origin was identified directly behind the MR, and the comparison with the NMI2092/09 genome revealed that this entire region has replaced ~ 83 kb of the original *K. oxytoca* chromosome, comprising bla_{OXY}.

'Phage 1' was identified in all of the Polish ST145-In237-like outbreak and five ST145-In916/In238 non-outbreak isolates and in 5/8 international ST145 isolates from Spain, the USA and China, indicating no strict correlation between this structure and the MR with the In237-like-Tn*21* element. It was inserted always in the $tRNA^{Arg}$ gene, but as revealed in two long-read sequenced non-outbreak ST145-In916/In238 isolates (NMI253/19 and NMI2990/17; described below), their 'Phage 1' was shorter (~ 48 kb) and had no any AMR region associated. Otherwise, apart from the NMI776/09 isolate, 'Phage 2' was detected in two isolates only, both bla_{OXY} positive, and not in any other isolate with the bla_{OXY} deletion.

Plasmid profiles; bla_{VIM}-carrying plasmids

At least one of 23 different plasmid replicon types was detected in 64 isolates of all STs (60.4%), producing profiles of 0–5 replicons per isolate (Table S6). The 42 isolates with no or no typeable plasmid belonged exclusively to the ST145 outbreak cluster with chromosomal In237-like elements.

Detailed structure of plasmids with bla_{VIM}-carrying integrons was revealed for two long-read sequenced non-outbreak *K. oxytoca* ST145 isolates, having In916 (isolate NMI253/19) or In238 (NMI2990/17). In916 was identified on a ~ 134 kb IncA-type plasmid (p253A). The comparative analysis of p253A against public sequence databases showed its high identity to the previously published In916-harbouring IncA plasmids p743A, p7753A, p5955A and p9546_2 from Polish *Enterobacter* spp. or *K. pneumoniae* [12, 29], as well as a series of such plasmids from Italian Enterobacterales [30], and Dutch *Aeromonas* sp. (MH220284) (Figure S3). Main differences between all these plasmids arose from multiple rearrangements within the AMR mosaic region, containing a IS*26*–bla_{SHV-12}–In916–IS*26* module, described originally in Italy [30]. In p253A, this region was

significantly smaller than in the previously published plasmids (~ 23 kb versus ~ 37.8 to ~ 51.8 kb, respectively), had less AMR genes and no mercury resistance operon and was flanked by IS*Kpn19*-like elements on both sides (Figure S4).

In238 was located in the NMI2990/17 isolate on a ~ 23 kb plasmid (p2990) of unknown replicon. The BLASTn-based comparison revealed its low overall similarity (coverage, ~ 35%; identity, > 99%) to few international plasmids only. p2990 comprised regions encoding plasmid replication and stability, conjugal transfer (TraK, J-like and TrbJ, K, L-like families) and type I and II toxin-antitoxin systems (ptaRNA1-, RelE/StbE- and VapC-families) (results not shown).

The analysis of the short-read data for the remaining isolates with the likely plasmidic location of bla_{VIM} integrons was not able to associate these elements with individual plasmids in a number of these. It demonstrated In916 to reside on IncA-type plasmids in all other isolates with this integron ($n = 12$), regardless the species and ST (Table S6). The only other case was the *K. oxytoca* ST2 isolate with In238-79 (bla_{VIM-79}) which was assigned to an IncM1-like plasmid.

Serotypes and virulence genes

The CPS K-antigen biosynthesis locus was identified sporadically ($n = 5$, 4.7%), whereas that of the LPS O-antigen was common ($n = 104$; 98.1%), with four variants detected (Table S6). The entire *K. oxytoca* ST145 clone and single ST2, ST346 and ST347 isolates were characterised by OL104. The yersiniabactin locus was observed broadly ($n = 94$; 88.7%), including all *K. oxytoca* organisms (Table S6). The kleboxymycin biosynthesis gene cluster was detected less frequently ($n = 40$; 37.7%), being present in various genotypes, including 27 K. *oxytoca* ST145-In237-like outbreak isolates (Table S6).

Discussion

KoSC has been an important producer of VIM-like MBLs in Poland, occupying the third position among all VIM CPE from 2006 to 2019 (11.3%), behind *Enterobacter* spp. (40.1%) and the *K. pneumoniae* species complex, KpSC (23.1%) [12]. The VIM-positive KoSC population has been mainly *K. oxytoca* itself (86.8%), which then to a similar extent has been dominated by the ST145-In237-like lineage (84.8%). Its clonal expansion has been one of the most spectacular phenomena in epidemiology of VIM CPE in Poland so far.

Originally recorded in 2009, subsequently, the *K. oxytoca* ST145-In237-like genotype has spread first in three provinces, mainly the central Mazowieckie with Warsaw [11], and then almost all over the country. It has had several specific characteristics, rarely or not observed in other Polish VIM CPE. The In237-like integron is a member of the In238 type, multiple variants of which have been identified in Enterobacterales and other Gram-negative rods in Poland and mid-Southern Europe since the 1990/2000s [11, 27, 28, 31, 32]. This individual element had been found originally in *Escherichia coli* from 2001 in Greece, the first VIM CPE ever reported [27], and in Poland, it has been recorded since 2009 mainly in the *K. oxytoca* ST145 outbreak lineage so far [11, 12, 32]. The second distinct feature of the genotype has been the location of the integron with its Tn*21*-like transposon inside the chromosomal MR linked to the putative phage, 'Phage 1'. 'Phage 1' has been common in ST145 genomes, including those having no MRs with In237-like integrons, and both the scenario and mechanisms of acquisition of these individual elements remain unclear. Moreover, apart from the In237-like cassettes, the MR carried up to nine other AMR genes, with the uncommon bla_{CMY-31}, conferring multidrug resistance (MDR) altogether. This single chromosomal island was the major or even the only AMR source in the outbreak isolates which is rare in Enterobacterales, exploiting plasmids as main AMR genetic platforms. Overall, the isolates showed similarity to VIM-1 + CMY-31-producing *K. oxytoca* isolates from community-onset infections in Greece from 2005 to 2007, including the identical bla_{OXY} allele [33]. This indicated the likely origin of the ST145-In237-like lineage; however, the Greek strains have not been typed by MLST, and their genomes seem to have not been sequenced or available.

The other interesting observation regarding the epidemic ST145-In237-like genotype referred to the frequent chromosomal deletions containing bla_{OXY} genes, observed in 30.8% of the outbreak isolates. The bla_{OXY} genes encode intrinsic KoSC-specific β-lactamases, estimated to have evolved along with individual phylogenetic lineages over approximately 100 million years [25, 26]. The bla_{OXY} deletions have been found only in three other KoSC genomes deposited in the international databases, including one ST145 from China. Therefore, it is possible that these might have been occurring preferentially in the ST145 genetic background, which is supported also by multiplicity of independent deletion events in the study isolates. The detailed structural analysis of the index bla_{OXY}-negative ST145-In237-like isolate suggested that the deletion could have been caused by the other putative phage ('Phage 2'), inserted in the neighbourhood of the 'Phage 1'–MR (In237-like) locus, and that there might have been an association between various rearrangements in this peculiar part of the genome. However, the lack of 'Phage 2' in other bla_{OXY}-negative isolates excluded the hypothesis of a single deletion mechanism and has left the phenomenon of the repetitive deletions without explanation.

The second, though less important factor of the bla_{VIM} genes' spread in the Polish KoSC population were In916-carrying IncA-type plasmids, observed in four non-outbreak ST145 isolates and nine isolates of various species/STs, documenting horizontal transmission. In contrast to other European countries where In916 has been associated with several plasmid incompatibility groups such as IncA, $IncFII_K$, IncHI2 or IncN [34–36], in Poland, the integron has been found exclusively on IncA plasmids so far. These have been of high identity rates to those analysed in Italy [12, 29], indicating the actual origin and large-scale, successful expansion of these molecules among Enterobacterales in Europe.

In contrast to KpSC, the knowledge on KoSC virulence factors and their genetic determinants has been scarce so far. Reports on those have been usually based on homologous sequences, being extrapolations of the KpSC data, and non-including evidence from functional studies [24, 37]. Similarly, the study isolates have been checked only for the presence of several putative virulence determinants. Like in other recent studies [24, 38], the CPS K-antigen locus was of low incidence (~7.3%), in contrast to the LPS O-antigen biosynthesis locus (~97.2%) and the siderophore yersiniabactin *ybt* loci (88.7%). However, despite the high nucleotide sequence identity to the KpSC *ybt* loci, those in KoSC had different genetic context and no identifiable mobility-associated genes [39]. The gene cluster of the best-known KoSC-specific virulence factor, i.e. the toxin kleboxymycin, involved in the antibiotic-associated haemorrhagic colitis (AAHC) [40–42], was recorded in ~42% of the isolates. These were mainly *K. oxytoca* but also *K. michiganensis*, *K. grimontii* and *K. pasteurii*, and similar to the observations of Long et al., the cluster was absent in *K. spallanzanii*, possibly not causing the AAHC [38].

Although limited to the single country, this work has provided a remarkable amount of broader scale relevant data on epidemiology and genetics of KoSC, especially *K. oxytoca*, which has rarely been an object of specific studies. *K. oxytoca* has been a significant opportunistic pathogen and reservoir of AMR genes, and in Polish hospitals, it has been one of the most important producers of VIM-type carbapenemases. The critical part of our study concerned the *K. oxytoca* ST145-In237-like outbreak lineage of the most likely Greek origin, which since 2009 has been disseminating over the almost entire Poland's territory. The detailed genomic analysis has revealed a number of specific characteristics of the organism, including the chromosomal MDR island carrying the In237-like integron and the bla_{CMY-31} cephalosporinase gene. The epidemic potential of the lineage creates a risk of its further expansion into other regions in Europe.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-023-04682-x.

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Data availability Genomic sequences have been deposited in the NCBI under the BioProject number PRJNA983967, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA983967, and BioSample numbers SAMN35742457-35,742,562. Plasmid sequences have been assigned the following accession numbers: p253A, OR232699 and p2990, OR232700.

Code availability Not applicable.

Declarations

Ethics approval The isolates used in the current study were obtained during a routine national surveillance activity of the National Reference Centre for Susceptibility Testing, under the mandate of the Ministry of Health. The study was performed in a retrospective manner with an anonymisation of patients' data; thus, ethical approval and informed consent were not required.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Lee CS, Doi Y (2014) Therapy of infections due to carbapenem-resistant Gram-negative pathogens. Infect Chemother 46(3):149–164
2. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL (2012) Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev 25(4):682–707

3. Walsh TR, Toleman MA, Poirel L, Nordmann P (2005) Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev 18(2):306–325
4. Psichogiou M, Tassios PT, Avlamis A, Stefanou I, Kosmidis C, Platsouka E, Paniara O, Xanthaki A, Toutouza M, Daikos GL, Tzouvelekis LS (2008) Ongoing epidemic of *bla*VIM-1-positive *Klebsiella pneumoniae* in Athens, Greece: a prospective survey. J Antimicrob Chemother 61(1):59–63
5. Miriagou V, Douzinas EE, Papagiannitsis CC, Piperaki E, Legakis NJ, Tzouvelekis LS (2008) Emergence of *Serratia* liquefaciens and *Klebsiella oxytoca* with metallo-β-lactamase-encoding IncW plasmids: further spread of the bla_{VIM-1}-carrying integron In-e541. Int J Antimicrob Agents 32(6):540–541
6. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P (2012) Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect 18(5):413–431
7. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL (2015) European survey of carbapenemase-producing *Enterobacteriaceae* working g (2015) carbapenemase-producing *Enterobacteriaceae* in Europe: assessment by national experts from 38 countries. Euro Surveill 20(45):pii=30062. https://doi.org/10.2807/1560-7917.ES.2015.20.45.30062
8. Nordmann P, Poirel L (2014) The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect 20(9):821–830
9. Oteo J, Ortega A, Bartolome R, Bou G, Conejo C, Fernandez-Martinez M, Gonzalez-Lopez JJ, Martinez-Garcia L, Martinez-Martinez L, Merino M, Miro E, Mora M, Navarro F, Oliver A, Pascual A, Rodriguez-Bano J, Ruiz-Carrascoso G, Ruiz-Garbajosa P, Zamorano L, Bautista V, Perez-Vazquez M, Campos J (2015) Prospective multicenter study of carbapenemase-producing *Enterobacteriaceae* from 83 hospitals in Spain reveals high in vitro susceptibility to colistin and meropenem. Antimicrob Agents Chemother 59(6):3406–3412
10. Sekowska A, Gospodarek E, Kruszynska E, Hryniewicz W, Gniadkowski M, Duljasz W, Kusza K, Wawrzyniak K (2010) First isolation of metallo-β-lactamase producing *Klebsiella pneumoniae* strain in Poland. Anestezjol Intens Ter 42(1):27–30
11. Izdebski R, Baraniak A, Żabicka D, Sękowska A, Gospodarek E, Hryniewicz W, Gniadkowski M (2018) VIM/IMP carbapenemase-producing *Enterobacteriaceae* in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother 73(10):2675–2681
12. Izdebski R, Biedrzycka M, Urbanowicz P, Żabicka D, Gniadkowski M (2023) Genome-based epidemiologic analysis of VIM/IMP carbapenemase-producing *Enterobacter* spp., Poland. Emerg Infect Dis 29(8):1618–1626
13. Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 18(9):1503–1507
14. Baraniak A, Machulska M, Zabicka D, Literacka E, Izdebski R, Urbanowicz P, Bojarska K, Herda M, Kozinska A, Hryniewicz W, Gniadkowski M, Group N-PS (2019) Towards endemicity: large-scale expansion of the NDM-1-producing *Klebsiella pneumoniae* ST11 lineage in Poland, 2015–16. J Antimicrob Chemother 74(11):3199–3204
15. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19(5):455–477
16. Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13(6):e1005595
17. Jain C, Rodriguez RL, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9(1):5114. https://doi.org/10.1038/s41467-018-07641-9
18. Darling AC, Mau B, Blattner FR, Perna NT (2004) Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14(7):1394–1403
19. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS (2016) PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44(W1):W16-21
20. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H (2014) In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58(7):3895–3903
21. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67(11):2640–2644
22. Wick RR, Heinz E, Holt KE, Wyres KL (2018) Kaptive Web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. J Clin Microbiol 56(6):e00197-e218
23. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE (2021) A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. Nat Commun 12(1):4188. https://doi.org/10.1038/s41467-021-24448-3
24. Stewart J, Judd LM, Jenney A, Holt KE, Wyres KL, Hawkey J (2022) Epidemiology and genomic analysis of *Klebsiella oxytoca* from a single hospital network in Australia. BMC Infect Dis 22(1):704. https://doi.org/10.1186/s12879-022-07687-7
25. Fevre C, Jbel M, Passet V, Weill FX, Grimont PA, Brisse S (2005) Six groups of the OXY β-lactamase evolved over millions of years in *Klebsiella oxytoca*. Antimicrob Agents Chemother 49(8):3453–3462
26. Yang J, Long H, Hu Y, Feng Y, McNally A, Zong Z (2022) *Klebsiella oxytoca* complex: update on taxonomy, antimicrobial resistance, and virulence. Clin Microbiol Rev 35(1):e0000621
27. Scoulica EV, Neonakis IK, Gikas AI, Tselentis YJ (2004) Spread of bla_{VIM-1}-producing *E. coli* in a university hospital in Greece. Genetic analysis of the integron carrying the bla_{VIM-1} metallo-β-lactamase gene. Diagn Microbiol Infect Dis 48(3):167–172
28. Patzer J, Toleman MA, Deshpande LM, Kaminska W, Dzierzanowska D, Bennett PM, Jones RN, Walsh TR (2004) *Pseudomonas aeruginosa* strains harbouring an unusual *bla*VIM-4 gene cassette isolated from hospitalized children in Poland (1998–2001). J Antimicrob Chemother 53(3):451–456
29. Biedrzycka M, Izdebski R, Urbanowicz P, Polanska M, Hryniewicz W, Gniadkowski M, Literacka E (2022) MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009–19. J Antimicrob Chemother 77(12):3367–3375
30. Arcari G, Di Lella FM, Bibbolino G, Mengoni F, Beccaccioli M, Antonelli G, Faino L, Carattoli A (2020) A multispecies cluster of VIM-1 carbapenemase-producing Enterobacterales linked by a novel, highly conjugative, and broad-host-range IncA plasmid forebodes the reemergence of VIM-1. Antimicrob Agents Chemother 64(4):e02435-e2519
31. Libisch B, Gacs M, Csiszar K, Muzslay M, Rokusz L, Fuzi M (2004) Isolation of an integron-borne blaVIM-4 type metallo-β-lactamase gene from a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate in Hungary. Antimicrob Agents Chemother 48(9):3576–3578

32. Urbanowicz P, Izdebski R, Baraniak A, Zabicka D, Hryniewicz W, Gniadkowski M (2021) Molecular and genomic epidemiology of VIM/IMP-like metallo-β-lactamase-producing *Pseudomonas aeruginosa* genotypes in Poland. J Antimicrob Chemother 76(9):2273–2284
33. Tsakris A, Poulou A, Markou F, Pitiriga V, Piperaki ET, Kristo I, Pournaras S (2011) Dissemination of clinical isolates of *Klebsiella oxytoca* harboring CMY-31, VIM-1, and a new OXY-2-type variant in the community. Antimicrob Agents Chemother 55(7):3164–3168
34. Arcari G, Raponi G, Sacco F, Bibbolino G, Di Lella FM, Alessandri F, Coletti M, Trancassini M, Deales A, Pugliese F, Antonelli G, Carattoli A (2021) *Klebsiella pneumoniae* infections in COVID-19 patients: a 2-month retrospective analysis in an Italian hospital. Int J Antimicrob Agents 57(1):106245
35. Emeraud C, Petit C, Gauthier L, Bonnin RA, Naas T, Dortet L (2022) Emergence of VIM-producing *Enterobacter cloacae* complex in France between 2015 and 2018. J Antimicrob Chemother 77(4):944–951
36. Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabak J, Derde LP, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, Mosar Wp WP, groups WPs, Mosar Wp WP, groups WPs, (2015) Survey of metallo-β-lactamase-producing *Enterobacteriaceae* colonizing patients in European ICUs and rehabilitation units, 2008–11. J Antimicrob Chemother 70(7):1981–1988
37. Moradigaravand D, Martin V, Peacock SJ, Parkhill J (2017) Population structure of multidrug resistant *Klebsiella oxytoca* within hospitals across the UK and Ireland identifies sharing of virulence and resistance genes with *K. pneumoniae*. Genome Biol Evol 9(3):574–587
38. Long H, Hu Y, Feng Y, Zong Z (2022) Genome analysis of *Klebsiella oxytoca* complex for antimicrobial resistance and virulence genes. Antimicrob Agents Chemother 66(3):e0218321
39. Lam MMC, Wick RR, Wyres KL, Gorrie CL, Judd LM, Jenney AWJ, Brisse S, Holt KE (2018) Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICE*Kp* in *Klebsiella pneumoniae* populations. Microb Genom 4(9):e000196
40. Tse H, Gu Q, Sze KH, Chu IK, Kao RY, Lee KC, Lam CW, Yang D, Tai SS, Ke Y, Chan E, Chan WM, Dai J, Leung SP, Leung SY, Yuen KY (2017) A tricyclic pyrrolobenzodiazepine produced by *Klebsiella oxytoca* is associated with cytotoxicity in antibiotic-associated hemorrhagic colitis. J Biol Chem 292(47):19503–19520
41. Dornisch E, Pletz J, Glabonjat RA, Martin F, Lembacher-Fadum C, Neger M, Hogenauer C, Francesconi K, Kroutil W, Zangger K, Breinbauer R, Zechner EL (2017) Biosynthesis of the enterotoxic pyrrolobenzodiazepine natural product tilivalline. Angew Chem 56(46):14753–14757
42. Hogenauer C, Langner C, Beubler E, Lippe IT, Schicho R, Gorkiewicz G, Krause R, Gerstgrasser N, Krejs GJ, Hinterleitner TA (2006) *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. N Engl J Med 355(23):2418–2426

Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009-2019

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Table S1. Taxonomy, clonality, *bla*$_{OXY}$ gene variants and acquired AMR genes in the study VIM-producing KoSC isolates, 2009-2019

Species	Isolate[a]	*bla*$_{OXY}$	ST[b]	*bla*$_{VIM}$-carrying integron[c]	hospital	acquired AMR genes									
						β-lactams[d]	aminoglycosides	colistin	fluoroquinolones	macrolide	phenicol	rifampicin	sulphonamide	tetracycline	trimethoprim
K. oxytoca	**776/09**	-	145	In237-like	Warsaw I	***bla*$_{VIM-1}$**, *bla*$_{CTX-M-3}$, ***bla*$_{CMY-31}$**, *bla*$_{TEM-1}$	*aac(3)-IId*, ***aacA4, aph(3')-Ia, strA, strB, aadA1***, *aadA2, armA*	-	-	*msr(E)*	***catA1***	-	***sul1*(x2)**, *sul1*	-	***dfrA1***, *dfrA12*
	2092/09	2-22	145	In237-like	Radom I	***bla*$_{VIM-1}$**, ***bla*$_{CMY-31}$**	***aacA4, aph(3')-Ia, strA, strB, aadA1***	-	-	-	-	-	***sul1*(x2)**	-	***dfrA1***
	2168/09	-	145	In237-like	Warsaw VII	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$, *bla*$_{TEM-1}$	*aac(3)-IId, aacA4, aph(3')-Ia, strA, strB, armA, aadA1, aadA2-like*	-	-	*mph(E), msr(E)*	-	-	*sul1*	-	*dfrA12*
	2833/09	2-22	145	In237-like	Ziel. Góra	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	-
	1674/10	2-22	145	In237-like	Nowa Sól	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA strB, armA, aadA1, aadA2*	-	-	*msr(E), mph(E)*	-	-	*sul1*	-	*dfrA1, dfrA12*
	3809/10	2-22	145	In237-like	Warsaw XIII	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1,*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	4204/10	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, ***bla*$_{CMY-177}$**, *bla*$_{DHA-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	*qnrB4*	-	*catA1*	*arr-3*	*sul1*	-	*dfrA1, dfrA27*
	5944/10	2-22	145	In237-like	Mińsk Maz.	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6421/10	-	145	In237-like	Lublin III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	377/11	2-22	145	In237-like	Otwock	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	445/11	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	1095/11	-	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{DHA-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	*qnrB4*	-	*catA1*	*arr-3*	*sul1*	-	*dfrA1, dfrA27*
	1765/11	-	145	In237-like	Warsaw VII	*bla*$_{VIM-1}$, *bla*$_{CTX-M-15}$,*bla*$_{OXA-1}$	*aac(3')-IIa, aacA4, aadA1*	-	*qnrB1*	-	*catA1*	-	*sul1*	*tet(A)*	*dfrA14*
	5190/11	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	9347/11	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	588/12	2-22	145	In237-like	Kostrzyn n.O.	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	3934/12	2-22	145	In237-like	Ziel. Góra	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	3936/12	2-22	145	In237-like	Ziel. Góra	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	6679/12	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{OXA-9}$, *bla*$_{OXA-10}$, *bla*$_{TEM-29}$	*aacA4, strA, strB*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	294/13	-	145	In237-like	Poznań II	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	1544/13	-	145	In237-like	Ziel. Góra	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB,aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	4464/13	-	145	In237-like	Katowice	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	4806/13	2-22	145	In237-like	Warsaw I	*bla*$_{VIM-1}$	*aacA4*	-	-	-	-	-	*sul1*	-	-
	124/14	-	145	In237-like	Katowice	*bla*$_{VIM-1}$,*bla*$_{CTX-M-3}$,*bla*$_{CMY-31}$, *bla*$_{TEM-1}$, *bla*$_{OXA-1}$	*aac(3)-IId, aacA4, aph(3')-Ia, armA, aadA1*	-	-	*msr(E), mph(E)*	*catA1, catB3*	*arr-3*	*sul1*	-	*dfrA1*
	626/14	2-22	145	In237-like	Osrtołęka I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	815/14	-	145	In237-like	Wrocław III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{CARB-2}$	*aacA4, aph(3')-Ia, strA, aphA6, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	972/14	-	145	In237-like	Wrocław III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{CARB-2}$	*aacA4,aph(3')-Ia, strA, aphA6, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	1362/14	2-22	145	In237-like	Kielce	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1, sul2*	-	*dfrA1*
	3325/14	-	145	In237-like	Sadowne	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	85/15	-	145	In237-like	Katowice	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	601/15	2-22	145	In237-like	Warsaw XV	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	1879/15	2-22	145	In237-like	Ostrołęka I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	2641/15	-	145	In237-like	Żary	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{OXA-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	*mph(A)*	*catB3*	*arr-3*	*sul1*	*tet(A)*	*dfrA1*
	4116/15	-	145	In237-like	Poznań II	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	-
	4566/15	2-22	145	In237-like	Warsaw IX	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	347/16	2-22	145	In237-like	Warsaw V	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA* (x2)*, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	723/16	2-22	145	In237-like	Grodzisk Maz.	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	-
	1231/16	-	145	In237-like	Grudziądz	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$, *bla*$_{TEM-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1, armA*	-	-	*msr(E), mph(E)*	*catA1, catB3*	-	*sul1*	-	*dfrA1*
	2260/16	2-22	145	In237-like	Warsaw III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	2579/16	-	145	In237-like	Warsaw III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	2672/16	-	145	In237-like	Cieszyn	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	4025/16	2-22	145	In237-like	Warsaw VII	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{OXA-1}$	*acc(3)-IIa, aacA4, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	5491/16	2-22	145	In237-like	Warsaw IX	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$, *bla*$_{TEM-1}$	*aac(3)-IId, aacA4, aph(3')-Ia, strA, strB, aadA1*	-	*qnrB19*	-	*catA1*	-	*sul1*	-	*dfrA1*
	6209/16	2-22	145	In237-like	Rudka	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, adA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6428/16	-	145	In237-like	Wołomin	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6695/16	2-22	145	In237-like	Przeworsk	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-170}$, *bla*$_{TEM-1}$	*aac(3)-IId, aacA4*	-	-	-	-	-	*sul1*	-	-
	7212/16	2-22	145	In237-like	Grodzisk Maz.	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	196/17	2-22	145	In237-like	Wrocław IV	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$, *bla*$_{TEM-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	460/17	2-22	145	In237-like	Białystok I	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	905/17	2-22	145	In237-like	Warsaw VII	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	1323/17	-	145	In237-like	Szczecin	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	3362/17	-	145	In237-like	Zamość	*bla*$_{VIM-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CMY-31}$, *bla*$_{TEM-1}$	*aac(3)-IId, aacA4, aph(3')-Ia, strA, strB, armA, aadA1, aadA2*	-	-	*mph(E), msr(E)*	*catA1*	-	*sul1*	-	*dfrA1, dfrA12*
	4062/17	2-22	145	In237-like	Warsaw III	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{OXA-1}$	*aacA4, acc(3)-IIa, aph(3')-Ia, aadA1*	-	*qnrB1, qnrS2*	-	*catA1*	-	*sul1*	*tet(A)*	*dfrA1, dfrA14*
	4759/17	2-22	145	In237-like	Wolomin	*bla*$_{VIM-1}$, *bla*$_{CMY-31}$, *bla*$_{OXA-1}$	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*

	4793/17	2-22	145	In237-like	Warsaw I	*bla*VIM-1, *bla*KPC-2, *bla*TEM-1	*aacA4*	-	-	-	*catA1*	-	*sul1*	-	-
	4794/17	2-22	145	In237-like	Warsaw I	*bla*VIM-1, *bla*KPC-2, *bla*TEM-1	*aacA4*	-	-	-	*catA1*	-	*sul1*	-	-
	5138/17	2-22	145	In237-like	Majdan	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	5947/17	2-22	145	In237-like	Limanowa	*bla*VIM-1, *bla*CTX-M-3, *bla*CMY-31, *bla*TEM-1	*aacA4, aac(3)-IId, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6676/17	2-22	145	In237-like	Warsaw IV	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6751/17	2-22	145	In237-like	Ziel. Góra	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	939/18	-	145	In237-like	Warsaw XVI	*bla*VIM-1, *bla*CMY-31	*aacA4, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	2383/18	2-22	145	In237-like	Opole	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	2437/18	2-22	145	In237-like	Piotrków Tryb.	*bla*VIM-1, *bla*CMY-31, *bla*OXA-1	*acc(3)-IIa, aacA4, aph(3')-Ia*	-	*qnrB1*	-	*catA1*	-	*sul1*	*tet(A)*	*dfrA1, dfrA14*
	5438/18	2-22	145	In237-like	Radom II	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	5439/18	2-22	145	In237-like	Radom II	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6107/18	2-22	145	In237-like	Ostrołęka II	*bla*VIM-1, *bla*CMY-31	*aacA43, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	7009/18	2-22	145	In237-like	Radom II	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	7702/18	2-22	145	In237-like	Warsaw VI	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	7904/18	2-22	145	In237-like	Radom I	*bla*VIM-1, *bla*CMY-31, *bla*OXA-1	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	*qnrB1*	-	-	-	*sul1*	*tet(A)*	*dfrA1, dfrA14*
	8748/18	2-22	145	In237-like	Radom I	*bla*VIM-1, *bla*CMY-31, *bla*OXA-1	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	*qnrB1*	-	-	-	*sul1*	*tet(A)*	*dfrA1, dfrA14*
	221/19	2-22	145	In237-like	Radom I	*bla*VIM-1, *bla*CTX-M-15, *bla*CMY-31, *bla*OXA-1, *bla*TEM-206	*aacA4, aph(3')-Ia, strB, aadA1*	-	*qnrB1*	-	-	-	*sul1, sul2*	*tet(A)*	*dfrA1, dfrA14*
	2990/17	2-22	145	In238	Białystok II	*bla*VIM-4, ***bla*CTX-M-251**, *bla*OXA-1, *bla*TEM-1	*aac(3)-IId, aacA4, aac(6')-Ib-cr, aadA5*	-	*qnrS2*	*mph(A)*	*catB3*	*arr-3*	*sul1(3)*	*tet(B)*	*dfrA17*
	1636/19	2-22	145	In237-like	Warsaw XIV	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	2226/19	2-22	145	In237-like	Ziel. Góra	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	-	-	*sul1*	-	*dfrA1*
	3634/19	-	145	In237-like	Katowice	*bla*VIM-1, *bla*CMY-31	*aac(6')-b, aph(3')-Ia, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	6337/19	2-22	145	In237-like	Radom II	*bla*VIM-1, *bla*CTX-M-15, *bla*OXA-1, *bla*TEM-206	*aac(3a)-IIa, aacA4, strA, strB*	-	*qnrB1*	-	-	-	*sul1, sul2*	*tet(A)*	*dfrA14*
	6955/19	2-22	145	In237-like	Warsaw VIII	*bla*VIM-1, *bla*CMY-31	*aacA4, aph(3')-Ia, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	7512/19	2-22	145	In237-like	Warsaw X	*bla*VIM-1, *bla*CMY-31	*aacA4, strA, strB, aadA1*	-	-	-	*catA1*	-	*sul1*	-	*dfrA1*
	8138/19	-	145	In237-like	Radom I	*bla*VIM-1, *bla*CTX-M-15, *bla*CMY-31, *bla*TEM-206	*aacA4, aph(3')-Ia, strB, aadA1*	-	*qnrB1*	-	-	-	*sul1, sul2*	*tet(A)*	*dfrA1, dfrA14*
	253/19	2-22	145	In916	Wrocław IV	*bla*VIM-1	*aacA4, aphA15, aadA1*	-	-	-	*catB2*	-	*sul1*	-	-
	768/19	2-22	145	In916	Wrocław IV	*bla*VIM-1	*aacA4, aphA15, aadA1*	-	-	-	*catB2*	-	*sul1*	-	-
	8015/19	2-22	145	In916	Wrocław I	*bla*VIM-1	*aacA4, aphA15, aadA5*	-	-	*mph(A)*	*catB2*	-	*sul1*	*tet(B)*	*dfrA17*
	10246/19	2-22	145	In916	Wrocław II	*bla*VIM-1	*aacA4 , aphA15, aadA5*	-	*qnrS1*	*mph(A)*	*catA1, catB2*	-	*sul1*	*tet(B)*	*dfrA17*
	1536/13	2-16	2	**In238-79**	Lublin I	***bla*VIM-79**, *bla*SHV-5	*aacA43, aadA1*	-	-	-	-	-	*sul1*	*tet(A)*	*dfrA1*
	7406/19	2-11	36	In916	Łodź II	*bla*VIM-1, *bla*SHV-12	*aacA4, strA, aphA15, strB, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14*
	10316/19	2-12	37	In71-like	Łódź I	*bla*VIM-1, *bla*SHV-12	*aacA4, strA, aphA15, strB*	-	*qnrS1*	*mph(A)*	-	-	*sul1,sul2*	-	*dfrA14*
	5820/12	2-20	282	In238	Lublin I	*bla*VIM-4, *bla*SHV-5	*aacA4, strA, strB, sat2*	-	-	-	-	-	*sul1, sul2*	*tet(A)*	*dfrA1*
	2978/13	2-12	**346**	In916	Gdańsk I	*bla*VIM-1, *bla*SHV-12	*aacA4, aphA15, strA, strB, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14*
	5636/16	2-2	**348**	In916	Gdańsk I	*bla*VIM-1, *bla*SHV-12	*aacA4, aphA15, strA, strB, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14*
	8275/17	2-6	285	In916	Warsaw XII	*bla*VIM-1	*aacA4, strA, aphA15, strB, aadA1*	-	*qnrS1*	-	*catB2*	-	*sul1, sul2*	-	-
	388/18	2-2	**347**	In916	Cracow	*bla*VIM-1, *bla*SHV-12	*aacA4, aphA15, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1*	-	*dfrA14*
	10340/19	2-10	**345**	In238	Lublin II	*bla*VIM-4, *bla*CTX-M-15, *bla*TEM-1	*aac(3)-IId, aacA4*	-	*qnrS1*	-	*catA2*	-	*sul1*	-	-
K. michiganensis	5810/12	1-1	146	In238a	Warsaw II	*bla*VIM-4, *bla*CTX-M-3	*aacA4, aph(3')-Ia*	-	-	-	-	-	*sul1*	*tet(A)*	-
	6620/12	1-1	146	In238a	Warsaw XI	*bla*VIM-4, *bla*CTX-M-3	*aacA4*	-	-	-	-	-	*sul1*	*tet(A)*	-
	1752/13	5-6	310	In41-like	Poznań I	*bla*VIM-2	*aacA4*	-	-	-	-	-	*sul1*	-	-
	3664/13	5-6	310	In41-like	Kościan	*bla*VIM-2	*aacA4*	-	-	-	-	-	*sul1*	-	-
	3524/15	1-14	213	In916	Gdańsk I	*bla*VIM-1, *bla*CTX-M-3, *bla*SHV-12	*aacA4, aph(3')-Ia, strA, aphA15, strB, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14*
	1085/17	5-1	95	In237-like	Warsaw I	*bla*VIM-1, *bla*OXA-1	*aacA4*	-	*qnrS2*	*mph(A)*	*catB3*	*arr-3*	*sul1*	-	-
	1086/17	5-1	95	In237-like	Warsaw I	*bla*VIM-1, *bla*OXA-1	*aacA4*	-	*qnrS2*	*mph(A)*	*catB3*	*arr-3*	*sul1*	-	-
	10279/19	5-1	95	In237-like	Warsaw I	*bla*VIM-1 *bla*OXA-1	*aacA4*	-	*qnrS2*	-	*catB3*	*arr-3*	*sul1*	-	-
	4445/19	5-9	210	In238	Olecko	*bla*VIM-4, *bla*LAP-2	*aacA4*	-	*qnrS1, qnrB19*	-	-	-	*sul1*	*tet(A)*	*dfrA1*
	8545/19	1-13	180	In916	Elbląg	*bla*VIM-1, *bla*SHV-12, *bla*GES-7	*aph(3')-Ia, strA, aphA15, strB, aadA1*	-	*qnrS2*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14, dfrB3*
	9543/19	1-13	180	In916	Elbląg	*bla*VIM-1, *bla*SHV-12, *bla*GES-7	*aph(3')-Ia, strA, aphA15, strB, aadA1*	-	*qnrS1, qnrS2*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14, dfrB3*
K. grimontii	5535/16	6-5	172	In110	Ciechanów	*bla*VIM-1, *bla*ACC-1 *bla*LAP-2, *bla*OXA-10	*aacA4, aadA1, strA, strB*	*mcr-9.1*	*qnrS1*	-	*catA1, cmlA5*	*arr-2*	*sul1*	-	*dfrA14*
K. pasteurii	6490/18	4-3	229	In238a	Wrocław II	*bla*VIM-4	*aacA4*	-	-	-	-	-	*sul1*	-	-
K. spallanzanii	7090/18	3-3	NA[e]	In916	Poznań I	*bla*VIM-1, *bla*SHV-12	*aacA4, aphA15, strB, aac(3'')-Ib, aadA1*	-	*qnrS1*	*mph(A)*	*catB2*	-	*sul1, sul2*	-	*dfrA14*

[a] – isolates selected to long-read sequencing are indicated in bold; total numbers of acquired AMR genes in these isolates are based on long-read sequencing; AMR genes indicated in bold are located within the chromosomal AMR islands; [b] – new STs are indicated in bold; [c] – new *bla*VIM-carrying integron variant is indicated in bold; [d] – new *bla*CMY, *bla*CTX-M and *bla*VIM genes are indicated in bold; nucleotide sequences of new genes are available under the following GenBank accession numbers: *bla*CMY-177, OK217282; *bla*CTX-M-251, OK217281; *bla*VIM-79, OK217280.

[e] – NA, non-applicable; *K. spallanzanii* is not included in the MLST scheme.

Table S2. VIM-encoding class 1 integrons identified in the KoSC study isolates

Integron type	Integron variant[a]	Gene cassette array	Species and STs	Country, year and species of the first identification[b, c]	GenBank entry
with *bla*$_{VIM-1}$-like genes					
In238 (n=89)	In237-like[d] (n=81)	5'CS_*aacA4*_*bla*$_{VIM-1rpt}$_3'CS	*K. oxytoca* ST145 *K. michiganensis* ST95	Greece, 2001, *E. coli* Greece, 2005, *A. baumanii* Poland, 2009, *K. oxytoca*	AY152821 EF690695
	In238-79[e] (n=1)	5'CS_*aacA4*_*bla*$_{VIM-79}$_3'CS	*K. oxytoca* ST2	Poland, 2013, *K. oxytoca*	OR258282
	In238 (n=5)	5'CS_*aacA4*_*bla*$_{VIM-4rpt}$_3'CS	*K. oxytoca* ST145, ST282, ST345 *K. michiganensis* ST146, ST210	Poland, 1998, *P. aeruginosa* Poland, 2008, *K. pneumoniae* Poland, 2012, *K. michiganensis*	AJ585042/AY702100[f]
	In238a[g] (n=2)	5'CS_*aacA4*_*bla*$_{VIM-4}$_3'CS	*K. michiganensis* ST146 *K. pasteurii* ST229	Poland, 2009, *E. hormaechei* Poland, 2012, *K. michiganensis*	JQ003906 (Hungary 2010)
In916 (n=13)	In916 (n=13)	5'CS_*bla*$_{VIM-1}$_*aacA4*_*aphA15*_*aadA1*_*catB2*_3'CS	*K. oxytoca* ST36, ST145, ST285, ST346, ST347, ST348 *K. michiganensis* ST180, ST231 *K. spallanzanii* ND	Spain, before 2014, *E. coli* Poland, 2013, *E. coli* & *C. freundii* Poland, 2013, *K. oxytoca*	KF856617
In70 (n=1)	In71-like[h]	5'CS_*bla*$_{VIM-1}$_*aacA4*_*aphA15*_3'CS	*K. oxytoca* ST137	Italy, 2016, *E. cloacae* Poland, 2019, *K. oxytoca*	AM183120
In110 (n=1)	In110	5'CS_*bla*$_{VIM-1}$_*aacA4*_*aadA1*_3'CS	*K. grimontii* ST172	Italy, 1999, *P. putida* Poland, 2006, *P. aeruginosa* Poland, 2016, *E. hormaechei* & *K. grimontii*	AJ439689
with *bla*$_{VIM-2}$-like genes					
In41 (n=2)	In41-like[i] (n=2)	5'CS_*bla*$_{VIM-2}$_*aacA4*_3'CS	*K. michiganensis* ST310	Argentina, 2014, *P.putida* Poland, 2013, *K. michiganensis*	KF840720

[a] – the new integron is indicated in bold.
[b] – when the first report was from another country, then it is followed by the first Polish case(s); if the first Polish record was from non-Enterobacterales and/or non-KoSC, it is then followed by the first Polish Enterobacterales and KoSC, respectively.
[c] – date of isolation of the first Polish organism with a given integron may be earlier than that of the first isolate reported ever in another country.
[d] – the In237-like GenBank entry EF690695 of an *A. baumannii* isolate stands for In237 in the INTEGRALL database (www.integrall.bio.ua.pt); the enterobacterial In237-like integrons from Greece AY152821 and Poland differ from that by two SNPs in the *bla*$_{VIM}$ 59-be element.
[e] – In238-79 differs from In238 by one point mutation converting *bla*$_{VIM-4}$ to *bla*$_{VIM-79}$; due to temporary suspended activity of the INTEGRALL database In238-79 has not been numbered according to that.
[f] – the original In238 record (AJ585042) contains a 2 nt error in the *bla*$_{VIM-4}$ coding sequence; the subsequent *P. aeruginosa* In238 entry from Hungary from 2003 has been provided.
[g] – In238a differs from the In238 element by having no 3'-terminal 169bp tandem repeat in the *bla*$_{VIM-4}$ gene cassette.
[h] – In71-like differs from the In71 element by *bla*$_{VIM-1}$ gene cassette instead of *bla*$_{VIM-1b}$.
[i] – In41-like differs from the In41 element by having the 3'CS region.

Table S3. SNP scores between *K. oxytoca* ST145 isolates

Isolate	Region	Hospital	Number of SNPs	OXY variant	Integron variant	Remarks
776/09[a]	Mazowieckie	Warsaw I	0	-	In237-like	ST145-In237-like-VIM-1 outbreak
445/11	Mazowieckie	Warsaw I	14	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
5190/11	Mazowieckie	Warsaw I	14	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
601/15	Mazowieckie	Warsaw XV	14	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
5944/10	Mazowieckie	Mińsk Maz.	16	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3809/10	Mazowieckie	Warsaw XIII	18	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1879/15	Mazowieckie	Ostrołęka I	18	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2833/09	Lubuskie	Ziel. Góra	19	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
626/14	Mazowieckie	Ostrołęka I	20	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4204/10	Mazowieckie	Warsaw I	21	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1674/10	Lubuskie	Nowa Sól	23	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4794/17	Mazowieckie	Warsaw I	23	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2092/09	Mazowieckie	Radom I	26	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3325/14	Mazowieckie	Sadowne	27	-	In237-like	ST145-In237-like-VIM-1 outbreak
905/17	Mazowieckie	Warsaw VII	28	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
377/11	Mazowieckie	Otwock	29	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1765/11	Mazowieckie	Warsaw VII	30	-	In237-like	ST145-In237-like-VIM-1 outbreak
4025/16	Mazowieckie	Warsaw VII	30	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2168/09	Mazowieckie	Warsaw VII	31	-	In237-like	ST145-In237-like-VIM-1 outbreak
5947/17	Małopolskie	Limanowa	31	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
7009/18	Mazowieckie	Radom II	31	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2437/18	Łódzkie	Piotrków Tryb.	32	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6421/10	Lubelskie	Lublin III	33	-	In237-like	ST145-In237-like-VIM-1 outbreak
588/12	Lubuskie	Kostrzyn n.O.	33	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3934/12	Lubuskie	Ziel. Góra	33	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3936/12	Lubuskie	Ziel. Góra	33	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2579/16	Mazowieckie	Warsaw III	33	-	In237-like	ST145-In237-like-VIM-1 outbreak
1095/11	Mazowieckie	Warsaw I	34	-	In237-like	ST145-In237-like-VIM-1 outbreak
723/16	Mazowieckie	Grodzisk Maz.	34	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4566/15	Mazowieckie	Warsaw IX	35	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
5438/18	Mazowieckie	Radom II	35	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6107/18	Mazowieckie	Ostrołęka II	35	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
347/16	Mazowieckie	Warsaw V	36	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1362/14	Świetokrzyskie	Kielce	36	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
294/13	Wielkopolskie	Poznań II	37	-	In237-like	ST145-In237-like-VIM-1 outbreak
9347/11	Mazowieckie	Warsaw I	39	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1544/13	Lubuskie	Ziel. Góra	39	-	In237-like	ST145-In237-like-VIM-1 outbreak
6428/16	Mazowieckie	Wołomin	40	-	In237-like	ST145-In237-like-VIM-1 outbreak
7212/16	Mazowieckie	Grodzisk Maz.	40	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
939/18	Mazowieckie	Warsaw XVI	40	-	In237-like	ST145-In237-like-VIM-1 outbreak
4116/15	Wielkopolskie	Poznań II	41	-	In237-like	ST145-In237-like-VIM-1 outbreak
460/17	Podlaskie	Białystok I	41	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6676/17	Mazowieckie	Warsaw IV	41	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6751/17	Lubuskie	Ziel. Góra	41	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
221/19	Mazowieckie	Radom I	41	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
8138/19	Mazowieckie	Radom I	42	-	In237-like	ST145-In237-like-VIM-1 outbreak
7904/18	Mazowieckie	Radom I	42	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6337/19	Mazowieckie	Radom II	44	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6679/12	Mazowieckie	Warsaw I	45	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4806/13	Mazowieckie	Warsaw I	46	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
8748/18	Mazowieckie	Radom I	46	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
5138/17	Mazowieckie	Majdan	47	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4759/17	Mazowieckie	Wolomin	47	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2383/18	Opolskie	Opole	48	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
5439/18	Mazowieckie	Radom II	49	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
7512/19	Mazowieckie	Warsaw X	50	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4464/13	Śląskie	Katowice	51	-	In237-like	ST145-In237-like-VIM-1 outbreak
85/15	Śląskie	Katowice	52	-	In237-like	ST145-In237-like-VIM-1 outbreak
1636/19	Mazowieckie	Warsaw XIV	52	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
124/14	Śląskie	Katowice	53	-	In237-like	ST145-In237-like-VIM-1 outbreak
2641/15	Lubuskie	Żary	53	-	In237-like	ST145-In237-like-VIM-1 outbreak
7702/18	Mazowieckie	Warsaw VI	53	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6955/19	Mazowieckie	Warsaw VIII	54	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2226/19	Lubuskie	Ziel. Góra	54	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3362/17	Lubelskie	Zamość	55	-	In237-like	ST145-In237-like-VIM-1 outbreak
196/17	Dolnośląskie	Wrocław IV	56	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
4062/17	Mazowieckie	Warsaw III	57	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
815/14	Dolnośląskie	Wrocław III	61	-	In237-like	ST145-In237-like-VIM-1 outbreak
1323/17	Zachodniopomorskie	Szczecin	61	-	In237-like	ST145-In237-like-VIM-1 outbreak
972/14	Dolnośląskie	Wrocław III	62	-	In237-like	ST145-In237-like-VIM-1 outbreak

5491/16	Mazowieckie	Warsaw IX	64	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
1231/16	Kujawsko-Pomorskie	Grudziądz	65	-	In237-like	ST145-In237-like-VIM-1 outbreak
2672/16	Śląskie	Cieszyn	69	-	In237-like	ST145-In237-like-VIM-1 outbreak
6209/16	Mazowieckie	Rudka	69	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
2260/16	Mazowieckie	Warsaw III	69	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
6695/16	Podkarpackie	Przeworsk	80	2-22	In237-like	ST145-In237-like-VIM-1 outbreak
3634/19	Śląskie	Katowice	90	-	In237-like	ST145-In237-like-VIM-1 outbreak
253/19	Dolnośląskie	Wrocław IV	110	2-22	In916	ST145-In916-VIM-1 hospital dissemination
768/19	Dolnośląskie	Wrocław IV	115	2-22	In916	ST145-In916-VIM-1 hospital dissemination
10246/19	Dolnośląskie	Wrocław II	125	2-22	In916	ST145-In916-VIM-1 local dissemination
8015/19	Dolnośląskie	Wrocław I	126	2-22	In916	ST145-In916-VIM-1 local dissemination
2990/17	Podlaskie	Białystok II	125	2-22	In238	ST145-In238-VIM4 single case

[a] – reference isolate, *i.e.* the Poland's index isolate of ST145 as confirmed by the National Reference Centre for Susceptibility Testing.

Table S4. Chromosomal deletions of the *bla*$_{OXY}$ gene regions in *K.oxytoca* ST145 *bla*$_{OXY}$-negative isolates

Isolate	Region	Hospital	Size of the chromosome deletion[a]	Group of isolates with similar size of deletion
2168/09	Mazowieckie	Warsaw VII	~161 kb	A
6421/10	Lubelskie	Lublin III	~161 kb	A
1765/11	Mazowieckie	Warsaw VII	~161 kb	A
294/13	Wielkopolskie	Poznań II	~161 kb	A
1544/13	Lubuskie	Ziel. Góra	~161 kb	A
4116/15	Wielkopolskie	Poznań II	~161 kb	A
2579/16	Mazowieckie	Warsaw III	~161 kb	A
6428/16	Mazowieckie	Wołomin	~161 kb	A
1323/17	Zachodniopomorskie	Szczecin	~159 kb	A
3362/17	Lubelskie	Zamość	~161 kb	A
4464/13	Śląskie	Katowice	~216 kb	B
124/14	Śląskie	Katowice	~216 kb	B
85/15	Śląskie	Katowice	~216 kb	B
2672/16	Śląskie	Cieszyn	~216 kb	B
815/14	Dolnośląskie	Wrocław III	~152 kb	C
972/14	Dolnośląskie	Wrocław III	~152 kb	C
1231/16	Kujawsko-Pomorskie	Grudziądz	~152 kb	C
2641/15	Lubuskie	Żary	~54 kb	single
776/09	Mazowieckie	Warsaw I	82,724 bp[b]	single
8138/19	Mazowieckie	Radom I	~112 kb	single
939/18	Mazowieckie	Warsaw XVI	~123 kb	single
1095/11	Mazowieckie	Warsaw I	~219 kb	single
3325/14	Mazowieckie	Sadowne	~224 kb	single
3634/19	Śląskie	Katowice	~258 kb	single

[a] – sizes of the chromosomal deletions have been estimated by the comparison of individual *bla*$_{OXY}$-negative genomes, to the reference *bla*$_{OXY}$-positive ST145-In237-like isolate NMI2092/09; for all isolates but NMI776/09, the sizes were estimated by the progressive Mauve algorithm, using Geneious Prime v.2022.0.1 (Biomatters, Auckland, New Zealand).

[b] – the precise size of the chromosome deletion in the NMI776/09 isolate was determined by the comparison of long-read sequences.

Table S5. MICs of antimicrobials for the KoSC isolates

Isolates[a]		MIC (mg/L)[b,c]																	
		PIP	TZP[d]	CAZ	FEP	ATM	IPM	MEM	CZA[d]	AZA[d]	I-R[d]	MVB[d]	FDC	AMK	GEN	TOB	CIP	LVX	SXT	CST
776/09	*Kox* ST145	**>32**	**>32**	**>32**	**>16**	**16**	2	2	**>16**	≤0.06	2	2	2	**>32**	**>32**	**>4**	**>8**	**>8**	**>8**	1
2092/09	*Kox* ST145	**>32**	**>32**	**>32**	**8**	**8**	≤1	0.5	**>16**	0.25	1	0.25	0.5	8	2	**>4**	**8**	**4**	**>8**	≤0.5
2833/09	*Kox* ST145	**32**	**>32**	**>32**	**8**	*4*	2	0.5	**>16**	0.25	2	0.25	0.5	**16**	0.5	**>4**	**4**	**2**	**>8**	≤0.5
2990/17	*Kox* ST145	**>32**	**>32**	**16**	*4*	*4*	**8**	*8*	**16**	0.25	**8**	4	0.25	**16**	**>32**	**>4**	**>8**	**>8**	**>8**	≤0.5
3634/19	*Kox* ST145	**>32**	**>32**	**>32**	**16**	**8**	*4*	*4*	**>16**	0.25	**4**	4	0.125	**16**	2	**>4**	**>8**	**>8**	**>8**	≤0.5
5820/12	*Kox* ST282	**>32**	**>32**	**>32**	*2*	**>32**	*4*	1	**16**	≤0.06	**4**	1	0.5	**16**	≤0.25	**>4**	≤0.06	≤0.125	**>8**	≤0.5
1536/13	*Kox* ST2	**>32**	**>32**	**>32**	**8**	**>32**	**>8**	*4*	**16**	0.5	**>8**	4	2	**16**	≤0.25	**>4**	≤0.06	≤0.125	**>8**	≤0.5
2978/13	*Kox* ST346	**>32**	**>32**	**>32**	**16**	**>32**	**8**	2	**>16**	0.125	**8**	2	0.5	≤2	1	**>4**	**1**	*1*	**>8**	≤0.5
5636/16	*Kox* ST348	**>32**	**>32**	**>32**	**16**	**>32**	*4*	2	**>16**	≤0.06	**4**	2	0.5	≤2	1	**>4**	*0.5*	0.5	**>8**	≤0.5
388/18	*Kox* ST347	**>32**	**>32**	**>32**	**>16**	**>32**	**8**	*8*	**>16**	≤0.06	**8**	8	2	≤2	2	**>4**	*0.5*	0.5	**>8**	≤0.5
7406/19	*Kox* ST36	**>32**	**>32**	**>32**	**>16**	**>32**	**8**	*8*	**>16**	0.25	**8**	8	**4**	≤2	1	**>4**	*0.5*	0.5	**>8**	≤0.5
10340/19	*Kox* ST345	**>32**	**32**	**16**	*4*	**32**	2	0.25	4	≤0.06	2	0.25	0.5	4	**32**	**>4**	0.125	0.25	≤1	≤0.5
10316/19	*Kox* ST37	**>32**	**>32**	**>32**	**>16**	**32**	**8**	*4*	**>16**	≤0.06	**8**	4	2	≤2	1	**>4**	**1**	0.5	**>8**	≤0.5
8275/17	*Kox* ST285	**>32**	**>32**	**>32**	**>16**	≤1	**8**	**16**	**>16**	0.125	**8**	**16**	1	≤2	1	**>4**	*0.5*	0.5	≤1	≤0.5
5810/12	*Kmi* ST146	**>32**	**>32**	**32**	*4*	**8**	**8**	*8*	**16**	0.125	**8**	4	0.5	**16**	≤0.25	**>4**	*0.5*	0.5	**>8**	1
1752/13	*Kmi* ST310	**32**	**32**	*4*	≤1	≤1	*4*	0.5	4	≤0.06	**4**	0.5	0.5	≤2	**4**	**>4**	≤0.06	≤0.125	≤1	≤0.5
3524/15	*Kmi* ST213	**>32**	**>32**	**>32**	**>16**	**>32**	**8**	**16**	**>16**	0.25	**8**	**16**	1	≤2	1	**>4**	**>8**	**>8**	**>8**	≤0.5
4445/19	*Kmi* ST210	**>32**	**>32**	*2*	≤1	≤1	2	0.5	4	0.25	**4**	0.5	0.125	≤2	0.5	**>4**	**4**	*1*	**>8**	≤0.5
8545/19	*Kmi* ST180	**>32**	**>32**	**>32**	**16**	**32**	**8**	*4*	**>16**	≤0.06	**8**	4	1	≤2	1	**>4**	**1**	0.5	**>8**	≤0.5
1085/17	*Kmi* ST95	**>32**	**>32**	**8**	*4*	≤1	*4*	0.5	**>16**	≤0.06	**4**	0.5	1	8	0.5	**>4**	**2**	0.5	≤1	≤0.5
5535/16	*Kgr* ST172	**>32**	**>32**	**>32**	**16**	*2*	*4*	2	**>16**	0.5	**4**	2	0.5	≤2	1	**4**	**4**	**2**	**>8**	≤0.5
6490/18	*Kpa* ST229	**32**	**32**	1	≤1	≤1	*4*	0.5	4	≤0.06	**4**	0.25	0.125	4	≤0.25	**>4**	≤0.06	≤0.125	≤1	≤0.5
7090/18	*Ksp*	**>32**	**>32**	**>32**	**>16**	**>32**	*4*	**16**	**>16**	0.125	**8**	8	2	≤2	1	**>4**	*0.5*	0.5	**>8**	≤0.5

[a] – *Kox*, *K. oxytoca*; *Kmi*, *K. michiganensis*; *Kgr*, *K. grimontii*; *Kpa*, *K. pasteurii*; *Ksp*, *K. spallanzanii*

[b] – abbreviations: PIP, piperacillin; TZP, piperacillin-tazobactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; CZA, ceftazidime-avibactam; AZA, aztreonam-avibactam; I-R, imipenem-relebactam; MVB, meropenem-vaborbactam; FDC, cefiderocol; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; CST, colistin.

[c]– bold, italic and normal styles refer to resistance, susceptibility increased exposure and susceptibility, respectively, according to EUCAST (http://eucast.org); the results for aztreonam-avibactam were not interpreted owing to the lack of criteria, however, the MICs against all of the isolates were in the category ‘susceptible’ for aztreonam alone.

[d] – tazobactam, avibactam, relebactam and vaborbactam were at fixed concentrations of 4, 4, 4 and 8 mg/L, respectively.

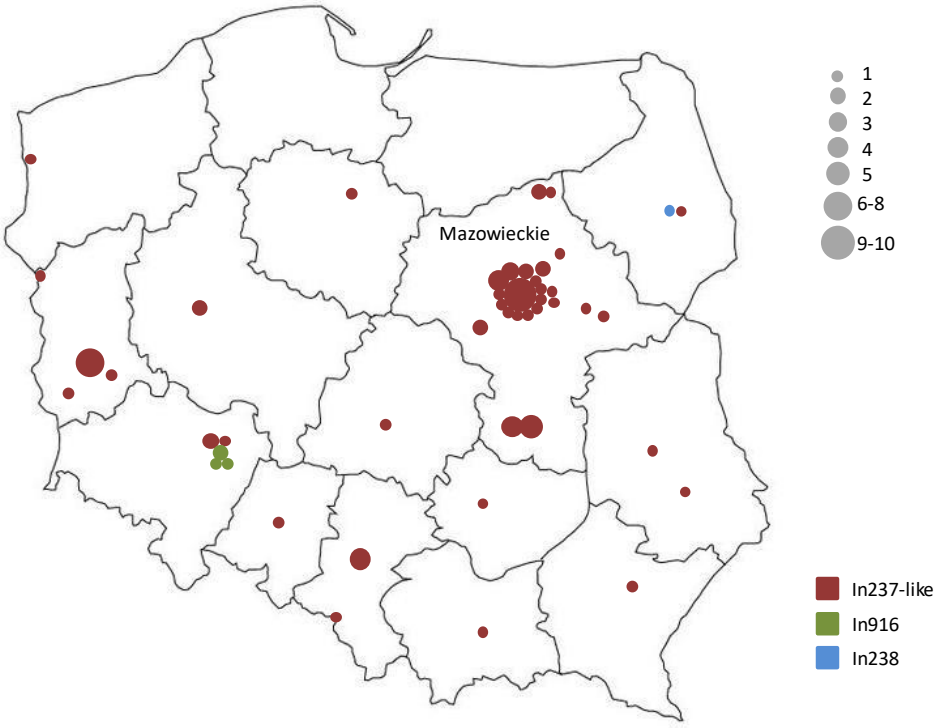
Table S6. Virulence loci, serotypes and plasmid replicon profiles in the study VIM-producing KoSC isolates

Species	Isolate	ST	*bla*$_{VIM}$ integron	Yersini abactin	Klebox ymycin	K locus	O locus	Plasmid replicons
K. oxytoca	776/09	145	In237-like	+	-	-	OL104	IncM2
	2092/09	145	In237-like	+	+	-	OL104	-
	2168/09	145	In237-like	+	+	-	OL104	IncM2
	2833/09	145	In237-like	+	-	-	OL104	-
	1674/10	145	In237-like	+	+	-	OL104	IncM2
	3809/10	145	In237-like	+	-	-	OL104	-
	4204/10	145	In237-like	+	-	-	OL104	-
	5944/10	145	In237-like	+	-	-	OL104	-
	6421/10	145	In237-like	+	+	-	OL104	-
	377/11	145	In237-like	+	-	-	OL104	-
	445/11	145	In237-like	+	-	-	OL104	-
	1095/11	145	In237-like	+	+	-	OL104	-
	1765/11	145	In237-like	+	+	-	OL104	IncHI2, IncHI2A
	5190/11	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	9347/11	145	In237-like	+	-	-	OL104	-
	588/12	145	In237-like	+	-	-	OL104	-
	3934/12	145	In237-like	+	-	-	OL104	-
	3936/12	145	In237-like	+	-	-	OL104	-
	6679/12	145	In237-like	-	+	-	OL104	-
	294/13	145	In237-like	+	-	-	OL104	IncFIA (HI1)
	1544/13	145	In237-like	+	-	-	OL104	-
	4464/13	145	In237-like	+	+	-	OL104	-
	4806/13	145	In237-like	+	+	-	OL104	-
	124/14	145	In237-like	+	+	-	OL104	IncM2
	626/14	145	In237-like	+	-	-	OL104	-
	815/14	145	In237-like	+	-	-	OL104	-
	972/14	145	In237-like	+	-	-	OL104	-
	1362/14	145	In237-like	+	+	-	OL104	-
	3325/14	145	In237-like	+	-	-	OL104	-
	85/15	145	In237-like	+	+	-	OL104	-
	601/15	145	In237-like	+	-	-	OL104	-
	1879/15	145	In237-like	+	-	-	OL104	IncpKPC-CAV1321
	2641/15	145	In237-like	+	+	-	OL104	IncpKPC-CAV1321
	4116/15	145	In237-like	+	-	-	OL104	-
	4566/15	145	In237-like	+	-	-	OL104	-
	347/16	145	In237-like	+	-	-	OL104	-
	723/16	145	In237-like	+	-	-	OL104	-
	1231/16	145	In237-like	+	-	-	OL104	IncM2
	2260/16	145	In237-like	+	-	-	OL104	-
	2579/16	145	In237-like	+	-	-	OL104	-
	2672/16	145	In237-like	+	+	-	OL104	-
	4025/16	145	In237-like	+	-	-	OL104	IncFIB$_K$
	5491/16	145	In237-like	+	-	-	OL104	Col (pHAD28), IncFIB$_K$, IncFII$_K$, IncM2
	6209/16	145	In237-like	+	-	-	OL104	-
	6428/16	145	In237-like	+	+	-	OL104	-
	6695/16	145	In237-like	+	+	-	OL104	IncFIB (pNDM-Mar), IncHI1B (pNDM-Mar), IncM2
	7212/16	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	196/17	145	In237-like	+	+	-	OL104	IncM2
	460/17	145	In237-like	+	+	-	OL104	-
	905/17	145	In237-like	+	+	-	OL104	IncN, IncpKPC-CAV1321
	1323/17	145	In237-like	+	+	-	OL104	-
	3362/17	145	In237-like	+	+	-	OL104	IncM2, IncpKPC-CAV1321
	4062/17	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncQ2
	4759/17	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	4793/17	145	In237-like	+	-	-	OL104	IncFII$_K$, IncFIBK, IncHI1A (NDM-CIT), IncHI1B (pNDM-CIT)
	4794/17	145	In237-like	+	-	-	OL104	IncFII(K), IncFIBK, IncHI1A (NDM-CIT), IncHI1B (pNDM-CIT)
	5138/17	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	5947/17	145	In237-like	+	-	-	OL104	IncM2
	6676/17	145	In237-like	+	-	-	OL104	-
	6751/17	145	In237-like	+	-	-	OL104	-
	939/18	145	In237-like	+	-	-	OL104	-
	2383/18	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	2437/18	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$, IncHI2, IncHI2A
	5438/18	145	In237-like	+	+	-	OL104	-
	5439/18	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	6107/18	145	In237-like	+	-	-	OL104	-
	7009/18	145	In237-like	+	+	-	OL104	-
	7702/18	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$

	7904/18	145	In237-like	+	+	-	OL104	IncFIB$_K$
	8748/18	145	In237-like	+	+	-	OL104	IncFIB$_K$
	221/19	145	In237-like	+	-	-	OL104	IncFIB$_K$
	1636/19	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	2226/19	145	In237-like	+	+	-	OL104	IncpKPC-CAV1321
	3634/19	145	In237-like	+	+	-	OL104	-
	6337/19	145	In237-like	+	+	-	OL104	IncFIB$_K$, IncM2
	6955/19	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	7512/19	145	In237-like	+	-	-	OL104	IncFIB$_K$, IncFII$_K$
	8138/19	145	In237-like	+	+	-	OL104	IncFIB$_K$
	2990/17	145	In238	+	+	-	OL104	IncFIB$_K$ (pCAV1099- 114), IncM2, IncU
	253/19	145	In916	+	-	-	OL104	IncA, IncFIBK
	768/19	145	In916	+	+	-	OL104	IncA, IncFIBK
	8015/19	145	In916	+	+	-	OL104	IncA, IncFIB$_K$ (pCAV1099-114)
	10246/19	145	In916	+	+	-	OL104	IncA, IncFIB$_K$ (pCAV1099-114)
	1536/13	2	In238-79	+	+	KL74	OL104	IncM1, IncrepA (pKOX)
	7406/19	36	In916	+	+	-	O5	IncA
	10316/19	37	In71-like	+	-	-	O3/O3a	IncA, IncFIB$_K$ (pCAV1099-114), IncN
	5820/12	282	In238	+	+	-	-	IncM1
	2978/13	346	In916	+	+	-	OL104	IncA
	5636/16	348	In916	+	-	-	O5	IncA, IncFIB$_K$
	8275/17	285	In916	+	+	-	O5	IncA
	388/18	347	In916	+	+	-	OL104	IncA
	10340/19	345	In238	+	+	-	O5	IncFIB (pNDM-Mar), IncHI1B (pNDM-Mar)
K. michiganensis	5810/12	146	In238a	+	-	-	O1/O2v1	IncFIB$_K$, IncFII$_K$, IncM1
	6620/12	146	In238a	-	-	-	O1/O2v1	IncFIB$_K$, IncFII$_K$, IncM1
	1752/13	310	In41-like	-	-	KL74	O1/O2v1	IncFIB$_K$
	3664/13	310	In41-like	-	-	KL74	O1/O2v1	IncFIB$_K$
	3524/15	213	In916	+	-	KL152	O1/O2v1	IncA
	1085/17	95	In237-like	-	-	-	O1/O2v1	IncFIB$_K$, IncFII (pKPX1), IncFII$_K$, IncU, repB(R1701)
	1086/17	95	In237-like	-	-	-	O1/O2v1	IncFIB$_K$, IncFII (pKPX1), IncFII$_K$, IncU, repB (R1701)
	10279/19	95	In237-like	-	-	-	O1/O2v1	IncFIB$_K$, IncFII (pKPX1), IncFII$_K$, IncU, repB (R1701)
	4445/19	210	In238	-	-	-	O1/O2v1	Col (pHAD28), IncHI1A (NDM-CIT), IncHI1B (NDM-CIT), IncN3
	8545/19	180	In916	-	-	-	O1/O2v1	IncA, IncU
	9543/19	180	In916	-	-	-	O1/O2v1	IncA, IncU
K. grimontii	5535/16	172	In110	-	+	-	O1/O2v1	IncHI2, IncHI2A
K. pasteurii	6490/18	229	In238a	+	+	-	O1/O2v1	IncFIB$_K$ (pCAV1099-114), IncHI1B (pNDM-Mar), IncR
K. spallanzanii	7090/18	-	In916	-	-	KL24	-	IncA

Figure S1. Geographic distribution and clonal analysis of *K. oxytoca* ST145 in Poland. **A.** Geographic distribution of the isolates shown on the map with main administrative regions. Circles represent medical centres where the isolates were recorded. Sizes of the circles are proportional to numbers of cases. **B.** SNP-based minimum spanning tree of the *K. oxytoca* ST145 isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots.

A



B

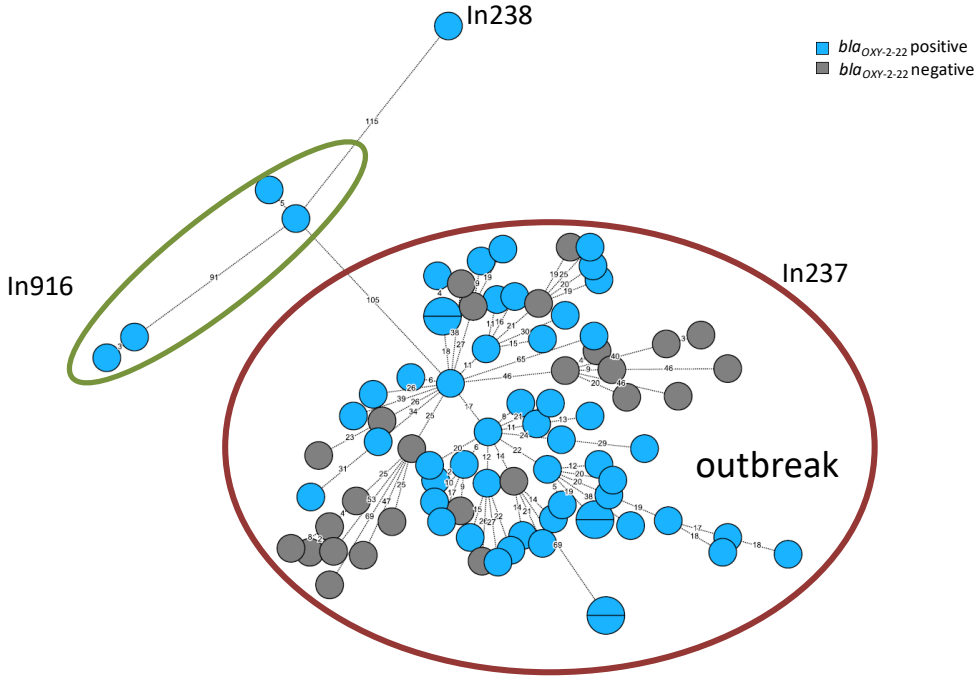


Figure S2. SNP-based phylogenetic tree of Polish *K. oxytoca* ST2 isolate compared with the international ST2 genomes available in RefSeq. Numbers in the inner circle correspond to original numbers of the study isolates or RefSeq assembly numbers. The presence of carbapenemases is indicated in the outer circles using corresponding colors. The country of origin is presented with country codes: AR, Argentina, CH, Switzerland; CN, China; DE, Germany; ES, Spain; GB, Great Britain; PL, Poland; US, USA. The tree was constructed using Parsnp and visualized with iTOL.

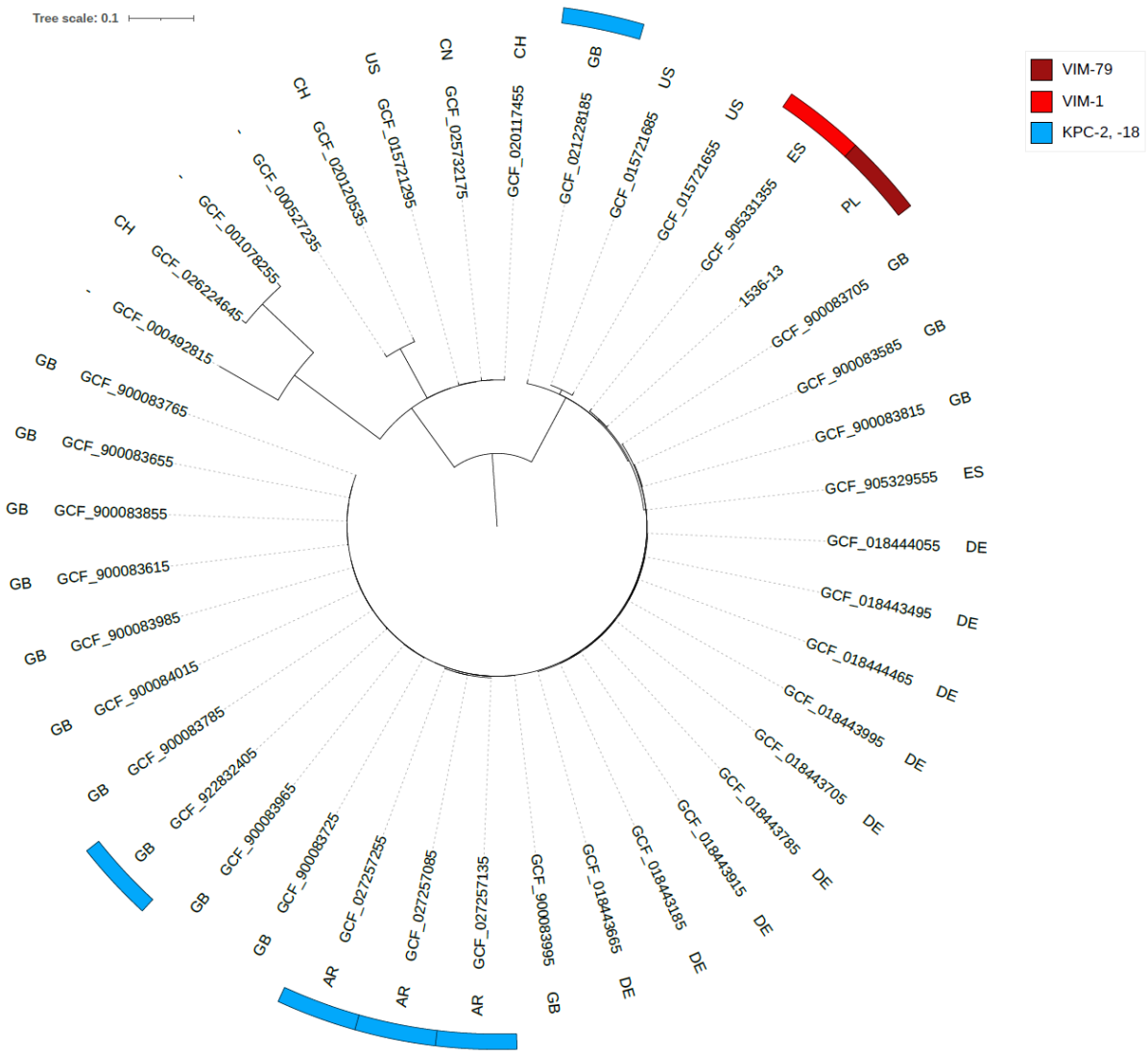


Figure S3. Comparison of the VIM-1-encoding (integron In916) IncA p253A plasmid to previously reported plasmids of the highest homology: p743A (Poland, OQ111274; inner, thin black circle), p5955A (Poland; OQ111275), p7753A (Poland, OQ111276), p9546_2 (Poland; ONO081626), p550_IncA_VIM (Italy; CP058224), PRIVM0001_VIM-1 (The Netherlands; MH220284), pFDL-VIM (Italy; MN783744), pKC-BO-N1_VIM (Italy; MG228427) and pGA_VIM (Italy; MN783743). The outer rings refers to the annotation of p743A, with the selected genes indicated. The percentage of sequence identity is reflected by color intensity. The picture was created using BRIG software

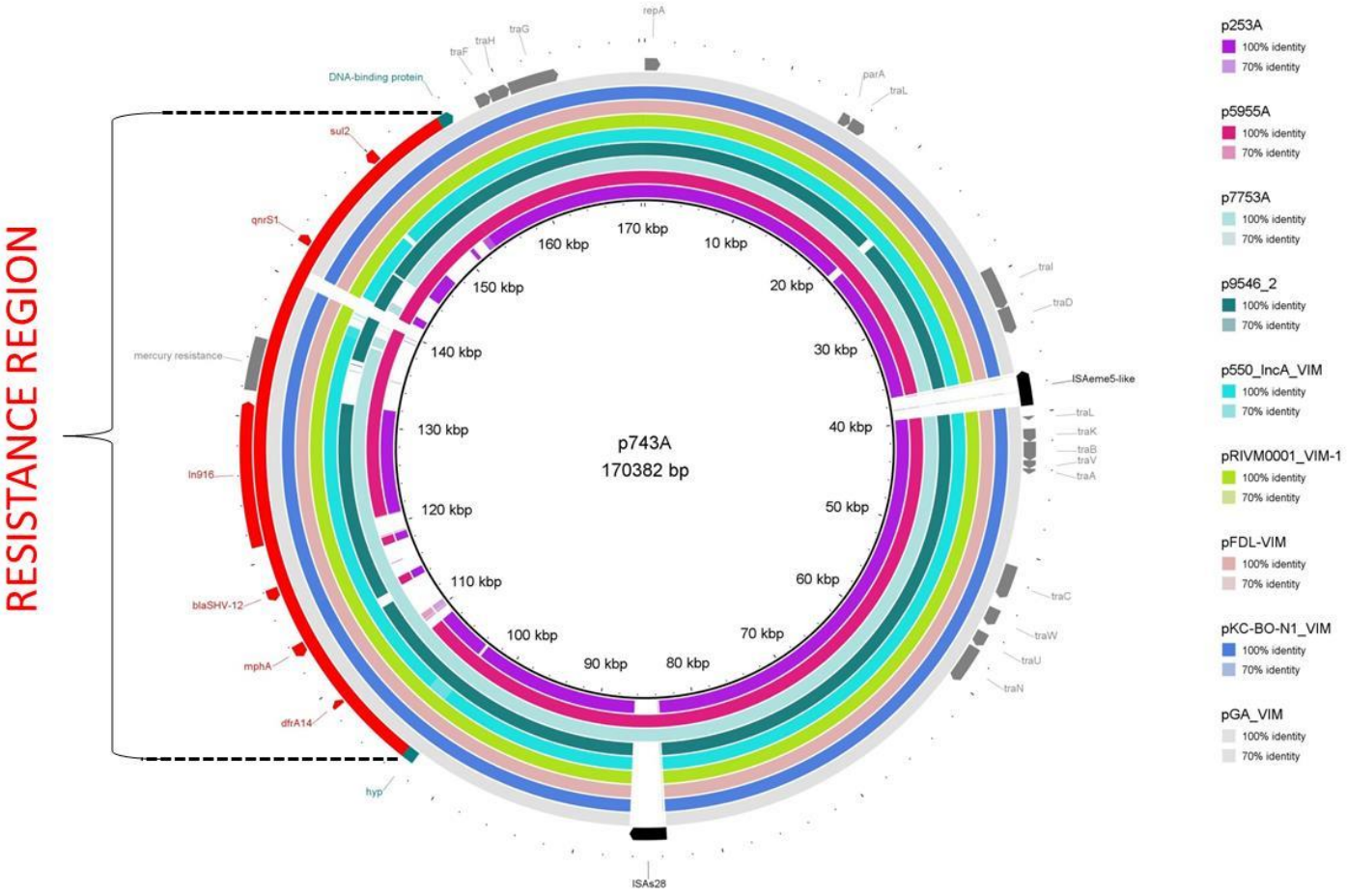
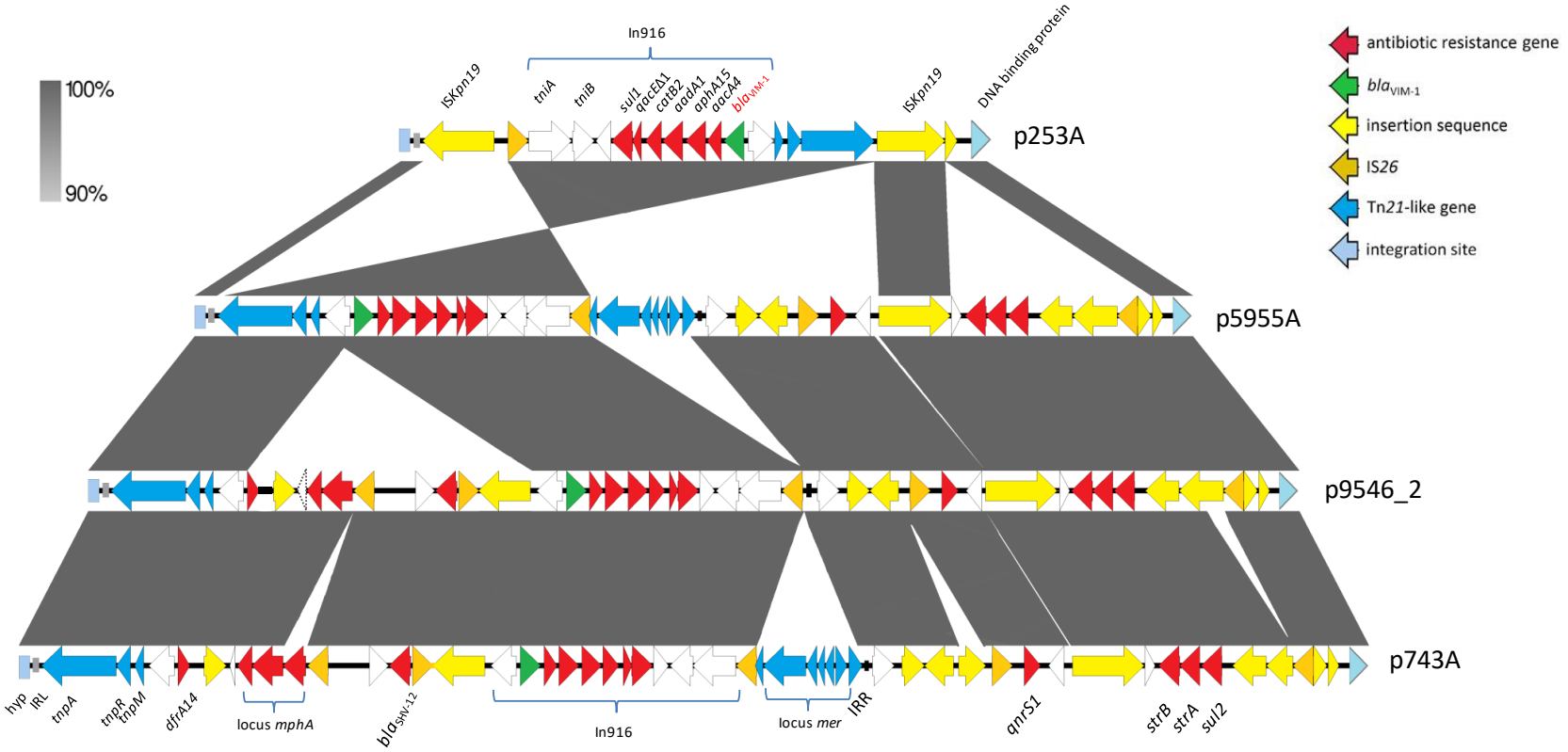


Figure S4. Comparison of the AMR region of the In916-carrying IncA-like plasmid p253A with the corresponding part of p5955A (Poland, *E. hormaechei*, OQ111275), p9546/19_2 (Poland, *K. pneumoniae*, ON081626) and p743A (Poland, *E. hormaechei*, OQ111274). The percentage of sequence identity is reflected by the gray color intensity. Individual loci (antibiotic AMR genes, mobile genetic elements and integration sites) are marked by coloured arrows or triangles as explained below. The picture was created using the Easyfig 2.2.5 software



6.4 Carbapenemase-producing *Enterobacterales* from patients arriving from Ukraine in Poland, March 2022-February 2023

Infect Dis Ther
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ORIGINAL RESEARCH

Carbapenemase-Producing Enterobacterales from Patients Arriving from Ukraine in Poland, March 2022–February 2023

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ABSTRACT

Introduction: Despite a scarcity of data, before 2022 Ukraine was already considered a high-prevalence country for carbapenemase-producing Enterobacterales (CPE), and the situation has dramatically worsened during the full-scale war with Russia. The aim of this study was to analyse CPEs isolated in Poland from victims of war in Ukraine.

Methods: The study included 65 CPE isolates from March 2022 till February 2023, recovered in 36 Polish medical centres from 57 patients arriving from Ukraine, differing largely by age and reason for hospitalisation. All isolates were sequenced by MiSeq and ten *Klebsiella pneumoniae* isolates also by MinION. Taxonomy, clonality and resistomes were analysed for all CPEs, whereas phylogeny, serotypes, virulomes and plasmids were characterised for *K. pneumoniae*, and partially for *Escherichia coli* ST131, using various bioinformatic tools.

Results: Multifactorial diversity of the isolates reflected the patients' clinical-epidemiological heterogeneity. The CPEs represented six species. *Klebsiella pneumoniae* was the most prevalent with 50 isolates and 15 sequence types (STs), mainly ST395, ST307, ST11, ST147 and ST23, producing NDM (-1/-5), OXA-48 (-48/-1242) or KPC (-2/-3)-like carbapenemases. Each of the STs produced groups of loosely related isolates, clusters of close relatives and/or unique isolates, correlating with K serotypes and carbapenemases. Many of these, especially NDM-1- and/or OXA-48-producing ST395 and ST307, were related to Russian organisms. Others, for example, NDM-1-producing ST11, clustered with those from Poland. Numerous *K. pneumoniae* isolates had specific virulence genes, including aerobactin *iuc*, largely due to spread of pNDM-MAR plasmids, showing both resistance and virulence. Two *E. coli* ST131 isolates belonged to clades B or C1 and produced KPC-3 or NDM-1, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40121-024-01097-9.

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Conclusions: Together with similar studies from Germany and The Netherlands, this work has documented broad dissemination of CPE in Ukraine, driven by a number of specific *K. pneumoniae* lineages circulating over a large territory of Eastern Europe.

Keywords: Carbapenemase; Carbapenemase-producing Enterobacterales; CPE; *Klebsiella pneumoniae*; Ukraine

Key Summary Points

Why carry out this study?

Epidemiology of carbapenemase-producing Enterobacterales (CPE) in Ukraine has not been systematically studied; however, partial data from different sources indicated their broad spread and high prevalence before 2022. The full-scale war that started in February 2022 has only worsened the situation and caused exportations of CPEs to other European countries with patients arriving from Ukraine. The organisms need to be characterized in detail, especially assessing their antimicrobial resistance (AMR), virulence and epidemic potential.

What did the study ask?

The study was carried out on a group of CPEs collected all over Poland from March 2022 to February 2023 from patients from Ukraine, including soldiers injured in warfare and children evacuated from hospitals. Using a broad genome analysis, we aimed to identify organisms with apparently increased epidemicity and their relevant characteristics. These were compared with isolates deposed in international sequence databases to assess their possible origins and actual geographic spread.

What were the study outcomes/conclusions?

The CPEs were highly diverse at both species and clonal levels. *Klebsiella pneumoniae* prevailed, comprising different clones and subclones. Several of these were represented by multiple isolates, indicating successful spread in Ukraine. Most of the genotypes were related to isolates from Russia, suggesting their broad circulation in Eastern Europe; from 2022, these were also recovered in Germany and The Netherlands from Ukrainian patients. All CPEs contained extensive sets of AMR genes, and a remarkable group also had some of the genes associated with hypervirulence in *K. pneumoniae*.

What has been learned from the study?

Ukraine has a difficult epidemiological situation regarding CPE due to long-term shortcomings in infection control and surveillance. This has been dramatically worsened by the war. A number of specific, potentially epidemic and virulent CPE genotypes have emerged, circulated over Eastern Europe, and may significantly affect CPE epidemiology in other European countries.

INTRODUCTION

Since the onset of the full-scale war in Ukraine in February 2022, millions of Ukrainians have looked for shelter abroad. Approximately 1.5 million refugees, mostly women and children, have stayed in Poland, where over 1.3 million received access to the free healthcare system [1]. This resulted in health services for ~ 350,000 patients arriving from Ukraine in 2022 [2], including treatment in hospitals of various types and reference levels [3]. The inpatients varied by demographic factors, regions of origin, health status and reason for hospitalisation. The group comprised individuals evacuated from Ukrainian hospitals, including severely ill children and soldiers injured in warfare.

Management of antimicrobial resistance (AMR), one of the major global threats to public health today [4], has shown remarkable shortcomings in Ukraine. The problem refers to all types of infections and multiple factors or activities, such as surveillance and research [5]. Recent inclusion of fragmentary data from Ukraine into international surveillance networks showed that a difficult situation already existed there before 2022 regarding all clinically relevant AMR types. For instance, the percentage of resistance to carbapenems in *Klebsiella pneumoniae* in 2018–2020 ranged from ~ 43% to ~ 61% [6] and might have been as high as ~ 70% in 2021 [7]. Similarly, a multicentre study in Ukraine in 2021 assessed this resistance rate to be ~ 54% [8]. A follow-up analysis revealed diversity of carbapenem-hydrolysing β-lactamases (carbapenemases) in resistant isolates, demonstrating spread of NDM-, VIM-, OXA- and KPC-like enzymes [9]. Higher quality molecular data on multidrug-resistant (MDR) organisms of Ukrainian origin had been scarce before 2022 [10], referring also to single isolates identified elsewhere, for example, in Poland [11, 12].

The full-scale war has deteriorated the AMR situation in Ukraine, causing non-controlled massive spread of MDR pathogens [13]. This has been reflected by reports on carbapenemase-producing Enterobacterales (CPE) from Ukrainian patients in Germany, The Netherlands and other countries [14–21]. Studies of this type are necessary to definitely characterize CPE and other MDR organisms circulating in Ukraine, since their implementation on a broader scale there is not feasible now. Such works are essential to assess the threat associated with these pathogens in Ukraine, and possibly elsewhere upon importation, especially considering the extent of trans-border movement and its potential escalation in the future. Here, we present the in-depth genomic analysis of 65 CPE isolates from patients arriving from Ukraine collected from March 2022 to February 2023 in Poland.

METHODS

Bacterial Isolates

From March 2022 to February 2023, the National Reference Centre for Susceptibility Testing (NRCST) in Warsaw, responsible for AMR surveillance in Poland, confirmed 74 non-duplicate CPE isolates of Ukrainian origin from 64 individuals. Nine NDM-positive *Providencia stuartii* isolates from nine patients were included in a separate study [14]. This analysis concerns 65 isolates from 57 patients treated in 34 hospitals and 2 outpatient clinics in 25 cities all over Poland.

Most of the 65 isolates ($n = 45$; ~ 69.2%) were from infections, often from wounds ($n = 19$; ~ 42.2% of infections) or the bloodstream ($n = 6$; ~ 13.3%); a smaller fraction represented intestinal carriage ($n = 20$; 30.8%). Male patients prevailed ($n = 47$; 82.4%). The median age of the patients was 31.5 years, and at least 17 patients (29.8%) were children (2 weeks to 17 years), treated in specialist paediatric centres, mainly in Krakow and Warsaw. At least 12 patients were soldiers with war wounds. The history of recent hospitalisation in Ukraine, including field hospitals, was documented for 22 patients. More than one unique CPE isolate of the same or another species was cultivated from seven patients, and three patients were co-infected/colonized with other MDR species (NDM/VIM-producing *Pseudomonas aeruginosa* or vancomycin-resistant *Enterococcus faecium*) (Table S1).

All of the isolates were tested for carbapenemases phenotypically and by PCRs for bla_{KPC}-, bla_{VIM}-, bla_{NDM}- and bla_{OXA-48}-like genes by a previously described procedure [22]. The preliminary species confirmation was carried out with VITEK 2 (bioMérieux, Marcy l'Etoile, France).

This article does not contain any study on human or animal subjects, material or data. The study was considered to be exempt from approval by a Polish ethics commission since it was an in vitro retrospective study on bacterial isolates cultured during routine medical procedures and collected for epidemiological purposes, not involving patients or their personal data.

Whole Genome Sequencing

All of the 65 CPE isolates were sequenced by Miseq (Illumina, San Diego, CA, USA), and 10 selected *K. pneumoniae* isolates were also sequenced by MinION (Oxford Nanopore Technologies, Oxford, UK). Hybrid assemblies were obtained with Unicycler v.0.4.7 [23].

Taxonomy, Clonality and Phylogeny

Species were identified using FastANI v.1.32 based on average nucleotide identity (ANI) scores with ≥95% cutoff [24] and RefSeq reference genomes. The seven-loci multi-locus sequence typing (MLST) of *K. pneumoniae*, *Escherichia coli*, *Enterobacter hormaechei* and *Citrobacter* spp was performed by the mlst tool (https://github.com/tseemann/mlst) and *Proteus mirabilis* using PubMLST [25]. The single-nucleotide polymorphism (SNP)-based clonality analysis in the sample was done for *K. pneumoniae* sequence types (STs) ST11, ST23, ST147, ST307 and ST395 and for *E. coli* ST131 by BioNumerics v.7.6.3 (Applied Maths NV, Sint-Martens-Latem, Belgium), with the chronologically first isolate of each ST as a reference. The SNP-based phylogenetic analysis in the international context was carried out for the five *K. pneumoniae* STs against all genomes available in the RefSeq database (as of February 1, 2023), using Parsnp v.1.2. [26]. Phylotrees were visualised by ITOL (https://itol.embl.de/). Comparisons of the study *K. pneumoniae* isolates of the major STs with those of the same STs of Ukrainian origin in other European countries [15–18] were done by CSI Phylogeny [27].

Resistomes, Genetic Context of Carbapenemase-Encoding Genes, Serotypes and Virulomes

AMR genes were detected with AMRFinderPlus with the 99.5% identity criterion [28]. The genetic context of bla_{KPC}-, bla_{VIM}-, bla_{NDM}- and bla_{OXA-48}-like genes was analysed manually using Geneious Prime v.2023.1.2 (Biomatters, Auckland, New Zealand). *Klebsiella pneumoniae* capsular (K) and LPS (O) serotypes and virulence genes were identified by Kaptive [29], Kleborate [30] and BIGSdb [31]. Serotypes and virulomes of *E. coli* ST131 were identified with SerotypeFinder 2.0, FimTyper-1.0 and VirulenceFinder-2.0 (https://cge.food.dtu.dk/services/).

Plasmid Replicon Types and Structural Analysis of Plasmids

Plasmid replicon types were detected by PlasmidFinder 2.1 [32, 33]. The structural analysis of carbapenemase-encoding plasmids was performed manually by Geneious Prime v.2023.1.2. AMR, and virulence genes/loci were assigned to plasmids with ResFinder 4.1 [34], Kleborate [30] and BLAST [32]. Plasmid comparisons were executed using BLASTn, and visualised with Easyfig v2.2.5 [35] and BRIG tools (http://brig.sourceforge.net/).

Antimicrobial Susceptibility Testing

Susceptibility to antimicrobials was tested using broth microdilution Sensititre EUGNF and EUMDRXXF plates (Thermo Fisher Scientific, Waltham, USA) and the ComASP Cefiderocol test (Liofilchem, Roseto degli Abruzzi, Italy). Results were interpreted according to EUCAST breakpoints v.14.0 (http://eucast.org).

Nucleotide Sequence Accession Numbers

Genomic sequences were deposited in the NCBI under the Bio-Project and Bio-Samples numbers PRJNA1152760 and SAMN43377775–SAMN43377839, respectively. Plasmid sequences were assigned the following accession numbers: p2468_1, PQ284023; p7242_1, PQ284024; p8087_1, PQ284025; p6772, PQ284026; p1195, PQ284027; p8597, PQ284028; p8287, PQ284029; p2858, PQ284030; p2468_2, PQ284031; p8087_2, PQ284032; p5378, PQ284033; p7242_2, PQ284034; p4247, PQ284035.

RESULTS

Taxonomy and MLST

The 65 Enterobacterales isolates were *K. pneumoniae* ($n=50$; 76.9%), *E. coli* ($n=7$; 10.8%), *E. hormaechei* ($n=5$; 7.7%) and *Citrobacter portucalensis*, *Citrobacter telavivensis* and *P. mirabilis* ($n=1$ each; 1.5%) (Table 1). *Klebsiella pneumoniae* isolates were classified into 15 STs, 6 having > 1 isolate: ST395 ($n=14$; 28.0% of *K. pneumoniae* isolates), ST307 ($n=11$; 22.0%), ST11 ($n=6$; 12.0%), ST147 ($n=5$; 7.6%), ST23 ($n=3$; 6.0%) and ST15 ($n=2$; 4.0%) (Table 1). Four *E. coli* STs were discerned, including ST46 ($n=3$) and ST131 ($n=2$), and three STs in *E. hormaechei*, including ST91 and ST231 ($n=2$ each).

Carbapenemase-Encoding Genes

Carbapenemase genes were of four types, namely bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} (Table 1). The most numerous were bla_{NDM}s, comprising bla_{NDM-1} ($n=34$; 52.3%) and bla_{NDM-5} ($n=6$; 9.2%). The second most common type, bla_{OXA-48}s, were bla_{OXA-48} itself ($n=16$; 24.6%) and $bla_{OXA-232}$, $bla_{OXA-244}$ and a new $bla_{OXA-1242}$ variant ($n=1$ each; 1.5%). $bla_{OXA-1242}$ differed from bla_{OXA-48} [36] by one nucleotide (G379T), causing the A127S amino-acid substitution in the protein sequence. bla_{KPC}s were represented by bla_{KPC-3} ($n=12$; 18.5%) and bla_{KPC-2} ($n=4$; 6.2%). One isolate had the bla_{VIM-1} gene. Genetic context of the carbapenemase genes varied depending on the carbapenemase type and host organism (Table S2).

The prevalent carbapenemases were distributed across the species and STs, and most of the STs with more than one isolate varied in carbapenemase content (Table 1). For example, *K. pneumoniae* ST395 expressed NDM-1 ($n=4$), NDM-5 ($n=1$), OXA-48 ($n=3$), OXA-1242 ($n=1$) or NDM-1 + OXA-48 ($n=5$); ST307 produced NDM-1 ($n=7$), KPC-3 ($n=2$), KPC-2 ($n=1$) or NDM-1 + OXA-48 ($n=1$); ST147 had NDM-1 ($n=2$), NDM-5 ($n=2$) or NDM-1 + OXA-48 ($n=1$). Apart from the seven ST147, ST307 and ST395 isolates with NDM-1 + OXA-48 mentioned above, all *K. pneumoniae* ST23 isolates ($n=3$) had this enzyme combination, and a single ST5859 isolate expressed NDM-1 + OXA-232. The two *E. coli* ST131 isolates produced NDM-1 or KPC-3.

Different CPE isolates (*K. pneumoniae* of different STs or *K. pneumoniae* plus 1–3 other species, including *E. coli* in four cases) identified from a single patient produced the same or another carbapenemase, specified by a gene located in the same or another genetic context (Table S1). The transmission of such a gene (bla_{NDM-1}, bla_{OXA-48} or bla_{KPC-3}) between two different organisms was possible in four cases, based on the identical gene context. One of these patients was infected/colonized by NDM-1-producing *K. pneumoniae* ST23 (plus OXA-48), *E. coli* ST131 and *P. stuartii*; the bla_{NDM-1} transmission was possible only between the two latter strains.

Serotypes and In-Sample Clonality of *K. pneumoniae* and *E. coli* ST131 Isolates

K and O serotypes of all *K. pneumoniae* isolates are shown in Tables 1 and S3. The analysis revealed their high variety, with 16K- and seven O-antigen biosynthetic loci variants. KL102 ($n=11$; 22.0%), K2 ($n=8$; 16.0%), K64 ($n=6$; 12.0%), K24 and KL108 (both $n=4$; 8.0%) prevailed among the putative K serotypes, whereas O1v.1 ($n=17$; 34.0%) and O2v.2 ($n=15$; 30.0%) dominated among the O serotypes.

Isolates of five *K. pneumoniae* STs, ST395, ST307, ST11, ST147 and ST23, were subjected to the SNP-based clonal in-sample analysis. In general, the SNP distribution within each ST correlated well with K serotypes and (less so) carbapenemases. ST395 isolates presented 30–3207 SNPs between any individual and the reference genome (isolate NMI2468_22) and comprised groups of the K2 ($n=7$), K39 ($n=2$) and KL108 ($n=4$) and one distant K64 isolate (Fig. 1a; Table S4A). Whereas the K39 and KL108 groups were clusters of 2–4 isolates with 8–32 SNPs between closest relatives, the K2 group was more differentiated and comprised two clusters (2–3 isolates; 18–40 SNPs) and two orphan isolates (101–169 SNPs from the clusters). Whereas the K2 and K39 groups had mainly OXA-48 types alone or in combination with NDM-1, KL108 basically had NDM-1, occasionally combined

Table 1 Carbapenemase-producing Enterobacterales (CPE) isolates from patients arriving from Ukraine identified in Poland, March 2022–February 2023: basic epidemiological data, carbapenemases, sequence types (STs) and *Klebsiella pneumoniae* K serotypes

Species[a,b]	Isolate	Isolation date	Specimen[c]	Sex	Age[c]	Centre	Ward[d]	ST	*Kpn* K serotype	*Eco* ST131 characteristics	Carbapenemase
Kpn	2468/22	03.22	Blood	M	65	HR1	Urology	ST395	K2	–	NDM-1 + OXA-48
	6991/22	08.22	Wound	M	–	HS1	Surgery	ST395	K2	–	NDM-1 + OXA-48
	9040/22	09.22	Urine	M	–	HP5	–	ST395	K2	–	NDM-1
	2858/22	03.22	Rectal swab	M	9	HP2	Paediatric oncology	ST395	K2	–	OXA-48
	10,443/22	04.22	Wound	M	34	HS4	Surgery	ST395	K2	–	OXA-48
	4263/22	05.22	Rectal swab	M	1	HS3	Paediatrics	ST395	K2	–	OXA-48
	5378/22	06.22	Blood	F	91	HP4	Internal medicine	ST395	K2	–	OXA-1242
	7242/22	08.22	Blood	M	44	HL2	–	ST395	K39	–	NDM-1 + OXA-48
	3420/22	04.22	Wound	M	48	HP3	Orthopedics	ST395	K39	–	NDM-1 + OXA-48
	8087/22	09.22	Wound	M	23	HK3	–	ST395	KL108	–	NDM-1 + OXA-48
	7025/22	07.22	Wound	M	30	HE1	Orthopedics	ST395	KL108	–	NDM-1
	302/23	01.23	Wound	M	52	HD2	Orthopedics	ST395	KL108	–	NDM-1
	10,423/22	11.22	Wound	M	34	HG1	Orthopedics	ST395	KL108	–	NDM-1
	3635/22	04.22	Blood	F	41	HD1	Oncology	ST395	K64	–	NDM-5
	10,440/22	11.22	Wound	M	68	HS5	Surgery	ST307	KL102	–	NDM-1
	1197/23	03.22	Wound	M	38	HW3	Orthopedics	ST307	KL102	–	NDM-1
	5015/22	03.22	Rectal swab	F	6	HW1	Pediatric ICU	ST307	KL102	–	NDM-1

Table 1 continued

Species[a,b]	Isolate	Isolation date	Specimen[c]	Sex	Age[c]	Centre	Ward[d]	ST	*Kpn* K serotype	*Eco* ST131 characteristics	Carbapenemase
	6772/22	07.22	Urine	M	–	AG1	Outpatients clinic	ST307	KL102	–	NDM-1
	2814/22	03.22	Urine	M	71	HK2	Internal medicine	ST307	KL102	–	NDM-1
	9816/22	11.22	Rectal swab	M	–	HK1	Paediatric nephrology	ST307	KL102	–	NDM-1 + OXA-48
	1195/23	11.22	Wound	M	43	HW3	Surgery	ST307	KL102	–	NDM-1
	1196/23	11.22	Wound	M	37	HW3	Orthopedics	ST307	KL102	–	NDM-1
	4247/22	05.22	Rectal swab	M	9	HK1	Paediatric nephrology	ST307	KL102	–	KPC-3
	5059/22	05.22	Rectal swab	M	–	HK1	Paediatric cardiology	ST307	KL102	–	KPC-3
	10,100/22	10.22	BAL	F	77	HW4	ICU	ST307	KL102	–	KPC-2
	8597/22	10.22	Bile	M	41	HN3	Surgery	ST11	K24	–	NDM-1
	6741/22	05.22	Urine	M	55	HW5	Neurology	ST11	K24	–	NDM-1
	6912/22	07.22	Wound	M	26	HN2	ICU	ST11	K24	–	NDM-1
	10,432/22	05.22	BAL	M	58	HG1	ICU	ST11	K24	–	NDM-1
	2466/22	03.22	Pus	F	38	HR1	Emergency	ST11	K15	–	OXA-48
	5008/22	03.22	Rectal swab	M	11	HW1	Paediatrics	ST11	K15	–	KPC-2
	8287/22	09.22	Wound	F	48	HS4	Surgery	ST147	K64	–	NDM-1
	9702/22	10.22	Rectal swab	M	67	HK4	ICU	ST147	K64	–	NDM-1
	2674/22	03.22	Pharyngeal swab	M	1,5	HK1	Paediatric surgery	ST147	K64	–	NDM-5

Table 1 continued

Species[a,b]	Isolate	Isolation date	Specimen[c]	Sex	Age[c]	Centre	Ward[d]	ST	*Kpn* K serotype	*Eco* ST131 characteristics	Carbapenemase
	2564/22	03.22	Blood	M	3 weeks	HN1	Neonatalogy	ST147	K64	–	NDM-1 + OXA-48
	10,718/22	12.22	BAL	M	59	HO1	ICU	ST147	K10	–	NDM-5
	2727/22	03.22	rectal swab	M	39	HP1	Hematology	ST23	K57	–	NDM-1 + OXA-48
	6228/22	05.22	Wound	M	35	HW2	–	ST23	K57	–	NDM-1 + OXA-48
	1198/22	05.22	Rectal swab	M	–	HW3	Orthopedics	ST23	K57	–	NDM-1 + OXA-48
	2369/22	03.22	Urine	F	8	HS2	Paediatrics	ST15	KL112	–	KPC-3
	2662/22	03.22	Faeces	M	7	HK1	Paediatric nephrology	ST15	KL112	–	KPC-3
	2821/22	03.22	Rectal swab	M	8	HW6	Paediatric orthopedics	ST14	K2	–	KPC-3
	2673/22	03.22	BAL	M	2 weeks	HK1	Pediatric ICU	ST17	K55	–	NDM-1
	8289/22	09.22	Wound	M	43	HS4	Surgery	ST39	K23	–	KPC-2
	5016/22	05.22	Wound	M	5	HW1	Paediatric nephrology	ST45	K52	–	KPC-3
	6914/24	07.22	Rectal swab	M	–	HD2	Neurology	ST152	KL149	–	KPC-2
	5010/22	04.22	Urine	M	6	HW1	Paediatrics	ST219	KL114	–	KPC-3
	5012/22	03.22	BAL	F	2 months	HW1	Paediatric ICU	ST253	K39	–	NDM-1
	10,441/22	11.22	Wound	M	78	HS5	Surgery	ST512	KL107	–	KPC-3
	5018/22	04.22	Urine	F	17	HW1	Paediatric ICU	ST5859	K64	–	NDM-1 + OXA-232

Table 1 continued

Species[a,b]	Isolate	Isolation date	Specimen[c]	Sex	Age[c]	Centre	Ward[d]	ST	*Kpn* K serotype	*Eco* ST131 characteristics	Carbapenemase
Eco	3494/22	04.22	Rectal swab	M	43	HP3	Surgery	ST46	–	–	NDM-5
	3495/22	04.22	Rectal swab	M	28	HP3	Surgery	ST46	–	–	NDM-5
	9036/22	09.22	Rectal swab	M	32	HP5	–	ST46	–	–	NDM-5
	5220/22	05.22	Wound	M	35	HW2	–	ST131	–	O25b:H4, *fimH*30	NDM-1
	6352/22	06.22	Urine	F	49	AE1	Outpatients clinic	ST131	–	O25b:H4, *fimH*22	KPC-3
	6179/22	06.22	Rectal swab	M	–	HD2	Nephrology	ST167	–	–	OXA-48
	5058/22	05.22	Faeces	M	9	HK1	Paediatric nephrology	ST224	–	–	KPC-3
Eho	6750/22	07.22	Rectal swab	M	45	HL1	ICU	ST91	–	–	KPC-3
	9723/22	10.22	Blood	M	43	HL3	ICU	ST91	–	–	VIM-1
	3657/22	04.22	Wound	M	44	HP3	Orthopedics	ST231	–	–	NDM-1
	8875/22	10.22	Wound	M	28	HZ1	Orthopedics	ST231	–	–	NDM-1
	6990/22	07.22	Wound	M	–	HS1	Vascular surgery	ST182	–	–	OXA-244
Cpo	4020/22	05.22	Nasal sinus aspirate	M	5	HW1	Paediatric ICU	ST728	–	–	KPC-3
Cte	6180/22	07.22	Rectal swab	M	–	HD2	Neurology	ST1282	–	–	OXA-48
Pmi	9856/22	11.22	Wound	M	22	HP6	Orthopedics	ST269	–	–	NDM-1

[a] Isolates are ordered according to species, STs (from the most prevalent one) and in case of *K. pneumoniae*–K serotypes

[b] *Kpn, Klebsiella pneumoniae*; *Eco, Escherichia coli*; *Eho, Enterobacter hormaechei*; *Cpo, Citrobacter portucalensis*; *Cte, Citrobacter telavivensis*; *Pmi, Proteus mirabilis*

[c] *BAL* bronchoalveolar lavage

[d] – No data available

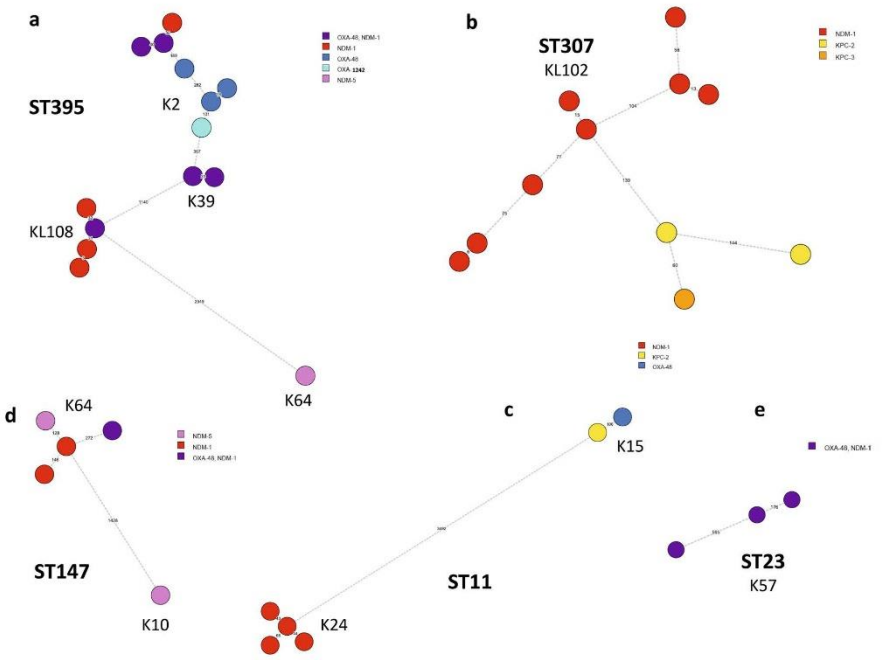

Fig. 1 Single-nucleotide polymorphism (SNP)-based minimum spanning trees of *Klebsiella pneumoniae* sequence types (STs): ST395 (**a**), ST307 (**b**), ST11 (**c**), ST147 (**d**) and ST23 (**e**) isolates. K serotypes and carbapenemases are shown. Trees were constructed and visualised with BioNumerics

with OXA-48. The K64 isolate was unique in having NDM-5.

The ST307 isolates were more compact genetically, with 15–174 SNPs with the reference (NMI2814_22) and the KL102 serotype locus only (Fig. 1b; Table S4B). These were divided into an NDM-1 group ($n=8$) with three clusters of two isolates each (6–15 SNPs) and three KPC-2/–3 producers of looser relationships. The ST11 isolates varied by 38–3045 SNPs from the reference (NMI5008_22) and were split into four related K24 NDM-1 isolates and two far-distant K15 isolates with OXA-48 or KPC-2 (Fig. 1c; Table S4C). The ST147 isolates were of serotypes K64 or K10 and were remarkably diverse, differing by 272–1504 SNPs from the reference (NMI2564_22) (Fig. 1d; Table S4D). These produced NDM-1 or NDM-5, occasionally together with OXA-48. Finally, the ST23 isolates had the serotype K57 and NDM-1 plus OXA-48 but were relatively distant from each other by SNPs (595 and 596 SNPs from the reference) (Fig. 1e; Table S4E).

The *E. coli* ST131 isolates were of the O25b:H4 serotype. The isolate with KPC-3 (NMI6352_22) had the *fimH* allele 22, indicative of clade B, whereas the one with NDM-1 (NMI5220_22) had *fimH*30, characteristic for clade C [37]. Consistently, the isolates were 2657 SNPs distant from each other.

Phylogeny of *Klebsiella pneumoniae* Isolates

The comparison of the *K. pneumoniae* ST395, ST307, ST11, ST147 and ST23 isolates with

genomes from RefSeq positioned their groups, clusters and single isolates within phylogenetic trees of the individual STs. The ST395 isolates were distributed into four clades according to their K2, K39, K64 and KL108 serotypes and further into six branches, corresponding to the clusters and orphans (Figure S1). In each of these, the Ukrainian isolates were most closely related to isolates from Russia. The ST307 NDM-1 group was split into three minor branches, each also shared with Russian isolates; the ST307 KPC-2/–3 producers were separate and related to isolates from the USA or Italy and France (Figure S2). The ST11 NDM-1 group belonged to a specific lineage recorded, for example, in Poland, Czechia, Bulgaria and Greece (Table S5). The ST147 organisms were scattered clonally, each related to another set of international isolates, including those from Russia, Egypt, India, Iran and Denmark (Table S6). Finally, the ST23 K57 isolates were loosely related to strains from Russia, Germany and Poland, including some of Ukrainian origin (Table S7) [12]. The separate comparison of the *K. pneumoniae* isolates with those of Ukrainian origin reported elsewhere revealed clear, even close relationships (5–100 SNPs) between most of the ST395, ST307, ST147 and ST23 isolates with those recovered in Germany and The Netherlands [15–18] (data not shown).

Klebsiella pneumoniae and *E. coli* ST131 Virulomes

A number of the isolates ($n=43$; 86.0%) carried 1–3 of the major virulence loci of *K. pneumoniae*, namely those specifying siderophores aerobactin (Iuc; locus *iuc*), yersiniabactin (Ybt; *ybt*) and capsule biosynthesis regulators (RmpA/A2; *rmpA/A2*) (Table S3). Loci *iuc* and *rmpA/A2* occurred in 23 (46.0%) isolates each and together in 20 isolates. Kleborate virulence scores 3 or 4, suggesting increased virulence [30], were assigned to 23 isolates. Almost all these belonged to ST23, ST307 and ST395, and their largest set of relatives were the eight ST307 NDM-1 isolates. Each of the virulence loci occurred in several STs, and *iuc* and *ybt* had variants, represented by specific sequence types, AbSTs and YbSTs (Table S3).

The *E. coli* ST131 isolates differed from each other in virulomes. The clade B isolate (NMI6352_22) carried, for example, genes *papA*, *papC*, *cnf1*, *hlyA*, *kpsMII_K5*, *iss* and *ibeA*, indicating virotype D [38]. The clade C isolate (NMI5220_22) had mostly non-specific virulence genes; the presence of *kpsMII_K5* and *iss* might suggest virotypes B or D.

Resistomes

Resistomes of the isolates varied largely, consisting of 2–22 acquired AMR genes per isolate, most often ≥ 10 genes ($n=46$; 70.7%) (Table S2). The diversity occurred within the species and STs; however, the groups and clusters of related isolates had similar or identical resistomes. Of the major *K. pneumoniae* STs, ST11 carried seven AMR genes on average, whereas ST23, ST147, ST307 and ST395 had means of 14.6–16.7 AMR genes. The most expanded resistomes of 19–22 genes were, for example, in the ST307 NDM-1 group or a pair of ST395 K39 NDM-1 + OXA-48. Similar to other characteristics, the two *E. coli* ST131s had different resistomes. The clade B isolate (NMI6352_22) had only two acquired AMR genes, $bla_{\text{KPC-3}}$ and $bla_{\text{TEM-1}}$, whereas the clade C isolate (NMI5220_22) carried 17 genes, including the extended-spectrum β-lactamase (ESBL) gene $bla_{\text{CTX-M-27}}$, indicative of subclade C1 [37].

The overall complexity of resistomes was largely due to β-lactamase genes, which, along with carbapenemases, commonly encoded CTX-M-type ESBLs ($n=55$, $bla_{\text{CTX-M-15}}$ mainly). Another source of variety were aminoglycoside resistance genes, including 16S rRNA methylase *armA* ($n=23$) and *rmtB/C/F* ($n=10$) genes. Most of the isolates ($n=53$) contained mobile genes conferring resistance to fluoroquinolones [*aac(6′)-Ib-cr*, *qnrB*, *qnrD*, *qnrS*, *oqxA/B*].

Plasmid Replicon Types; Structure of Carbapenemase-Encoding Plasmids in *K. pneumoniae*

Plasmid replicon type profiles (Table S8) were largely variations of FI types [$n=108$; 36% of

all replicons, for example, FIB_K and FIB(pNDM-MAR)], FII ($n=56$; 18.8%; usually FII_K and FII) and HI [$n=32$; 10.7%; predominantly HI1B(pNDM-MAR)]. Structures of 13 plasmids with bla_{NDM-1}, bla_{OXA-48} or bla_{KPC-3} were revealed for 10 long-read-sequenced *K. pneumoniae* isolates of ST395 ($n=5$), ST307 ($n=3$), ST11 ($n=1$) and ST147 ($n=1$) (Tables S1 and S7).

Seven bla_{NDM-1} plasmids included five IncFIB + IncHI1B pNDM-MAR-type molecules [39], one IncFII(pKPX-1) and one IncR. The pNDM-MAR-like plasmids p2468_1 (267,820 bp), p7242_1 (353,328 bp), p8087_1 (346,897 bp), p6772 (345,615 bp) and p1195 (358,699 bp) were identified in three ST395 and two ST307 isolates. These were compared to each other and to the GenBank database, with the largest p7242_1 used as a reference (Figure S3). The comparison revealed high similarity among the five plasmids, and between these and a number of others (coverage and identity, > 99%) identified in *K. pneumoniae* of various STs, for example, in Poland and Germany, of Ukrainian origin, and in Russia. The plasmids combine parts homologous to the MDR plasmid pNDM-MAR [39] with segments of pK2044/KpVP-1, the typical *K. pneumoniae* virulence plasmid [40]. Most of these molecules had a single MDR mosaic region with bla_{NDM-1}, *armA* and several other AMR genes interwoven with mobile elements, as described previously (plasmid p4313_1 [12]). Otherwise, the pK2044/KpVP-1 part includes the virulence loci *iuc*, *rmpA*/*A2*, defining these plasmids as chimeras combining MDR with virulence potential. Two molecules in this study, p2468_1 and p8087_1, lacked the entire virulence segment or *rmpA* only, respectively.

The bla_{NDM-1}-carrying IncFII(pKPX-1) plasmid p8597 (109,464 bp) from an ST11 isolate belonged to a family of plasmids of the *K. pneumoniae* ST11 NDM-1 lineage, spread in Central and Southeastern Europe [41]. p8597 was highly similar (coverage, 91%: identity, 100%) to the family prototype pB3002cz from Czechia [42]. Apart from bla_{NDM-1}, this MDR plasmid has other AMR genes, including $bla_{CTX-M-15}$, *aac(6')-Ib-cr5* and *aac(3)-IIa*. The bla_{NDM-1} IncR plasmid p8287 (46,642 bp) from an ST147 isolate was related (coverage, 85–95%; identity, 99.9–100%) to parts of several multireplicon molecules from *K. pneumoniae* isolates, for example, Norway (pK66-45-1) [43] and Germany, of Ukrainian origin (strain 110,821; plasmid p1) [44]. The latter one, mentioned also above, is an IncFIB(pNDM-MAR) + IncHI1B(pNDM-MAR) + IncR hybrid coding for NDM-1 and OXA-48.

Five plasmids carrying bla_{OXA-48}-like genes, all identified in *K. pneumoniae* ST395 isolates, were assigned to pNDM-MAR-like IncFIB + IncHI1B, IncL and IncR types. The pNDM-MAR-type plasmid p2858 (286,077 bp) was smaller than most of the related bla_{NDM-1} molecules (Figure S4), lacking, for example, the bla_{NDM-1}-containing MDR region. It carried the bla_{OXA-48} plus the *iuc* and *rmpA2* virulence loci. The *iuc* variant in p2858 (AbST95) differed from that in the bla_{NDM-1} pNDM-MAR-like plasmids (AbST63). A set of similar plasmids were identified in GenBank, including a closely related though bla_{OXA-48}/bla_{NDM-1}-negative one from Russia (GenBank accession no. CP125160).

Two bla_{OXA-48} IncL plasmids, p2468_2 and p8087_2 (63,589 bp each), represented the major pOXA-48 lineage [45]. Consistently, these shared high similarity with multiple plasmids in public resources, including one of Ukrainian origin in Poland [12]. Analysis of two IncR-type plasmids with bla_{OXA-48}-like genes showed that p7242_2 (75,176 bp) was very similar to the IncR part of the hybrid plasmid from Germany (strain 110,821; plasmid p1) [44], mentioned twice above. It should be underlined that the pNDM-MAR-like IncFIB + IncHI1B plasmid p7242_1 and p7242_2 resided in a single ST395 K39 isolate; therefore, it is likely that fusion of these two molecules might have produced the triple-replicon hybrid observed in Germany, in an ST395 *K. pneumoniae* from Ukraine [44].

The bla_{KPC-3} plasmid from an ST307 isolate contained an $IncFII_K$ + FIB_K plasmid p4247 (114,865 bp) of the pKpQIL lineage, being highly colinear and homologous to the prototypes from Israel and Italy [46].

Antimicrobial Susceptibility

Resistance patterns of the isolates reflected well their resistomes (Table S9). Only two *E. coli* isolates, including the KPC-3-producing clade B

ST131 (NMI6352_22) and the OXA-48-positive *C. telavivensis* (NMI6180_22), were not MDR, showing just the β-lactamase (carbapenemase)-mediated resistance to β-lactams. All of the isolates were β-lactam-resistant according to their carbapenemase types and ESBL presence/absence, and this also referred to new β-lactamase inhibitor combinations that targeted minorities of the isolates only. Ceftazidime-avibactam was active against all sole KPC- or OXA-48-type producers, whereas imipenem-relebactam and meropenem-vaborbactam had the best activity against KPC producers. Cefiderocol was the most active β-lactam against all isolates, including NDM producers. However, ten NDM-producing isolates repeatedly tested resistant to cefiderocol (MICs, 4–8 mg/l), of which one *E. coli* isolate (NMI3495_22) had the YRIN duplication in the PBP3 protein [47]. Resistance was commonly observed for ciprofloxacin (90.8%), tobramycin (87.7%), co-trimoxazole (83.1%), amikacin (66.2%) and gentamicin (64.6%) but not for colistin (15.4%) or tigecycline (13.8%). All of the ten colistin-resistant isolates had mutations in the *pmrB* gene, and three had additional mutations or disruption of *mgrB* [48].

DISCUSSION

With the escalation of war in Ukraine in February 2022, Europe has experienced the largest refugee crisis since WWII [1]. Considering the apparently broad spread of AMR in Ukraine [6–8], the European Centre for Disease Prevention and Control recommended that European hospitals should screening patients arriving from Ukraine for MDR organisms [49]. Like several European countries providing shelter and health care to war victims [15–18], Poland has recorded CPE and other MDR strains from these patients.

The study sample was epidemiologically random. The patients differed in age and reason for hospitalisation, presented mostly with different medical histories and locations in Ukraine and were distributed all across Poland. Information on the time of culturing CPE in Poland was available only for a fraction of cases; therefore, the origin of some isolates was not confirmed by the clinical data. However, the genomic analysis revealed that, except for four *K. pneumoniae* ST11 NDM-1 isolates, most of the genotypes have not been recorded in Poland so far but were related to Ukrainian CPE from other countries [15–18]. It was assumed that most of the study isolates originated from Ukraine. Like elsewhere [16, 18, 19], a distinct group of CPE-positive patients were children, which has occurred rarely in Poland. Their CPE isolates varied greatly and belonged mainly to lineages prevalent in the sample, as described below, reflecting the difficult AMR/CPE epidemiology in Ukraine. This complexity was also documented by the relatively frequent cases of co-infection/co-colonization with more than one different CPE or a CPE and other MDR organisms.

The sample showed high taxonomic and clonal variety, comprising seven species (with *P. stuartii* [14]) and multiple STs, including *E. coli* ST131 with two non-related isolates, and 15 STs of *K. pneumoniae*. Similar *K. pneumoniae* populations, made largely of ST147, ST307 and ST395, were observed in other European studies on Ukrainian CPE [16–19]. These three STs have been emerging MDR clones, expanding globally with various AMR mechanisms, like CTX-M-type ESBLs or KPC-, NDM-, VIM- or OXA-48-type carbapenemases [50, 51]. Diversity of carbapenemases (and entire resistomes) occurred in multiple STs, including the frequent combination of NDM-1 + OXA-48 types, noticed also in Ukrainian strains in other countries [16–19].

Isolates of the same ST varied also in the SNP analysis, segregating groups, clusters and unique isolates, often with specific genotypes, characterized by carbapenemase and other AMR genes, and in case of *K. pneumoniae* by K serotype and virulence loci as well. Some genotypes, especially of *K. pneumoniae* ST395 or ST307, were represented by several related isolates in the sample and by Ukrainian isolates in Germany and The Netherlands [15–18]. This indicated their broad spread in Ukraine and apparently epidemic potential. Probably most of the patients got infected or colonised with these organisms independently in Ukraine; only in a few cases was the relationship between the two isolates close enough (<20 SNPs) to consider transmission in

a Ukrainian or Polish hospital. The phylogenetic analysis revealed that numerous organisms, including all ST395 and ST307 NDM-1, and some ST147 and ST23, had their closest relatives in Russia, revealing circulation of the specific CPE lineages over a large territory in Eastern Europe. Some others, like ST147 NDM-1/–5 or ST307 KPC-2/–3, were more closely related to organisms from primary endemic regions, like Egypt or India, or the USA or Italy, respectively, suggesting imports. Finally, four ST11 NDM-1 isolates belonged to the lineage circulating since 2012 in Central and Southeastern Europe, with high-level endemicity in Poland [41]. Their presence in the sample might be due to the spread of this organism also in Ukraine or acquisition in Polish hospitals.

A remarkable fraction of *K. pneumoniae* isolates carried the *iuc*, *ybt* and *rmpA/A2* virulence loci, including 20 isolates with the hypervirulence markers *iuc* and *rmpA/A2* together [52]. This has been a notably higher representation of such isolates than in Poland, for example, where most carbapenemase-producing *K. pneumoniae* isolates have had no *iuc* and/or *rmpA/A2* [41, 53]. Higher virulence scores characterised the ST307 NDM-1 group, several ST395 genotypes and all ST23 isolates; similar organisms, also of Ukrainian origin, have been reported elsewhere [16, 17, 54, 55], including relatively often in Russia [56–59]. A distinct group were the ST23 K57 NDM-1 + OXA-48 isolates. ST23, associated with serotype K1 and unambiguous hypervirulence, has been acquiring AMR in recent years, posing a powerful threat to public health [44, 52, 60, 61]. However, the study ST23 K57 isolates, together with their relatives from Russia, Germany and Poland, including some of Ukrainian origin [12, 16–18, 56–58, 62], represent a specific lineage, non-related to ST23 K1, and their virulence potential remains to be assessed [12, 63].

Consistent with the overall diversity, the CPE isolates carried various carbapenemase-encoding plasmids, including the family of pNDM-MAR-type IncFIB + IncHI1B hybrids. These contained not only AMR genes/regions but also virulence loci *iuc* and *rmpA/A2*, and their broad presence, for example, in ST395 and ST307 isolates, explained the high prevalence of virulence loci in the study isolates. Interestingly, two lineages of the pNDM-MAR-like plasmids, marked by different *iuc* variants, and carrying either $bla_{\text{OXA-48}}$ or $bla_{\text{NDM-1}}$, were present in two ST395 K2 isolates. The pNDM-MAR types were found in *K. pneumoniae* in numerous countries, notably often in Central and Southeastern Europe, including isolates of Ukrainian origin and from Russia [12, 44, 59, 64].

CONCLUSIONS

Together with similar studies in other countries [15, 17–19], this work provided a set of high-quality data on CPE in Ukraine that had been not available before the full-scale war. The sample corresponded well to collections of isolates from Ukrainian patients analysed especially in Germany and The Netherlands [15–18]. All these studies revealed a set of specific epidemic *K. pneumoniae* ST395, ST307, ST147 and ST23 lineages producing NDM-1 and/or OXA-48-like enzymes, circulating broadly in Eastern Europe. A remarkable fraction of these, mainly ST307 and ST395, carried pNDM-MAR-like plasmids with not only $bla_{\text{NDM-1}}$ or $bla_{\text{OXA-48}}$ genes but also with *iuc* and *rmpA/A2* virulence loci. The apparently broad spread of these organisms indicates their significant epidemic potential, creating a risk for public health when spread further in Europe.

This work has several limitations, mainly the gaps in relevant epidemiological information, such as patients' medical history in Ukraine or the time when their CPEs were cultured in Polish hospitals. Therefore, the main objective of this study was the detailed microbiological and genomic analysis of the isolates collected. Another limitation was that long-read sequencing was only performed for *K. pneumoniae* isolates, making it impossible to demonstrate interspecies transmissions of plasmids with carbapenemase genes between organisms in single patients. However, our results suggested the possibility of such events, for example, $bla_{\text{KPC-3}}$

between *K. pneumoniae* and *E. coli* or bla_{NDM-1} between *E. coli* and *P. stuartii* (Table S1).

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Waleria Hryniewicz is an Editorial Board member of Infectious Diseases and Therapy. Waleria Hryniewicz was not involved in the selection of peer reviewers for the manuscript or any of the subsequent editorial decisions. Other authors have nothing to disclose.

Ethical Approval. This article does not contain any study on human or animal subjects, material or data. The study was considered to be exempt for approval by a Polish ethical commission since it was an in vitro retrospective study on bacterial isolates cultured during routine medical procedures and collected for epidemiological purposes, not involving patients or their personal data.

REFERENCES

1. World Health Organization (2023) Health of refugees from Ukraine in Poland 2022; household survey and behavioural insights research.

2. Gumułka A (2023) News CwZ: Rok wojny w Ukrainie – ilu pacjentów przyjął polski system? https://www.cowzdrowiu.pl/aktualnosci/post/rok-wojny-w-ukrainie-ilu-pacjentow-przyjal-polski-system. In Polish

3. European Commision (2022) Ukraine: 1,000 Ukrainian patients transferred to European hospitals. https://www.ec.europa.eu/commission/presscorner/detail/en/IP_22_4882. Accessed 10 Sept 2024

4. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55.

5. Patel J, Harant A, Fernandes G, Mwamelo AJ, Hein W, Dekker D, Sridhar D. Measuring the global response to antimicrobial resistance, 2020–21: a systematic governance analysis of 114 countries. Lancet Infect Dis. 2023;23(6):706–18.

6. European Centre for Disease Prevention and Control (2022) Antimicrobial Resistance Surveillance in Europe 2022–2020 data. https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data.

7. European Centre for Disease Prevention and Control, World Health Organization (2023) Antimicrobial resistance surveillance in Europe 2023–2021 data

8. Salmanov A, Shchehlov D, Svyrydiuk O, Bortnik I, Mamonova M, Korniyenko S, Rud V, Artyomenko V, Gudym M, Maliarchuk R, Bondar T. Epidemiology of healthcare-associated infections and mechanisms of antimicrobial resistance of responsible pathogens in Ukraine: a multicentre study. J Hosp Infect. 2023;131:129–38.

9. Salmanov A, Shchehlov D, Artyomenko V, Svyrydiuk O, Maliarchuk R, Bortnik I, Mamonova M, Korniyenko S, Rud V, Gudym M, Shuba V, Loskutov O. Nosocomial transmission of multi-drug-resistant organisms in Ukrainian hospitals: results of a multi-centre study (2019–2021). J Hosp Infect. 2023;132:104–15.

10. Kondratiuk V, Jones BT, Kovalchuk V, Kovalenko I, Ganiuk V, Kondratiuk O, Frantsishko A. Phenotypic and genotypic characterization of antibiotic resistance in military hospital-associated bacteria from war injuries in the Eastern Ukraine conflict between 2014 and 2020. J Hosp Infect. 2021;112:69–76.

11. Izdebski R, Baraniak A, Zabicka D, Machulska M, Urbanowicz P, Fiett J, Literacka E, Bojarska K, Kozinska A, Zieniuk B, Hryniewicz W, Gniadkowski M, Group O-PS. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. J Antimicrob Chemother. 2018;73(3):620–5.

12. Biedrzycka M, Izdebski R, Urbanowicz P, Polanska M, Hryniewicz W, Gniadkowski M, Literacka E. MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009–19. J Antimicrob Chemother. 2022;77(12):3367–75.

13. Loban G, Faustova M, Dobrovolska O, Tkachenko P. War in Ukraine: incursion of antimicrobial resistance. Ir J Med Sci. 2023;192(6):2905–7.

14. Witteveen S, Hans JB, Izdebski R, Hasman H, Samuelsen O, Dortet L, Pfeifer Y, Delappe N, Oteo-Iglesias J, Zabicka D, Cormican M, Sandfort M, Reichert F, Pontinen AK, Fischer MA, Verkaik N, Perez-Vazquez M, Pfennigwerth N, Hammerum AM, Hallstrom S, Biedrzycka M, Raisanen K, Wielders CC, Urbanowicz P, de Haan A, Westmo K, Landman F, van der Heide HG, Lansu S, Zwittink RD, Notermans DW, Guzek A, Kondratiuk V, Salmanov A, Haller S, Linkevicius M, Gatermann S, Kohlenberg A, Gniadkowski M, Werner G, Hendrickx AP. Dissemination of extensively drug-resistant NDM-producing *Providencia stuartii* in Europe linked to patients transferred from Ukraine, March 2022 to March 2023. Euro Surveill. 2024;29(23):2300616.

15. van Almsick VF, Sobkowiak A, Scherff N, Schuler F, Mellmann A, Schwierzeck V. Characterization of bla_{OXA-48}-carrying plasmids and small non-AMR-coding plasmids collected from Ukrainian patients. Infection. 2023;52(2):661–5.

16. Schultze T, Hogardt M, Velazquez ES, Hack D, Besier S, Wichelhaus TA, Rochwalsky U, Kempf VA, Reinheimer C. Molecular surveillance of multidrug-resistant Gram-negative bacteria in Ukrainian patients, Germany, March to June 2022. Euro Surveill. 2023;28(1):2200850.

17. Sandfort M, Hans JB, Fischer MA, Reichert F, Cremanns M, Eisfeld J, Pfeifer Y, Heck A, Eckmanns T, Werner G, Gatermann S, Haller S, Pfennigwerth N. Increase in NDM-1 and NDM-1/OXA-48-producing *Klebsiella pneumoniae* in Germany associated with the war in Ukraine, 2022. Euro Surveill. 2022;27(50):2200926.

18. Zwittink RD, Wielders CC, Notermans DW, Verkaik NJ, Schoffelen AF, Witteveen S, Ganesh VA, de Haan A, Bos J, Bakker J, Schneeberger-van der Linden C, Kuijper EJ, de Greeff SC, Hendrickx

AP, Dutch CPE, Groups MSS. Multidrug-resistant organisms in patients from Ukraine in the Netherlands, March to August 2022. Euro Surveill. 2022;27(50):2200896.

19. Stein C, Zechel M, Spott R, Pletz MW, Kipp F. Multidrug-resistant isolates from Ukrainian patients in a German health facility: a genomic surveillance study focusing on antimicrobial resistance and bacterial relatedness. Infection. 2023;51(6):1731–8.

20. Stolberg RS, Hansen F, Porsbo LJ, Karstensen KT, Roer L, Holzknecht BJ, Hansen KH, Schonning K, Wang M, Justesen US, Roder BL, Thomsen P, Skov MN, Hammerum AM, Hasman H. Genotypic characterisation of carbapenemase-producing organisms obtained in Denmark from patients associated with the war in Ukraine. J Glob Antimicrob Resist. 2023;34:15–7.

21. Mc Gann PT, Lebreton F, Jones BT, Dao HD, Martin MJ, Nelson MJ, Luo T, Wyatt AC, Smedberg JR, Kettlewell JM, Cohee BM, Hawley-Molloy JS, Bennett JW. Six extensively drug-resistant bacteria in an injured soldier. Ukraine Emerg Infect Dis. 2023;29(8):1692–5.

22. Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabak J, Derde LP, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, Mosar Wp WP, groups WPs, Mosar Wp WP, groups WPs. Survey of metallo-β-lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008–11. J Antimicrob Chemother. 2015;70(7):1981–8.

23. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13(6): e1005595.

24. Jain C, Rodriguez RL, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9(1):5114.

25. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018;3:124.

26. Treangen TJ, Ondov BD, Koren S, Phillippy AM. The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol. 2014;15(11):524.

27. Kaas RS, Leekitcharoenphon P, Aarestrup FM, Lund O. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. PLoS ONE. 2014;9(8): e104984.

28. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep. 2021;11(1):12728.

29. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive Web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. J Clin Microbiol. 2018;56(6):e00197-e1118.

30. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. Nat Commun. 2021;12(1):4188.

31. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decre D, Brisse S. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis. 2014;20(11):1812–20.

32. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformat. 2009;10:421.

33. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58(7):3895–903.

34. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykasenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020;75(12):3491–500.

35. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011;27(7):1009–10.

36. Poirel L, Heritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48(1):15–22.

37. Pitout JDD, Finn TJ. The evolutionary puzzle of *Escherichia coli* ST131. Infect Genet Evol. 2020;81: 104265.

38. Blanco J, Mora A, Mamani R, Lopez C, Blanco M, Dahbi G, Herrera A, Marzoa J, Fernandez V, de la Cruz F, Martinez-Martinez L, Alonso MP, Nicolas-Chanoine MH, Johnson JR, Johnston B, Lopez-Cerero L, Pascual A, Rodriguez-Bano J, Spanish Group for Nosocomial I. Four main virotypes among extended-spectrum-β-lactamase-producing isolates of *Escherichia coli* O25b:H4-B2-ST131: bacterial, epidemiological, and clinical characteristics. J Clin Microbiol. 2013;51(10):3358–67.

39. Villa L, Poirel L, Nordmann P, Carta C, Carattoli A. Complete sequencing of an IncH plasmid carrying the bla_{NDM-1}, $bla_{CTX-M-15}$ and *qnrB1* genes. J Antimicrob Chemother. 2012;67(7):1645–50.

40. Lam MMC, Wyres KL, Judd LM, Wick RR, Jenney A, Brisse S, Holt KE. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. Genome Med. 2018;10(1):77.

41. Izdebski R, Sitkiewicz M, Urbanowicz P, Krawczyk M, Brisse S, Gniadkowski M. Genomic background of the *Klebsiella pneumoniae* NDM-1 outbreak in Poland, 2012–18. J Antimicrob Chemother. 2020;75(11):3156–62.

42. Studentova V, Dobiasova H, Hedlova D, Dolejska M, Papagiannitsis CC, Hrabak J. Complete nucleotide sequences of two NDM-1-encoding plasmids from the same sequence type 11 *Klebsiella pneumoniae* strain. Antimicrob Agents Chemother. 2015;59(2):1325–8.

43. Heikal A, Samuelsen O, Kristensen T, Okstad OA (2017) Complete genome sequence of a multidrug-resistant, bla_{NDM-1}-expressing *Klebsiella pneumoniae* K66-45 clinical isolate from Norway. Genome Announc 5(27)

44. Russo TA, Alvarado CL, Davies CJ, Drayer ZJ, Carlino-MacDonald U, Hutson A, Luo TL, Martin MJ, Corey BW, Moser KA, Rasheed JK, Halpin AL, McGann PT, Lebreton F (2024) Differentiation of hypervirulent and classical *Klebsiella pneumoniae* with acquired drug resistance. mBio 15(2):e0286723

45. Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 β-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. Euro Surveill. 2013;18(31):20549.

46. Garcia-Fernandez A, Villa L, Carta C, Venditti C, Giordano A, Venditti M, Mancini C, Carattoli A. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob Agents Chemother. 2012;56(4):2143–5.

47. Le Terrier C, Nordmann P, Buchs C, Poirel L. Effect of modification of penicillin-binding protein 3 on susceptibility to ceftazidime-avibactam, imipenem-relebactam, meropenem-vaborbactam, aztreonam-avibactam, cefepime-taniborbactam, and cefiderocol of *Escherichia coli* strains producing broad-spectrum β-lactamases. Antimicrob Agents Chemother. 2024;68(4): e0154823.

48. Baron S, Hadjadj L, Rolain JM, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents. 2016;48(6):583–91.

49. European Centre for Disease Prevention and Control (2022) Operational public health considerations for the prevention and control of infectious diseases in the context of Russia's aggression towards Ukraine. 8 March 2022. Stockholm. https://www.ecdc.europa.eu/en/publications-data/operational-public-health-considerations-prevention-and-control-infectious.

50. Peirano G, Chen L, Kreiswirth BN, Pitout JDD. Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. Antimicrob Agents Chemother. 2020;64(10):e01148-e1220.

51. Shamina OV, Kryzhanovskaya OA, Lazareva AV, Alyabieva NM, Polikarpova SV, Karaseva OV, Mayanskiy NA. Emergence of a ST307 clone carrying a novel insertion element MITEKpn1 in the *mgrB* gene among carbapenem-resistant *Klebsiella pneumoniae* from Moscow, Russia. Int J Antimicrob Agents. 2020;55(2): 105850.

52. Russo TA, Marr CM. Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev. 2019;32(3):e00001-00019.

53. Biedrzycka M, Urbanowicz P, Guzek A, Brisse S, Gniadkowski M, Izdebski R. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015–19. J Antimicrob Chemother. 2021;76(10):2538–45.

54. Heiden SE, Hubner NO, Bohnert JA, Heidecke CD, Kramer A, Balau V, Gierer W, Schaefer S, Eckmanns T, Gatermann S, Eger E, Guenther S, Becker K, Schaufler K. A *Klebsiella pneumoniae* ST307 outbreak clone from Germany demonstrates features of extensive drug resistance, hypermucoviscosity, and enhanced iron acquisition. Genome Med. 2020;12(1):113.

55. Tavoschi L, Forni S, Porretta A, Righi L, Pieralli F, Menichetti F, Falcone M, Gemignani G, Sani S,

Vivani P, Bellandi T, Tacconi D, Turini L, Toccafondi G, Privitera G, Lopalco P, Baggiani A, Gemmi F, Luchini G, Petrillo M, Roti L, Pezzotti P, Pantosti A, Iannazzo S, Mechi MT, Rossolini GM, On Behalf Of The Tuscan Clinical Microbiology Laboratory N. Prolonged outbreak of New Delhi metallo-β-lactamase-producing carbapenem-resistant *Enterobacterales* (NDM-CRE), Tuscany, Italy, 2018 to 2019. Euro Surveill. 2020;25(6):2000085.

56. Fursova NK, Astashkin EI, Ershova ON, Aleksandrova IA, Savin IA, Novikova TS, Fedyukina GN, Kislichkina AA, Fursov MV, Kuzina ES, Biketov SF, Dyatlov IA. Multidrug-resistant *Klebsiella pneumoniae* causing severe infections in the neuro-ICU. Antibiotics. 2021;10(8):979.

57. Shaidullina E, Shelenkov A, Yanushevich Y, Mikhaylova Y, Shagin D, Alexandrova I, Ershova O, Akimkin V, Kozlov R, Edelstein M. Antimicrobial resistance and genomic characterization of OXA-48- and CTX-M-15-co-producing hypervirulent *Klebsiella pneumoniae* ST23 recovered from nosocomial outbreak. Antibiotics. 2020;9(12):862.

58. Lev AI, Astashkin EI, Kislichkina AA, Solovieva EV, Kombarova TI, Korobova OV, Ershova ON, Alexandrova IA, Malikov VE, Bogun AG, Borzilov AI, Volozhantsev NV, Svetoch EA, Fursova NK. Comparative analysis of *Klebsiella pneumoniae* strains isolated in 2012–2016 that differ by antibiotic resistance genes and virulence genes profiles. Pathog Glob Health. 2018;112(3):142–51.

59. Starkova P, Lazareva I, Avdeeva A, Sulian O, Likholetova D, Ageevets V, Lebedeva M, Gostev V, Sopova J, Sidorenko S. Emergence of hybrid resistance and virulence plasmids harboring New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* in Russia. Antibiotics. 2021;10(6):691.

60. European Centre for Disease Prevention and Control (2024) Risk Assessment: emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries—first update. https://www.ecdc.europa.eu/en/publications-data/risk-assessment-emergence-hypervirulent-klebsiella-pneumoniae-eu-eea.

61. Lam MMC, Wyres KL, Duchene S, Wick RR, Judd LM, Gan YH, Hoh CH, Archuleta S, Molton JS, Kalimuddin S, Koh TH, Passet V, Brisse S, Holt KE. Population genomics of hypervirulent *Klebsiella pneumoniae* clonal-group 23 reveals early emergence and rapid global dissemination. Nat Commun. 2018;9(1):2703.

62. Schwanbeck J, Bohne W, Hasdemir U, Gross U, Pfeifer Y, Bunk B, Riedel T, Sproer C, Overmann J, Frickmann H, Zautner AE. Detection of a new resistance-mediating plasmid chimera in a bla_{OXA-48}-positive *Klebsiella pneumoniae* strain at a German University Hospital. Microorganisms. 2021;9(4):720.

63. Lam MMC, Holt KE, Wyres KL. Comment on: MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009–19. J Antimicrob Chemother. 2023;78(4):1132–4.

64. Chudejova K, Kraftova L, Mattioni Marchetti V, Hrabak J, Papagiannitsis CC, Bitar I. Genetic plurality of OXA/NDM-encoding features characterized from Enterobacterales recovered from Czech hospitals. Front Microbiol. 2021;12: 641415.

Carbapenemase-producing Enterobacterales from patients arriving from Ukraine in Poland, March 2022-February 2023

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Table S1. Patients from whom more than one carbapenemase-producing Enterobacterales (CPE) or other multi-drug-resistant (MDR) isolate were recovered

Patient ID	Specimen	Isolate	Species	ST	Carbapenemase/main AMR mechanism	Possible transmission of a carbapenemase gene[a]
Patient 1	urine	9040/22	*K. pneumoniae*	ST395	NDM-1	NA
	rectal swab	9036/22	*E. coli*	ST46	NDM-5	
Patient 2	wound	10440/22	*K. pneumoniae*	ST307	NDM-1	NA
	wound	10441/22	*K. pneumoniae*	ST512	KPC-3	
Patient 3	wound	1197/23	*K. pneumoniae*	ST307	NDM-1	no
	rectal swab	1198/23	*K. pneumoniae*	ST23	NDM-1+OXA-48	
Patient 4	rectal swab	4247/23	*K. pneumoniae*	ST307	KPC-3	yes
	stool	5058/22	*E. coli*	ST224	KPC-3	
Patient 5	wound	6228/22	*K. pneumoniae*	ST23	NDM-1+OXA-48	no
	wound	5220/22	*E. coli*	ST131	NDM-1	yes[b]
	wound	5219/22	*P. stuartii*[c]	ST23	NDM-1	
Patient 6	wound	5016/22	*K. pneumoniae*	ST45	KPC-3	yes
	nasal sinus aspirate	4020/22	*C. portucalensis*	ST728	KPC-3	
Patient 7	rectal swab	6914/24	*K. pneumoniae*	ST152	KPC-2	NA
	rectal swab	6179/22	*E. coli*	ST167	OXA-48	yes[d]
	rectal swab	6180/22	*C. telavivensis*	ST1282	OXA-48	
	urine	6178/22	*P. stuartii*[c]	ST11	NDM-5	NA
Patient 8	blood	2564/22	*K. pneumoniae*	ST147	NDM-1+OXA-48	NA
	blood	3158/22	*P. aeruginosa*[e]	-	VIM	
Patient 9	blood	5378/22	*K. pneumoniae*	ST395	OXA-1242	NA
	blood	5637/22	*E. faecium*[e]	-	VanA	
Patient 10	wound	9856/22	*P. mirabilis*	ST269	NDM-1	NA
	wound	7946/22	*P. aeruginosa*[e]	-	NDM	

[a] – possibility of transmission of a carbapenemase gene was based on the identity of the gene, its genetic context, and the presence of the same plasmid replicons in the two isolates; NA, non-applicable.

[b] – transmission of the $bla_{\text{NDM-1}}$ gene only between the *E. coli* and *P. stuartii* isolates was possible.

[c] – the *P. stuartii* isolates were described in a separate, international report [1]; however, their STs were determined by PubMLST [2] in this study.

[d] – only the transmission of the $bla_{\text{OXA-48}}$ gene only between the *E. coli* and *C. telavivensis* isolates was possible.

[e] – the *P. aeruginosa* and *E. faecium* isolates were not subjected to WGS.

Table S2. Resistomes [acquired antimicrobial resistance (AMR) genes only] in the carbapenemase-producing Enterobacterales (CPE) isolates from patients arriving from Ukraine

Isolate[a]	Species[a]	ST[a]	Carbapenemase gene context[a,c]	β-Lactams[b]	Aminoglycosides[b]	Fluoroquinolones[b,e]	Phenicols	Sulphonamides[b]	Trimethoprim	Tetracycline	Rifampin	Fosfamycin[b]
2468/22	*Kpn*	ST395	Tn*125*K, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, $bla_{TEM-257}$[f]	*aac(6')-Ib-cr5*, *aph(3'')-VI*, *armA*	*aac(6')-Ib-cr5*, *qnrS1*(x2)	*catA1*	*sul1*, *sul2*	*dfrA5*	-	-	-
6991/22	*Kpn*	ST395	Tn*125*K, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, $bla_{TEM-257}$[f]	*aac(6')-Ib-cr5*, *aph(3')-VI*, *armA*	*aac(6')-Ib-cr5*,*qnrS1*	*catA1*	*sul1*, *sul2*	*dfrA5*	-	-	-
9040/22	*Kpn*	ST395	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, $bla_{TEM-257}$[f]	*aac(6')-Ib-cr5*, *aph(3')-VI*, *armA*	*aac(6')-Ib-cr5*,*qnrS1*	*catA1*	*sul1*, *sul2*	*dfrA5*	-	-	-
2858/22	*Kpn*	ST395	Tn*1999.2*	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{IYA-48}(x2)	*aac(6')-Ib-cr5*	*aac(6')-Ib-cr5*, *qnrS1*	-	-	-	-	-	-
10443/22	*Kpn*	ST395	Tn*1999*[a]	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib*	*qnrS1*	*catA1*	*sul1*	*dfrA1*	*tet(A)*	-	-
4263/22	*Kpn*	ST395	Tn*1999.2*	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib*	*qnrS1*	*catA1*	*sul1*	*dfrA1*	*tet(A)*	-	-
5378/22	*Kpn*	ST395	Tn*1999.2*-like	$bla_{CTX-M-15}$, bla_{OXA-1}, $bla_{IYA-176}$[c]	*aac(6')-Ib-cr5*	-	-	*sul1*	*dfrA1*	*tet(A)*	-	-
7242/22	*Kpn*	ST395	Tn*125*K,Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aph(3')-VI*, *aadA1*, *armA*, *ant(2'')-Ia*	*aac(6')-Ib-cr5*, *qnrS1*	*catA1*	*sul1*(x3), *sul2*	*dfrA1*, *dfrA5*	*tet(A)*	-	-
3420/22	*Kpn*	ST395	Tn*125*K,Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aph(3')-VI*, *ant(2'')-Ia*, *aadA1*, *armA*	*aac(6')-Ib-cr5*	*catA1*	*sul1*, *sul2*	*dfrA1*, *dfrA5*	*tet(A)*	-	-
8087/22	*Kpn*	ST395	Tn*125*K, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{IYA-48}, bla_{IYA-1}	*aac(6')-Ib-cr5*, *aac(3)-IIe*, *aph(3')-VI*, *armA*	*aac(6')-Ib-cr5*, *qnrS1*(x2)	*catA1*	*sul1*(x2), *sul2*	*dfrA1*, *dfrA5*	*tet(A)*	-	-
7025/22	*Kpn*	ST395	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{TEM-1}	*aac(3)-IIe*, *aac(6')-Ib-cr5*, *aph(3')-VI*, *armA*	*aac(6')-Ib-cr5*, *qnrS1*	*catA1*	*sul1*, *sul2*	*dfrA1*, *dfrA5*	*tet(A)*	-	-
302/23	*Kpn*	ST395	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{TEM-1}	*aac(3)-IIe*, *aac(6')-Ib-cr5*, *aph(3')-VI*	*aac(6')-Ib-cr5*, *qnrS1*	*catA1*	*sul1*	*dfrA1*,*dfrA5*	*tet(A)*	-	-
10423/22	*Kpn*	ST395	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{IYA-1}	*aac(6')-Ib-cr5*, *aac(3)-IIe*, *aph(3')-VI*	*aac(6')-Ib-cr5*, *qnrS1*	*catA1*	*sul1*	*dfrA1*, *dfrA5*	*tet(A)*	-	-
3635/22	*Kpn*	ST395	Tn*125*R	$bla_{CTX-M-15}$, bla_{NDM-5}, bla_{OXA-1}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aac(3)-IId*, *aadA2*, *rmtB1*	*aac(6')-Ib-cr5*, *qnrB1*	*catA1*	*sul1*	*dfrA12*	-	-	-
10440/22	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{TEM-1}	*aac(6')-Ib*, *aph(3'')-Ib*, *aph(6)-Id*, *aph(3')-VI*, *armA*, *rmtF1*	*qnrB1*, *qnrS1*	*catB*,	*sul1*, *sul2*	*dfrA5*	-	*arr-2*	-
1197/23	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{IYA-1}	*aac(3)-IIe*, *aac(6')-Ib*, *aph(3')-VI*, *aph(3'')-Ib*, *aph(6)-Id*, *rmtF1*	*qnrB1*, *qnrS1*	*catB*,	*sul1*, *sul2*	*dfrA5*, *dfrA14*	-	*arr-2*	-
5015/22	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{TEM-1}	*aac(6')-Ib-like*, *aph(3'')Ib*, *aph(6)-Id*, *aph(3')-VI*, *rmtE*	*qnrB1*, *qnrS1*	-	*sul1*, *sul2*	*dfrA5*, *dfrA14*	-	-	-
6772/22	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aph(3')-VI*, *aph(3'')-Ib*, *aph(6)-Id*, *armA*	*aac(6')-Ib-cr5*, *qnrB1*, *qnrS1*	-	*sul1*, *sul2*(x2)	*dfrA5*, *dfrA14*	*tet(A)*	-	-
2814/22	*Kpn*	ST307	Tn*125*K	bla_{NDM-5}	*aph(3')-VI*, *armA*	*qnrS1*	-	*sul1*, *sul2*	*dfrA5*	-	-	-
9816/22	*Kpn*	ST307	Tn*125*K, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aph(3'')-Ib*, *aph(3')-VI*, *aph(6)-Id*, *armA*	*aac(6')-Ib-cr5*, *qnrB1*, *qnrS1*	-	*sul1*, *sul2*	*dfrA5*, *dfrA14*	*tet(A)*	-	-
1195/23	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{IYA-1}	*aac(6')-Ib-cr5*, *aph(3')-VI*, *aph(3'')-Ib*, *aph(6)-Id*, *armA*	*aac(6')-Ib-cr5*, *qnrB1*, *qnrS1*	-	*sul1*, *sul2*	*dfrA5*, *dfrA14*	*tet(A)*	-	-
1196/23	*Kpn*	ST307	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-9}, bla_{TEM-1}	*aac(6')-Ib*, *aph(3')-VI*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA1*, *armA*	*qnrS1*	-	*sul1*, *sul2*	*dfrA5*, *dfrA14*	*tet(A)*	-	-
4247/22	*Kpn*	ST307	Tn*4401a*	$bla_{CTX-M-15}$(x2), bla_{KPC-3}, bla_{TEM-1}(x3)	*aph(3'')-Ib*, *aph(6)-Id*, *aadA5*, *armA*	*qnrB1*	-	*sul1*, *sul2*	*dfrA14*, *dfrA17*	-	-	-
5059/22	*Kpn*	ST307	Tn*4401a*	$bla_{CTX-M-15}$, bla_{IYA-1}, bla_{TEM-1}	*aph(3'')-Ib*, *aph(6)-Id*	*qnrB1*	-	*sul2*	*dfrA14*	*tet(A)*	-	-
10100/22	*Kpn*	ST307	Tn*4401a*	bla_{KPC-3}, bla_{OXA-1}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aac(3)-IIe*, *aph(3'')-Ib*, *aph(6)-Id*	*aac(6')-Ib-cr5*, *qnrB1*	-	*sul2*	*dfrA14*	-	-	-
8597/22	*Kpn*	ST11	ΔTn*125*A	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}	*aac(6')-Ib*, *aac(6')-Ib-cr5*, *aac(3)-IIa*	*aac(6')-Ib-cr5*	-	-	*dfrA14*	-	-	-
6741/22	*Kpn*	ST11	ΔTn*125*A	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}	*aac(6')-Ib*, *aac(6')-Ib-cr5*	*aac(6')-Ib-cr5*	-	-	*dfrA14*	-	-	-
6912/22	*Kpn*	ST11	Tn*125*A	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}	*aadA1*	-	*catA1*	*sul1*	*dfrA1*	-	-	-
10432/22	*Kpn*	ST11	Tn*125*A	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}	*aac(3)-IIe*	-	-	-	*dfrA14*	-	-	-
2466/22	*Kpn*	ST11	Tn*1999.2*	$bla_{CTX-M-15}$, bla_{OXA-48}	*aac(6')-Ib*, *aph(3')-Ia*,*rmtF1*	*qnrB1*	*catB*,	*sul1*, *sul2*	*dfrA12*	-	*arr-2*	-
5008/22	*Kpn*	ST11	Tn*4401a*	bla_{KPC-2}, bla_{TEM-1}	*aac(6')-Ib*, *rmtF1*	-	*catB*,	-	*dfrA12*	-	-	-
8287/22	*Kpn*	ST147	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-9}, bla_{TEM-1}	*aac(6')-Ib*, *aac(6')-Ib-cr5*, *aph(3')-VI*, *aadA1*, *aadA2*	*qnrS1*	*catA2*, *catB3*	*sul1*(x2)	*dfrA12*	-	*arr-3*	-
9702/22	*Kpn*	ST147	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{IYA-9}, bla_{TEM-1}	*aac(6')-Ib*, *aph(3')-VI*, *aadA1*	*qnrS1*	*catB3*	*sul1*	-	-	*arr-3*	-
2674/22	*Kpn*	ST147	ΔTn*125*K	$bla_{CTX-M-15}$, bla_{NDM-5}, bla_{OXA-1}, bla_{TEM-1}	*aph(3')-Ia*, *aph(3')-VI*, *armA*, *aadA1*	*qnrS1*	*catB3*	*sul1*, *sul2*	*dfrA5*	-	*arr-3*	-
2564/22	*Kpn*	ST147	Tn125M, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, bla_{OXA-48}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *rmtC*	*aac(6')-Ib-cr5*	*catB3*	*sul1*	-	-	-	-
10718/22	*Kpn*	ST147	Tn*125*R	bla_{NDM-5}, bla_{IYA-1}	*aadA1*,*aadA2*, *armA*, *rmtB1*	*qnrB1*	*cmlA5*	*sul1*	*dfrA12*	-	*arr-2*	-
2727/22	*Kpn*	ST23	Tn*125*K, Tn*1999.2*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-48}	*aac(3)-IIe*, *aac(6')-Ib*, *aph(3')-VI*, *rmtF1*	*qnrB1*, *qnrS1*	*catA1*	*sul1*, *sul2*	*dfrA5*	-	*arr-2*	-
6228/22	*Kpn*	ST23	Tn*125*K, Tn*1999*[e]	$bla_{CTX-M-55}$, bla_{NDM-1}, bla_{OXA-48}	*aac(3)-IIa*, *aph(3')-VI*, *armA*	*qnrS1*	-	*sul1*, *sul2*	*dfrA5*	-	-	-
1198/23	*Kpn*	ST23	Tn*125*K, Tn*1999*[e]	$bla_{CTX-M-55}$, bla_{NDM-5}, bla_{IYA-1}, bla_{OXA-48}	*aac(6')-Ib*, *aac(6')-Ib-cr5*, *aph(3')-VI*, *armA*, *rmtF1*	*aac(6')-Ib-cr5*, *qnrB1*, *qnrS1*	*catA1*, *catB*,	*sul1*, *sul2*	*dfrA5*	-	*arr-2*	-
2369/22	*Kpn*	ST15	Tn*4401a*	bla_{KPC-3}, bla_{TEM-1}	*aac(3)-IIe*, *aac(6')-Ib*, *aadA1*	-	-	-	*dfrA14*	-	-	-
2662/22	*Kpn*	ST15	Tn*4401a*	bla_{KPC-3}, bla_{TEM-1}	*aac(6')-Ib*, *aac(3)-IIe*, *aadA1*	-	-	-	*dfrA14*	-	-	-
2821/22	*Kpn*	ST14	Tn*4401a*	$bla_{CTX-M-15}$, bla_{KPC-3}, bla_{OXA-1}, bla_{TEM-1}	*aac(6')-Ib-cr5*	*aac(6')-Ib-cr5*, *qnrB1*	-	-	*dfrA14*	-	-	-
2673/22	*Kpn*	ST17	Tn*125*F	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, $bla_{TEM-255}$[f]	*aac(3)-IId*, *aac(3)-IIe*, *aac(6')-Ib*, *aac(6')-Ib-cr5*, *rmtF1*, *aadA1*	*aac(6')-Ib-cr5*	*catB*, *catB3*	*sul1*	*dfrA15*	-	*arr-2*	-
8289/22	*Kpn*	ST39	Tn*4401a*	$bla_{CTX-M-15}$, bla_{IMP-1}, bla_{IYA-1}, bla_{TEM-1}	*aac(6')-Ib-cr5*, *ant(2'')-Ia*, *aadA1*	*aac(6')-Ib-cr5*,*qnrS1*	*catA1*	*sul1*	-	-	-	-
5016/22	*Kpn*	ST45	Tn*4401a*	$bla_{CTX-M-15}$, bla_{KPC-3}, bla_{TEM-1}	*aph(3'')Ib*, *aph(6)-Id*	*qnrB1*	-	*sul2*	*dfrA14*	*tet(A)*	-	-
6914/24	*Kpn*	ST152	non-Tn*4401*[f]	$bla_{CTX-M-15}$, bla_{KPC-2}, bla_{OXA-1}, $bla_{TEM-256}$[f]	*aac(6')-Ib-cr5*, *aph(3')Ia*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA16*	*aac(6')-Ib-cr5*	*catA1*	*sul1*, *sul2*	*dfrA27*	-	*arr-3*	-
5010/22	*Kpn*	ST219	Tn*4401a*	$bla_{CTX-M-15}$, bla_{IMP-1}, bla_{TEM-1}	*aph(3'')-Ib*, *aph(6)-Id*, *aadA2*	*qnrS1*	-	*sul1*, *sul2*	*dfrA12*	-	-	-
5012/22	*Kpn*	ST253	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}	*aph(3')-VI*, *armA*	*qnrS1*	-	*sul1*, *sul2*	*dfrA5*	-	-	-
10441/22	*Kpn*	ST512	Tn*4401a*	bla_{KPC-3}	*aac(6')-Ib*, *aph(3')-Ia*, *aadA2*	-	*catA1*	*sul1*	*dfrA12*	-	-	-
5018/22	*Kpn*	ST5859	Tn*125*K, Tn*2013*	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}, $bla_{IYA-737}$	*aac(6')-Ib-cr5*, *aph(3')-VI*	*aac(6')-Ib-cr5*, *qnrB1*,*qnrS1*	*catA1*	*sul1*, *sul2*	*dfrA5*	-	-	-
3494/22	*Eco*	ST46	Tn*125*R	$bla_{CTX-M-15}$, bla_{NDM-5}	*aadA2*	-	-	*sul1*	*dfrA12*	-	-	-
3495/22	*Eco*	ST46	Tn*125*R	$bla_{CTX-M-15}$, bla_{NDM-5}	*aadA2*	-	-	*sul1*	*dfrA12*	-	-	-
9036/22	*Eco*	ST46	Tn*125*R	$bla_{CTX-M-15}$, bla_{NDM-5}, bla_{TEM-1}	*aadA2*, *rmtB1*	-	-	*sul1*	*dfrA12*	*tet(A)*	-	-
5220/22	*Eco*	ST131	Tn*125*J	$bla_{CTX-M-27}$, bla_{CMY-16}, bla_{NDM-1}, bla_{OXA-10}	*aac(6')-Ib3*, *aph(3'')-Ib*, *aph(6)-Id*, *aadA1*, *aadA5*, *armA*, *rmtC*	-	*cmlA5*, *floR*	*sul1*, *sul2*	*dfrA17*	-	*arr-2-like*	-
6352/22	*Eco*	ST131	Tn*4401a*	bla_{KPC-3}, bla_{TEM-1}	-	-	-	-	-	-	-	-
6179/22	*Eco*	ST167	Tn*1999.2*	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{IYA-48}, bla_{TEM-1}	*aac(3)-IId*, *aac(6')-Ib-cr5*, *aadA5*	*aac(6')-Ib-cr5*	*catA1*	*sul1*	*dfrA17*	*tet(A)*	-	-
5058/22	*Eco*	ST224	Tn*4401a*	$bla_{CTX-M-15}$, bla_{KPC-3}, bla_{TEM-1}	*aph(6)-Id*, *aadA5*, *armA*	-	-	*sul1*, *sul2*	*dfrA14*, *dfrA17*	-	-	-
6750/22	*Ehe*	ST91	Tn*4401b*	bla_{KPC-3}	*aac(6')-Ib-cr5*, *aph(3')-Ia*, *aadA16*	*aac(6')-Ib-cr5*, *qnrB6*	-	*sul1*	*dfrA27*	*tet(A)*	-	*fosA*-like
9723/22	*Ehe*	ST91	In*916*	bla_{OXA-1}	*aac(6')-Ib4*, *aph(3')-VIa*, *aph(3')-XV*, *aph(3'')-Ib*, *aph(6)-Id*, *ant(2'')-Ia*, *aadA1*	*qnrS1*	*catB2*	*sul1*, *sul2*	*dfrA14*	-	-	-
3657/22	*Ehe*	ST231	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-9}, bla_{TEM-1}	*aph(3'')-Ib*, *aph(3')-VI*, *aph(6)-Id*, *aadA1*	*qnrS1*, *oqxA*, *oqxB*,	*catA2*	*sul2*	*dfrA14*	*tet(A)*	-	*fosA*-like
8875/22	*Ehe*	ST231	Tn*125*K	$bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-9}, bla_{TEM-1}	*aac(6')-Ib*, *aph(3'')-Ib*, *aph(3')-VI*, *aph(6)-Id*, *aadA1*	*oqxA*-like, *oqxB*-like *qnrB7*, *qnrS1*	-	*sul1*, *sul2*	*dfrA5*, *dfrA14*	-	-	*fosA*-like
6990/22	*Ehe*	ST182	Tn*1999.2*	$bla_{CTX-M-15}$, $bla_{IYA-2ab}$, bla_{TEM-1}	*aac(6')-Ib-cr5*, *aac(3)-IIe*, *aph(6)-Id*, *aph(3'')-Ib*, *aadA1*	*aac(6')-Ib-cr5*, *qnrB1*, *oqxA*-like, *oqxB*-like	-	*sul2*	*dfrA14*	*tet(A)*	-	*fosA*-like
4020/22	*Cpn*	ST728	Tn*4401a*	$bla_{CTX-M-3}$, bla_{CMY-30}, bla_{KPC-3}, bla_{TEM-1}	*aadA5*, *armA*	*qnrB1*	-	*sul1*	*dfrA14*, *dfrA17*	-	-	-
6180/22	*Cfe*	ST1282	Tn*1999.2*	bla_{IYA-48}	-	*oqxB*,	-	-	-	-	-	-
9856/22	*Psyl*	ST269	Tn*125*G	$bla_{CTX-M-55}$, bla_{NDM-1}, bla_{TEM-1}	*aac(3)-IIe*, *aph(3')-Ia*, *aph(3'')-Ib*, *aph(6)-Id*,*aadA1*, *armA*, *sat2*	*qnrD1*	*catA1*	*sul1*, *sul2*	*dfrA1*	*tet(J)*	-	-

[a] – isolates selected to long-read sequencing are indicated in bold; total numbers of acquired AMR genes in these isolates are based on long-read sequencing

[b] – *Kpn, Klebsiella pneumoniae*; *Eco*, *Escherichia coli*; *Eho*, *Enterobacter hormaechei*; *Cpo*, *Citrobacter portucalensis*; *Cte*, *Citrobacter telavivensis*; *Pmi*, *Proteus mirabilis*.
[c] – ST, sequence type

[d] – Tn*1999*, sequence assembly has not allowed to distinguish between Tn*1999.1* and Tn*1999.2* in these isolates.

[e] – the nomenclature of Tn*125*-like elements is specific and refers to the structures identified in NDM-producing Enterobacterales in Poland [3, 4].
[f] – "x2" and "x3" mean two or three copies of a gene, respectively.
[g] – according to the aim of this analysis, only the acquired AMR genes are shown; therefore, the AMR genes intrinsic in a given species, *e.g.* *bla*$_{SHV}$, *oqxA/B* or *fosA* in *K. pneumoniae* have not been shown.
[h] – *aac(6')-Ib-cr* shown in the 'Aminoglycosides' and 'Fluoroquinolones' columns is the same gene.
[i] – this *bla*$_{KPC-2}$-carrying structure, containing a remnant of Tn*4401*, was identical to those identified in a series of CPE from China (*e.g.* GenBank acc. No. CP047966.1)
[j] –new *bla*$_{TEM}$ gene variants identified in this study: *bla*$_{TEM-255}$, *bla*$_{TEM-256}$ and *bla*$_{TEM-257}$ (GenBank accession numbers: PQ284151-53)

Table S3. K and O serotypes, and sequence types (STs) of the *ybt* (YbSTs), *iuc* (AbSTs), *rmpA* (RmSTs) and *rmpA2* alleles in the *K. pneumoniae* isolates, determined by Kaptive [5] and Kleborate [6]

Isolate	ST	K serotype	O serotype	YbST[a]	AbST[b]	RmST	*rmpA2* allele
2468/22	ST395	K2	O1v1	**604**	-	-	-
6991/22	ST395	K2	O1v1	**604**	63	-	66
9040/22	ST395	K2	O1v1	-	63	147	9
2858/22	ST395	K2	O1v1	-	95	-	28
10443/22	ST395	K2	O1v1	-	95	-	-
4263/22	ST395	K2	O1v1	-	95	-	-
5378/22	ST395	K2	O1v1	**604**	-	-	-
7242/22	ST395	K39	O1v1	**604**	63	147	28
3420/22	ST395	K39	O1v1	**604**	63	147	28
8087/22	ST395	KL108	O1v2	-	63	-	28
7025/22	ST395	KL108	O1v2	-	-	-	28
302/23	ST395	KL108	O1v2	-	**101**	-	28
10423/22	ST395	KL108	O1v2	-	-	-	28
3635/22	ST395	K64	O1v1	280	-	-	-
10440/22	ST307	KL102	O2v2	384	63	147	28
1197/23	ST307	KL102	O2v2	384	63	147	28
5015/22	ST307	KL102	O2v2	384	63	-	28
6772/22	ST307	KL102	O2v2	384	63	147	28
2814/22	ST307	KL102	O2v2	384	63	147	28
9816/22	ST307	KL102	O2v2	384	63	147	28
1195/23	ST307	KL102	O2v2	384	63	147	28
1196/23	ST307	KL102	O2v2	384	63	147	28
4247/22	ST307	KL102	O2v2	**606**	-	-	-
5059/22	ST307	KL102	O2v2	-	-	-	-
10100/22	ST307	KL102	O2v2	**601**	-	-	-
8597/22	ST11	K24	O2v1	230	-	-	-
6741/22	ST11	K24	O2v1	230	-	-	-
6912/22	ST11	K24	O2v1	230	-	-	-
10432/22	ST11	K24	O2v1	230	-	-	-
2466/22	ST11	K15	O4	**603**	**102**	147	28
5008/22	ST11	K15	O4	183	-	-	-
8287/22	ST147	K64	O2v1	**605**	-	-	-
9702/22	ST147	K64	O2v1	**607**	-	-	-
2674/22	ST147	K64	O2v1	**605**	**103**	147	-
2564/22	ST147	K64	O2v1	-	-	-	-
10718/22	ST147	K10	O3/O3a	-	-	-	-
2727/22	ST23	K57	O2v2	**202 2LV**	1	-	27
6228/22	ST23	K57	O2v2	**202 1LV**	63	-	6
1198/23	ST23	K57	O2v2	**202 1LV**	63	-	66
2369/22	ST15	KL112	O1v1	**602**	-	-	-
2662/22	ST15	KL112	O1v1	**602**	-	-	-
2821/22	ST14	K2	O1v1	**606**	-	-	-
2673/22	ST17	K55	O1v2	-	-	-	-
8289/22	ST39	K23	O1v1	151	95	-	-
5016/22	ST45	K52	OL101	80	-	-	-
6914/24	ST152	KL149	O4	325	-	-	-
5010/22	ST219	KL114	O1v1	-	-	-	-
5012/22	ST253	K39	O1v1	-	-	-	-
10441/22	ST512	KL107	O2v2	-	-	-	-
5018/22	ST5859	K64	O1v1	-	-	-	28

[a] – new YbST profiles identified in this study are indicated in bold style; "202 1LV" and "202 2LV" mean one- and two-locus variants of YbST202

[b] – new AbST profiles identified in this study are indicated in bold style

Table S4. Single-nucleotide polymorphism (SNP) scores between the study *K. pneumoniae* isolates within individual STs

A. ST395 isolates

Isolate	n SNPs	K serotype	Carbapenemase
2468/22[a]	0	K2	NDM-1, OXA-48
9040/22	30	K2	NDM-1
6991/22	40	K2	NDM-1, OXA-48
2858/22	169	K2	OXA-48
10443/22	285	K2	OXA-48
4263/22	288	K2	OXA-48
5378/22	302	K2	OXA-1242
7242/22	510	K39	NDM-1, OXA-48
3420/22	525	K39	NDM-1, OXA-48
8087/22	1295	KL108	NDM-1, OXA-48
302/23	1298	KL108	NDM-1
10423/22	1300	KL108	NDM-1
7025/22	1301	KL108	NDM-1
3635/22	3207	K64	NDM-5

[a] – reference isolate.

B. ST307 isolates

Isolate	n SNPs	K serotype	Carbapenemase
2814/22[a]	0	KL102	NDM-1
6772/22	15	KL102	NDM-1
9816/22	82	KL102	NDM-1, OXA-48
1195/23	91	KL102	NDM-1
1196/23	95	KL102	NDM-1
1197/23	115	KL102	NDM-1
10440/22	118	KL102	NDM-1
5015/22	122	KL102	NDM-1
4247/22	150	KL102	KPC-3
10100/22	167	KL102	KPC-2
5059/22	174	KL102	KPC-3

[a] – reference isolate.

C. ST11 isolates

Isolate	n SNPs	K serotype	Carbapenemase
5008/22[a]	0	K15	KPC-2
2466/22	106	K15	OXA-48
8597/22	3492	K24	NDM-1
10432/22	3515	K24	NDM-1
6912/22	3516	K24	NDM-1
6741/22	3524	K24	NDM-1

[a] – reference isolate.

D. ST147 isolates

Isolate	n SNPs	K serotype	Carbapenemase
2564/22[a]	0	K64	NDM-1, OXA-48
8287/22	272	K64	NDM-1
2674/22	314	K64	NDM-5
9702/22	334	K64	NDM-1
10718/22	1504	K10	NDM-5

[a] – reference isolate.

E. ST23 isolates

Isolate	n SNPs	K serotype	Carbapenemase
2727/22[a]	0	K57	NDM-1, OXA-48
1198/23	595	K57	NDM-1, OXA-48
6228/22	596	K57	NDM-1, OXA-48

[a] – reference isolate.

Table S5. SNP scores between selected *K. pneumoniae* ST11 K15 and K24 isolates, being the closest relatives to the six study ST11 isolates. The study isolates are indicated by original numbers and in bold, whereas those from RefSeq by assembly numbers

Isolates	K serotype	Number of SNPs	Country of origin
8597/22 [a]	K24	0	Ukraine
GCF_017976045.1	K24	16	Poland
GCF_013134055.1	K24	17	Poland
GCF_013134435.1	K24	17	Poland
GCF_013134965.1	K24	17	Poland
GCF_013134275.1	K24	18	Poland
GCF_013134475.1	K24	18	Poland
GCF_013134935.1	K24	20	Poland
GCF_013134325.1	K24	20	Poland
GCF_013135065.1	K24	21	Poland
GCF_013134675.1	K24	24	Poland
GCF_013133955.1	K24	26	Poland
GCF_013134375.1	K24	26	Poland
GCF_013134255.1	K24	27	Poland
GCF_013134585.1	K24	27	Poland
GCF_013134105.1	K24	28	Poland
GCF_013134625.1	K24	28	Poland
GCF_013133965.1	K24	29	Poland
GCF_013134415.1	K24	30	Poland
10432/22	K24	31	Ukraine
GCF_013134975.1	K24	31	Poland
6741/22	K24	32	Ukraine
GCF_013135225.1	K24	33	Poland
GCF_013135015.1	K24	34	Poland
GCF_900511495.1	K24	34	Poland
GCF_013135155.1	K24	34	Poland
GCF_013135085.1	K24	34	Poland
GCF_900500745.1	K24	35	Greece
GCF_900500735.1	K24	35	Greece
GCF_013134515.1	K24	36	Poland
GCF_013134535.1	K24	36	Poland
GCF_013134195.1	K24	36	Poland
GCF_013134775.1	K24	36	Poland
GCF_013134315.1	K24	37	Poland
GCF_013134065.1	K24	37	Poland
GCF_013134365.1	K24	37	Poland
GCF_900500715.1	K24	37	Greece
GCF_013134715.1	K24	38	Poland
GCF_013134095.1	K24	38	Poland
GCF_900501545.1	K24	38	Greece
GCF_013134205.1	K24	38	Poland
GCF_013134165.1	K24	39	Poland
GCF_013134425.1	K24	39	Poland
GCF_013133855.1	K24	39	Poland
GCF_900501265.1	K24	39	Greece
GCF_900500895.1	K24	40	Greece
GCF_900501515.1	K24	41	Greece
GCF_900502385.1	K24	41	Greece
GCF_900502325.1	K24	42	Greece
GCF_013133885.1	K24	43	Poland
6912/22	K24	43	Ukraine
GCF_013134285.1	K24	44	Poland
GCF_900501575.1	K24	45	Greece
GCF_900502635.1	K24	46	Greece
GCF_902156165.1	K24	46	Greece

GCF_013134155.1	K24	46	Poland
GCF_013134615.1	K24	46	Poland
GCF_013135335.1	K24	47	Poland
GCF_013135215.1	K24	48	Poland
GCF_013134355.1	K24	48	Poland
2466/22[a]	K15	0	Ukraine
GCF_900503115.1	K15	54	United Kingdom
GCF_900512285.1	K15	55	Germany
GCF_003977125.1	K15	57	Egypt
GCF_003977085.1	K15	61	Egypt
GCF_019660105.1	K15	67	China
GCF_015831425.1	K15	67	Sweden
GCF_016651515.1	K15	68	Egypt
GCF_902166935.1	K15	69	Spain
GCF_003977205.1	K15	69	Egypt
5008/22[a]	K15	0	Ukraine
GCF_900512285.1	K15	43	Germany
GCF_900503115.1	K15	43	United Kingdom
GCF_003977125.1	K15	44	Egypt
GCF_003977085.1	K15	49	Egypt
GCF_019660105.1	K15	57	China
GCF_016651515.1	K15	57	Egypt
GCF_015831425.1	K15	57	Sweden
GCF_902166935.1	K15	59	Spain
GCF_003977205.1	K15	59	Egypt

[a] – reference study isolates.

Table S6. SNP scores between selected *K. pneumoniae* ST147 isolates, being the closest relatives to five individual ST147 isolates of Ukrainian origin identified in Poland. The study isolates are indicated by original numbers and in bold, whereas those from RefSeq by assembly numbers

Isolates	Number of SNPs	Country of origin
2564/22[a]	0	Ukraine
GCF_002591075.1	47	Iran
GCF_019433715.1	62	Iran
GCF_019433735.1	64	Iran
GCF_019433755.1	70	Iran
GCF_900504585.1	86	Romania
GCF_900504225.1	88	Romania
GCF_008693405.1	89	United States
GCF_900504855.1	91	Romania
GCF_900504685.1	92	Romania
GCF_002263345.1	96	Russia
2674/22[a]	0	Ukraine
GCF_004803235.1	20	Egypt
GCF_004803225.1	22	Egypt
GCF_016651815.1	23	Egypt
GCF_900502585.1	24	Denmark
GCF_022369925.1	26	Italy
GCF_025392585.1	26	Italy
GCF_025392575.1	27	Italy
GCF_947390165.1	28	United Kingdom
GCF_022370195.1	29	Italy
GCF_022369815.1	29	Italy
GCF_020406995.1	29	Italy
GCF_002202235.1	30	United States
GCF_002202215.1	30	United States
8287/22[a]	0	Ukraine
GCF_900502585.1	21	Denmark
GCF_004803235.1	27	Egypt
GCF_002202235.1	27	United States
GCF_002202215.1	27	United States
GCF_004832395.1	29	Thailand
GCF_024998405.1	29	India
GCF_016652065.1	29	Egypt
GCF_004803225.1	29	Egypt
GCF_016651815.1	30	Egypt
9702/22 [a]	0	Ukraine
GCF_021229935.1	32	Russia
GCF_021229975.1	40	Russia
GCF_021229955.1	42	Russia
GCF_900502585.1	46	Denmark
GCF_004803235.1	52	Egypt
GCF_002202235.1	52	United States
GCF_002202215.1	52	United States
GCF_004832395.1	54	Thailand
GCF_024998405.1	54	India
GCF_016652065.1	54	Egypt
GCF_004803225.1	54	Egypt
GCF_016651815.1	55	Egypt
GCF_018068405.1	56	United States
GCF_018068465.1	56	United States
GCF_018068525.1	57	United States
GCF_018068355.1	58	United States

GCF_022369925.1	58	Italy
GCF_025392585.1	58	Italy
GCF_018068485.1	58	United States
GCF_018068435.1	58	United States
GCF_018068425.1	58	United States
GCF_018068535.1	58	United States
GCF_011030125.1	58	Lebanon
GCF_018068385.1	59	United States
GCF_025392575.1	59	Italy
GCF_023146895.1	59	Lebanon
GCF_018068345.1	60	United States
10718/22 [a]	0	Ukraine
GCF_020830005.1	29	India
GCF_016762195.1	30	Bangladesh
GCF_011759465.1	31	India
GCF_011742595.1	31	India
GCF_024998475.1	31	India
GCF_011742455.1	31	India
GCF_024198005.1	32	India
GCF_011742755.1	32	India
GCF_011742335.1	32	India
GCF_016761745.1	33	Bangladesh
GCF_023275575.1	34	France
GCF_025377505.1	34	India
GCF_023275185.1	36	France
GCF_022029075.1	39	India
GCF_025377285.1	40	India

[a] – reference study isolates.

Table S7. SNP scores between selected *K. pneumoniae* ST23 isolates, being the closest relatives of the three ST23 isolates of Ukrainian origin identified in Poland. The study isolates are indicated by original numbers and in bold, whereas those from RefSeq by assembly numbers

Isolates	Number of SNPs	Country of origin
2727/22 [a]	0	Ukraine
GCF_022748775.1	356	Poland
GCF_022748875.1	973	Poland
GCF_022748955.1	1010	Poland
GCF_022748895.1	1043	Poland
GCF_022748755.1	1104	Poland
GCF_015645935.1	1174	Russia
GCF_015645965.1	1208	Russia
GCF_001902335.1	1209	Germany
GCF_001970155.2	1214	Russia
GCF_022748835.1	1218	Poland
GCF_022748795.1	1221	Poland
GCF_009661655.1	1231	Russia
6228/22 [a]	0	Ukraine
GCF_001902335.1	44	Germany
GCF_022748835.1	45	Poland
GCF_022748795.1	56	Poland
GCF_015645935.1	76	Russia
GCF_015645965.1	79	Russia
GCF_001970155.2	82	Russia
GCF_018138645.1	93	Russia
GCF_009661655.1	98	Russia
GCF_009661575.1	109	Russia
1198/23 [a]	0	Ukraine
GCF_001902335.1	95	Germany
GCF_022748835.1	103	Poland
GCF_022748795.1	107	Poland
GCF_015645935.1	116	Russia
GCF_015645965.1	120	Russia
GCF_001970155.2	121	Russia
GCF_018138645.1	134	Russia
GCF_009661655.1	139	Russia
GCF_009661575.1	150	Russia

[a] – reference study isolates.

Table S8. Plasmid replicon types in the CPE isolates from patients arriving from Ukraine

Isolate[a,b]	Species	ST	Plasmid replicon types
2468/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncL
6991/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncL
9040/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR)
2858/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), Col440II,Col(pHAD28), ColpVC
10443/22	*Kpn*	ST395	IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), IncR
4263/22	*Kpn*	ST395	IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), IncR, Col440II, Col(pHAD28), ColRNAI
5378/22	*Kpn*	ST395	IncR, ColRNAI, Col440II-like, Col(pHAD28)-like
7242/22	*Kpn*	ST395	IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR),IncN, IncR, ColRNAI, Col440II(x2), Col(pHAD28)
3420/22	*Kpn*	ST395	IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), IncR, Col440II, Col(pHAD28)
8087/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncFII-like, IncHI1B(pNDM-MAR), IncN, IncL, IncR, Col440II, Col(pHAD28)
7025/22	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncR
302/23	*Kpn*	ST395	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncR
10423/22	*Kpn*	ST395	IncFIB$_K$,IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncR
3635/22	*Kpn*	ST395	IncFIB(pQil), Col440II, Col(pHAD28)
10440/22	*Kpn*	ST307	IncFIBK,IncFIB(pNDM-MAR), IncFIB(pQil), IncFIIK, IncHI1B(pNDM-MAR)
1197/23	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncFIB(pQil), IncFII$_K$, IncHI1B(pNDM-MAR)
5015/22	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncFIB(pQil), IncFII$_K$, IncHI1B(pNDM-MAR)
6772/22	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR)
2814/22	*Kpn*	ST307	IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR)
9816/22	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), IncL
1195/23	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), ColRNAI
1196/23	*Kpn*	ST307	IncFIB$_K$, IncFIB(pNDM-MAR), IncFIB(pQil), IncHI1B(pNDM-MAR), ColRNAI
4247/22	*Kpn*	ST307	IncFIB$_K$, IncFIB(pQil), IncFII$_K$ (x2), IncM2, Col(pHAD28) (x2)
5059/22	*Kpn*	ST307	IncFIB$_K$, IncFIB(pQil), IncFII$_K$
10100/22	*Kpn*	ST307	IncFIB$_K$,IncFIB(pQil), IncFII$_K$
8597/22	*Kpn*	ST11	IncFII(pKPX1), repB(R1701), Col440I, Col440II, Col(pHAD28)
6741/22	*Kpn*	ST11	IncFII(pKPX1), repB(R1701)
6912/22	*Kpn*	ST11	IncFIB$_K$, IncFII$_K$, IncFII(pKX1), repB(R1701)
10432/22	*Kpn*	ST11	IncFIB$_K$, IncFII$_K$, IncFII(pKX1), repB (R1701)
2466/22	*Kpn*	ST11	IncFIB(pNDM-MAR), IncFIB(pQil), IncFII, IncFII$_K$, IncHI1B(pNDM-MAR), IncR, Col440I, Col(pHAD28)
5008/22	*Kpn*	ST11	IncFIB(pQil), IncFII, IncFII$_K$, IncR, Col440I
8287/22	*Kpn*	ST147	IncFIB$_K$, IncFIB(pKPHS1), IncFIB(pQil), IncFII$_K$, IncR
9702/22	*Kpn*	ST147	IncFIB(pKPHS1), IncFIB(pQil), IncR, Col(pHAD28)
2674/22	*Kpn*	ST147	IncFIB(pKPHS1), IncFIB(pNDM-MAR), IncFIB(pQil), IncHI1B(pNDM-MAR), IncR, Col(pHAD28)
2564/22	*Kpn*	ST147	IncFIA(HI1), IncFIB$_K$(pCAV1099-114), IncFIB(pQil), InFII$_K$, IncFII(Yp), IncHI1B(pNDM-MAR), IncL, IncR
10718/22	*Kpn*	ST147	IncFIB(pNDM-MAR), IncFII, IncHI1B(pNDM-MAR), IncR
2727/22	*Kpn*	ST23	IncFIB(pQil), IncFII$_K$, IncFII(pAR0022)-like, IncL, repB, Col(pHAD28), ColpVC
6228/22	*Kpn*	ST23	IncFIA(HI1), IncFII, IncFII$_K$, repB
1198/23	*Kpn*	ST23	IncFIA(HI1), IncFII, IncFII$_K$, IncL, repB
2369/22	*Kpn*	ST15	IncFIB(pQil), IncFII$_K$, Col440I, Col(BS512)
2662/22	*Kpn*	ST15	IncFIB(pQil), IncFII$_K$, Col(BS512), Col440I
2821/22	*Kpn*	ST14	IncFIB$_K$, IncFIB(pQil), IncFII$_K$, IncFII(pKP91), Col(pHAD28)
2673/22	*Kpn*	ST17	IncFIB$_K$, IncFIB(pNDM-MAR), IncFIB(pQil), IncFII$_K$, IncFII(pKP91), IncHI1B(pNDM-MAR), IncR, Col440I, repB(R1701)
8289/22	*Kpn*	ST39	IncFIB(pQil), IncFII$_K$, IncHI1B(pNDM-MAR), ColRNAI
5016/22	*Kpn*	ST45	IncFIB$_K$, IncFIB(pQil), IncFII$_K$, IncFII(Yp)
6914/24	*Kpn*	ST152	IncFIB$_K$, IncM1, repB(R1701), ColRNAI
5010/22	*Kpn*	ST219	IncFIB$_K$(pCAV1099-114), IncFIB(pQil), IncFII$_K$
5012/22	*Kpn*	ST253	IncFIB$_K$, IncFIB(pNDM-MAR), IncFII$_K$, IncFII(pBK30683), IncHI1B(pNDM-MAR)
10441/22	*Kpn*	ST512	IncFIB$_K$,IncFIB(pNDM-MAR), IncFII$_K$, IncHI1B(pNDM-MAR), IncX3, ColRNAI
5018/22	*Kpn*	ST5859	IncFIB(pNDM-MAR), IncFIB(pQil), IncHI1B(pNDM-MAR),ColKP3
3494/22	*Eco*	ST46	IncFIA, IncFIB(AP001918), IncFII(pRSB107), IncI1-I(Alpha), Col(BS512)
3495/22	*Eco*	ST46	IncFIA, IncFIB(AP001918), IncFII(pRSB107), IncI-1(Alpha), Col(BS512)
9036/22	*Eco*	ST46	IncFIA, IncFIB(AP001918), IncFII(pRSB107), Col(BS512), Col(KPHS6)
5220/22	*Eco*	ST131	IncC, IncFIA, IncFIB(AP001918), IncFII(pRSB107)
6352/22	*Eco*	ST131	IncFIB(pQil), IncFII$_K$
6179/22	*Eco*	ST167	IncC, IncFIA, IncFII, Inc1-I(Alpha), IncL, IncX4, ColpEC648
5058/22	*Eco*	ST224	IncFIB(pQil), IncFII$_k$, IncM2
6750/22	*Ehor*	ST91	IncN, ColpVC
9723/22	*Ehor*	ST91	IncA, IncN5,ColpVC
3657/22	*Ehor*	ST231	IncFIA(HI1), IncFIB(pHCM2), IncR, repA(dsdm701b), repB(R1701), Col440I
8875/22	*Ehor*	ST231	IncFIA, IncFIB(pNDM-MAR), IncHI1B(pNDM-MAR), IncR, IncX3, repB(R1701), Col440I
6990/22	*Ehor*	ST182	IncFIB(pECLA), IncFII(pECLA), IncHI2, IncHI2A, IncM2
4020/22	*Cpor*	ST728	IncFIB(pQil), IncFII$_K$, IncM2
6180/22	*Ctel*	ST1282	IncL
9856/22	*Pmi*	ST269	IncC, Col3M

[a] – *Kpn, Klebsiella pneumoniae*; *Eco*, *Escherichia coli*; *Eho*, *Enterobacter hormaechei*; *Cpo*, *Citrobacter portucalensis*; *Cte*, *Citrobacter telavivensis*; *Pmi*, *Proteus mirabilis*.

[b] – isolates selected to long-read sequencing are indicated in bold; total numbers of plasmid replicons in these isolates are based on long-read sequencing, including double replicons of the same type (x2).

Table S9. Antimicrobial susceptibility of the CPE isolates from patients arriving in Poland from Ukraine

				MIC (mg/L)[a,b]																			
Isolate ID	Species[c]	ST[d]	Carbapenemase/main AMR mechanism	AMP	AMC	TZP	CTX	CAZ	FEP	FDC	ATM	IMP	MEM	ERT	CZA	MVB	IPR	CIP	AMK	GEN	TOB	TGC	CST	SXT
2468/22	*Kpn*	ST395	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
6991/22	*Kpn*	ST395	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
9040/22	*Kpn*	ST395	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
2858/22	*Kpn*	ST395	OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	≤0.25	**>16**	**>8**	**>1**	≤2	≤0.5	**8**	≤0.5	≤0.5	2
10443/22	*Kpn*	ST395	OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**>8**	**>16**	**>2**	2	**>16**	**8**	**>1**	**16**	≤0.5	**>8**	≤0.5	≤0.5	**>8**
4263/22	*Kpn*	ST395	OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**8**	**>16**	0.5	1	**>16**	**8**	**>1**	8	≤0.5	**>8**	1	1	**>8**
5378/22	*Kpn*	ST395	OXA-1242	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	*4*	**>16**	**>2**	1	8	2	**>1**	4	≤0.5	**8**	≤0.5	≤0.5	**>8**
7242/22	*Kpn*	ST395	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	1	**8**	**>8**
3420/22	*Kpn*	ST395	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	1	**8**	**>8**
8087/22	*Kpn*	ST395	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**8**	**16**	**>2**	**>16**	8	**8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
7025/22	*Kpn*	ST395	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**16**	**>2**	**>16**	**16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
302/23	*Kpn*	ST395	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**8**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	8	**>8**	**>8**	≤0.5	1	**>8**
10423/22	*Kpn*	ST395	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**8**	**>32**	**8**	**16**	**>2**	**>16**	**16**	**8**	**>1**	4	**>8**	**>8**	≤0.5	≤0.5	**>8**
3635/22	*Kpn*	ST395	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
10440/22	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**8**	**16**	**>2**	**>16**	**16**	**8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
1197/23	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
5015/22	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	**>16**	**>8**
6772/22	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**16**	1	**>8**	≤0.5	≤0.5	**>8**
2814/22	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	≤1	**8**	**16**	**>2**	**>16**	8	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	**8**	**>8**
9816/22	*Kpn*	ST307	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
1195/23	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	*4*	**16**	**>2**	**>16**	8	**8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
1196/23	*Kpn*	ST307	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**8**	**16**	**>2**	**>16**	8	**8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
4247/22	*Kpn*	ST307	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.25	**>32**	**8**	*8*	**>2**	2	≤0.06	0.12	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
5059/22	*Kpn*	ST307	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**16**	1	**>32**	**8**	*8*	**>2**	2	≤0.06	0.12	**>1**	≤2	≤0.5	≤0.5	≤0.5	≤0.5	**>8**
10100/22	*Kpn*	ST307	KPC-2	**>16**	**>32**	**>32**	**>4**	**>8**	**16**	0.5	**>32**	*4*	*8*	**>2**	0.5	≤0.06	0.12	**>1**	≤2	**>8**	**>8**	≤0.5	≤0.5	**>8**
8597/22	*Kpn*	ST11	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	8	**>8**	**>8**	≤0.5	≤0.5	≤1
6741/22	*Kpn*	ST11	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	8	≤0.5	**>8**	1	1	*4*
6912/22	*Kpn*	ST11	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**16**	1	**>8**	≤0.5	**>16**	**>8**
10432/22	*Kpn*	ST11	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**8**	**16**	**>2**	**>16**	**16**	**8**	**>1**	**16**	**>8**	**>8**	≤0.5	≤0.5	2
2466/22	*Kpn*	ST11	OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	≤1	2	**>2**	1	2	1	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
5008/22	*Kpn*	ST11	KPC-2	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**>8**	**>16**	**>2**	2	0.12	0.5	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
8287/22	*Kpn*	ST147	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**16**	**>8**	**>1**	**16**	≤0.5	**>8**	≤0.5	≤0.5	**>8**
9702/22	*Kpn*	ST147	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**16**	**1**	**>8**	≤0.5	≤0.5	≤1
2674/22	*Kpn*	ST147	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
2564/22	*Kpn*	ST147	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	**>16**	≤1
10718/22	*Kpn*	ST147	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	≤1	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
2727/22	*Kpn*	ST23	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	**16**	**>8**
6228/22	*Kpn*	ST23	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
1198/23	*Kpn*	ST23	NDM-1+OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
2369/22	*Kpn*	ST15	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**8**	**16**	**>2**	2	≤0.06	0.5	**>1**	**16**	**8**	**>8**	≤0.5	≤0.5	**>8**
2662/22	*Kpn*	ST15	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**16**	0.25	**>32**	**8**	*8*	**>2**	2	≤0.06	0.25	**>1**	**16**	**>8**	**>8**	≤0.5	≤0.5	≤1
2821/22	*Kpn*	ST14	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	4	2	1	**>1**	4	≤0.5	**>8**	≤0.5	≤0.5	2
2673/22	*Kpn*	ST17	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**32**	**8**	**>16**	**>2**	**>16**	8	**8**	≤0.12	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**

8289/22	*Kpn*	ST39	KPC-2	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.5	**>32**	**>8**	**>16**	**>2**	2	0.5	0.25	**>1**	≤2	**>8**	**>8**	1	≤0.5	≤1
5016/22	*Kpn*	ST45	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	8	8	**>8**	**>1**	≤2	≤0.5	≤0.5	**2**	**8**	**>8**
6914/24	*Kpn*	ST152	KPC-2	**>16**	**>32**	**>32**	**>4**	**>8**	**8**	0.5	**>32**	**8**	*4*	**>2**	1	≤0.06	0.12	**>1**	8	≤0.5	**>8**	≤0.5	≤0.5	**>8**
5010/22	*Kpn*	ST219	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**8**	*8*	**>2**	1	≤0.06	0.25	*0.5*	≤2	≤0.5	≤0.5	≤0.5	≤0.5	**>8**
5012/22	*Kpn*	ST253	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	*4*	*4*	**>2**	**>16**	4	**4**	*0.5*	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
10441/22	*Kpn*	ST512	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**>32**	**>8**	**>16**	**>2**	2	0.25	0.12	**>1**	**32**	1	**>8**	≤0.5	≤0.5	**>8**
5018/22	*Kpn*	ST5859	NDM-1+OXA-232	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	1	**32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	≤2	≤0.5	2	≤0.5	**4**	**>8**
3494/22	*Eco*	ST46	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	4	2	2	≤0.5	≤0.5	**>8**
3495/22	*Eco*	ST46	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**4**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	4	1	2	≤0.5	≤0.5	**>8**
9036/22	*Eco*	ST46	NDM-5	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
5220/22	*Eco*	ST131	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
6352/22	*Eco*	ST131	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**8**	0.5	**>32**	2	*4*	**>2**	≤0.25	≤0.06	0.12	≤0.12	≤2	≤0.5	1	≤0.5	≤0.5	≤1
6179/22	*Eco*	ST167	OXA-48	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	*4*	*8*	**>2**	2	4	2	**>1**	8	**>8**	**>8**	≤0.5	≤0.5	**>8**
5058/22	*Eco*	ST224	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.25	**>32**	2	*4*	**>2**	≤0.25	≤0.06	0.12	**>1**	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
6750/22	*Eho*	ST91	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**16**	0.5	**>32**	**8**	*8*	**>2**	2	0.12	0.25	**>1**	4	1	**8**	**2**	≤0.5	**>8**
9723/22	*Eho*	ST91	VIM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>8**	2	**>32**	*4*	*4*	**>2**	**>16**	2	2	**>1**	**16**	8	**>8**	**2**	≤0.5	**>8**
3657/22	*Eho*	ST231	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**16**	1	**>8**	≤0.5	≤0.5	**>8**
8875/22	*Eho*	ST231	NDM-1	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	**8**	**>32**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**16**	≤0.5	**8**	≤0.5	≤0.5	**>8**
6990/22	*Eho*	ST182	OXA-244	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	2	**>32**	≤1	1	**>2**	0.5	0.5	1	**>1**	≤2	**>8**	**>8**	≤0.5	≤0.5	**>8**
4020/22	*Cpo*	ST728	KPC-3	**>16**	**>32**	**>32**	**>4**	**>8**	**>16**	0.25	**>32**	2	2	**2**	0.5	≤0.06	0.25	0.25	**>32**	**>8**	**>8**	≤0.5	≤0.5	**>8**
6180/22	*Cte*	ST1282	OXA-48	**>16**	**>32**	**>32**	**4**	≤0.5	2	0.064	**8**	*4*	*8*	**>2**	≤0.25	2	**4**	≤0.12	≤2	≤0.5	≤0.5	≤0.5	≤0.5	≤1
9856/22	*Pmi*	ST269	NDM-1	**>16**	**>32**	**32**	**>4**	**>8**	**>16**	1	**16**	**>8**	**>16**	**>2**	**>16**	**>16**	**>8**	**>1**	**>32**	**>8**	**>8**	**2**	**>16**	**>8**

[a] – abbreviations: AMC: amoxicillin-clavulanic acid; AMK: amikacin; AMP: ampicillin; ATM: aztreonam; CAZ: ceftazidime; CIP: ciprofloxacin; CST: colistin; CTX: cefotaxime; CZA: ceftazidime-avibactam; ERT: ertapenem; FDC: cefiderocol; FEP: cefepime; GEN: gentamicin; IMP: imipenem; IPR: imipenem-relebactam; MEM: meropenem; MVB: meropenem-vaborbactam; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; TOB: tobramycin; TZP: piperacillin-tazobactam.

[b] – the bold and normal styles refer to resistance and susceptibility, respectively, according to EUCAST (http://eucast.org), including tigecycline for which the EUCAST ECOFF values were used, and italics refer to increased exposure.

[c] – *Kpn, Klebsiella pneumoniae*; *Eco*, *Escherichia coli*; *Eho*, *Enterobacter hormaechei*; *Cpo*, *Citrobacter portucalensis*; *Cte*, *Citrobacter telavivensis*; *Pmi*, *Proteus mirabilis*.

[d] – ST, sequence type

Figure S1. SNP-based minimum spanning tree of the *K. pneumoniae* ST395 isolates available in RefSeq (as of February 1, 2023). Isolates of Ukrainian origin identified in Poland are indicated by original numbers, whereas those from RefSeq by assembly numbers. The country of origin is presented with the following codes: BD, Bangladesh; BR, Brazil; CH, Switzerland; CN, China; DE, Germany; EE, Estonia; EG, Egipt; FR, France; GB, United Kingdom; IL, Israel; IN, India; IT, Italy; LB, Lebanon; LU, Luxembourg; NL, Netherlands; PT, Portugal; RO, Romania; RU, Russia; SP, Spain; TR, Turkey; UA, Ukraine; US, United States; VN, Viet Nam. The presence of carbapenemases is indicated using corresponding colours, K serotype is indicated in the outer circle. The tree was constructed using Parsnp and visualised with ITOL.

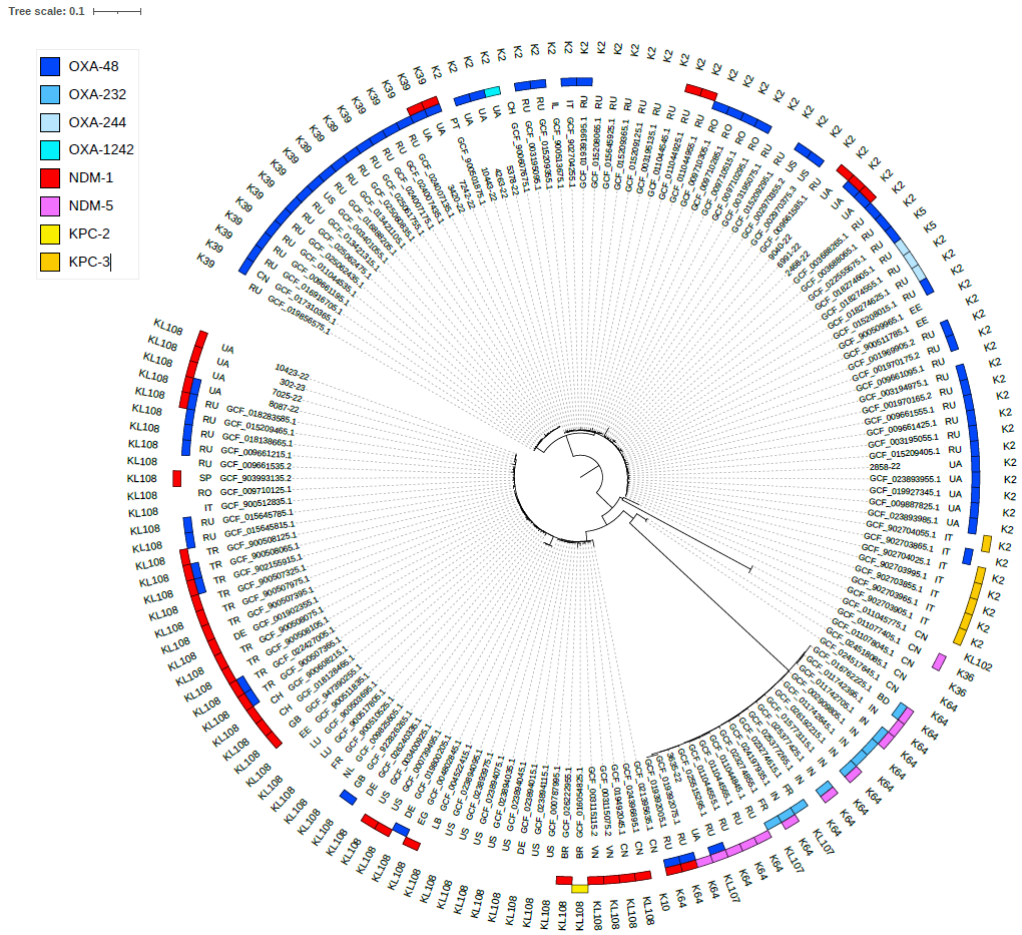


Figure S2. SNP-based minimum spanning tree of the *K. pneumoniae* ST307 isolates available in RefSeq (as of February 1, 2023). Isolates of Ukrainian origin identified in Poland are indicated by original numbers, whereas those from RefSeq by assembly numbers. The country of origin is presented with the following codes: BD, Bangladesh; BE, Belgium; BR, Brazil; CH, Switzerland; CM, Cameroon; CN, China; CO, Colombia; CZ, Czechia; DE, Germany; EG, Egypt; ES, Spain; FR, France; GB, United Kingdom; GH, Ghana; GN, Guinea; GR, Greece; HU, Hungary; IL, Israel; IR, Iran; IT, Italy; KE, Kenya; KR, Korea; LB, Lebanon; MT, Malta; MW, Malawi; NG, Nigeria; NL, Netherlands; NO, Norway; PE, Peru; PK, Pakistan; PT, Portugal; RO, Romania; RU, Russia; SI, Slovenia; SN, Senegal; TH, Thailand; TN, Tunisia; TR, TT, Trinidad and Tobago; Turkey; UA, Ukraine; US, United States; ZA, South Africa; ZM, Zambia. The presence of carbapenemases is indicated using corresponding colors, K serotype is indicated in the outer circle. The tree was constructed using Parsnp and visualised with ITOL.

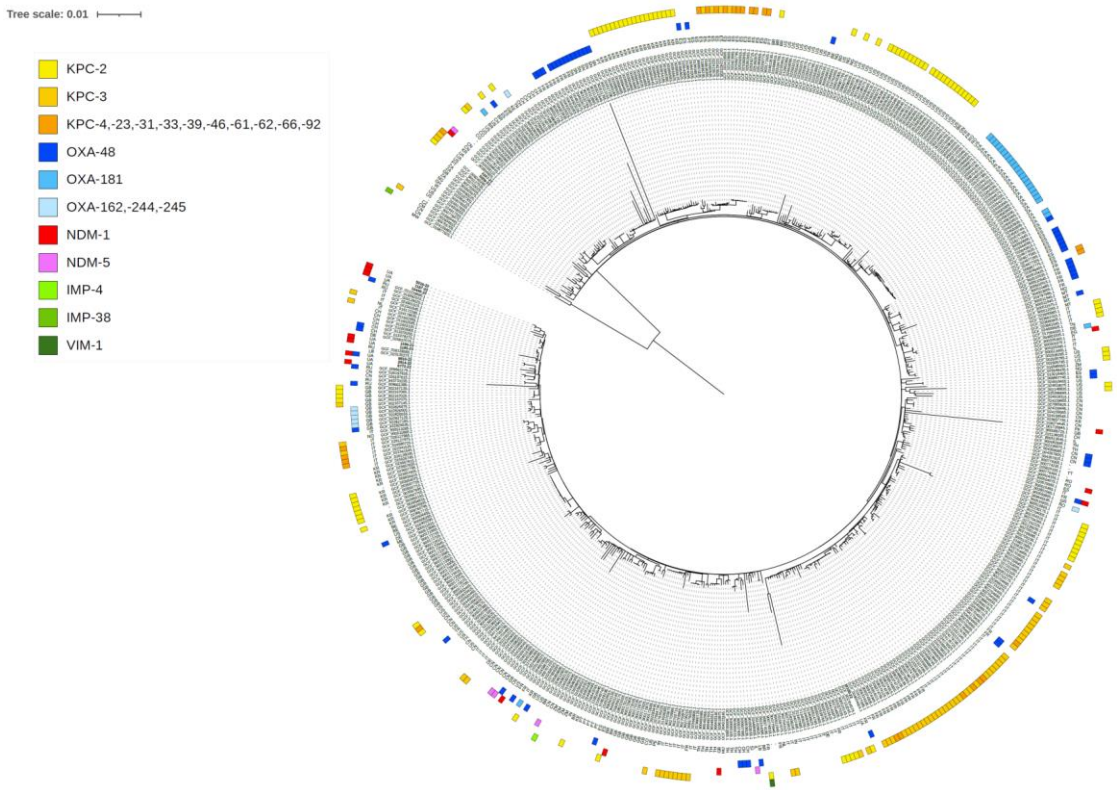


Figure S3. Comparison of the study NDM-1-encoding pNDM-MAR-like IncFIB+IncHI1B plasmid p7242_1 (inner black circle) to p6772, p1195, p8087_1, p2468_1, and previously reported homologous plasmids: p1 (Germany, CP132632) [7], p4313/18_19 (Poland, ON081621) [8], phvKpST147_NDM1_1659 (Russia, CP072810) [9], p51015_NDM_1 (Czechia, CP050380) [10], pSI646A-ARMA-Vir_NDM (Italy, CP084395) [11], pFQ61_ST383_NDM-5 (Qatar, CP091814) [12], pKpvST383L (United Kingdom, CP034201) [13]. The outer thick black ring refers to the annotation of p7242_1, with the selected genes indicated. The percentage of sequence identity is reflected by colour intensity. The picture was created using BRIG software (http://brig.sourceforge.net/).

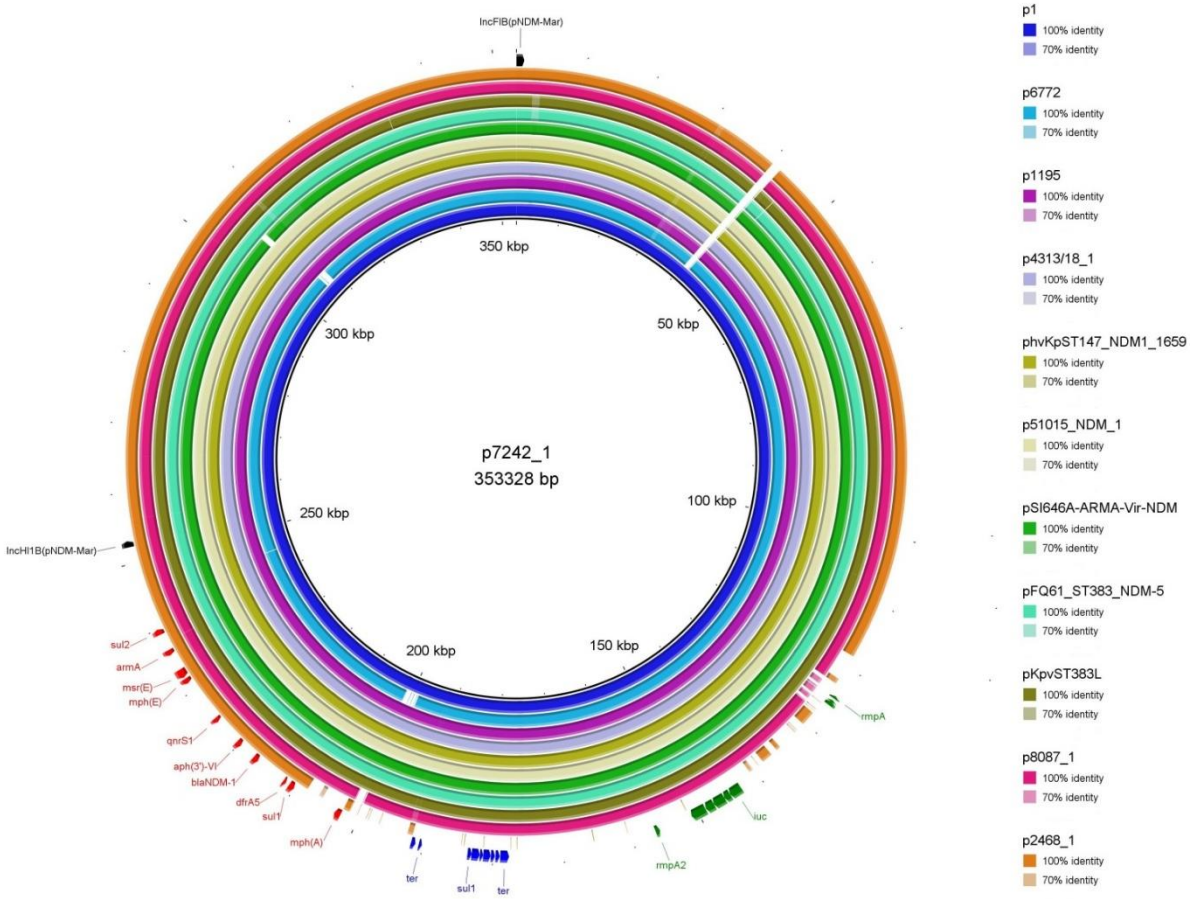
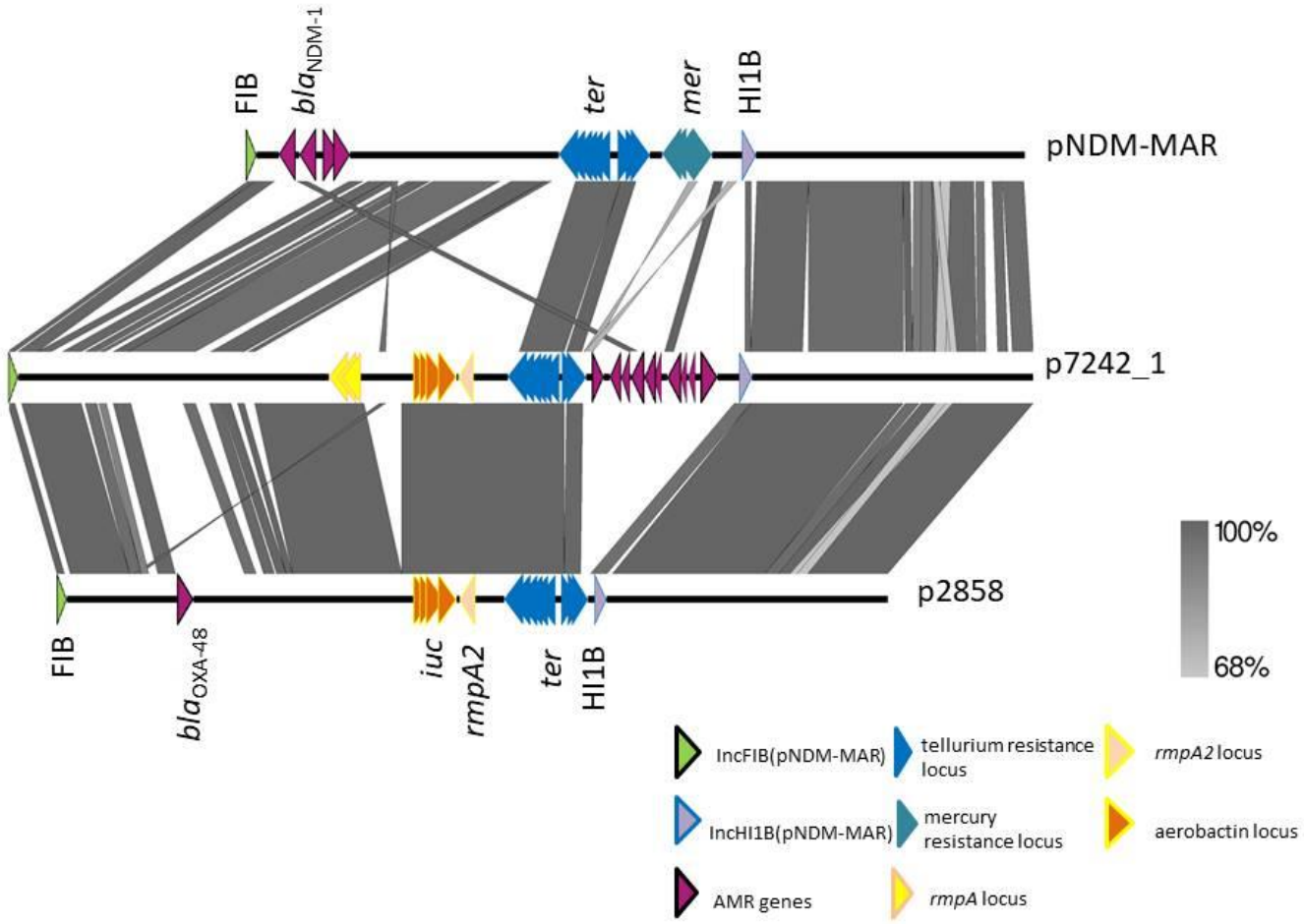


Figure S4. Comparison of the study OXA-48 encoding plasmid p2858 and NDM-1-carrying plasmid p7242_1 with the reference plasmid pNDM-MAR. The percentage of sequence identity is reflected by gray color intensity. Individual loci (replicons, virulence genes, AMR and heavy metal resistance genes) are marked by coloured triangles as explained below. The picture was created using the Easyfig 2.2.5 software.



References

1. Witteveen S, Hans JB, Izdebski R, Hasman H, Samuelsen O, Dortet L, et al. Dissemination of extensively drug-resistant NDM-producing *Providencia stuartii* in Europe linked to patients transferred from Ukraine, March 2022 to March 2023. Euro Surveill. 2024;29(23):2300616.
2. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome open research. 2018;3:124.
3. Baraniak A, Machulska M, Zabicka D, Literacka E, Izdebski R, Urbanowicz P, et al. Towards endemicity: large-scale expansion of the NDM-1-producing *Klebsiella pneumoniae* ST11 lineage in Poland, 2015-16. J Antimicrob Chemother. 2019;74(11):3199-204.
4. Biedrzycka M, Urbanowicz P, Guzek A, Brisse S, Gniadkowski M, Izdebski R. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19. J Antimicrob Chemother. 2021;76(10):2538-45.
5. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive Web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. J Clin Microbiol. 2018;56(6):e00197-18.
6. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. Nature communications. 2021;12(1):4188.
7. Russo TA, Alvarado CL, Davies CJ, Drayer ZJ, Carlino-MacDonald U, Hutson A, et al. Differentiation of hypervirulent and classical *Klebsiella pneumoniae* with acquired drug resistance. mBio. 2024;15(2):e0286723.
8. Biedrzycka M, Izdebski R, Urbanowicz P, Polanska M, Hryniewicz W, Gniadkowski M, et al. MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009-19. J Antimicrob Chemother. 2022;77(12):3367-75.
9. Starkova P, Lazareva I, Avdeeva A, Sulian O, Likholetova D, Ageevets V, et al. Emergence of hybrid resistance and virulence plasmids harboring New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* in Russia. Antibiotics. 2021;10(6):691.
10. Chudejova K, Kraftova L, Mattioni Marchetti V, Hrabak J, Papagiannitsis CC, Bitar I. Genetic plurality of OXA/NDM-encoding features characterized from Enterobacterales recovered from Czech hospitals. Front Microbiol. 2021;12:641415.
11. Martin MJ, Corey BW, Sannio F, Hall LR, MacDonald U, Jones BT, et al. Anatomy of an extensively drug-resistant *Klebsiella pneumoniae* outbreak in Tuscany, Italy. Proc Natl Acad Sci U S A. 2021;118(48):e2110227118.
12. Tsui CK, Ben Abid F, Al Ismail K, McElheny CL, Al Maslamani M, Omrani AS, et al. Genomic epidemiology of carbapenem-resistant *Klebsiella* in Qatar: emergence and dissemination of hypervirulent *Klebsiella pneumoniae* sequence type 383 strains. Antimicrob Agents Chemother. 2023;67(7):e0003023.
13. Turton J, Davies F, Turton J, Perry C, Payne Z, Pike R. Hybrid resistance and virulence plasmids in "high-risk" clones of *Klebsiella pneumoniae*, including those carrying $bla_{\text{NDM-5}}$. Microorganisms. 2019;7(9):326.

7. Conclusions

A number of specific selected questions regarding the epidemiological situation of CP *Klebsiella* spp. in Poland were addressed in this thesis. In order to answer these, several molecular and genomic-bioinformatic analyses were carried out on representative collections of KpSC and KoSC isolates from medical institutions from all over the country. The studies comprised characterization of clonal structures of the bacterial populations, phylogenies of major epidemic genotypes, their carbapenemase gene contents and genetic contexts, AMR and virulence gene compositions (resistomes and virulomes), and structures of plasmids carrying carbapenemase and hvKp genes. These efforts allowed for drawing a set of conclusions as follows:

- The *K. pneumoniae* ST147-Tn*125*F genotype of the Tunisian origin, introduced to Poland in March 2015, has settled down in the country, causing a regional outbreak in the metropolitan area of Warsaw and Mazowsze mainly; however, its contribution to the expansion of NDM-1 producers in Poland in 2015-2019 was limited in the context of the *K. pneumoniae* ST11 Tn*125*A overwhelming expansion all over the country.
- A number of related, though non-outbreak *K. pneumoniae* ST147-Tn*125*F organisms recovered in different towns most likely represented repeated imports of basically the same genotype from its endemic reservoirs in the Mediterranean basin. Like in other works, this finding shows the risk of multiple importations of dangerous organisms from popular touristic destinations.
- The dissemination of several high-risk *K. pneumoniae* genotypes, namely ST147, ST437, ST392 or ST15, contributed significantly to the spread of VIM-type carbapenemases in Poland in 2014-2019 along with *Enterobacter* spp. The *K. pneumoniae* genotypes with VIM-4/-1/-20-encoding integrons In238, In916 or In1444 were responsible for eight regional outbreaks in such areas as Lubelskie, Małopolskie, Śląskie, Wielkopolskie, Świetokrzyskie, and Kujawsko-Pomorskie. Poland belongs to only several European countries in which VIM CPE constitute a significant epidemiological problem, and this study has provided important original data to the growing knowledge on the *K. pneumoniae* clones listed above.
- The 10-year period of the clonal spread of the *K. oxytoca* ST145 genotype carrying the In237-like integron has been one of the most spectacular phenomena in the entire epidemiology of VIM CPE in Poland so far. In the context of the lack of the data on the VIM-producing *K. oxytoca* strains from other countries, including Greece, this study has

revealed a remarkable specificity and possible local significance of the epidemic genotype. It has also demonstrated interesting original observations regarding molecular genetics of *K. oxytoca*, which may result in the definition of useful molecular markers in the epidemiological studies of this species.

- This thesis has been one of a number of recent reports from Europe, mainly Germany or The Netherlands, documenting frequent colonization or infection of the patients evacuated from Ukraine by dangerous microorganisms. This has been due to several factors, including the difficult epidemiological situation of AMR in that country before the war with Russia, and then all of the war-associated disruptions of the health-care system in there.
- The CP *K. pneumoniae* genotypes identified in the patients from Ukraine turned out to represent "high-risk" clones of increased epidemic potential, carrying numerous AMR mechanisms and hypervirulence genes with a strikingly high frequency. The multiple ST395, ST307, ST147 and ST23 organisms of the East European (Ukrainian and Russian) origins constitute a severe danger for public health in Europe.
- The KpSC and KoSC isolates characterized in this thesis showed a high variety of genetic elements carrying carbapenemase genes, including integrons (*e. g.* In238 and In916) and transposons (*e. g.* Tn*125* and Tn*1999*). Their horizontal transfer mediated mainly by multiple types of plasmids, like IncFII$_K$+IncFIB$_K$, IncA, IncL or IncFIB+HI1B, has been one of the critical mechanisms of the dissemination of the carbapenemase genes in populations of pathogens in Polish hospitals.
- A remarkable number of "Ukrainian" strains carried a specific lineage of hybrid IncFIB+HI1B-like plasmids of the pNDM-MAR type, encoding MDR and hvKp determinants simultaneously; horizontal expansion of these molecules may constitute a detrimental threat to public health in Poland and other European countries.

8. References

1. Adeolu M, Alnajar S, Naushad S, R SG. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol. 2016;66(12):5575–99.
2. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998;11(4):589–603.
3. Bagley ST, Seidler RJ, Talbot HW, Jr., Morrow JE. Isolation of *Klebsielleae* from within living wood. Appl Environ Microbiol. 1978;36(1):178–85.
4. Brown C, Seidler RJ. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. Appl Microbiol. 1973;25(6):900–4.
5. Edberg SC, Piscitelli V, Cartter M. Phenotypic characteristics of coliform and noncoliform bacteria from a public water supply compared with regional and national clinical species. Appl Environ Microbiol. 1986;52(3):474–8.
6. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis. 2010;51 Suppl 1:S81–7.
7. Hansen DS, Gottschau A, Kolmos HJ. Epidemiology of *Klebsiella bacteraemia*: a case control study using *Escherichia coli* bacteraemia as control. J Hosp Infect. 1998;38(2):119–32.
8. Brisse S, Verhoef J. Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, *gyrA* and *parC* genes sequencing and automated ribotyping. Int J Syst Evol Microbiol. 2001;51(Pt 3):915–24.
9. Bagley ST. Habitat association of *Klebsiella* species. Infect Control. 1985;6(2):52–8.
10. Vergara-Lopez S, Dominguez MC, Conejo MC, Pascual A, Rodriguez-Bano J. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*. Clin Microbiol Infect. 2013;19(11):E490–8.
11. Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol. 2020;18(6):344–59.
12. Rodrigues C, Passet V, Rakotondrasoa A, Diallo TA, Criscuolo A, Brisse S. Description of *Klebsiella africanensis* sp. nov., *Klebsiella variicola* subsp. *tropicalensis* subsp. nov. and *Klebsiella variicola* subsp. *variicola* subsp. nov. Res Microbiol. 2019;170(3):165–70.
13. Wyres KL, Holt KE. *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr Opin Microbiol. 2018;45:131–9.
14. Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, et al. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. Clin Infect Dis. 2017;65(2):208–15.
15. Conlan S, Kong HH, Segre JA. Species-level analysis of DNA sequence data from the NIH Human Microbiome Project. PLoS One. 2012;7(10):e47075.
16. Martin RM, Cao J, Brisse S, Passet V, Wu W, Zhao L, et al. Molecular epidemiology of colonizing and infecting isolates of *Klebsiella pneumoniae*. mSphere. 2016;1(5):e00261-16.
17. Chung DR, Lee H, Park MH, Jung SI, Chang HH, Kim YS, et al. Fecal carriage of serotype K1 *Klebsiella pneumoniae* ST23 strains closely related to liver abscess isolates in Koreans living in Korea. Eur J Clin Microbiol Infect Dis. 2012;31(4):481–6.
18. Brisse S, Duijkeren E. Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. Vet Microbiol. 2005;105(3-4):307–12.

19. Friedländer C. Ueber die Schizomyceten bei der acuten fibrösen Pneumonie. Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin. 1882;87(2):319–24.
20. Shon AS, Bajwa RP, Russo TA. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence. 2013;4(2):107–18.
21. Ko WC, Paterson DL, Sagnimeni AJ, Hansen DS, Von Gottberg A, Mohapatra S, et al. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. Emerg Infect Dis. 2002;8(2):160–6.
22. Montgomerie JZ. Epidemiology of *Klebsiella* and hospital-associated infections. Rev Infect Dis. 1979;1(5):736–53.
23. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, et al. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. J Clin Microbiol. 2018;56(9):e00776-18.
24. Russo TA, Marr CM. Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev. 2019;32(3):e00001–19.
25. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci U S A. 2015;112(27):E3574–81.
26. Wyres KL, Wick RR, Judd LM, Froumine R, Tokolyi A, Gorrie CL, et al. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of *Klebsiella pneumoniae*. PLoS genetics. 2019;15(4):e1008114.
27. Lam MMC, Wyres KL, Wick RR, Judd LM, Fostervold A, Holt KE, et al. Convergence of virulence and MDR in a single plasmid vector in MDR *Klebsiella pneumoniae* ST15. J Antimicrob Chemother. 2019;74(5):1218–22.
28. Gu D, Dong N, Zheng Z, Lin D, Huang M, Wang L, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis. 2018;18(1):37–46.
29. Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017;41(3):252–75.
30. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682–707.
31. WHO. Prioritization of pathogens to guide discovery, research and development of a new antibiotics for drug-resistant bacterial infections, including tuberculosis 2017.
32. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther. 2013;11(3):297–308.
33. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56–66.
34. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*. Ann Clin Microbiol Antimicrob. 2017;16(1):18.
35. Liu YC, Cheng DL, Lin CL. *Klebsiella pneumoniae* liver abscess associated with septic endophthalmitis. Arch Intern Med. 1986;146(10):1913–6.
36. Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis. 2007;45(3):284–93.
37. Lee HC, Chuang YC, Yu WL, Lee NY, Chang CM, Ko NY, et al. Clinical implications of hypermucoviscosity phenotype in *Klebsiella pneumoniae* isolates: association with

invasive syndrome in patients with community-acquired bacteraemia. J Intern Med. 2006;259(6):606–14.
38. Rafat C, Messika J, Barnaud G, Dufour N, Magdoud F, Billard-Pomarès T, et al. Hypervirulent *Klebsiella pneumoniae*, a 5-year study in a French ICU. J Med Microbiol. 2018;67(8):1083–9.
39. Fazili T, Sharngoe C, Endy T, Kiska D, Javaid W, Polhemus M. *Klebsiella pneumoniae* liver abscess: an emerging disease. Am J Med Sci. 2016;351(3):297–304.
40. Decre D, Verdet C, Emirian A, Le Gourrierec T, Petit JC, Offenstadt G, et al. Emerging severe and fatal infections due to *Klebsiella pneumoniae* in two university hospitals in France. J Clin Microbiol. 2011;49(8):3012–4.
41. Fang CT, Chen YC, Chang SC, Sau WY, Luh KT. *Klebsiella pneumoniae* meningitis: timing of antimicrobial therapy and prognosis. Qjm. 2000;93(1):45–53.
42. Cheng DL, Liu YC, Yen MY, Liu CY, Wang RS. Septic metastatic lesions of pyogenic liver abscess. Their association with *Klebsiella pneumoniae* bacteremia in diabetic patients. Arch Intern Med. 1991;151(8):1557–9.
43. Han SH. Review of hepatic abscess from *Klebsiella pneumoniae*. An association with diabetes mellitus and septic endophthalmitis. West J Med. 1995;162(3):220–4.
44. Yang J, Long H, Hu Y, Feng Y, McNally A, Zong Z. *Klebsiella oxytoca* complex: update on taxonomy, antimicrobial resistance, and virulence. Clin Microbiol Rev. 2022;35(1):e0000621.
45. Podschun R, Pietsch S, Höller C, Ullmann U. Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. Appl Environ Microbiol. 2001;67(7):3325–7.
46. Lautrop H. Gelatin-liquefying *Klebsiella* strains (*Bacterium oxytocum* (Flügge)). Acta Pathol Microbiol Scand. 1956;39(5):375–84.
47. Long H, Hu Y, Feng Y, Zong Z. Genome analysis of *Klebsiella oxytoca* complex for antimicrobial resistance and virulence genes. Antimicrob Agents Chemother. 2022;66(3):e0218321.
48. Baker JL, Hendrickson EL, Tang X, Lux R, He X, Edlund A, et al. *Klebsiella* and *Providencia* emerge as lone survivors following long-term starvation of oral microbiota. Proc Natl Acad Sci U S A. 2019;116(17):8499–504.
49. Netsvyetayeva I, Marusza W, Olszanski R, Szyller K, Krolak-Ulinska A, Swoboda-Kopec E, et al. Skin bacterial flora as a potential risk factor predisposing to late bacterial infection after cross-linked hyaluronic acid gel augmentation. Infect Drug Resist. 2018;11:213–22.
50. Hogenauer C, Langner C, Beubler E, Lippe IT, Schicho R, Gorkiewicz G, et al. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. N Engl J Med. 2006;355(23):2418–26.
51. Beaugerie L, Metz M, Barbut F, Bellaiche G, Bouhnik Y, Raskine L, et al. *Klebsiella oxytoca* as an agent of antibiotic-associated hemorrhagic colitis. Clin Gastroenterol Hepatol. 2003;1(5):370–6.
52. Zollner-Schwetz I, Herzog KA, Feierl G, Leitner E, Schneditz G, Sprenger H, et al. The toxin-producing pathobiont *Klebsiella oxytoca* is not associated with flares of inflammatory bowel diseases. Dig Dis Sci. 2015;60(11):3393–8.
53. Lee KM, Morris-Love J, Cabral DJ, Belenky P, Opal SM, Jamieson AM. Coinfection with influenza A virus and *Klebsiella oxytoca*: an underrecognized impact on host resistance and tolerance to pulmonary infections. Front Immunol. 2018;9:2377.
54. Baier C, Pirr S, Ziesing S, Ebadi E, Hansen G, Bohnhorst B, et al. Prospective surveillance of bacterial colonization and primary sepsis: findings of a tertiary neonatal intensive and intermediate care unit. J Hosp Infect. 2019;102(3):325–31.

55. Neog N, Phukan U, Puzari M, Sharma M, Chetia P. *Klebsiella oxytoca* and emerging nosocomial infections. Curr Microbiol. 2021;78(4):1115–23.
56. Kim BN, Ryu J, Kim YS, Woo JH. Retrospective analysis of clinical and microbiological aspects of *Klebsiella oxytoca* bacteremia over a 10-year period. Eur J Clin Microbiol Infect Dis. 2002;21(6):419–26.
57. Lin RD, Hsueh PR, Chang SC, Chen YC, Hsieh WC, Luh KT. Bacteremia due to K*lebsiella oxytoca*: clinical features of patients and antimicrobial susceptibilities of the isolates. Clin Infect Dis. 1997;24(6):1217–22.
58. Reiss I, Borkhardt A, Füssle R, Sziegoleit A, Gortner L. Disinfectant contaminated with *Klebsiella oxytoca* as a source of sepsis in babies. Lancet. 2000;356(9226):310.
59. Moradigaravand D, Martin V, Peacock SJ, Parkhill J. Population structure of multidrug resistant *Klebsiella oxytoca* within hospitals across the UK and Ireland identifies sharing of virulence and resistance genes with *K. pneumoniae*. Genome Biol Evol. 2017;9(3):574–87.
60. Leitner E, Zarfel G, Luxner J, Herzog K, Pekard-Amenitsch S, Hoenigl M, et al. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing *Klebsiella oxytoca* on a hematology ward. Antimicrob Agents Chemother. 2015;59(1):714–6.
61. Berthelot P, Grattard F, Patural H, Ros A, Jelassi-Saoudin H, Pozzetto B, et al. Nosocomial colonization of premature babies with *Klebsiella oxytoca*: probable role of enteral feeding procedure in transmission and control of the outbreak with the use of gloves. Infect Control Hosp Epidemiol. 2001;22(3):148–51.
62. Stewart J, Judd LM, Jenney A, Holt KE, Wyres KL, Hawkey J. Epidemiology and genomic analysis of *Klebsiella oxytoca* from a single hospital network in Australia. BMC Infect Dis. 2022;22(1):704.
63. Nesme J, Cécillon S, Delmont TO, Monier JM, Vogel TM, Simonet P. Large-scale metagenomic-based study of antibiotic resistance in the environment. Curr Biol. 2014;24(10):1096–100.
64. Saga T, Yamaguchi K. History of antimicrobial agents and resistant bacteria. JMAJ. 2009;52(2):103–8.
65. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother. 2010;54(3):969–76.
66. Matthew M, Hedges RW, Smith JT. Types of β-lactamase determined by plasmids in gram-negative bacteria. J Bacteriol. 1979;138(3):657–62.
67. Fevre C, Jbel M, Passet V, Weill FX, Grimont PA, Brisse S. Six groups of the OXY β-lactamase evolved over millions of years in *Klebsiella oxytoca*. Antimicrob Agents Chemother. 2005;49(8):3453–62.
68. Livermore DM. β-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev. 1995;8(4):557–84.
69. Fournier B, Arlet G, Lagrange PH, Philippon A. *Klebsiella oxytoca*: resistance to aztreonam by overproduction of the chromosomally encoded β-lactamase. FEMS Microbiol Lett. 1994;116(1):31–6.
70. Fournier B, Lagrange PH, Philippon A. β-Lactamase gene promoters of 71 clinical strains of *Klebsiella oxytoca*. Antimicrob Agents Chemother. 1996;40(2):460–3.
71. Fournier B, Gravel A, Hooper DC, Roy PH. Strength and regulation of the different promoters for chromosomal β-lactamases of *Klebsiella oxytoca*. Antimicrob Agents Chemother. 1999;43(4):850–5.
72. Fournier B, Lu CY, Lagrange PH, Krishnamoorthy R, Philippon A. Point mutation in the pribnow box, the molecular basis of beta-lactamase overproduction in *Klebsiella oxytoca*. Antimicrob Agents Chemother. 1995;39(6):1365–8.

73. Sato T, Hara T, Horiyama T, Kanazawa S, Yamaguchi T, Maki H. Mechanism of resistance and antibacterial susceptibility in extended-spectrum β-lactamase phenotype *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated between 2000 and 2010 in Japan. J Med Microbiol. 2015;64(Pt 5):538–43.
74. Ito R, Mustapha MM, Tomich AD, Callaghan JD, McElheny CL, Mettus RT, et al. Widespread fosfomycin resistance in Gram-negative bacteria attributable to the chromosomal fosA gene. mBio. 2017;8(4) :e00749-17.
75. Li J, Zhang H, Ning J, Sajid A, Cheng G, Yuan Z, et al. The nature and epidemiology of OqxAB, a multidrug efflux pump. Antimicrobial resistance and infection control. 2019;8:44.
76. Wong MH, Chan EW, Chen S. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of *Enterobacteriaceae*. Antimicrob Agents Chemother. 2015;59(6):3290–7.
77. Bernardini A, Cuesta T, Tomás A, Bengoechea JA, Martínez JL, Sánchez MB. The intrinsic resistome of *Klebsiella pneumoniae*. Int J Antimicrob Agents. 2019;53(1):29–33.
78. Jana B, Cain AK, Doerrler WT, Boinett CJ, Fookes MC, Parkhill J, et al. The secondary resistome of multidrug-resistant *Klebsiella pneumoniae*. Scientific reports. 2017;7:42483.
79. Cain AK, Boinett CJ, Barquist L, Dordel J, Fookes M, Mayho M, et al. Morphological, genomic and transcriptomic responses of *Klebsiella pneumoniae* to the last-line antibiotic colistin. Scientific reports. 2018;8(1):9868.
80. Nicolas-Chanoine MH, Mayer N, Guyot K, Dumont E, Pagès JM. Interplay between membrane permeability and enzymatic barrier leads to antibiotic-dependent resistance in *Klebsiella pneumoniae*. Front Microbiol. 2018;9:1422.
81. Xu Q, Jiang J, Zhu Z, Xu T, Sheng ZK, Ye M, et al. Efflux pumps AcrAB and OqxAB contribute to nitrofurantoin resistance in an uropathogenic *Klebsiella pneumoniae* isolate. Int J Antimicrob Agents. 2019;54(2):223–7.
82. He F, Fu Y, Chen Q, Ruan Z, Hua X, Zhou H, et al. Tigecycline susceptibility and the role of efflux pumps in tigecycline resistance in KPC-producing *Klebsiella pneumoniae*. PLoS One. 2015;10(3):e0119064.
83. Fajardo-Lubian A, Ben Zakour NL, Agyekum A, Qi Q, Iredell JR. Host adaptation and convergent evolution increases antibiotic resistance without loss of virulence in a major human pathogen. PLoS Pathog. 2019;15(3):e1007218.
84. Wong JLC, Romano M, Kerry LE, Kwong HS, Low WW, Brett SJ, et al. OmpK36-mediated carbapenem resistance attenuates ST258 *Klebsiella pneumoniae in vivo*. Nature communications. 2019;10(1):3957.
85. Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. Trends Microbiol. 2016;24(12):944–56.
86. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. J Antimicrob Chemother. 2018;73(5):1121–37.
87. Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother. 1985;28(2):302–7.
88. Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A, et al. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel beta-lactamase. J Antimicrob Chemother. 1987;20(3):323–34.
89. Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. Korean J Intern Med. 2012;27(2):128–42.

90. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother. 2007;59(2):165–74.
91. Rossolini GM, D'Andrea MM, Mugnaioli C. The spread of CTX-M-type extended-spectrum β-lactamases. Clin Microbiol Infect. 2008;14 Suppl 1:33–41.
92. Naseer U, Sundsfjord A. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. Microb Drug Resist. 2011;17(1):83–97.
93. Hawkey PM. Prevalence and clonality of extended-spectrum β-lactamases in Asia. Clin Microbiol Infect. 2008;14 Suppl 1:159–65.
94. Bush K. Extended-spectrum β-lactamases in North America, 1987-2006. Clin Microbiol Infect. 2008;14 Suppl 1:134–43.
95. Evans BA, Amyes SG. OXA β-lactamases. Clin Microbiol Rev. 2014;27(2):241–63.
96. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14(4):933–51.
97. WHO. Antimicrobial resistance: global report on surveillance. 2014.
98. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22(1):161–82.
99. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20(3):440–58.
100. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect. 2012;18(5):413–31.
101. Knothe H, Antal M, Krcméry V. Imipenem and ceftazidime resistance in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. J Antimicrob Chemother. 1987;19(1):136–8.
102. Baumgartner JD, Glauser MP. Comparative study of imipenem in severe infections. J Antimicrob Chemother. 1983;12 Suppl D:141–8.
103. Haruta S, Yamaguchi H, Yamamoto ET, Eriguchi Y, Nukaga M, O'Hara K, et al. Functional analysis of the active site of a metallo-β-lactamase proliferating in Japan. Antimicrob Agents Chemother. 2000;44(9):2304–9.
104. Ambler RP, Coulson AF, Frère JM, Ghuysen JM, Joris B, Forsman M, et al. A standard numbering scheme for the class A β-lactamases. Biochem J. 1991;276 (Pt 1)(Pt 1):269–70.
105. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2001;45(4):1151–61.
106. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. Clin Microbiol Rev. 2015;28(3):565–91.
107. Papp-Wallace KM, Bethel CR, Distler AM, Kasuboski C, Taracila M, Bonomo RA. Inhibitor resistance in the KPC-2 β-lactamase, a preeminent property of this class A β-lactamase. Antimicrob Agents Chemother. 2010;54(2):890–7.
108. Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, et al. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Infect Control Hosp Epidemiol. 2009;30(10):972–6.
109. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol Med. 2012;18(5):263–72.
110. Peirano G, Pitout JDD. Rapidly spreading *Enterobacterales* with OXA-48-like carbapenemases. J Clin Microbiol. 2025;63(2):e0151524.
111. Poirel L, Heritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48(1):15–22.

112. Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev. 2019;33(1):e00102-19.
113. Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P, Fortineau N, et al. OXA-244-producing *Escherichia coli* isolates, a challenge for clinical microbiology laboratories. Antimicrob Agents Chemother. 2017;61(9):e00818-17.
114. Kuwabara S, Abraham EP. Some properties of two extracellular β-lactamases from *Bacillus cereus* 569/H. Biochem J. 1967;103(3):27c–30c.
115. Iaconis JP, Sanders CC. Purification and characterization of inducible β-lactamases in *Aeromonas* spp. Antimicrob Agents Chemother. 1990;34(1):44–51.
116. Saino Y, Inoue M, Mitsuhashi S. Purification and properties of an inducible cephalosporinase from *Pseudomonas maltophilia* GN12873. Antimicrob Agents Chemother. 1984;25(3):362–5.
117. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? Lancet Infect Dis. 2011;11(5):381–93.
118. Rolain JM, Parola P, Cornaglia G. New Delhi metallo-β-lactamase (NDM-1): towards a new pandemia? Clin Microbiol Infect. 2010;16(12):1699–701.
119. Palzkill T. Metallo-β-lactamase structure and function. Ann N Y Acad Sci. 2013;1277:91–104.
120. Ito H, Arakawa Y, Ohsuka S, Wacharotayankun R, Kato N, Ohta M. Plasmid-mediated dissemination of the metallo-β-lactamase gene *bla*$_{IMP}$ among clinically isolated strains of *Serratia marcescens*. Antimicrob Agents Chemother. 1995;39(4):824–9.
121. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev. 2005;18(2):306–25.
122. Lauretti L, Riccio ML, Mazzariol A, Cornaglia G, Amicosante G, Fontana R, et al. Cloning and characterization of *bla*$_{VIM}$, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother. 1999;43(7):1584–90.
123. Psichogiou M, Tassios PT, Avlamis A, Stefanou I, Kosmidis C, Platsouka E, et al. Ongoing epidemic of *bla*$_{VIM-1}$-positive *Klebsiella pneumoniae* in Athens, Greece: a prospective survey. J Antimicrob Chemother. 2008;61(1):59–63.
124. Miriagou V, Douzinas EE, Papagiannitsis CC, Piperaki E, Legakis NJ, Tzouvelekis LS. Emergence of *Serratia* liquefaciens and *Klebsiella oxytoca* with metallo-β-lactamase-encoding IncW plasmids: further spread of the *bla*$_{VIM-1}$-carrying integron In-e541. Int J Antimicrob Agents. 2008;32(6):540–1.
125. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:249856.
126. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla$_{NDM-1}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046–54.
127. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of *bla*$_{NDM-1}$-positive *Enterobacteriaceae*. Antimicrob Agents Chemother. 2011;55(11):5403–7.
128. Naeem A, Badshah SL, Muska M, Ahmad N, Khan K. The current case of quinolones: synthetic approaches and antibacterial activity. Molecules. 2016;21(4):268.
129. Poirel L, Rodriguez-Martinez JM, Mammeri H, Liard A, Nordmann P. Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob Agents Chemother. 2005;49(8):3523–5.
130. Jacoby GA, Griffin CM, Hooper DC. *Citrobacter* spp. as a source of *qnrB* alleles. Antimicrob Agents Chemother. 2011;55(11):4979–84.

131. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet. 1998;351(9105):797–9.
132. Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, et al. *qnrB*, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother. 2006;50(4):1178–82.
133. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. Microbiology spectrum. 2014;2(5):10.
134. Ruiz E, Sáenz Y, Zarazaga M, Rocha-Gracia R, Martínez-Martínez L, Arlet G, et al. *qnr*, *aac(6')-Ib-cr* and *qepA* genes in *Escherichia coli* and *Klebsiella* spp.: genetic environments and plasmid and chromosomal location. J Antimicrob Chemother. 2012;67(4):886–97.
135. Fàbrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. Microb Biotechnol. 2009;2(1):40–61.
136. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. Cold Spring Harb Perspect Med. 2016;6(6):a027029.
137. Zhang Y, Zhang N, Wang M, Luo M, Peng Y, Li Z, et al. The prevalence and distribution of aminoglycoside resistance genes. Biosaf Health. 2023;5(1):14–20.
138. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis. 2005;40(9):1333–41.
139. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161–8.
140. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016;71(8):2066–70.
141. Zhang XW, Huang XY, Zhou ZY, Li BL, Lu JH, Song JJ, et al. Genetic framework and evolutionary dynamics of *mcr*-positive *Klebsiella pneumoniae* from 2000 to 2023. Int J Antimicrob Agents. 2025;66(3):107533.
142. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, et al. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis. 2014;20(11):1812–20.
143. Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, et al. Identification of *Klebsiella* capsule synthesis loci from whole genome data. Microb Genom. 2016;2(12):e000102.
144. Khater F, Balestrino D, Charbonnel N, Dufayard JF, Brisse S, Forestier C. In silico analysis of usher encoding genes in *Klebsiella pneumoniae* and characterization of their role in adhesion and colonization. PLoS One. 2015;10(3):e0116215.
145. Murphy CN, Mortensen MS, Krogfelt KA, Clegg S. Role of *Klebsiella pneumoniae* type 1 and type 3 fimbriae in colonizing silicone tubes implanted into the bladders of mice as a model of catheter-associated urinary tract infections. Infect Immun. 2013;81(8):3009–17.
146. Lam MMC, Wick RR, Judd LM, Holt KE, Wyres KL. Kaptive 2.0: updated capsule and lipopolysaccharide locus typing for the *Klebsiella pneumoniae* species complex. Microb Genom. 2022;8(3):000800.
147. Lee IR, Molton JS, Wyres KL, Gorrie C, Wong J, Hoh CH, et al. Differential host susceptibility and bacterial virulence factors driving Klebsiella liver abscess in an ethnically diverse population. Scientific reports. 2016;6:29316.
148. Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebize R, Diancourt L, et al. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. PLoS One. 2009;4(3):e4982.

149. Wang W, Tian D, Hu D, Chen W, Zhou Y, Jiang X. Different regulatory mechanisms of the capsule in hypervirulent *Klebsiella pneumonia*: "direct" *wcaJ* variation vs. "indirect" *rmpA* regulation. Frontiers in cellular and infection microbiology. 2023;13:1108818.
150. Follador R, Heinz E, Wyres KL, Ellington MJ, Kowarik M, Holt KE, et al. The diversity of *Klebsiella pneumoniae* surface polysaccharides. Microb Genom. 2016;2(8):e000073.
151. Martin RM, Bachman MA. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. Frontiers in cellular and infection microbiology. 2018;8:4.
152. Holden VI, Breen P, Houle S, Dozois CM, Bachman MA. *Klebsiella pneumoniae* siderophores induce inflammation, bacterial dissemination, and HIF-1α stabilization during pneumonia. mBio. 2016;7(5):e01397-16.
153. Wyres KL, Nguyen TNT, Lam MMC, Judd LM, van Vinh Chau N, Dance DAB, et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. Genome medicine. 2020;12(1):11.
154. Lam MMC, Wyres KL, Judd LM, Wick RR, Jenney A, Brisse S, et al. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. Genome medicine. 2018;10(1):77.
155. Lam MMC, Wick RR, Wyres KL, Gorrie CL, Judd LM, Jenney AWJ, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICE*Kp* in *Klebsiella pneumoniae* populations. Microb Genom. 2018;4(9):e000196.
156. Lai YC, Lin AC, Chiang MK, Dai YH, Hsu CC, Lu MC, et al. Genotoxic *Klebsiella pneumoniae* in Taiwan. PLoS One. 2014;9(5):e96292.
157. Lu MC, Chen YT, Chiang MK, Wang YC, Hsiao PY, Huang YJ, et al. Colibactin contributes to the hypervirulence of pks(+) K1 CC23 *Klebsiella pneumoniae* in mouse meningitis infections. Frontiers in cellular and infection microbiology. 2017;7:103.
158. Arakawa Y, Ohta M, Wacharotayankun R, Mori M, Kido N, Ito H, et al. Biosynthesis of *Klebsiella* K2 capsular polysaccharide in *Escherichia coli* HB101 requires the functions of *rmpA* and the chromosomal *cps* gene cluster of the virulent strain *Klebsiella pneumoniae* Chedid (O1:K2). Infect Immun. 1991;59(6):2043–50.
159. Wacharotayankun R, Arakawa Y, Ohta M, Tanaka K, Akashi T, Mori M, et al. Enhancement of extracapsular polysaccharide synthesis in *Klebsiella pneumoniae* by RmpA2, which shows homology to NtrC and FixJ. Infect Immun. 1993;61(8):3164–74.
160. Walker KA, Miner TA, Palacios M, Trzilova D, Frederick DR, Broberg CA, et al. A *Klebsiella pneumoniae* regulatory mutant has reduced capsule expression but retains hypermucoviscosity. mBio. 2019;10(2):e00089-19.
161. Lin YC, Lu MC, Tang HL, Liu HC, Chen CH, Liu KS, et al. Assessment of hypermucoviscosity as a virulence factor for experimental *Klebsiella pneumoniae* infections: comparative virulence analysis with hypermucoviscosity-negative strain. BMC Microbiol. 2011;11:50.
162. Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT. A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. J Exp Med. 2004;199(5):697–705.
163. Biedrzycka M, Urbanowicz P, Zabicka D, Hryniewicz W, Gniadkowski M, Izdebski R. Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009-2019. Eur J Clin Microbiol Infect Dis. 2023;42(12):1449–57.
164. Dornisch E, Pletz J, Glabonjat RA, Martin F, Lembacher-Fadum C, Neger M, et al. Biosynthesis of the enterotoxic pyrrolobenzodiazepine natural product tilivalline. Angewandte Chemie. 2017;56(46):14753–7.
165. Tse H, Gu Q, Sze KH, Chu IK, Kao RY, Lee KC, et al. A tricyclic pyrrolobenzodiazepine produced by *Klebsiella oxytoca* is associated with cytotoxicity in antibiotic-associated hemorrhagic colitis. The Journal of biological chemistry. 2017;292(47):19503–20.

166. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018;31(4):e00088-17.
167. Dortet L, Nordmann P, Poirel L. Association of the emerging carbapenemase NDM-1 with a bleomycin resistance protein in *Enterobacteriaceae* and *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2012;56(4):1693–7.
168. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother. 2012;56(1):559–62.
169. Carrer A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. Antimicrob Agents Chemother. 2008;52(8):2950–4.
170. Giani T, Conte V, Di Pilato V, Aschbacher R, Weber C, Larcher C, et al. *Escherichia coli* from Italy producing OXA-48 carbapenemase encoded by a novel Tn*1999* transposon derivative. Antimicrob Agents Chemother. 2012;56(4):2211–3.
171. Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 β-lactamase-producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011. Euro Surveill. 2013;18(31).
172. Skalova A, Chudejova K, Rotova V, Medvecky M, Studentova V, Chudackova E, et al. Molecular characterization of OXA-48-like-producing *Enterobacteriaceae* in the Czech Republic and evidence for horizontal transfer of pOXA-48-like plasmids. Antimicrob Agents Chemother. 2017;61(2):e01889-16.
173. Naas T, Cuzon G, Truong HV, Nordmann P. Role of IS*Kpn7* and deletions in *bla*$_{KPC}$ gene expression. Antimicrob Agents Chemother. 2012;56(9):4753–9.
174. Hall RM, Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol Microbiol. 1995;15(4):593–600.
175. Domingues S, da Silva GJ, Nielsen KM. Integrons: vehicles and pathways for horizontal dissemination in bacteria. Mob Genet Elements. 2012;2(5):211–23.
176. Moura A, Soares M, Pereira C, Leitao N, Henriques I, Correia A. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics. 2009;25(8):1096–8.
177. Severino P, Magalhães VD. Integrons as tools for epidemiological studies. Clin Microbiol Infect. 2004;10(2):156–62.
178. Leavitt A, Chmelnitsky I, Ofek I, Carmeli Y, Navon-Venezia S. Plasmid pKpQIL encoding KPC-3 and TEM-1 confers carbapenem resistance in an extremely drug-resistant epidemic *Klebsiella pneumoniae* strain. J Antimicrob Chemother. 2010;65(2):243–8.
179. Chen L, Chavda KD, Melano RG, Jacobs MR, Levi MH, Bonomo RA, et al. Complete sequence of a *bla*$_{KPC-2}$-harboring IncFII(K1) plasmid from a *Klebsiella pneumoniae* sequence type 258 strain. Antimicrob Agents Chemother. 2013;57(3):1542–5.
180. Potron A, Poirel L, Nordmann P. Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. Antimicrob Agents Chemother. 2014;58(1):467–71.
181. Nordmann P, Couard JP, Sansot D, Poirel L. Emergence of an autochthonous and community-acquired NDM-1-producing *Klebsiella pneumoniae* in Europe. Clin Infect Dis. 2012;54(1):150–1.
182. Miriagou V, Papagiannitsis CC, Kotsakis SD, Loli A, Tzelepi E, Legakis NJ, et al. Sequence of pNL194, a 79.3-kilobase IncN plasmid carrying the *bla*$_{VIM-1}$ metallo-β-lactamase gene in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2010;54(10):4497–502.
183. Arcari G, Di Lella FM, Bibbolino G, Mengoni F, Beccaccioli M, Antonelli G, et al. A multispecies cluster of VIM-1 carbapenemase-producing *Enterobacterales* linked by a

novel, highly conjugative, and broad-host-range IncA plasmid forebodes the reemergence of VIM-1. Antimicrob Agents Chemother. 2020;64(4):e02435-19.
184. Yang X, Wai-Chi Chan E, Zhang R, Chen S. A conjugative plasmid that augments virulence in *Klebsiella pneumoniae*. Nat Microbiol. 2019;4(12):2039–43.
185. Breurec S, Guessennd N, Timinouni M, Le TA, Cao V, Ngandjio A, et al. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. Clin Microbiol Infect. 2013;19(4):349–55.
186. Hennart M, Guglielmini J, Bridel S, Maiden MCJ, Jolley KA, Criscuolo A, et al. A dual barcoding approach to bacterial strain nomenclature: genomic taxonomy of *Klebsiella pneumoniae* strains. Molecular biology and evolution. 2022;39(7):msac135.
187. Arcari G, Carattoli A. Global spread and evolutionary convergence of multidrug-resistant and hypervirulent *Klebsiella pneumoniae* high-risk clones. Pathogens and global health. 2023;117(4):328–41.
188. Chen L, Chavda KD, Melano RG, Jacobs MR, Koll B, Hong T, et al. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York Hospitals. Antimicrob Agents Chemother. 2014;58(5):2871–7.
189. Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, et al. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. Proc Natl Acad Sci U S A. 2014;111(13):4988–93.
190. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol. 2005;43(8):4178–82.
191. Liao W, Liu Y, Zhang W. Virulence evolution, molecular mechanisms of resistance and prevalence of ST11 carbapenem-resistant *Klebsiella pneumoniae* in China: A review over the last 10 years. J Glob Antimicrob Resist. 2020;23:174–80.
192. Baraniak A, Machulska M, Zabicka D, Literacka E, Izdebski R, Urbanowicz P, et al. Towards endemicity: large-scale expansion of the NDM-1-producing *Klebsiella pneumoniae* ST11 lineage in Poland, 2015-16. J Antimicrob Chemother. 2019;74(11):3199–204.
193. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother. 2015;59(10):5873–84.
194. Wyres KL, Gorrie C, Edwards DJ, Wertheim HF, Hsu LY, Van Kinh N, et al. Extensive capsule locus variation and large-scale genomic recombination within the *Klebsiella pneumoniae* clonal group 258. Genome Biol Evol. 2015;7(5):1267–79.
195. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. Antimicrob Agents Chemother. 2011;55(5):2420–3.
196. Shaidullina ER, Schwabe M, Rohde T, Shapovalova VV, Dyachkova MS, Matsvay AD, et al. Genomic analysis of the international high-risk clonal lineage *Klebsiella pneumoniae* sequence type 395. Genome medicine. 2023;15(1):9.
197. Rodrigues C, Desai S, Passet V, Gajjar D, Brisse S. Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. Microb Genom. 2022;8(1):000737.
198. Damjanova I, Toth A, Paszti J, Hajbel-Vekony G, Jakab M, Berta J, et al. Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type β-lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005 - the new 'MRSAs'? J Antimicrob Chemother. 2008;62(5):978–85.

199. Peirano G, Chen L, Kreiswirth BN, Pitout JDD. Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. Antimicrob Agents Chemother. 2020;64(10):e01148-20.
200. Messaoudi A, Haenni M, Mansour W, Saras E, Bel Haj Khalifa A, Chaouch C, et al. ST147 NDM-1-producing *Klebsiella pneumoniae* spread in two Tunisian hospitals. J Antimicrob Chemother. 2017;72(1):315–6.
201. Gamal D, Egea P, Elías C, Fernández-Martínez M, Causse M, Pérez-Nadales E, et al. High-risk clones and novel sequence type ST4497 of *Klebsiella pneumoniae* clinical isolates producing different alleles of NDM-type and other carbapenemases from a single tertiary-care centre in Egypt. Int J Antimicrob Agents. 2020;56(6):106164.
202. Habeeb MA, Haque A, Nematzadeh S, Iversen A, Giske CG. High prevalence of 16S rRNA methylase RmtB among CTX-M extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* from Islamabad, Pakistan. Int J Antimicrob Agents. 2013;41(6):524–6.
203. Wyres KL, Hawkey J, Hetland MAK, Fostervold A, Wick RR, Judd LM, et al. Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. J Antimicrob Chemother. 2019;74(3):577–81.
204. Bonura C, Giuffrè M, Aleo A, Fasciana T, Di Bernardo F, Stampone T, et al. An update of the evolving epidemic of bla_{KPC} carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014: emergence of multiple non-ST258 clones. PLoS One. 2015;10(7):e0132936.
205. Castanheira M, Farrell SE, Wanger A, Rolston KV, Jones RN, Mendes RE. Rapid expansion of KPC-2-producing *Klebsiella pneumoniae* isolates in two Texas hospitals due to clonal spread of ST258 and ST307 lineages. Microb Drug Resist. 2013;19(4):295–7.
206. Ocampo AM, Chen L, Cienfuegos AV, Roncancio G, Chavda KD, Kreiswirth BN, et al. A two-year surveillance in five Colombian tertiary care hospitals reveals high frequency of non-CG258 clones of carbapenem-resistant *Klebsiella pneumoniae* with distinct clinical characteristics. Antimicrob Agents Chemother. 2016;60(1):332–42.
207. Yoon EJ, Yang JW, Kim JO, Lee H, Lee KJ, Jeong SH. Carbapenemase-producing *Enterobacteriaceae* in South Korea: a report from the National Laboratory Surveillance System. Future Microbiol. 2018;13:771–83.
208. Long SW, Olsen RJ, Eagar TN, Beres SB, Zhao P, Davis JJ, et al. Population genomic analysis of 1,777 extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates, Houston, Texas: unexpected abundance of Clonal Group 307. mBio. 2017;8(3):e00489-17.
209. Villa L, Feudi C, Fortini D, Brisse S, Passet V, Bonura C, et al. Diversity, virulence, and antimicrobial resistance of the KPC-producing *Klebsiella pneumoniae* ST307 clone. Microb Genom. 2017;3(4):e000110.
210. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, et al. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. Nat Microbiol. 2019;4(11):1919–29.
211. Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, et al. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. Euro Surveill. 2013;18(22):20489.
212. Gonçalves GB, Furlan JPR, Vespero EC, Pelisson M, Stehling EG, Pitondo-Silva A. Spread of multidrug-resistant high-risk *Klebsiella pneumoniae* clones in a tertiary hospital from southern Brazil. Infect Genet Evol. 2017;56:1–7.
213. Cejas D, Elena A, Guevara Nuñez D, Sevillano Platero P, De Paulis A, Magariños F, et al. Changing epidemiology of KPC-producing *Klebsiella pneumoniae* in Argentina: emergence of hypermucoviscous ST25 and high-risk clone ST307. J Glob Antimicrob Resist. 2019;18:238–42.

214. Lomonaco S, Crawford MA, Lascols C, Timme RE, Anderson K, Hodge DR, et al. Resistome of carbapenem- and colistin-resistant *Klebsiella pneumoniae* clinical isolates. PLoS One. 2018;13(6):e0198526.
215. Kedišaletše M, Phumuzile D, Angela D, Andrew W, Mae NF. Epidemiology, risk factors, and clinical outcomes of carbapenem-resistant *Enterobacterales* in Africa: A systematic review. J Glob Antimicrob Resist. 2023;35:297–306.
216. Hu Y, Yang Y, Feng Y, Fang Q, Wang C, Zhao F, et al. Prevalence and clonal diversity of carbapenem-resistant *Klebsiella pneumoniae* causing neonatal infections: a systematic review of 128 articles across 30 countries. PLoS medicine. 2023;20(6):e1004233.
217. Potron A, Nordmann P, Rondinaud E, Jaureguy F, Poirel L. A mosaic transposon encoding OXA-48 and CTX-M-15: towards pan-resistance. J Antimicrob Chemother. 2013;68(2):476–7.
218. Giske CG, Froding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore D, et al. Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of *bla*NDM-1 in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother. 2012;56(5):2735–8.
219. Jain A, Hopkins KL, Turton J, Doumith M, Hill R, Loy R, et al. NDM carbapenemases in the United Kingdom: an analysis of the first 250 cases. J Antimicrob Chemother. 2014;69(7):1777–84.
220. Yoon EJ, Kang DY, Yang JW, Kim D, Lee H, Lee KJ, et al. New Delhi metallo-β-lactamase-producing *Enterobacteriaceae* in South Korea between 2010 and 2015. Front Microbiol. 2018;9:571.
221. Lam MMC, Wyres KL, Duchene S, Wick RR, Judd LM, Gan YH, et al. Population genomics of hypervirulent *Klebsiella pneumoniae* clonal-group 23 reveals early emergence and rapid global dissemination. Nature communications. 2018;9(1):2703.
222. Turton JF, Payne Z, Coward A, Hopkins KL, Turton JA, Doumith M, et al. Virulence genes in isolates of *Klebsiella pneumoniae* from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and 'non-hypervirulent' types ST147, ST15 and ST383. J Med Microbiol. 2018;67(1):118–28.
223. Gundestrup S, Struve C, Stahlhut SG, Hansen DS. First case of liver abscess in Scandinavia due to the international hypervirulent *Klebsiella pneumoniae* clone ST23. Open Microbiol J. 2014;8:22–4.
224. Vila A, Cassata A, Pagella H, Amadio C, Yeh KM, Chang FY, et al. Appearance of *Klebsiella pneumoniae* liver abscess syndrome in Argentina: case report and review of molecular mechanisms of pathogenesis. Open Microbiol J. 2011;5:107–13.
225. Hirai J, Sakanashi D, Kinjo T, Haranaga S, Fujita J. The first case of community-acquired pneumonia due to capsular genotype K2-ST86 hypervirulent *Klebsiella pneumoniae* in Okinawa, Japan: a case report and literature review. Infect Drug Resist. 2020;13:2237–43.
226. Matsuda N, Aung MS, Urushibara N, Kawaguchiya M, Ohashi N, Taniguchi K, et al. Prevalence, clonal diversity, and antimicrobial resistance of hypervirulent *Klebsiella pneumoniae* and *Klebsiella variicola* clinical isolates in northern Japan. J Glob Antimicrob Resist. 2023;35:11–8.
227. Fursova NK, Astashkin EI, Ershova ON, Aleksandrova IA, Savin IA, Novikova TS, et al. Multidrug-resistant *Klebsiella pneumoniae* causing severe infections in the neuro-ICU. Antibiotics. 2021;10(8):979.
228. Pinpimai K, Roe WD, Biggs PJ, Dittmer KE. Draft whole-genome sequences of seven isolates of *Klebsiella pneumoniae* from New Zealand Sea Lions. Microbiol Resour Announc. 2018;7(20):e01270-18.

229. Li L, Li S, Wei X, Lu Z, Qin X, Li M. Infection with carbapenem-resistant hypervirulent *Klebsiella pneumoniae:* clinical, virulence and molecular epidemiological characteristics. Antimicrobial resistance and infection control. 2023;12(1):124.
230. Yonekawa S, Mizuno T, Nakano R, Nakano A, Suzuki Y, Asada T, et al. Molecular and epidemiological characteristics of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates in Japan. mSphere. 2020;5(5):e00490-20.
231. Ikhimiukor OO, Souza SSR, Akintayo IJ, Marcovici MM, Workman A, Martin IW, et al. Phylogenetic lineages and antimicrobial resistance determinants of clinical *Klebsiella oxytoca* spanning local to global scales. Microbiology spectrum. 2023;11(5):e0054923.
232. Herzog KA, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Zollner-Schwetz I, et al. Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated hemorrhagic colitis. J Clin Microbiol. 2014;52(5):1607–16.
233. Izdebski R, Fiett J, Urbanowicz P, Baraniak A, Derde LP, Bonten MJ, et al. Phylogenetic lineages, clones and β-lactamases in an international collection of *Klebsiella oxytoca* isolates non-susceptible to expanded-spectrum cephalosporins. J Antimicrob Chemother. 2015;70(12):3230–7.
234. Eriksen HD, Porsbo LJ, Bang B, Ank N, Hasman H. New Delhi metallo-β-lactamase-1 - producing *Klebsiella oxytoca* sequence type 2 from toilets as the likely source of nosocomial transmission to patients - a root cause analysis. Infect Prev Pract. 2025;7(4):100487.
235. Álvarez VE, Knecht C, Piekar M, García Allende N, Gonzales Machuca A, Campos J, et al. Genomic characterization reveals infections and silent dissemination of carbapenemase-producing *Klebsiella oxytoca* complex associated with ST2 lineage in a hospital setting. Curr Microbiol. 2025;82(10):451.
236. Pérez-Vazquez M, Oteo-Iglesias J, Sola-Campoy PJ, Carrizo-Manzoni H, Bautista V, Lara N, et al. Characterization of carbapenemase-producing *Klebsiella oxytoca* in Spain, 2016-2017. Antimicrob Agents Chemother. 2019;63(6):e02529-18.
237. Dietrich J, LeCuyer TE, Hendrix GK, Burbick CR, Jacob ME, Byrne BA, et al. Prevalence and molecular epidemiology of carbapenemase-producing *Enterobacterales* isolated from dog and cat faeces submitted to veterinary laboratories in the USA. Zoonoses Public Health. 2024;71(5):538–48.
238. Saxenborn P, Baxter J, Tilevik A, Fagerlind M, Dyrkell F, Pernestig AK, et al. Genotypic characterization of clinical *Klebsiella* spp. isolates collected from patients with suspected community-onset sepsis, Sweden. Front Microbiol. 2021;12:640408.
239. López-Hernández I, Delgado-Valverde M, Fernández-Cuenca F, López-Cerero L, Machuca J, Pascual Á. Carbapenemase-producing Gram-negative bacteria in Andalusia, Spain, 2014-2018. Emerg Infect Dis. 2020;26(9):2218–22.
240. Wan W, Yang X, Yu H, Wang M, Jia W, Huang B, et al. Genomic characterization of carbapenem-resistant *Klebsiella oxytoca* complex in China: a multi-center study. Front Microbiol. 2023;14:1153781.
241. Sekowska A, Hryniewicz W, Gniadkowski M, Deptula A, Kusza K, Gospodarek E. Antimicrobial susceptibility of metallo-β-lactamase positive and negative *Klebsiella pneumoniae* strains isolated from intensive care unit patients. Pol J Microbiol. 2010;59(1):67–9.
242. Izdebski R, Baraniak A, Żabicka D, Sękowska A, Gospodarek E, Hryniewicz W, et al. VIM/IMP carbapenemase-producing Enterobacteriaceae in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother. 2018;73(10):2675–81.

243. Scoulica EV, Neonakis IK, Gikas AI, Tselentis YJ. Spread of *bla*$_{VIM-1}$-producing *E. coli* in a university hospital in Greece. Genetic analysis of the integron carrying the *bla*$_{VIM-1}$ metallo-β-lactamase gene. Diagn Microbiol Infect Dis. 2004;48(3):167–72.
244. Tsakris A, Poulou A, Markou F, Pitiriga V, Piperaki ET, Kristo I, et al. Dissemination of clinical isolates of *Klebsiella oxytoca* harboring CMY-31, VIM-1, and a new OXY-2-type variant in the community. Antimicrob Agents Chemother. 2011;55(7):3164–8.
245. Izdebski R, Biedrzycka M, Urbanowicz P, Zabicka D, Gniadkowski M. Genome-based epidemiologic analysis of VIM/IMP carbapenemase-producing *Enterobacter* spp., Poland. Emerg Infect Dis. 2023;29(8):1618–26.
246. Baraniak A, Grabowska A, Izdebski R, Fiett J, Herda M, Bojarska K, et al. Molecular characteristics of KPC-producing *Enterobacteriaceae* at the early stage of their dissemination in Poland, 2008-2009. Antimicrob Agents Chemother. 2011;55(12):5493–9.
247. Baraniak A, Izdebski R, Zabicka D, Bojarska K, Gorska S, Literacka E, et al. Multiregional dissemination of KPC-producing *Klebsiella pneumoniae* ST258/ST512 genotypes in Poland, 2010-14. J Antimicrob Chemother. 2017;72(6):1610–6.
248. Literacka E, Grzegorczyk M, Biedrzycka M. Badanie przeglądowe oporności na antybiotyki populacji szczepów bakterii izolowanych z zakażeń, z gatunków istotnych za względu na oporność na antybiotyki - *Klebsiella pneumoniae*. Narodowy Program Zdrowia na lata 2021-2025. www.korld.nil.gov.pl. 2024.
249. Baraniak A, Izdebski R, Fiett J, Gawryszewska I, Bojarska K, Herda M, et al. NDM-producing Enterobacteriaceae in Poland, 2012-14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. J Antimicrob Chemother. 2016;71(1):85–91.
250. Izdebski R, Bojarska K, Baraniak A, Literacka E, Herda M, Zabicka D, et al. NDM-1- or OXA-48-producing *Enterobacteriaceae* colonising Polish tourists following a terrorist attack in Tunis, March 2015. Euro Surveill. 2015;20(23):21150.
251. Izdebski R, Sitkiewicz M, Urbanowicz P, Krawczyk M, Brisse S, Gniadkowski M. Genomic background of the *Klebsiella pneumoniae* NDM-1 outbreak in Poland, 2012-18. J Antimicrob Chemother. 2020;75(11):3156–62.
252. Izdebski R, Baraniak A, Zabicka D, Machulska M, Urbanowicz P, Fiett J, et al. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. J Antimicrob Chemother. 2018;73(3):620–5.
253. Biedrzycka M, Urbanowicz P, Guzek A, Brisse S, Gniadkowski M, Izdebski R. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19. J Antimicrob Chemother. 2021;76(10):2538–45.
254. Biedrzycka M, Urbanowicz P, Brisse S, Palma F, Zabicka D, Gniadkowski M, et al. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019. Eur J Clin Microbiol Infect Dis. 2025;44(3):619–28.
255. Biedrzycka M, Izdebski R, Urbanowicz P, Polanska M, Hryniewicz W, Gniadkowski M, et al. MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009-19. J Antimicrob Chemother. 2022;77(12):3367–75.
256. Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, et al. Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. J Bacteriol. 2009;191(14):4492–501.
257. Villa L, Poirel L, Nordmann P, Carta C, Carattoli A. Complete sequencing of an IncH plasmid carrying the *bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$ and *qnrB1* genes. J Antimicrob Chemother. 2012;67(7):1645–50.

258. Fricke WF, Welch TJ, McDermott PF, Mammel MK, LeClerc JE, White DG, et al. Comparative genomics of the IncA/C multidrug resistance plasmid family. J Bacteriol. 2009;191(15):4750–7.
259. Biedrzycka M, Izdebski R, Hryniewicz W, Gniadkowski M, Żabicka D. Carbapenemase-producing *Enterobacterales* from patients arriving from Ukraine in Poland, March 2022-February 2023. Infectious diseases and therapy. 2025;14(2):401–19.
260. van Almsick VF, Sobkowiak A, Scherff N, Schuler F, Mellmann A, Schwierzeck V. Characterization of *bla*$_{\text{OXA-48}}$-carrying plasmids and small non-AMR-coding plasmids collected from Ukrainian patients. Infection. 2023;52(2):661-665.
261. Schultze T, Hogardt M, Velazquez ES, Hack D, Besier S, Wichelhaus TA, et al. Molecular surveillance of multidrug-resistant Gram-negative bacteria in Ukrainian patients, Germany, March to June 2022. Euro Surveill. 2023;28(1):2200850.
262. Sandfort M, Hans JB, Fischer MA, Reichert F, Cremanns M, Eisfeld J, et al. Increase in NDM-1 and NDM-1/OXA-48-producing *Klebsiella pneumoniae* in Germany associated with the war in Ukraine, 2022. Euro Surveill. 2022;27(50):2200926.
263. Zwittink RD, Wielders CC, Notermans DW, Verkaik NJ, Schoffelen AF, Witteveen S, et al. Multidrug-resistant organisms in patients from Ukraine in the Netherlands, March to August 2022. Euro Surveill. 2022;27(50):2200896.
264. Shaidullina E, Shelenkov A, Yanushevich Y, Mikhaylova Y, Shagin D, Alexandrova I, et al. Antimicrobial resistance and genomic characterization of OXA-48- and CTX-M-15-co-producing hypervirulent *Klebsiella pneumoniae* ST23 recovered from nosocomial outbreak. Antibiotics. 2020;9(12):862.
265. Lev AI, Astashkin EI, Kislichkina AA, Solovieva EV, Kombarova TI, Korobova OV, et al. Comparative analysis of *Klebsiella pneumoniae* strains isolated in 2012-2016 that differ by antibiotic resistance genes and virulence genes profiles. Pathogens and global health. 2018;112(3):142–51.
266. Starkova P, Lazareva I, Avdeeva A, Sulian O, Likholetova D, Ageevets V, et al. Emergence of hybrid resistance and virulence plasmids harboring New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* in Russia. Antibiotics. 2021;10(6):691.
267. Coelho A, Mirelis B, Alonso-Tarrés C, Nieves Larrosa M, Miró E, Clivillé Abad R, et al. Detection of three stable genetic clones of CTX-M-15-producing *Klebsiella pneumoniae* in the Barcelona metropolitan area, Spain. J Antimicrob Chemother. 2009;64(4):862–4.
268. Di Pilato V, Henrici De Angelis L, Aiezza N, Baccani I, Niccolai C, Parisio EM, et al. Resistome and virulome accretion in an NDM-1-producing ST147 sublineage of *Klebsiella pneumoniae* associated with an outbreak in Tuscany, Italy: a genotypic and phenotypic characterisation. The Lancet Microbe. 2022;3(3):e224–e34.
269. Hasan CM, Turlej-Rogacka A, Vatopoulos AC, Giakkoupi P, Maatallah M, Giske CG. Dissemination of *bla*$_{\text{VIM}}$ in Greece at the peak of the epidemic of 2005-2006: clonal expansion of *Klebsiella pneumoniae* clonal complex 147. Clin Microbiol Infect. 2014;20(1):34–7.
270. Rodrigues C, Desai S, Passet V, Gajjar D, Brisse S. Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. Microb Genom. 2022;8(1):000737.
271. Guo L, An J, Ma Y, Ye L, Luo Y, Tao C, et al. Nosocomial outbreak of OXA-48-producing *Klebsiella pneumoniae* in a Chinese hospital: clonal transmission of ST147 and ST383. PLoS One. 2016;11(8):e0160754.
272. Naha S, Sands K, Mukherjee S, Roy C, Rameez MJ, Saha B, et al. KPC-2-producing *Klebsiella pneumoniae* ST147 in a neonatal unit: clonal isolates with differences in colistin susceptibility attributed to AcrAB-TolC pump. Int J Antimicrob Agents. 2020;55(3):105903.

273. Guerra AM, Lira A, Lameirão A, Selaru A, Abreu G, Lopes P, et al. Multiplicity of carbapenemase-producers three years after a KPC-3-producing *K. pneumoniae* ST147-K64 hospital outbreak. Antibiotics. 2020;9(11):806.
274. Hernández-García M, García-Fernández S, García-Castillo M, Bou G, Cercenado E, Delgado-Valverde M, et al. WGS characterization of MDR *Enterobacterales* with different ceftolozane/tazobactam susceptibility profiles during the SUPERIOR surveillance study in Spain. JAC-antimicrobial resistance. 2020;2(4):dlaa084.
275. Liu J, Du SX, Zhang JN, Liu SH, Zhou YY, Wang XR. Spreading of extended-spectrum β-lactamase-producing *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST11 in patients with pneumonia: a molecular epidemiological study. Chin Med J (Engl). 2019;132(16):1894–902.
276. Ko KS, Lee JY, Baek JY, Suh JY, Lee MY, Choi JY, et al. Predominance of an ST11 extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* clone causing bacteraemia and urinary tract infections in Korea. J Med Microbiol. 2010;59(Pt 7):822–8.
277. Yang Y, Qin J, Hu Y, Wang J, Feng Y, Zong Z. Carbapenem-resistant *Klebsiella pneumoniae* of sequence type 11: a scoping review. J Infect Dis. 2026;233(Supplement_1):S72–s80.
278. Yang X, Sun Q, Li J, Jiang Y, Li Y, Lin J, et al. Molecular epidemiology of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in China. Emerg Microbes Infect. 2022;11(1):841–9.
279. Guo L, Wang L, Zhao Q, Ye L, Ye K, Ma Y, et al. Genomic analysis of KPC-2-producing *Klebsiella pneumoniae* ST11 isolates at the respiratory department of a tertiary care hospital in Beijing, China. Front Microbiol. 2022;13:929826.
280. Machuca J, López-Cerero L, Fernández-Cuenca F, Mora-Navas L, Mediavilla-Gradolph C, López-Rodríguez I, et al. OXA-48-like-producing *Klebsiella pneumoniae* in Southern Spain in 2014-2015. Antimicrob Agents Chemother. 2019;63(1):e01396-18.
281. Lazaro-Perona F, Dahdouh E, Sotillo A, Perez-Blanco V, Villa J, Viedma E, et al. Dissemination of a single ST11 clone of OXA-48-producing *Klebsiella pneumoniae* within a large polyclonal hospital outbreak determined by genomic sequencing. Microb Genom. 2022;8(4):000808.
282. Wang G, Huang T, Surendraiah PK, Wang K, Komal R, Zhuge J, et al. CTX-M β-lactamase-producing *Klebsiella pneumoniae* in suburban New York City, New York, USA. Emerg Infect Dis. 2013;19(11):1803–10.
283. Aires CAM, Pereira PS, Rocha-de-Souza CM, Silveira MC, Carvalho-Assef APD, Asensi MD. Population structure of KPC-2-producing *Klebsiella pneumoniae* isolated from surveillance rectal swabs in Brazil. Microb Drug Resist. 2020;26(6):652–60.
284. Emeraud C, Birer A, Girlich D, Jousset AB, Creton E, Naas T, et al. Polyclonal dissemination of OXA-232 carbapenemase-producing *Klebsiella pneumoniae*, France, 2013-2021. Emerg Infect Dis. 2022;28(11):2304–7.
285. Benulič K, Pirš M, Couto N, Chlebowicz M, Rossen JWA, Zorec TM, et al. Whole genome sequencing characterization of Slovenian carbapenem-resistant *Klebsiella pneumoniae*, including OXA-48 and NDM-1 producing outbreak isolates. PLoS One. 2020;15(4):e0231503.
286. Zhu C, Liyanapathirana V, Li C, Pinto V, Hui M, Lo N, et al. Characterizing mobilized virulence factors and multidrug resistance genes in carbapenemase-producing *Klebsiella pneumoniae* in a Sri Lankan Hospital. Front Microbiol. 2018;9:2044.
287. Sahoo S, Sahoo RK, Dixit S, Behera DU, Subudhi E. NDM-5-carrying *Klebsiella pneumoniae* ST437 belonging to high-risk clonal complex (CC11) from an urban river in eastern India. 3 Biotech. 2023;13(5):139.

288. Andrade LN, Curiao T, Ferreira JC, Longo JM, Climaco EC, Martinez R, et al. Dissemination of *bla*$_{KPC-2}$ by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* species in Brazil. Antimicrob Agents Chemother. 2011;55(7):3579–83.
289. Tijet N, Sheth PM, Lastovetska O, Chung C, Patel SN, Melano RG. Molecular characterization of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* in Ontario, Canada, 2008-2011. PLoS One. 2014;9(12):e116421.
290. Seara N, Oteo J, Carrillo R, Perez-Blanco V, Mingorance J, Gomez-Gil R, et al. Interhospital spread of NDM-7-producing *Klebsiella pneumoniae* belonging to ST437 in Spain. Int J Antimicrob Agents. 2015;46(2):169–73.
291. Ranieri SC, Fabbrizi V, AM DA, Frascella MG, Di Biase V, Di Francesco C, et al. First report of a *bla*$_{NDM}$-producing extensively drug resistant *Klebsiella pneumoniae* ST437 in Italy. Frontiers in cellular and infection microbiology. 2024;14:1426817.
292. Di Marcantonio S, Perilli M, Alloggia G, Segatore B, Miconi G, Bruno G, et al. Coexistence of *bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-232}$, *bla*$_{SHV-182}$ genes in multidrug-resistant *K. pneumoniae* ST437-carrying OmpK36 and OmpK37 porin mutations: first report in Italy. J Glob Antimicrob Resist. 2024;37:24–7.
293. Pirs M, Cerar Kisek T, Krizan Hergouth V, Seme K, Mueller Premru M, Jeverica S, et al. Successful control of the first OXA-48 and/or NDM carbapenemase-producing *Klebsiella pneumoniae* outbreak in Slovenia 2014-2016. J Hosp Infect. 2019;101(2):142–9.
294. Fuster B, Salvador C, Tormo N, García-González N, Gimeno C, González-Candelas F. Molecular epidemiology and drug-resistance mechanisms in carbapenem-resistant *Klebsiella pneumoniae* isolated in patients from a tertiary hospital in Valencia, Spain. J Glob Antimicrob Resist. 2020;22:718–25.
295. Novovic K, Trudic A, Brkic S, Vasiljevic Z, Kojic M, Medic D, et al. Molecular epidemiology of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in Serbia from 2013 to 2016. Antimicrob Agents Chemother. 2017;61(5):e02550–16.
296. Loconsole D, Accogli M, De Robertis AL, Capozzi L, Bianco A, Morea A, et al. Emerging high-risk ST101 and ST307 carbapenem-resistant *Klebsiella pneumoniae* clones from bloodstream infections in Southern Italy. Ann Clin Microbiol Antimicrob. 2020;19(1):24.
297. Hadjirin NF, van Tonder AJ, Blane B, Lees JA, Kumar N, Delappe N, et al. Dissemination of carbapenemase-producing *Enterobacterales* in Ireland from 2012 to 2017: a retrospective genomic surveillance study. Microb Genom. 2023;9(3):mgen000924.
298. Patzer J, Toleman MA, Deshpande LM, Kaminska W, Dzierzanowska D, Bennett PM, et al. *Pseudomonas aeruginosa* strains harbouring an unusual *bla*$_{VIM-4}$ gene cassette isolated from hospitalized children in Poland (1998-2001). J Antimicrob Chemother. 2004;53(3):451–6.
299. Urbanowicz P, Izdebski R, Biedrzycka M, Gniadkowski M. VIM-type metallo-β-lactamase (MBL)-encoding genomic islands in *Pseudomonas* spp. in Poland: predominance of clc-like integrative and conjugative elements (ICEs). J Antimicrob Chemother. 2024;79(5):1030–7.
300. Urbanowicz P, Izdebski R, Baraniak A, Zabicka D, Hryniewicz W, Gniadkowski M. Molecular and genomic epidemiology of VIM/IMP-like metallo-β-lactamase-producing *Pseudomonas aeruginosa* genotypes in Poland. J Antimicrob Chemother. 2021;76(9):2273–84.
301. Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabak J, et al. Survey of metallo-β-lactamase-producing *Enterobacteriaceae* colonizing patients in European ICUs and rehabilitation units, 2008-11. J Antimicrob Chemother. 2015;70(7):1981–8.

302. Izdebski R, Biedrzycka M, Urbanowicz P, Żabicka D, Gniadkowski M. NDM- or VIM-producing *Citrobacter* spp. in Poland, 2011-19: high genetic diversity with broader representation of ST8, ST18 and ST22 global clones. Eur J Clin Microbiol Infect Dis. 2025;44(7):1723–8.
303. Heiden SE, Hubner NO, Bohnert JA, Heidecke CD, Kramer A, Balau V, et al. A *Klebsiella pneumoniae* ST307 outbreak clone from Germany demonstrates features of extensive drug resistance, hypermucoviscosity, and enhanced iron acquisition. Genome medicine. 2020;12(1):113.
304. Tavoschi L, Forni S, Porretta A, Righi L, Pieralli F, Menichetti F, et al. Prolonged outbreak of New Delhi metallo-β-lactamase-producing carbapenem-resistant *Enterobacterales* (NDM-CRE), Tuscany, Italy, 2018 to 2019. Euro Surveill. 2020;25(6):2000085.
305. Lam MMC, Holt KE, Wyres KL. Comment on: MDR carbapenemase-producing *Klebsiella pneumoniae* of the hypervirulence-associated ST23 clone in Poland, 2009-19. J Antimicrob Chemother. 2023;78(4):1132–4.
306. European Centre for Disease Prevention and Control E. Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries. 2021.
307. European Centre for Disease Prevention and Control E. Risk Assessment: Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries - first update. Available from: https://www.ecdc.europa.eu/en/publications-data/risk-assessment-emergence-hypervirulent-klebsiella-pneumoniae-eu-eea. 2024.
308. WHO. Health of refugees from Ukraine in Poland 2022; household survey and behavioural insights research. 2023.
309. Gumułka A. News CwZ: Rok wojny w Ukrainie – ilu pacjentów przyjął polski system? Available from: https://www.cowzdrowiu.pl/aktualnosci/post/rok-wojny-w-ukrainie-ilu-pacjentow-przyjal-polski-system. In Polish. 2023.

9. Statements of co-authors of joint publications

Warszawa, 19.01.2026 r.
(miejscowość, data)

dr Paweł Urbanowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

wykonanie analiz bioinformatycznych oraz badanie lekowrażliwości izolatów.

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

konceptualizację pracy, dobór metodologii, wykonanie analiz molekularnych i bioinformatycznych, badanie lekowrażliwości, intepretację wyników oraz przygotowanie ostatecznej wersji manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.

(imię i nazwisko kandydata do stopnia)

Paweł Urbanowicz
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 20.03.2026
(miejscowość, data)

dr Aneta Guzek
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: zbiórka izolatów wraz z danymi epidemiologicznymi, analiza mikrobiologiczna i wykrycie mechanizmów oporności, wstępna selekcja izolatów oraz przesłanie ich do dalszych analiz.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy, dobór metodologii, wykonanie analiz molekularnych i bioinformatycznych, badanie lekowrażliwości, intepretację wyników oraz przygotowanie ostatecznej wersji manuskryptu.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

Aneta Guzek
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Paris, March 29th, 2026
(miejscowość, data)
(place and date)

prof. Sylvain Brisse
(imię i nazwisko)

OŚWIADCZENIE

STATEMENT

Jako współautor pracy pt. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19,

As co-author of the work entitled: Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19,

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
analiza bioinformatyczna i konsulatcja wyników.
I declare that my own substantive contribution in the preparation, conduct and develeopment of research and the presentation of the work in the form of a publication is:
Bioinformatic analysis and consulation of the results.

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
konceptualizację pracy, dobór metodologii, wykonanie analiz molekularnych i bioinformatycznych, badanie lekowrażliwości, intepretację wyników oraz przygotowanie ostatecznej wersji manuskryptu.
Contribution of MS Marta Biedrzycka in the creation of the publication included:
Conceptualization and selection of methodology, performance of molecular and bioinformatic analyses, antimicrobial susceptibility testing, interpretation of the results and preparation of the final version of the manuscript.
Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
At the same time, I consent the use of the above-mentioned work as part of the doctoral dissertation MS Marta Biedrzycka.

....

(podpis oświadczającego)
(signature of declarant)

Warszawa, 18.03.26
.....
(miejscowość, data)

prof. dr hab. Marek Gniadkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: dobór metodologii, analiza epidemiologiczna, interpretacja uzyskanych wyników oraz przygotowanie ostatecznej wersji manuskryptu.

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)

konceptualizację pracy, dobór metodologii, wykonanie analiz molekularnych i bioinformatycznych, badanie lekowrażliwości, intepretację wyników oraz przygotowanie ostatecznej wersji manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

M. Gniadkowski
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 18.03.2026
(miejscowość, data)

dr hab. Radosław Izdebski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Dissemination of *Klebsiella pneumoniae* ST147 NDM-1 in Poland, 2015-19, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: konceptualizacja pracy, dobór metodologii, wykonanie analiz bioinformatycznych, badanie lekowrażliwości, konsultacje uzyskanych wyników, napisanie roboczej wersji manuskryptu, przygotowanie ostatecznej wersji manuskryptu.

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)

konceptualizację pracy, dobór metodologii, wykonanie analiz molekularnych i bioinformatycznych, badanie lekowrażliwości, intepretację wyników oraz przygotowanie ostatecznej wersji manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

R Izdebski
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

7.10.2026 r. Warszawa
(miejscowość, data)

dr Paweł Urbanowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy i dobór metodologii, zbiorka izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów oraz analiza bioinformatyczna.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy i dobór metodologii, zbiórkę izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Paris, March 29th, 2026
(miejscowość, data)
(place and date)

prof. Sylvain Brisse
(imię i nazwisko)

OŚWIADCZENIE
STATEMENT

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019,

As co-author of the work entitled: Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019,

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy, dobór metodologii, analiza bioinformatyczna oraz przygotowanie ostatecznej wersji manuskryptu.
I declare that my own substantive contribution in the preparation, conduct and development of research and the presentation of the work in the form of a publication is:
conceptualization of the work, selection of methodology, bioinformatics analysis and preparation of the final version of the manuscript

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
konceptualizację pracy i dobór metodologii, zbiórka izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
Contribution of MS Marta Biedrzycka in the creation of the publication included:
conceptualization and selection of methodology, survey and microbiological analyses of the isolates, obtaining epidemiological data and bioinformatic analysis.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
At the same time, I consent the use of the above-mentioned work as part of the doctoral dissertation MS Marta Biedrzycka.

....
(podpis oświadczającego)

Teramo, 30/03/2026
(miejscowość, data)
(place and date)

dr Federica Palma
(imię i nazwisko)

OŚWIADCZENIE

STATEMENT

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019,

As co-author of the work entitled: Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019,

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy, dobór metodologii oraz analiza bioinformatyczna.
I declare that my own substantive contribution in the preparation, conduct and develeopment of research and the presentation of the work in the form of a publication is:
conceptualization of the work, selection of methodology and bioinformatics analysis.

Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
konceptualizację pracy i dobór metodologii, zbiórka izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
Contribution of MS Marta Biedrzycka in the creation of the publication included:
conceptualization and selection of methodology, survey and microbiological analyses of the isolates, obtaining epidemiological data and bioinformatic analysis.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
At the same time, I consent the use of the above-mentioned work as part of the doctoral dissertation MS Marta Biedrzycka.

Federica Palma

(signature of declarant)

Warszawa 18.03.2026
(miejscowość, data)

dr Dorota Żabicka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy i dobór metodologii, zbiorka izolatów wraz z danymi epidemiologicznymi oraz wstępne badania mikrobiologiczne izolatów.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy i dobór metodologii, zbiórkę izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

Dorota Żabicka
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 18.03.26
(miejscowość, data)

prof. dr hab. Marek Gniadkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy i dobór metodologii oraz przygotowanie ostatecznej wersji manuskryptu.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy i dobór metodologii, zbiórkę izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 18.03.2026
(miejscowość, data)

dr hab. Radosław Izdebski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Multiple regional outbreaks caused by global and local VIM-producing *Klebsiella pneumoniae* clones in Poland, 2006-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
konceptualizacja pracy i dobór metodologii, zbiórka izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analiza bioinformatyczna oraz przygotowanie ostatecznej wersji manuskryptu.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy i dobór metodologii, zbiórkę izolatów wraz z danymi epidemiologicznymi, wstępne badania mikrobiologiczne izolatów, analizę bioinformatyczną.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

R Izdebski
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 19.03.2026 r
(miejscowość, data)

dr Paweł Urbanowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
wykonanie analiz bioinformatycznych.
Wkład mgr Marty Biedrzyckiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
konceptualizację pracy, dobór metodologii, wykonanie analiz bioinformatycznych, badanie lekowrażliwości, interpretację wyników, napisanie roboczej wersji manuskryptu oraz przygotowanie ostatecznej wersji manuskryptu.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Marty Biedrzyckiej.
(imię i nazwisko kandydata do stopnia)

Paweł Urbanowicz
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 18.03.2026
(miejscowość, data)

dr Dorota Żabicka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Country-wide expansion of a VIM-1 carbapenemase-producing *Klebsiella oxytoca* ST145 lineage in Poland, 2009-2019, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
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Dorota Żabicka
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

WARSZAWA 23.03.2026
(miejscowość, data)

prof. dr hab. Waleria Hryniewicz
(imię i nazwisko)

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(podpis oświadczającego)

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