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Probiotyki w zapobieganiu działaniom niepożądanym antybiotykoterapii u dzieci

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

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Spis treści

Wykaz stosowanych skrótów

AAD *antibiotic-associated diarrhoea*, biegunka związana z antybiotykoterapią

ADHD *attention deficit hyperactivity disorder*, zespół nadpobudliwości psychoruchowej z deficytem uwagi

ASD *autism spectrum disorders*, zaburzenia ze spektrum autyzmu

CI *confidence interval*, przedział ufności

COS *core outcome set*, zestaw podstawowych punktów końcowych

ESPGHAN *European Society for Paediatric Gastroenterology, Hepatology and Nutrition*, Europejskie Towarzystwo Gastroenterologii, Hepatologii i Żywienia Dzieci

PRR *pattern recognition receptors*, receptory rozpoznające wzorce

RCT *randomised controlled trial*, badanie z randomizacją

RR *relative risk*, ryzyko względne

Streszczenie

Antybiotyki to jedne z najczęściej stosowanych oraz nadużywanych leków w populacji dziecięcej. Ich wpływ na mikrobiom jelitowy może prowadzić do wystąpienia zarówno ostrych, jak i przewlekłych zdarzeń niepożądanych. Jednym z najczęstszych jest biegunka związana z antybiotykoterapią, w zapobieganiu której zastosowanie znajdują probiotyki. W odniesieniu do większości z nich nie udokumentowano jednak korzystnego działania. W celu uzyskania jednoznacznych dowodów skuteczności probiotyków konieczne jest prowadzenie badań klinicznych oceniających przejrzyście zdefiniowane, klinicznie istotne punkty końcowe.

W pierwszym badaniu zawartym w niniejszej rozprawie oceniono skuteczność probiotyku wielogatunkowego składającego się z 8 szczepów bakterii (*Bifidobacterium bifidum* W23*, B. lactis* W51*, Lactobacillus acidophilus* W37*, L. acidophilus* W55*, Lacticaseibacillus paracasei* W20*, Lactiplantibacillus plantarum* W62*, Lacticaseibacillus rhamnosus* W71 oraz *Ligilactobacillus salivarius* W24) w zapobieganiu biegunce związanej z antybiotykoterapią u dzieci. W tym celu, zgodnie z opublikowanym wcześniej protokołem (Łukasik i wsp., BMJ Open, 2018; 8[5]:1-7) przeprowadzono badanie z randomizacją i poczwórnie ślepą próbą w grupie 350 dzieci w wieku od 3 miesięcy do 18 lat (mediana wieku 28 miesięcy) otrzymujących systemowo antybiotyki o szerokim spektrum działania. Uczestnicy w ciągu 24 h od rozpoczęcia antybiotykoterapii byli losowo przydzielani do grupy otrzymującej probiotyk (w dobowej dawce 10^{10} jednostek tworzących kolonie) lub placebo przez cały czas antybiotykoterapii oraz przez 7 kolejnych dni. Pierwotnym punktem końcowym była biegunka związana z antybiotykoterapią, zdefiniowana jako co najmniej 3 luźne lub wodniste stolce na dobę w okresie 24 godzin, wywołane przez *Clostridioides difficile* lub o nieustalonej etiologii. W analizie w grupach wyodrębnionych zgodnie z zaplanowanym leczeniem dzieci otrzymujące probiotyk (n = 158) w porównaniu z otrzymującymi placebo (n = 155) miały podobne ryzyko wystąpienia biegunki związanej z antybiotykoterapią (ryzyko względne, *relative risk,* RR 0,81, 95% przedział ufności, *confidence interval,* CI 0,49–1,33) ocenianej według najbardziej konserwatywnej definicji opartej na wykluczeniu etiologii rotawirusowej, norowirusowej i adenowirusowej oraz *Salmonella spp., Campylobacter spp.* i *Yersinia spp.* Jednocześnie, w grupie otrzymującej badany probiotyk stwierdzono istotnie mniejsze w porównaniu z grupą otrzymującą placebo całkowite ryzyko biegunki ocenianej niezależnie od etiologii (RR 0,65;

95% CI 0,44–0,94). Nie stwierdzono istotnych różnic w odniesieniu do większości wtórnych punktów końcowych. Zdarzenia niepożądane występowały w obu grupach z podobną częstością (Łukasik i wsp., JAMA Pediatrics, 2022; w druku).

W drugim badaniu systematycznie udokumentowano punkty końcowe raportowane w badaniach dotyczących stosowania probiotyków w trakcie antybiotykoterapii u dzieci. W 37 badaniach spełniających kryteria włączenia do przeglądu zidentyfikowano aż 16 różnych definicji biegunki. Kryteria rozpoznania biegunki związanej ze stosowaniem antybiotyków nie były jasno zdefiniowane w 12 spośród 33 badań oceniających ją jako punkt końcowy. Wykluczenie zakaźnej etiologii przyjęto jako warunek diagnozy biegunki związanej ze stosowaniem antybiotyków jedynie w 7 badaniach. Czas trwania biegunki oceniano jedynie w 9, a jej ciężkość w 4 badaniach. Tylko 2 badania oceniały punkty końcowe związane z jakością życia pacjentów (Łukasik i wsp. PLoS One, 2020; 15[5]:e0228824).

Podsumowując, na podstawie wyników przeprowadzonego badania z randomizacją można rozważać zastosowanie badanego wielogatunkowego probiotyku w trakcie antybiotykoterapii u dzieci. Wyniki przeglądu systematycznego wykazały znaczną heterogenność punktów końcowych oraz ich niedostateczne przełożenie na rzeczywiste funkcjonowanie pacjentów. Wnioski płynące z obu badań demonstrują istotność definicji punktu końcowego dla interpretacji wyników badań klinicznych dotyczących stosowania probiotyków w trakcie antybiotykoterapii.

Abstract

Probiotics for preventing harms during antibiotic therapy in children

Antibiotics are one of the most used and overused drugs in the pediatric population. Their influence on the gut microbiome may lead to both acute and chronic adverse effects. Probiotics are usually used to reduce the risk of antibiotic-associated diarrhea (AAD). However, the effectiveness of most of them is not scientifically proven. To acquire unequivocal evidence, it is vital to conduct randomized controlled trials assessing clearly defined, clinically relevant outcomes.

In the first study, a multispecies probiotic consisting of 8 bacterial strains (*Bifidobacterium bifidum* W23*, B. lactis* W51*, Lactobacillus acidophilus* W37*, L. acidophilus* W55*, Lacticaseibacillus paracasei* W20*, Lactiplantibacillus plantarum* W62*, Lacticaseibacillus rhamnosus* W71 and *Ligilactobacillus salivarius* W24) was assessed for its effectiveness in reducing the risk of AAD in children. A randomized, quadruple blind, placebo-controlled trial involving 350 children aged 3 months – 18 years (median age: 28 months) receiving broad-spectrum systemic antibiotics was conducted according to the previously published protocol (Łukasik et al. BMJ Open. 2018; 8[5]:1-7). Within 24 h following initiation of antibiotic therapy, the participants were randomized to receive either the multispecies probiotic (at a daily dose of 10^{10} colony-forming units) or a placebo for the duration of antibiotic treatment and for 7 days after. The primary outcome was AAD, defined as 3 or more loose or watery stools per day in a 24-hour period, caused either by *Clostridioides difficile* or of otherwise unexplained etiology. In the intention-to-treat analysis, children from the probiotic group (n=158) compared to the placebo group (n=155) had a similar AAD risk (relative risk [RR] 0.81, 95% confidence interval [CI] 0.49 to 1.33) when assessed according to the most stringent definition, based on the exclusion of rotaviruses, noroviruses, adenoviruses, *Salmonella spp., Campylobacter spp.,* and *Yersinia spp*. However, children in the probiotic group had a lower risk of diarrhea regardless of the etiology (RR 0.65; 95% CI 0.44 to 0.94). No differences were observed between the groups for most of the secondary outcomes. The risk of adverse events was similar in both groups (Łukasik et al. JAMA Pediatrics. 2022; accepted).

The second study, a scoping systematic review, documented outcomes reported in studies on probiotic use during antibiotic therapy in children. Among 37 included studies, as many as 16 different diarrhea definitions were identified. Diagnostic criteria were not clearly defined in 12 out of 33 studies assessing AAD as an outcome. Tests for common diarrheal pathogens were performed to confirm the AAD diagnosis only in 7 studies. Diarrhea duration was assessed in 9, and severity only in 4 studies. Only 2 studies assessed quality of life-related outcomes (Łukasik et al. PLoS One. 2020; 15[5]:e0228824).

In conclusion, the administration of the studied multispecies probiotic may be considered for diarrhea prevention during antibiotic treatment in children. The results of the scoping review show considerable outcome heterogeneity and a scarcity of patient-relevant outcomes reported in the studies on probiotic use during antibiotic therapy in children. The summarised results of the two studies demonstrate that the AAD outcome definition has a significant impact on clinical trial results and their interpretation.

1. Wstęp

1.1. Niekorzystne skutki antybiotykoterapii u dzieci

1.1.1. Antybiotykoterapia u dzieci w ujęciu epidemiologicznym

Antybiotyki to jedne z najczęściej stosowanych leków w populacji pediatrycznej. Szacuje się, że częstość ich stosowania u dzieci w różnych rejonach świata wynosi średnio od 0,66 do 1,05 antybiotykoterapii na jedno dziecko rocznie w USA[1] i od 0,52 do 1,04 antybiotykoterapii na jedno dziecko rocznie w Niemczech[1] oraz że do osiągnięcia dorosłości antybiotyki otrzymuje 43% dzieci w Australii[2] i 63,8% w Chinach[3]. Nasilenie i dynamika tego problemu są powiązane ze statusem ekonomicznym kraju. W krajach o niskich i średnich dochodach pomiędzy rokiem 2000 a 2015 całkowita konsumpcja antybiotyków w populacji zwiększyła się dwukrotnie,[4] a w latach 2005–2017 częstość ich stosowania u dzieci poniżej 5. roku życia z objawami pospolitych infekcji wzrosła z 36,8 do 43,1%.[5] Jednocześnie, w części państw o wysokich dochodach w ostatnich latach obserwowany jest korzystny trend stabilizacji lub nawet zmniejszenia częstości stosowania antybiotyków u dzieci.[4,6] Również w Polsce w latach 2017–2019 wskaźnik dawek dobowych definiowanych w przeliczeniu na 1000 mieszkańców na dzień utrzymywał się na stałym poziomie, nie zostały jednak opracowane osobne dane dla populacji dziecięcej. W roku 2020 obserwowano istotne zmniejszenie zużycia antybiotyków zarówno w Polsce, jak i wielu innych krajach.[6,7] Zjawisko to jest wiązane ze zmianami epidemiologii pospolitych chorób zakaźnych w czasie pandemii SARS-CoV-2 wynikającymi z obostrzeń sanitarnych, takich jak stosowanie masek ochronnych, dystans społeczny czy zdalne działanie szkół i innych placówek edukacyjnych.[8] W związku z powyższym, należy się spodziewać, że efekt ten będzie zależny od zmieniających się zaleceń epidemiologicznych i może okazać się przejściowy.

Ze względu na szeroką dostępność, niską cenę oraz silne powszechne przekonanie o skuteczności antybiotyków nawet w sytuacjach niestanowiących wskazań do ich stosowania są one jednymi z najczęściej nadużywanych produktów leczniczych.[9] W dużym badaniu

kohortowym antybiotyk otrzymało aż 70% amerykańskich dzieci z rozpoznaniem zapalenia oskrzeli o prawdopodobnie wirusowej etiologii i 48% dzieci z wirusowym zapaleniem ucha środkowego. Wykazano również, że niewłaściwa antybiotykoterapia istotnie zwiększa koszty ponoszone przez pojedynczych pacjentów, jak i system opieki zdrowotnej w danym kraju.[10] Najgroźniejszym, obejmującym wymiar cywilizacyjny skutkiem tego zjawiska jest „kryzys oporności na antybiotyki",[11] uznawany przez Światową Organizację Zdrowia za jedno z dziesięciu największych współczesnych zagrożeń dla zdrowia w skali globalnej.[12] Inicjatywy dedykowane monitorowaniu oraz walce z rozprzestrzenianiem się szczepów antybiotykoopornych zajmują priorytetowe miejsca w narodowych i międzynarodowych programach zdrowotnych.[13]

1.1.2. Mikrobiota i mikrobiom przewodu pokarmowego u dzieci

Mikrobiotę definiuje się jako wszystkie mikroorganizmy (bakterie, archeony, eukarionty i wirusy) obecne w danym środowisku.[14] Termin „mikrobiom" jest zgodnie z większością proponowanych definicji szerszym pojęciem, obejmującym mikrobiotę, jej genom oraz środowisko zewnętrzne.[15] Najbogatszym ekosystemem w ciele człowieka jest mikrobiom jelitowy – szacuje się, że zasiedla go od kilkuset[16] do około 2 tysięcy różnych gatunków drobnoustrojów,[17] zaś w 1 gramie treści jelitowej znajduje się 10^{11} komórek bakteryjnych.[18] Jego kompozycja zmienia się wraz z wiekiem, a najbardziej dynamiczny okres rozwoju przypada na pierwsze dwa lata życia.[19] Co najmniej do 10. roku życia mikrobiom jelitowy dziecka istotnie różni się od mikrobiomu osoby dorosłej.[20] Jego charakterystyka zależy ponadto od wielu indywidualnych czynników, takich jak typ porodu (poród naturalny, cesarskie cięcie[21]), dieta (mleko kobiece, mleko modyfikowane, pokarmy uzupełniające[22]), wiek ciążowy w chwili narodzin[23] czy stosowanie leków – zwłaszcza antybiotyków[24] i inhibitorów pompy protonowej.[25]

Zależności pomiędzy mikrobiomem jelitowym i zdrowiem człowieka są jednym z głównych przedmiotów współczesnych badań biomedycznych.[26] Rośnie liczba dowodów na związki pomiędzy zdrowiem dzieci a dysbiozą, czyli zmianami składników mikrobiomu gospodarza w stosunku do mikrobiomu identyfikowanego u zdrowych osób.[27] Nietypowe wzorce mikrobiomu opisano między innymi u dzieci otyłych, z alergiami, chorobami

autoimmunologicznymi, czynnościowymi zaburzeniami przewodu pokarmowego i zaburzeniami neurorozwojowymi.[28,29]

Badania nad mikrobiomem człowieka są stosunkowo młodą dziedziną nauki. Z tego powodu związana z nim nomenklatura oraz podstawowe koncepcje stanowią przedmiot dyskusji, włączając w to ustalenie precyzyjnej definicji mikrobiomu. Istniejące propozycje dowodzą wielości perspektyw, składając się na:

- definicje ekologiczne, oparte na analogiach mikrobiomu z ekosystemami organizmów wielokomórkowych;
- definicje oparte na interakcjach pomiędzy mikroorganizmami a gospodarzem;
- definicje oparte na wykorzystywanej metodzie badawczej – część z nich charakteryzuje mikrobiom jako całkowity genom mikrobioty, nie uwzględniając jej środowiska;
- definicje mieszane.[15]

W piśmiennictwie stosowane są także różnorodne definicje dysbiozy, w zależności od podejścia skupiające się na zmianach mikrobiomu w stosunku do stanu wyjściowego, jego zaburzonej równowadze, nadmiernej ekspansji lub zmniejszonym udziale konkretnych drobnoustrojów.[30] Niezależnie od przyjętej definicji, do określania dysbiozy mogą służyć rozmaite systemy oceny ilościowej, tzw. „indeksy dysbiozy", wśród których również nie wskazuje się jednej właściwej metody.[31] Ponadto, dotychczas nie sprecyzowano, jakie cechy powinien posiadać mikrobiom zdrowego człowieka, co wynika zarówno z niedostatecznej wiedzy na temat jego składowych, jak i ze znacznej zmienności osobniczej. Przedmiotem dyskusji pozostaje nawet tak podstawowa kwestia, jak realna możliwość wykonania i zasadność badań nad szczegółowym określeniem „zdrowego mikrobiomu".[32]

1.1.3. Biegunka związana z antybiotykoterapią

Biegunka związana z antybiotykoterapią (ang. *antibiotic-associated diarrhea*, AAD) jest jednym z najlepiej udokumentowanych powikłań farmakoterapii infekcji bakteryjnych.[33] Najczęściej jest ona definiowana jako „biegunka, która wystąpiła w powiązaniu z leczeniem antybiotykami, po wykluczeniu innych etiologii".[34] Pierwotną przyczyną AAD jest dysbioza przewodu pokarmowego, jednak bezpośredni patomechanizm prowadzący do wystąpienia

biegunki nie jest jednorodny. Jednym z wytłumaczeń jest wywołany antybiotykoterapią spadek oporności mikrobiomu na kolonizację oraz powstanie nowych nisz w ekosystemie jelitowym, prowadzące w efekcie do nadmiernego namnażania się patogennych drobnoustrojów.[35] Najlepiej udokumentowanym z nich jest *Clostridioides difficile.*[36] Opisywano również między innymi rolę kolonizacji przez *Staphylococcus aureus*,[37] *Clostridium perfringens*[38] czy *Klebsiella oxytoca*.[39] Innym potencjalnym patomechanizmem AAD są wynikające z dysbiozy zaburzenia funkcji metabolicznych mikrobioty, takich jak wytwarzanie krótkołańcuchowych kwasów tłuszczowych pełniących rolę w zapobieganiu biegunce osmotycznej oraz odżywianiu enterocytów.[40,41] Niektórzy autorzy rozszerzają definicję AAD na każdą biegunkę pozostającą w związku czasowym z antybiotykoterapią, włączając w to infekcje pospolitymi patogenami wirusowymi takimi jak rotawirusy, adenowirusy czy norowirusy.[42] Nie ma jednak dowodów na związek przyczynowo-skutkowy leczenia przeciwdrobnoustrojowego i zakażeń wirusowych przewodu pokarmowego poza zwiększonym ryzykiem wynikającym z okoliczności kontaktu z instytucjami opieki zdrowotnej.[43] Mechanizmem niezależnym od dysbiozy, mogącym odgrywać rolę w patogenezie AAD, jest ponadto bezpośrednie działanie prokinetyczne niektórych antybiotyków (makrolidów, amoksycyliny z kwasem klawulanowym).[44,45]

Przebieg i patogeneza AAD istotnie różnią się u dzieci i u osób dorosłych – w populacji pediatrycznej jest ona rzadziej związana z infekcją *C. difficile*, ma krótszy okres wylęgania, przeciętnie trwa krócej (od 3 do 9 dni) i częściej prowadzi do odwodnienia.[36] AAD u dzieci ma najczęściej lekki, samoograniczający się przebieg, a jego najcięższa postać (związane z *C. difficil*e rzekomobłoniaste zapalenie jelita grubego) występuje głównie u osób dorosłych i dzieci z zaburzeniami odporności.[33] Czas do wystąpienia AAD wynosi od poniżej jednej doby do aż 8 tygodni od rozpoczęcia antybiotykoterapii.[42] Inne związane z dysbiozą działania niepożądane antybiotykoterapii dotyczące przewodu pokarmowego, takie jak nudności, wymioty, ból brzucha i pogorszenie łaknienia, mogą towarzyszyć AAD lub występować niezależnie od niej.[46]

Częstość AAD zależy od przyjętej definicji i badanej populacji. W najbardziej aktualnym przeglądzie systematycznym badań z randomizacją dotyczącym stosowania probiotyków w trakcie antybiotykoterapii wykazano, że wystąpiła ona łącznie u 19% dzieci z grup kontrolnych włączonych do przeglądu,[47] przy czym odsetek ten wahał się od 2[48] do 80%.[49] Na

podstawie badania obserwacyjnego dedykowanego określeniu epidemiologii AAD zdefiniowanej jako „3 lub więcej luźne lub płynne stolce na dobę przez co najmniej 2 kolejne dni w trakcie antybiotykoterapii i przez 7 dni po zakończeniu leczenia" jej częstość u dzieci leczonych ambulatoryjnie oszacowano na 11%.[50] Do znanych czynników ryzyka wystąpienia powikłania należą: młodszy wiek (szczególnie poniżej 2 lat), klasa zastosowanego antybiotyku (największe ryzyko dla aminopenicylin z inhibitorami β-laktamaz, cefalosporyn i linkozamidów), przedłużony czas trwania antybiotykoterapii, hospitalizacja i wcześniejsze epizody AAD.[34,50]

1.1.4. Odległe niekorzystne skutki antybiotykoterapii u dzieci

Poza wczesnymi, najczęściej szybko ustępującymi działaniami niepożądanymi antybiotykoterapii manifestującymi się ostrymi objawami żołądkowo-jelitowymi przedmiotem badań w ostatnich latach był wpływ antybiotyków na ryzyko chorób przewlekłych w późniejszym wieku. Niektóre z nich zostały opisane w niniejszym rozdziale.

Zaburzenia neurorozwojowe

Według teorii „osi mikrobiom-jelito-mózg"[51] w ciele człowieka istnieje dwukierunkowa relacja pomiędzy mikrobiomem a ośrodkowym układem nerwowym. Według tego modelu „dialog" pomiędzy mózgiem i przewodem pokarmowym prowadzony jest za pośrednictwem zstępujących i wstępujących sygnałów przewodzonych przez nerwy błędne. Rola mikrobioty w tym mechanizmie ma wynikać z produkowanych przez mikroorganizmy neurotransmiterów, takich jak serotonina, dopamina, noradrenalina i kwas γ-aminomasłowy,[52] a także regulacji funkcji immunologicznych mikrogleju.[53]

Istnieją doniesienia o dysbiozie stwierdzanej w różnych zaburzeniach psychiatrycznych i neurorozwojowych, takich jak zaburzenia ze spektrum autyzmu (ang. *autism spectrum disorder*s, ASD)[54] i zespół nadpobudliwości psychoruchowej z deficytem uwagi (ang. *attention deficit hyperactivity disorder*, ADHD) u dzieci[55] czy depresja i zaburzenia lękowe u osób dorosłych.[56] Wpływ antybiotyków na ryzyko ADHD i ASD u dzieci był przedmiotem licznych badań obserwacyjnych. W 5 spośród 6 badań kohortowych poświęconych temu zagadnieniu

opisano pozytywny związek pomiędzy wczesnodziecięcą ekspozycją na antybiotyki a ryzykiem ADHD, który tracił jednak istotność statystyczną po ograniczeniu analizy porównawczej do rodzeństwa dzieci chorych.[57] Podobny związek obserwowano w części badań dotyczących ASD.[58-60] Również prenatalna ekspozycja na antybiotyki była przez część autorów wiązana z ryzykiem autyzmu.[61,62] Istniejące badania obserwacyjne na ten temat zostały podsumowane przy współudziale autora niniejszej rozprawy w przeglądzie systematycznym z metaanalizą.[63] Na jego podstawie oceniono, że dostępne dowody na istnienie związku pomiędzy wczesną ekspozycją na antybiotyki a ryzykiem autyzmu u dzieci są niejednoznaczne i niewystarczające do sformułowania silnych konkluzji.

Otyłość i nadwaga

Antybiotykoterapia prenatalna oraz stosowana w dzieciństwie były oceniane jako czynnik ryzyka nadmiernej masy ciała w licznych badaniach kohortowych oraz kilku przeglądach systematycznych. Najbardziej aktualny przegląd systematyczny z metaanalizą nie wykazał istotnego związku pomiędzy prenatalną antybiotykoterapią a późniejszym ryzykiem otyłości i nadwagi u dzieci.[64] W czterech przeglądach systematycznych opublikowanych w ostatnim czasie wykazano niewielki wzrost ryzyka otyłości i nadwagi u dzieci eksponowanych na antybiotyki we wczesnym dzieciństwie.[65-68] Zależność pomiędzy mikrobiomem a masą ciała może być tłumaczona różnymi mechanizmami, w tym: regulującymi anabolizm tkanki tłuszczowej receptorami dla krótkołańcuchowych kwasów tłuszczowych produkowanych przez bakterie,[69] wpływem mikrobiomu na trawienie, absorpcję, gromadzenie i wydzielanie lipidów w nabłonku jelitowym[70,71] czy regulacją centralnych ośrodków głodu i sytości za pośrednictwem osi mikrobiom-jelito-mózg.[72]

Choroby autoimmunologiczne

Przewód pokarmowy wraz z jego mikrobiomem jest największą powierzchnią ekspozycji na antygeny zewnętrzne w ciele człowieka, krytyczną dla rozwoju prawidłowo działającego układu odpornościowego.[73] Kluczową rolę w interakcji pomiędzy mikrobiomem a układem immunologicznym odgrywają receptory rozpoznające wzorce (ang. *pattern recognition receptors*, PRR), znajdujące się na powierzchni komórek odporności wrodzonej, takich jak

komórki dendrytyczne, makrofagi i komórki NK.[74] Receptory te posiadają zdolność rozpoznawania między innymi wzorców molekularnych związanych z mikroorganizmami (ang. *microbe associated molecular pattern*s) mikrobiomu.[75] Interakcja ta ma regulacyjny wpływ na tkankę limfatyczną związaną z jelitami (ang. *gut-associated lymphatic tissue*)[76] oraz na inne ośrodki układu odpornościowego, takie jak śledziona, grasica czy węzły chłonne.[77] Dysbioza wywołana antybiotykoterapią i wynikające z niej zaburzenia immunologiczne były przedmiotem licznych badań oceniających czynniki ryzyka chorób autoimmunologicznych.

W dwóch opublikowanych w ostatnich latach przeglądach systematycznych stwierdzono wzrost ryzyka choroby Leśniowskiego i Crohna u dzieci poddawanych uprzednio antybiotykoterapii. Podobnego efektu nie zaobserwowano w odniesieniu do wrzodziejącego zapalenia jelita grubego.[78,79] W jednym badaniu kohortowym i dwóch badaniach kliniczno-kontrolnych opisano zwiększone ryzyko młodzieńczego idiopatycznego zapalenia stawów po antybiotykoterapii w dzieciństwie.[80-82]

Dysbioza wywołana antybiotykami zwiększała ryzyko cukrzycy typu 1 w modelu zwierzęcym,[83] obserwacje te nie potwierdziły się jednak w badaniach obserwacyjnych u ludzi.[84]

Sprzeczne są dane na temat związku antybiotykoterapii z ryzykiem choroby trzewnej.[85,86]

Choroby alergiczne

W oparciu o podobne mechanizmy jak w przypadku chorób autoimmunologicznych badany jest związek pomiędzy wywołaną antybiotykami dysbiozą a chorobami na tle alergicznym. Metaanaliza 52 badań obserwacyjnych wykazała związek pomiędzy antybiotykoterapią we wczesnym dzieciństwie i późniejszym ryzykiem astmy.[87] Zależność obserwowano również w badaniach obserwacyjnych dotyczących ryzyka atopowego zapalenia skóry, alergii na pokarm i alergicznego nieżytu nosa.[84] Podobne obserwacje dotyczące świszczącego oddechu, astmy wczesnodziecięcej, wyprysku i atopowego zapalenia skóry uzyskiwano w badaniach dotyczących prenatalnej ekspozycji na antybiotyki.[88]

Badania na temat związku antybiotykoterapii z chorobami przewlekłymi – trudności interpretacyjne

Statystycznie istotny związek pomiędzy antybiotykoterapią a ryzykiem chorób przewlekłych wykazano w licznych badaniach obserwacyjnych. Do ich wyników należy jednak podchodzić z najwyższą ostrożnością, szczególnie w kwestii podejmowania decyzji klinicznych. Ocena związku przyczynowo-skutkowego za pomocą badań obserwacyjnych jest z definicji obarczona większym ryzykiem błędu i wymaga bardziej złożonego podejścia niż w przypadku badania z randomizacją.[89] W ocenie późnych skutków antybiotykoterapii trudnym do wyeliminowania źródłem błędu, nie zawsze obecnym w badaniach obserwacyjnych, jest „zakłócenie poprzez wskazanie do leczenia" (ang. *confounding by indication*).[90] Zdecydowana większość dzieci otrzymuje antybiotyki z powodu infekcji, więc ustalenie, czy na ryzyko choroby przewlekłej wypłynęła przebyta choroba zakaźna, czy jej leczenie, jest bardzo trudne nawet przy użyciu metod statystycznych uwzględniających czynniki zakłócające. Kolejnym zjawiskiem potencjalnie prowadzącym do błędnych konkluzji jest „błąd protopatyczny" (ang. *protopathic bias*).[91] Trudno jest na przykład ustalić, w jakim stopniu stosowanie antybiotyków w dzieciństwie zwiększa ryzyko astmy, a w jakim stopniu częste infekcje układu oddechowego wymagające antybiotykoterapii są jej wczesnym objawem.

Natura problemu powoduje, że w praktyce bardzo trudna lub wręcz niemożliwa jest eksploracja omawianego związku w badaniu interwencyjnym. Rodzaj interwencji (antybiotykoterapia we wczesnym dzieciństwie) z przyczyn etycznych znacznie ogranicza możliwość przeprowadzenia badania z randomizacją. Choroby przewlekłe mogą występować dłuższy czas po leczeniu i bywają stosunkowo rzadkie, co wymuszałoby utworzenie grupy badanej o dużej liczebności i długim okresie obserwacji. W czasie obserwacji istotna część dzieci zostałaby poddana antybiotykoterapii z innych wskazań, co dodatkowo utrudniłoby interpretację wyników.

Jednym z kierunków rozwoju naukowego mogącym przybliżyć odpowiedź na pytanie o zależność pomiędzy antybiotykoterapią a ryzykiem chorób przewlekłych są badania nad mikrobiomem człowieka.

1.1.5. Problem oporności na antybiotyki w pediatrii

Oporność na antybiotyki jest wielowymiarowym i niejednorodnym problemem, którego specyfika i nasilenie są unikalne dla każdego regionu geograficznego[92,93] i uzależnione od okoliczności socjoekonomicznych[94] oraz cech pacjentów wpływających na epidemiologię chorób zakaźnych w danej grupie (jak wiek czy choroby współistniejące). Powagę problemu w pediatrycznej grupie wiekowej dobrze obrazuje rozpowszechnienie opornych na podstawowe leczenie szczepów bakterii często wywołujących infekcje u dzieci, m.in.:

- *Streptococcus pneumoniae* – czynnik etiologiczny zapalenia ucha środkowego, zapalenia zatok, zapalenia płuc oraz zakażeń inwazyjnych. Według danych Europejskiego Centrum ds. Kontroli i Zapobiegania Chorób oraz Światowej Organizacji Zdrowia, odsetek wyizolowanych szczepów opornych na penicylinę w Polsce w roku 2020 wynosił około 11%, a szczepów opornych na makrolidy 23%.[93]
- *Haemophilus influenzae* w wyniku aktualnych programów szczepień w Europie jest przede wszystkim czynnikiem etiologicznym infekcji dróg oddechowych, w przeszłości również zakażeń inwazyjnych. Według danych projektu Respi-Net w roku 2019 w Polsce około 80% szczepów było wrażliwych na aminopenicyliny, a 70% na cefuroksym.[95]
- *Escherichia coli* – patogen najczęściej wywołujący u dzieci zakażenia układu moczowego. Brak danych polskich na temat aktualnej antybiotykooporności szczepów wywołujących zakażenia dróg moczowych u zdrowych dzieci. W innych krajach europejskich oceniono, że około 75–85% szczepów jest wrażliwych na amoksycylinę z kwasem klawulanowym, 83% na cefuroksym i 70% na trimetoprim lub kotrimoksazol.[96,97]

Wyżej wymieniono wybrane drobnoustroje, których lekowrażliwość jest szczególnie ważna w codziennej praktyce pediatrycznej. Wiele innych bakterii o wysokim potencjale oporności może być niebezpiecznych dla szczególnie narażonych grup pacjentów (m.in. *Staphylococcus spp., Klebsiella pneumoniae, Enterococcus spp.* czy *Pseudomonas aeruginosa).*[93] Tylko nieliczne spośród pospolitych patogenów sporadycznie rozwijają niewrażliwość na leczenie pierwszego rzutu (jak w przypadku *Neisseria meningitidis*)[98] lub nie rozwijają jej nigdy (jak *Streptococcus pyogenes*).[95]

Rozpowszechnienie antybiotykooporności jest jednoznacznie powiązane z częstością stosowania antybiotyków w danej populacji.[11] W wyniku ekspozycji dochodzi do selekcji szczepów o zmniejszonej wrażliwości, które następnie przekazują kluczowe geny organizmom potomnym lub horyzontalnie, za pomocą mobilnych elementów genetycznych, takich jak plazmidy.[99] Ważną rolę tym procesie odgrywa mikrobiom, stanowiący bogaty rezerwuar genów antybiotykooporności (tzw. rezystom), z którego patogeny mogą „zapożyczać" kluczowe dla przetrwania mechanizmy.[100] Zarówno krótkotrwała, jak i przewlekła antybiotykoterapia może prowadzić do selekcji niewrażliwych szczepów u dzieci. Wykazano, że nawet jednorazowe leczenie β-laktamami zwiększa oporność kolonizującego gardło *H. influenzae* na tę grupę leków w sposób przejściowy, lecz istotnie wpływający na populacyjny profil antybiotykowrażliwości.[101] W metaanalizie z 2019 roku ryzyko pojawienia się szczepów opornych u dzieci przyjmujących przewlekle profilaktykę antybiotykową w związku z zakażeniami układu moczowego było dwa i pół razy większe niż w grupie kontrolnej otrzymującej placebo.[102]

Nieracjonalnie wdrażane leczenie przeciwdrobnoustrojowe prowadzi do konsekwencji dotykających bezpośrednio pacjentów, zwiększenia kosztów opieki zdrowotnej oraz zagrożeń dla zdrowia publicznego w skali globalnej.[103] Niezależnie od już stosowanych i wciąż badanych interwencji mających przeciwdziałać niekorzystnym skutkom antybiotykoterapii, nie należy zapominać, że najlepszą metodą zapobiegawczą jest racjonalna polityka antybiotykowa polegająca na stosowaniu leczenia o możliwie najwęższym spektrum, przez najkrótszy skuteczny czas, we właściwych dawkach oraz potwierdzonych naukowo wskazaniach.[104]

1.2. Probiotyki

1.2.1. Terminologia

Według obowiązującej definicji probiotyki to „żywe mikroorganizmy, które podawane w odpowiednich ilościach wywierają korzystne skutki zdrowotne".[105] Do terminów pokrewnych należą „prebiotyki", „synbiotyki" oraz „postbiotyki" (**Tab. 1.**).

Tabela 1. Aktualnie obowiązująca terminologia dotycząca „biotyków”

Termin	**Obowiązująca definicja**
Probiotyki	Żywe mikroorganizmy, które podawane w odpowiednich ilościach wywierają korzystne skutki zdrowotne[105]
Prebiotyki	Substancje, które są selektywnie wykorzystywane przez mikroorganizmy zasiedlające organizm człowieka, pośrednio przynosząc korzyści zdrowotne[106]
Synbiotyki	Mieszanina składająca się z żywych drobnoustrojów i substratu/substratów selektywnie wykorzystywanych przez drobnoustroje gospodarza i korzystnie oddziałujących na jego organizm[107]
Postbiotyki	Preparaty składające się z nieożywionych mikroorganizmów i/lub ich składowych przynoszące korzyści zdrowotne[108]

1.2.2. Mechanizmy działania probiotyków

Najbardziej bezpośrednim mechanizmem działania probiotyków jest kolonizacja jelit prowadząca do zmiany składu oraz funkcjonowania mikrobiomu gospodarza. Warunkiem działania probiotyku w tym mechanizmie jest zdolność przetrwania istotnej liczby żywych komórek w niekorzystnych warunkach przewodu pokarmowego oraz posiadanie cech umożliwiających kolonizację, takich jak zdolność do adhezji.[109] Choć w powszechnej opinii mechanizm ten uznaje się za istotny, większość badań klinicznych z zastosowaniem probiotyków nie oceniało, czy i jak długo żywe drobnoustroje utrzymują się w organizmie gospodarza.[110] Międzynarodowe Towarzystwo Naukowe Probiotyków i Prebiotyków (ang. *International Scientific Association for Probiotics and Prebiotics*, ISAPP) stoi na stanowisku, że kolonizacja przewodu pokarmowego nie jest kryterium świadczącym o skuteczności probiotyku.[111] Jeśli nawet do niej dojdzie, ma ona najczęściej charakter przejściowy ze względu na oporność ekosystemu jelitowego na zasiedlenie nowymi gatunkami.[112] Ponadto, podatność na kolonizację szczepami probiotycznymi zależy od indywidualnej kompozycji mikrobiomu gospodarza.[113]

Innymi mechanizmami działania probiotyków mogącymi odgrywać rolę nawet w przypadku przejściowej kolonizacji są konkurencja o zasoby z drobnoustrojami patogennymi oraz bezpośrednie działanie antagonistyczne poprzez wytwarzanie bakteriocyn, zmiany pH i blokowanie adhezji patogenów.[110,114,115] Zjawiska te wydają się szczególnie istotne w przypadku stosowania probiotyków w leczeniu i zapobieganiu zakażeniom przewodu pokarmowego oraz biegunce związanej z antybiotykoterapią.[116]

Mikroorganizmy probiotyczne mogą również wpływać na zdrowie za pośrednictwem własnej aktywności enzymatycznej oraz indukując lub hamując enzymy mikrobiomu gospodarza;[117,118] wytwarzając i metabolizując działające wielokierunkowo na organizm krótkołańcuchowe kwasy tłuszczowe – masłowy, propionowy i octowy;[119,120] regulując działanie układu odpornościowego poprzez oddziaływanie na receptory PRR[121] czy modyfikując działanie osi mikrobiom-jelita-mózg.[122]

1.2.3. Stosowanie probiotyków – uwagi ogólne

Kliniczny efekt probiotyków zależy od zastosowanego gatunku i szczepu, dlatego każdy preparat probiotyczny powinien być poddany badaniom dotyczącym jego skuteczności i bezpieczeństwa w konkretnym wskazaniu. Ta sama zasada dotyczy poszczególnych preparatów wieloszczepowych i wielogatunkowych.[123] Skuteczność probiotyku zależy również od jego dawki, która powinna być ustalona w oparciu o badania kliniczne.[124] Kolejnym istotnym aspektem praktycznego zastosowania probiotyków jest jakość produkcyjna konkretnych preparatów. W stanowisku opublikowanym w 2017 roku Europejskie Towarzystwo Gastroenterologii, Hepatologii i Żywienia Dzieci (ang. *European Society for Paediatric Gastroenterology, Hepatology and Nutrition*, ESPGHAN) zwróciło uwagę na problem rejestracji większości probiotyków jako suplementów diety i wynikającą z niego mniej rygorystyczną w porównaniu z lekami kontrolę jakości.[125] W piśmiennictwie dostępne są liczne dowody niezgodności oficjalnych opisów preparatów probiotycznych z ich rzeczywistą zawartością dotyczące gatunków i szczepów,[126] deklarowanej przez producenta dawki probiotyku[127] czy kontaminacji produktu innymi drobnoustrojami.[128]

1.2.4. Probiotyki w zapobieganiu biegunce związanej z antybiotykoterapią

Zgodnie z najnowszym (2019) przeglądem systematycznym Cochrane obejmującym 33 badania z randomizacją probiotyki jako grupa wykazują umiarkowany efekt ochronny przed AAD.[47] Pomimo dowodów na skuteczność jednoznaczne zalecenie stosowania probiotyków u każdego dziecka przyjmującego antybiotyk budzi kontrowersje ze względu na najczęściej łagodny, samoograniczający się przebieg biegunki.[129] Wątpliwości te odzwierciedlają najaktualniejsze (2016) wytyczne ESPGHAN, według których stosowanie probiotyków o udowodnionej skuteczności można rozważać w przypadku występowania u dziecka czynników ryzyka AAD, takich jak antybiotykoterapia o szerokim spektrum, przedłużona antybiotykoterapia czy hospitalizacja. Szczepami o udowodnionej skuteczności są *Lacticaseibacillus* (wcześniejsza nazwa *Lactobacillus*) *rhamnosus* GG i *Saccharomyces boulardii.*[34] Jedynie 6 badań z randomizacją oceniało skuteczność probiotyków w zapobieganiu biegunce wywołanej przez *C. difficile* u dzieci.[130] Na ich podstawie w grupach szczególnie narażonych na ten rodzaj powikłania można rozważyć zastosowanie profilaktyczne szczepu *S. boulardii.*[34]

1.2.5. Ostry nieżyt żołądkowo-jelitowy

Ostry nieżyt żołądkowy u dzieci jest najczęściej powodowany przez infekcje wirusowe, rzadziej przez bakteryjne.[131] Według ostatniego przeglądu systematycznego Cochrane dowody na skuteczność probiotyków (analizowanych jako jedna grupa) w skracaniu czasu trwania ostrej biegunki są niejednoznaczne.[132] W aktualnych wytycznych ESPGHAN zawarte są zalecenia o słabej i bardzo słabej sile dowodu przemawiające za stosowaniem *S. boulardii*, *L. rhamnosus* GG, *Limosilactobacillus* (wcześniejsza nazwa *Lactobacillus*) *reuteri* DSM 17938 lub *L. rhamnosus* 19070-2 w połączeniu z *L. reuteri* DSM 12246 w celu skrócenia czasu trwania objawów ostrego nieżytu żołądkowo-jelitowego u dzieci.[133]

1.2.6. Biegunka szpitalna

Biegunka szpitalna jest heterogenną jednostką chorobową, obejmującą zarówno biegunki zakaźne, jak i związane ze stosowaniem antybiotyków w warunkach szpitalnych.[134,135]

Aktualne wytyczne ESPGHAN sformułowane na podstawie analizy 8 badań z randomizacją zalecają stosowanie *L. rhamnosus* GG w sytuacjach, gdy podanie probiotyku jest rozważane.[136]

1.2.7. Czynnościowe zaburzenia przewodu pokarmowego

Jednym z probiotyków o najlepiej udowodnionej naukowo skuteczności w leczeniu i zapobieganiu czynnościowym zaburzeniom przewodu pokarmowego u dzieci jest *L. reuteri* DSM 17938. Jego stosowanie zmniejszało czas trwania płaczu związanego z kolką niemowlęcą według wszystkich opublikowanych do tej pory przeglądów systematycznych, w tym przeglądu Cochrane z 2019 roku.[137] Niektóre probiotyki mogą być również stosowane w leczeniu czynnościowych bólów brzucha, szczególnie w zespole jelita drażliwego.[138] Istnieją badania z randomizacją wskazujące na korzystne działanie wybranych probiotyków w regurgitacjach niemowlęcych,[139] jednak dowody naukowe są niewystarczające, by uzasadniać ich stosowanie.[140] Nie ma również podstaw do ich stosowania w leczeniu zaparcia czynnościowego.[141]

1.2.8. Nieswoiste zapalenia jelit

Zaburzenia mikrobiomu odgrywają kluczową rolę w patogenezie choroby Leśniowskiego i Crohna i wrzodziejącego zapalenia jelita grubego.[142] Istnieją ograniczone i niepewne dowody na skuteczność konkretnych probiotyków (*L. reuteri* ATCC 55730, *Escherichia coli Nissle* 1917 i jednego z preparatów wielogatunkowych zawierającego 8 szczepów bakterii) jako terapii pomocniczej w indukcji remisji i terapii podtrzymującej wrzodziejącego zapalenia jelita grubego.[143,144] Stosowanie probiotyków w leczeniu choroby Leśniowskiego i Crohna nie jest zalecane.[143]

1.2.9. Martwicze zapalenie jelit, sepsa i inne powikłania wcześniactwa

Skuteczność probiotyków w poprawie rokowania u dzieci urodzonych przedwcześnie i z bardzo małą urodzeniową masą ciała (ang. *very low birth weight*) była przedmiotem ponad

60 badań klinicznych, które podsumowano w 2020 roku w metaanalizie sieciowej[145] oraz w przeglądzie systematycznym Cochrane.[146] Obie prace wykazały korzystny wpływ niektórych probiotyków na ryzyko martwiczego zapalenia jelit (ang. *necrotizing enterocolitis*, NEC). Ponadto, przegląd Cochrane wykazał zmniejszenie całkowitej śmiertelności oraz ryzyka sepsy o późnym początku w tej grupie pacjentów. W aktualnych wytycznych ESPGHAN sformułowano warunkowe zalecenie dotyczące stosowania *L. rhamnosus* GG ATCC 53103 lub kombinacji *Bifidobacterium infantis* Bb-02, *Bifidobacterium lactis* Bb-12 i *Streptococcus thermophilus* TH-4 w celu zmniejszania częstości NEC.[147] Warunkiem zastosowania tej interwencji jest ścisłe przestrzeganie zasad bezpieczeństwa w populacji dzieci urodzonych przedwcześnie, wyjątkowej pod względem ryzyka wystąpienia sepsy wywołanej szczepami zawartymi w probiotykach[148] oraz kwasicy d-mleczanowej.[149]

1.2.10. Choroby alergiczne

Skuteczność probiotyków w zapobieganiu i leczeniu chorób alergicznych oceniano w licznych badaniach, przede wszystkim dotyczących atopowego zapalenia skóry, alergicznego nieżytu nosa oraz astmy wczesnodziecięcej.[150] Według stanowiska Europejskiej Akademii Alergii i Immunologii Klinicznej (ang. *European Academy of Allergy and Clinical Immunology*, EAACI) nie ma wystarczających dowodów, aby wydać zalecenie za lub przeciw stosowaniu probiotyków w zapobieganiu[151] i leczeniu[152] alergii na pokarm. Ze względu na niejednoznaczne wyniki badań probiotyki nie są również zalecane w chorobach związanych z nadwrażliwością na alergeny wziewne.[150]

1.2.11. Probiotyki wielogatunkowe i wieloszczepowe

Probiotyki wieloszczepowe można definiować jako preparaty zawierające więcej niż jeden szczep tego samego gatunku lub blisko spokrewnionych gatunków, a wielogatunkowe jako zawierające co najmniej dwa szczepy pochodzące z różnych gatunków.[153] Koncepcja stosowania tego typu mieszanek wynika z wiedzy o unikalnych właściwościach różnych szczepów. Na przykład, jeśli uda się zidentyfikować dwa drobnoustroje wykazujące w danym wskazaniu korzystne działanie oparte na różnych mechanizmach, to w teorii zastosowanie ich

razem może przynieść lepszy efekt niż każdego osobno w monoterapii.[154] Indywidualne szczepy mogą również wykazywać między sobą synergizm poprzez wzajemną potencjalizację właściwości adhezyjnych czy hamowanie wzrostu patogenów.[155,156] Z drugiej strony, mogą też oddziaływać na siebie antagonistycznie,[157] dlatego każdy preparat wieloszczepowy i wielogatunkowy powinien zostać oceniony w badaniu klinicznym.

Probiotyki wielogatunkowe i wieloszczepowe wielokrotnie poddawano ocenie w badaniach klinicznych.[158] U dzieci najlepiej udowodniono ich skuteczność w skracaniu czasu utrzymywania się objawów ostrej biegunki (*L. rhamnosus* 19070-2 w połączeniu z *L. reuteri* DSM 12246),[133] indukcji remisji i jej podtrzymaniu we wrzodziejącym zapaleniu jelita grubego (probiotyk zawierający 8 szczepów)[143,144] oraz w profilaktyce NEC (kombinacja *Bifidobacterium infantis* Bb-02, *Bifidobacterium lactis* Bb-12 i *Streptococcus thermophilus* TH-4).[147] Zarazem nie ma przekonujących dowodów na to, że jakikolwiek pojedynczy szczep jest w danym zastosowaniu mniej skuteczny niż preparat wieloszczepowy zawierający ten sam szczep jako jeden z elementów. Jedyne dwa badania, w których bezpośrednio przeprowadzono takie porównanie, nie wykazały różnic pomiędzy grupami.[159,160] Różnic pomiędzy preparatami jedno- i wieloszczepowymi nie stwierdzono również w zdecydowanej większości przypadków, gdy porównywano do siebie wyniki różnych badań.[158]

1.4. Punkty końcowe w badaniach klinicznych

1.4.1. Problemy związane z definiowaniem punktów końcowych

Fundamentalną zasadą prowadzenia badań klinicznych jest precyzyjne sformułowanie pytania klinicznego.[161] W badaniach dotyczących interwencji terapeutycznych powszechnie przyjęto format PICO – akronim, w którym „P" oznacza populację (ang. *population*), „I"– interwencję (ang. *intervention*), „C" – porównanie z grupą kontrolną (ang. *comparison*), a „O" – punkt końcowy (ang. *outcome*).[162] Z projektowaniem tych ostatnich wiąże się szereg wyzwań o kluczowym znaczeniu dla wiarygodności i użyteczności wyników badań naukowych, które omówiono poniżej.

W odpowiedzi na utrwalony historycznie, paternalistyczny i arbitralny schemat relacji lekarz-pacjent pod koniec dwudziestego wieku zapoczątkowano inicjatywy mające na celu

wypracowanie bardziej partnerskiego, reaktywnego wobec potrzeb chorego modelu opieki, określanego aktualnie mianem „opieki skoncentrowanej na pacjencie".[163] Aby system ten mógł funkcjonować w zgodzie z wiedzą medyczną, konieczne jest projektowanie badań naukowych oceniających punkty końcowe istotne z perspektywy pacjenta.[164] Spektakularnym przykładem uzasadniającym uwzględnienie opinii chorych w planowaniu badań klinicznych stało się włączenie tej grupy w procesy decyzyjne inicjatywy OMERACT (Outcome Measures in Rheumatology), skupiającej się na definiowaniu punktów końcowych w badaniach klinicznych z zakresu reumatologii.[165] W ciągu 10 lat od zaangażowania pacjentów w planowanie reumatologicznych projektów badawczych trwale zmienił się ich obraz, między innymi poprzez wprowadzenie nie uwzględnianych wcześniej przez naukowców takich punktów końcowych, jak „zmęczenie" czy „jakość snu".

W celu uzyskania silniejszych dowodów na skuteczność interwencji konieczne jest przeprowadzenie syntezy danych z różnych opublikowanych wcześniej badań klinicznych i podsumowanie ich w przeglądzie systematycznym. Jednym z warunków umożliwiających ilościową analizę wyników wielu badań (metaanalizę) jest jednorodność raportowanych w nich punktów końcowych.[166] Jako przykład skrajnej niejednorodności utrudniającej lub wręcz uniemożliwiającej wiarygodną syntezę danych przytoczyć można przegląd systematyczny 10 000 badań na temat schizofrenii, w których łącznie użyto prawie 2200 różnych skal oceny objawów.[167]

Błąd systematyczny związany z selektywnym raportowaniem (ang. *selective reporting bias*) jest definiowany jako wybiórcze publikowanie danych jedynie dla części ze zmierzonych w trakcie badania punktów końcowych kierowane na przykład uzyskanymi wynikami.[166] Problem ten bierze się między innymi z niekorzystnego zjawiska większej szansy na publikację badań, których wyniki osiągnęły istotność statystyczną.[168] Selektywne raportowanie punktów końcowych powoduje, że decyzje kliniczne są podejmowane w oparciu o niepełne dane, co może prowadzić do wyboru nieskutecznych lub wręcz szkodliwych interwencji oraz marnowania środków finansowych systemów opieki zdrowotnej.[169]

Podsumowując, właściwie zdefiniowany, zmierzony i zaraportowany punkt końcowy jest nie tylko niezbędny do prawidłowej weryfikacji hipotezy badawczej – stanowi również wyraz odpowiedzialności badacza wobec społeczności naukowej, fundatorów badań i społeczeństwa.[170]

1.4.2. Zestawy podstawowych punktów końcowych

W odpowiedzi na opisane powyżej problemy powstała koncepcja zestawów podstawowych punktów końcowych (ang. *core outcome set*, COS), czyli pierwotnie uzgodnionych i zdefiniowanych punktów końcowych, które powinny znaleźć się w każdym badaniu klinicznym na dany temat.[171] Inicjatywa COMET (*Core Outcome Measures in Effectiveness Trials*) jest międzynarodowym przedsięwzięciem mającym na celu opracowywanie wytycznych i narzędzi tworzenia COS, ich dokumentację i popularyzację.[172] Zgodnie z opracowanym przez COMET podręcznikiem pierwszym krokiem w procesie tworzenia COS jest przeprowadzenie przeglądu systematycznego punktów końcowych raportowanych w opublikowanych badaniach klinicznych na dany temat w celu oceny ich heterogenności oraz istotności klinicznej. W kolejnych etapach przeprowadza się serie ankiet i dyskusji z udziałem istotnych dla danego problemu klinicznego grup interesów, ze szczególnym uwzględnieniem pacjentów. Po osiągnięciu konsensusu w kwestii „co mierzyć", wskazane jest przeprowadzenie kolejnego przeglądu systematycznego, dokumentującego poparte dowodami naukowymi metody pomiaru punktów końcowych uzgodnionych na wcześniejszym etapie. Finalnie, na podstawie powyższych kroków formułuje się rekomendacje, które nadają ostateczny kształt nowemu COS.[173]

2. Założenia i cel pracy

Stosowanie antybiotyków może prowadzić do wystąpienia działań niepożądanych ze strony przewodu pokarmowego, z których najlepiej udokumentowanym jest biegunka związana z antybiotykoterapią. Jedną z interwencji mogących zmniejszać jej ryzyko jest stosowanie probiotyków o udowodnionej naukowo skuteczności. Probiotyki wielogatunkowe nie są obecnie zalecane w trakcie antybiotykoterapii ze względu na niedostateczne dowody na ich korzystne działanie.

Wiarygodna weryfikacja hipotez badawczych oraz optymalna synteza dowodów z różnych badań naukowych dotyczących stosowania probiotyków w trakcie antybiotykoterapii wymaga zdefiniowania istotnych klinicznie, homogennych punktów końcowych.

Celem badań objętych rozprawą były:

- ocena skuteczności probiotyku wielogatunkowego w zapobieganiu biegunce związanej z antybiotykoterapią u dzieci;
- systematyczna dokumentacja i ocena metodologiczna punktów końcowych raportowanych w istniejących badaniach na temat stosowania probiotyków w trakcie antybiotykoterapii u dzieci.

3. Kopie opublikowanych prac

BMJ Open

Effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial

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ABSTRACT

Introduction Certain individual probiotic strains have been proven to be effective in reducing the risk of antibiotic-associated diarrhoea (AAD). However, the effects of using multispecies probiotics (MPs) remain unclear. We aim to assess the effectiveness of a specific MP preparation (Winclove 612) in reducing the incidence of AAD in children.

Methods and analysis A total of 350 children aged 6 months to 18 years, undergoing antibiotic treatment, will be randomly allocated to receive either a MP consisting of two strains of *Bifidobacterium* (*B. bifidum* W23 and *B. lactis* W51) and six strains of *Lactobacillus* (*L. acidophilus* W37, *L. acidophilus* W55, *L. paracasei* W20, *L. plantarum* W62, *L. rhamnosus* W71 and *L. salivarius* W24) at a total dose of 10^{10} colony-forming units daily, or a placebo, from the first day of antibiotic treatment until 7 days after antibiotic cessation, up to a maximum of 17 days. The primary outcome will be the incidence of AAD, defined as ≥3 loose or watery stools (a score of A on the Amsterdam Infant Stool Scale or a score of 5–7 on the Bristol Stool Form scale) in 24 hours, caused either by *Clostridium difficile* or of otherwise unexplained aetiology, occurring during the intervention period. The secondary outcomes will include the incidence of AAD according to alternative definitions; the incidence of any kind of diarrhoea; the duration of diarrhoea; the need for hospitalisation; intravenous rehydration or discontinuation of antibiotic treatment due to diarrhoea; adverse events; and the intestinal microbiota composition.

Ethics and dissemination The study protocol is approved by the Ethics Committee of the Medical University of Warsaw. The findings will be published in a peer-reviewed journal and submitted to relevant conferences.

Date and protocol version identifier 14/10/2017.

Trial registration number NCT03334604; Pre-results.

Strengths and limitations of this study

- This study's design is simple, with the intent to answer a precise and unambiguous clinical question.
- The study protocol closely follows the rules included in the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.
- This will be the first trial of this specific probiotic formulation in the paediatric population.
- The incidence of antibiotic-associated diarrhoea (AAD) in specific populations is difficult to predict and may turn out to be lower than expected, limiting the trial's statistical power.
- Since AAD may occur up to 8 weeks after antibiotic treatment, some cases may be missed in this study.

INTRODUCTION

Antibiotics are well known to cause disturbances in the composition of the intestinal microbiota, leading to the development of gastrointestinal (GI) symptoms.[1] Antibiotic-associated diarrhoea (AAD), which may be defined as diarrhoea that occurs in relation to antibiotic treatment with the exclusion of other aetiologies, is a common complication of antibiotic use in children.[2] Based on the analysis of data from randomised controlled trials (RCTs), the pooled risk of AAD in children was 19%.[3] However, the risk varies greatly from study to study, ranging from 2.1%[4] to 80%,[5] depending on factors such as the adopted definition of diarrhoea, the study population and the type of antibiotic treatment.[6] The underlying mechanism of AAD is not fully understood. It may be caused by a specific enteric pathogen (eg, *Clostridium difficile, C. perfringens, Staphylococcus aureus* or *Candida albicans*), metabolic consequences of altered intestinal microbiota or a direct effect of antibiotics on the mucosa.[7] AAD may vary both in severity (from uncomplicated diarrhoea to pseudomembranous colitis) and in incubation period (from the first day of antibiotic treatment to 8 weeks after discontinuation).[8]

The impact of antibiotics on commensal micro-organisms of the gut justifies the idea

of using probiotics to reduce the incidence of AAD. According to a consensus definition, probiotics are 'live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host'.[9] There are a number of potential mechanisms of their action, including activity in the intestinal lumen (eg, competition with, or direct suppression of, pathogenic micro-organisms), interaction with the mucosal barrier (eg, upregulation of tight junctions, modulation of water and ion channels) and influence on the intestinal immune system.[10]

Probiotic properties are species-specific and strain-specific, so each strain or their combinations should be examined separately.[2 11] In children, two probiotic strains with proven efficacy in the prevention of AAD are *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii*.[12 13] Both are currently recommended to reduce the incidence of AAD in children, if the use of probiotics is considered.[2] Probiotic preparations consisting of more than one strain are not yet recommended for reducing the incidence of AAD in children, despite some evidence of their effectiveness.[3 14]

In this trial, a preparation consisting of eight probiotic strains (Winclove 612, Winclove Probiotics, the Netherlands), including two strains of *Bifidobacterium* (*B. bifidum* W23, *B. lactis* W51) and six strains of *Lactobacillus* (*L. acidophilus* W37, *L. acidophilus* W55, *L. paracasei* W20, *L. plantarum* W62, *L. rhamnosus* W71 and *L. salivarius* W24), will be used. Hereafter, this probiotic strain combination is referred to as 'multispecies probiotic' (MP). None of the individual strains included in MP have been proven to be effective in reducing the incidence of AAD. However, studies on the effectiveness of a comparable preparation, Ecologic AAD, in reducing diarrhoeal symptoms have been performed.[15 16] The aforementioned preparation has a similar composition to MP; however, it additionally contains *Enterococcus faecium* W54. The species *E. faecium* is not recommended for use in children by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) due to unclear safety issues[17] and, therefore, is excluded from the current formulation. In one RCT conducted in 41 healthy adult volunteers receiving amoxicillin with either Ecologic AAD or placebo, subjects in the experimental group had a significantly lower rate of diarrhoea-like bowel movements compared with those in the placebo group (48% vs 79%, respectively, relative risk (RR)=0.61, $p<0.05$).[15] Another RCT conducted in 45 adult patients with a chronic obstructive pulmonary disease exacerbation who were treated with antibiotics did not reveal a difference the in rate of diarrhoea-like bowel movements between the Ecologic AAD and placebo groups (77% vs 70%, respectively, RR=1.1, $p>0.05$).[16] However, this study was carried out in a very specific group of patients, that is, those with a history of frequent and prolonged antibiotic use. So far, there have been no RCTs using this probiotic preparation carried out in larger groups of participants or in children.

METHODS AND ANALYSIS

Aim

The primary objective of this study is to test the hypothesis that the MP reduces the risk of AAD in children undergoing antibiotic treatment. Other objectives include investigating the MP's influence on the incidence of other types of diarrhoea, diarrhoea duration, intestinal microbiota composition and potential adverse events associated with the MP's use.

Trial design

The study is a randomised, double-blind, placebo-controlled, parallel group trial with an allocation ratio of 1:1.

Study setting

Participants in this study will be recruited among both the inpatients and outpatients of the Paediatric Hospital of the Medical University of Warsaw, Poland. In case of a low recruitment rate (defined as described in the 'Monitoring' section of this document), other hospitals and medical care centres would also be plausible sources of participants, providing the presence of adequately trained personnel. In case of the inclusion of additional recruitment centres, adequate information will be added to the protocol registry site, and the bioethics committee will be informed.

Eligibility criteria

Eligibility criteria will be as follows: (1) age between 6 months and 18 years, (2) therapy with oral or intravenous antibiotics for common infections, (3) ability to start the probiotic intervention within 24 hours after the start of antibiotic intake, (4) therapy with broad-spectrum antibiotics (broad-spectrum penicillins, cephalosporins, fluoroquinolones, clindamycin) and (5) signed informed consent.

The exclusion criteria will include the following: prior use of antibiotics within the previous 4 weeks, presence of a severe or generalised infection, history of severe chronic disease (eg, cancer, inflammatory bowel disease, tuberculosis), critical/life-threatening illness, immunodeficiency, history of pre-existing diarrhoea within the previous 4 weeks, exclusive breastfeeding, allergy or hypersensitivity to any component of the study product, tube-feeding, use of proton-pump inhibitors, laxatives, antidiarrhoeal drugs or any probiotics 14 days before or during the study.

Interventions

The experimental group will receive MP at a dose of 10^{10} colony-forming units (CFU) daily. This food supplement consists of the eight following bacterial strains:

- *B. bifidum* W23
- *B. lactis* W51
- *L. acidophilus* W37
- *L. acidophilus* W55
- *L. paracasei* W20
- *L. plantarum* W62
- *L. rhamnosus* W71
- *L. salivarius* W24

Apart from the probiotic strains, the active product consists of maize starch, maltodextrin, fructo-oligosaccharides P6, maize dextrin P9, potassium chloride, hydrolysed rice protein, magnesium sulfate, amylase and manganese sulfate. The dosage of MP to be used in this study is based on the aforementioned human studies with a comparable preparation.[15 16] The control group will receive a placebo product that is indistinguishable in colour, smell and taste from MP, and will have the same composition but without the live bacteria, fructo-oligosaccharides and maize dextrin. Both MP and placebo will be a powder, which has to be dissolved in water or milk before use. The interval between antibiotic intake and probiotic consumption has to be at least 2 hours. The study products (MP and placebo) will be manufactured and supplied by Winclove Probiotics B.V (Amsterdam, The Netherlands) free of charge.

The products will be transferred to the study site with a temperature control system, and the readings from a thermometer will be verified after their delivery. The study products will be stored at the study site in a locked, dark, and dry place, at room temperature.

Explanation for choice of comparators

To enable assessment of the occurrence of AAD in this study's population, a placebo will be used as a comparator. Contrary to the 'best available therapy' model, use of a placebo may lead to the development of a number of cases of theoretically avoidable AAD in the placebo group. However, overestimation of the MP's effectiveness will be avoided.[18] One may argue that probiotics with proven efficacy such as LGG or *S. boulardii* should be used in the control group. However, it is noteworthy that they are only recommended if the use of probiotics for preventing AAD is considered because of the existence of risk factors such as class of antibiotic(s), duration of antibiotic treatment, age, need for hospitalisation, comorbidities or previous episodes of AAD diarrhoea.[2 19] Due to these factors, no universal standard of care to reduce the risk of AAD in the paediatric population is defined.

Study procedure

The recruiting physician who is familiar with the study protocol will perform an eligibility screen on the prospective patients, who began therapy with antibiotics in the preceding 24 hours, based on their medical records. Then, during a face-to-face meeting with the patient's caregivers, the recruiter will obtain missing information concerning the inclusion and exclusion criteria, explain the study procedures, risks and benefits and supply them with a leaflet containing the study's description. After that, written informed consent in two copies will be obtained from the participant's caregivers. Consent will be also obtained from participants themselves if they are 15 years of age or older. Subsequently, the patient's case report form (CRF) will be created and archived along with one copy of the informed consent. Participants will be randomised to receive orally two times per day either MP at a dose of 5×10^9 CFU (total daily dosage of 1×10^{10} CFU) or a placebo during the antibiotic treatment and until 7 days after antibiotic cessation, up to a maximum of 17 days. This period is referred to as the intervention period later in the document. Data from earlier studies suggest that doses of $>5\times10^9$ CFU of probiotic micro-organisms are more effective than doses $<5\times10^9$ CFU in preventing AAD.[20]

During the intervention period (ie, the whole MP/placebo administration period), stool number and consistency will be recorded in a study diary, based on the Amsterdam Infant Stool Scale (AISS)[21] for children younger than 1 year and the Bristol Stool Form (BSF) scale[22] for children older than 1 year. The study diaries will be filled in by caregivers of participants younger than 14 years or by participants themselves, provided they are older than 14 years. A score of A on the AISS or 5–7 on the BSF scale will be considered as loose or watery stool. Caregivers also will be instructed to record any other observations concerning the health of the participants, including all adverse events involving the GI tract (such as vomiting, decreased appetite or abdominal pain) or other systems as well as information regarding compliance with treatment (ie, if the participant has taken the MP or not) in the study diary. The diary will be returned to the study site at the end of the intervention period. The outcome data for inpatients (eg, the occurrence of diarrhoea) will be verified using hospital charts.

The participants will be reminded not to use other treatments during the intervention period that may affect the incidence or course of the diarrhoea, namely other probiotics, diosmectite, loperamide, proton pump inhibitors or laxatives. Usage of any of the aforementioned preparations will be treated as a protocol violation, and such patients will not be included in the per protocol analysis. Caregivers will be asked to write down in the study diary any other medications or dietary supplements taken by the participants during the intervention period. Withdrawal of consent for participation in the study will be possible at any moment, with no consequences, and without an obligation to give reasons for the decision. In case of the occurrence of serious adverse events or new circumstances affecting the safety of the participants (eg, difficulty in swallowing, a new diagnosis of immunodeficiency), the intervention will be discontinued.

In cases of the occurrence of diarrhoea, stool samples will be obtained and examined for presence of common diarrhoeal pathogens—rotavirus, adenovirus, norovirus, *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, *and Yersinia* spp—via chromatographic immunoassay (for viruses) or isolation from stool culture (for bacteria). Additionally, *C. difficile* toxins A and B will be identified in the stool using immunoassay in cases involving children older than 1 year.

Additionally, as a part of an independent study, participants' microbiota composition will be tested in the stool at four time points: at baseline, at the day of antibiotic cessation, at the end of intervention and 1 month after

the intervention's cessation. The tests will be performed by analysing microbial gene sequences with 16S rRNA-based diversity methods. DNA will be extracted from the faecal samples by state-of-the art methods in a specialised laboratory. PCR amplificated 16S rRNA gene fragments will be analysed with the use of Illumina HiSeq Sequencer, and subsequent bioinformatic analyses will be performed by standardised pipelines within this laboratory. Next to this, microbial biomass will be measured with quantitative PCR and/or flow cytometry. Microbial functionality (metabolites produced) can be performed in addition to the composition analyses, and will be done by proteome analyses.

Follow-up

The primary and secondary outcomes (for details, see below) will be assessed during the intervention period. There will be no follow-up period. In cases of inpatients discharged before the end of the intervention period as well as in outpatients, the caregivers will be asked to bring the remaining product along with the study diary to the study site at the end of the 7-day intervention period.

Compliance

Compliance with the study protocol will be assessed by direct interview with the patient and/or caregiver, by analysing information from the study diary and by checking the number of returned non-consumed study products. Participants who receive <75% of the recommended dose of MP/placebo will be considered as non-compliant.

Outcome measures

The primary outcome measure will be AAD, defined as three or more loose or watery stools (a score of A on the AISS or 5–7 on the BSF scale) per day in a 24-hour period, caused by *C. difficile* infection or of otherwise unexplained aetiology (after testing for common diarrhoeal pathogens), occurring during the intervention period.

Secondary outcomes assessed during the intervention period will include AAD based on two other definitions of diarrhoea used in previous studies:

- ≥3 loose or watery stools per day for a minimum of a 48-hour period caused by *C. difficile* infection or of otherwise unexplained aetiology.
- ≥2 loose or watery stools per day for a minimum of a 24-hour period caused by *C. difficile* infection or of otherwise unexplained aetiology.

For both definitions, loose or watery stools will correspond to a score of A on the AISS or 5–7 on the BSF scale. AAD needs to be caused by *C. difficile* infection or of unexplained aetiology (after testing for common diarrhoeal pathogens), and it must occur during the intervention period.

Other secondary outcome measures will be as follows:

- Any diarrhoea (defined as ≥3 loose or watery stools per day for a minimum of 24 hours regardless of its aetiology).
- *C. difficile*-associated diarrhoea (diarrhoea defined as above caused by *C. difficile* confirmed by the presence of toxin-producing *C. difficile* in stools (positive toxin tests)).
- The duration of diarrhoea (defined as the time until the normalisation of stool consistency according to the BSF or AISS scale (on BSF scale, numbers 1, 2, 3 and 4; on AISS scale, letters B or C), and the presence of normal stools for 48 hours).
- Discontinuation of the antibiotic treatment due to severity of diarrhoea.
- Hospitalisation caused by diarrhoea in outpatients.
- Need for intravenous rehydration in any of the study groups.
- Adverse events.
- Intestinal microbiota composition, tested in stool samples as described above at four time points: at baseline, at the day of antibiotic cessation, at the end of intervention and 1 month after the intervention's cessation.

The timeline of the study is presented in table 1.

Sample size

The pooled risk of AAD determined from previous studies conducted at the Medical University of Warsaw[23 24] is 12.4%. However, in those studies, the definition of diarrhoea was more strict—loose or watery stools had to last for at least 48 hours, so AAD is expected to be more frequent in our proposed study. Consequently, we have chosen to perform a sample size calculation based on an expected AAD risk of 16%, which is a compromise between the results from the Medical University of Warsaw and the pooled AAD risk of 19% as reported in the Cochrane meta-analysis.[3] To show a difference of 11% in the treatment effect in the study groups with α=0.05% and 80% power (unpaired Student's t-test), and assuming a 20% withdrawal rate, a total of 337 participants will be needed. Sample size calculations were performed with StatsDirect (V.3.1.4, StatsDirect statistical software; StatsDirect, Chesire, UK).

Random sequence generation and allocation concealment

The randomisation will be performed centrally by Winclove Probiotics B.V. by a person not involved in the study. Blocked randomisation (blocks of four) will be used to ensure a good balance of participant characteristics in each group. Allocation will be determined by using a computerised random number generation process. All study products will be sequentially numbered. Coded study products will be handed over to the researchers. When the study has ended, participants will be divided into two blinded groups, which will be used in the statistical analyses. After performing the analyses, code numbers will be opened by the coordinating and principal investigators. Sealed envelopes containing the allocation of each number will be handed to the principal investigator ensuring that if a medical problem occurs for which treatment allocation is needed, the code can at all times be broken.

Table 1 The timeline of the study

	Intervention period														
	Days of antibiotic treatment							Days after antibiotic treatment							
	1	2	3	4	5	Every day	n (end of antibiotic treatment)	n+1	n+2	n+3	n+4	n+5	n+6	n+7	Close-out (n+37)
Enrolment															
Eligibility assessment	x														
Informed consent reception	x														
Allocation and randomisation	x														
Handing over of study diary	x														
Interventions															
Multistrain probiotic	←													→	
Placebo	←													→	
Data collection															
Study diary	←													→	
Stool tests in case of diarrhoea	←													→	
Stool microbiota examination	x						x							x	x
Reception of study diary and unused product															x

Blinding

The probiotic preparation and placebo will be stored in identical packages. The contents will look, smell and taste the same. Researchers, caregivers, participants, medical personnel and outcome assessors will all be blinded to the intervention until the study is completed and the data analysed.

Data collection and management

All study participants will receive a study identification number. CRFs containing each participant's identification number and baseline data will be filled in electronically and printed. Outcome data will be added to both the paper and electronic copies of the CRF after the reception of the study diary. Electronic data will be stored in a password-protected electronic database. The original paper copies of the CRFs and all study data will be stored in a locker within the study site. Both versions of the CRFs will be accessible to the involved researchers only. Overall, only the involved researchers will have access to the participant's personal information, and no personal data will be shared with the company performing the randomisation or with any other outside party.

Statistical analysis

Descriptive statistics will be used to summarise baseline characteristics. For continuous variables, comparison between groups will be done using the Student's t-test or Mann-Whitney U test, depending on whether or not the variables are distributed normally. The normality of the distribution will be checked using the Shapiro-Wilk test. The χ^2 test or Fisher's exact test will be used, as appropriate, to compare dichotomous variables. Differences between groups will be presented for continuous outcomes as differences in means or differences in medians (for normal or non-normal distribution, respectively) along with a 95% CI. For dichotomous outcomes, the RR and number needed to treat, calculated as the inverse of the absolute risk reduction, will be determined along with a 95% CI. In the second stage of analysis, the primary outcome will be analysed by logistic regression, controlling for five prespecified potential risk factors for AAD (age, sex, antibiotic class, duration of antibiotic treatment and duration of hospital stay). The difference between study groups will be considered significant when the p value is <0.05, when the 95% CI for RR (or OR) does not include 1.0 or when the 95% CI for mean difference does not include 0. All statistical

tests will be two-tailed and performed at the 5% level of significance.

An intention-to-treat model will be applied—data from all randomised participants will be used in the analysis, including those with low compliance or those who drop out or withdraw their consent. Per-protocol analysis will be performed as well, and it will include all participants who finish the study according to the protocol.

Monitoring

The study will be carried out in accordance with the protocol, as it will be registered. No changes in the study protocol are expected to be made after the study starts. However, in case of any unexpected circumstances requiring alterations of the protocol, changes will be immediately applied to the protocol registry site at clinicaltrials.gov, and, if relevant enough, reported to the Bioethics committee. An independent Data and Safety Monitoring Board (DSMB) will be created before the start of the study. The DSMB will review data after recruitment from 25%, 50% and 75% of participants to assess the study progress (including rate of recruitment, completeness of data and their appropriate collection) and all of the adverse events. The number of recruited patients will be monitored and kept up to date; appropriate changes (ie, training of the recruiting physicians, study leaflets, addition of new recruitment centres) will be applied to the study procedure and protocol if the pace of recruitment is not high enough to finish the study within the established time, which is 2 years.

Harms

All eight of the probiotic strains to be used in the study have the Qualified Presumption of Safety status established by the European Food Safety Authority.[25] The occurrence of serious adverse events in immunocompetent populations during oral use of probiotics is unlikely.[26]

The exact same product has not been assessed in previous studies. However, several clinical studies have been performed with a comparable product, in different populations (healthy volunteers and chronic obstructive pulmonary disease patients) in the Netherlands and Austria without any reported serious side effects.[15 16 27] Moreover, currently, a study is being performed with Ecologic AAD in patients with spinal cord injury who require antibiotic treatment during their inpatient rehabilitation (trial number: NTR5831).

In addition, the preparation is commercially available in several countries (Austria, Germany, Greece, Norway, Russia, Slovenia, Ukraine and the Netherlands) and since the market introduction in 2007, no serious adverse effects have been reported. In the Netherlands, probiotics are considered to be food or food supplements and, therefore, have to be produced under Hazard Analysis and Critical Control Point regulations, which is the Dutch regulation system for safety and hygiene in food and food supplements. All components are legally admitted as food additives or food components. Winclove is an NSF International Certified Good Manufacturing Practices Facility for manufacturing dietary supplements and works with the food safety management system ISO 22000:2005.

Overall, based on the literature and manufacturer's data, we assume that receiving the study product poses only a marginal risk to the participants. Nevertheless, during the whole study period, the participants will benefit from telephone and email contact with the primary investigator, so all the potential adverse events will be reported to and consulted by a physician. Moreover, patients at higher likelihood of experiencing severe adverse events (eg, critical/life-threatening illness, immunodeficiency or severe chronic illness) will not be recruited, as stated in the exclusion criteria.

Since adverse events of probiotic use are unlikely, no prespecified list will be a part of the study diary or CRF. Instead, a section entitled 'other symptoms' will be included, in which caregivers of the participants will be able to write down any other symptoms that occur during the intervention. Additionally, at the time of study diary reception, a physician will personally ask the caregiver about the occurrence of any symptoms during the study. As indicated in the Consolidated Standards of Reporting Trials (CONSORT) extension on harms document,[28] all of those symptoms will be reported for all of the randomised participants, including those who withdraw from the study. The data on adverse events will be presented for each study arm and each type of adverse event separately, with an exact count of each event, and distinction between patients with single and multiple events.

In case of suspected serious adverse events, the project leader will immediately notify the Ethics Committee, DSMB, all study personnel and the manufacturer of the product about the nature of the event. The decision regarding continuation or discontinuation of the trial will be made by the project leader in agreement with the Ethics Committee and DSMB. All adverse events also will be noted in the CRFs.

Patient and public involvement

Patients and public were not involved in the design of the study.

ETHICS AND DISSEMINATION

The protocol of the study was reviewed and approved by the Ethics Committee of the Medical University of Warsaw. Participants (or their legal representatives) will be fully informed about the study, and informed consent will be obtained. The manufacturer of the study products commented on the first draft of the protocol; however, all final decisions were made by the study team who also will be in charge of all study data.

The manufacturer will have no role in the conduct of the study, or in the analysis or interpretation of the data. The findings of this study, whether positive or negative,

will be published in a peer-reviewed journal in accordance with CONSORT. Abstracts will be submitted to relevant national and international conferences.

Contributors HS conceptualised the study. JŁ developed the first draft of the manuscript. Both authors contributed to and approved the final manuscript. HS is the guarantor.

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Competing interests None declared.

Patient consent Not required.

Ethics approval Ethics Committee of the Medical University of Warsaw.

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RESEARCH ARTICLE

Probiotics for the prevention of antibiotic-associated adverse events in children—A scoping review to inform development of a core outcome set

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Abstract

Introduction

Routine use of probiotics during antibiotic therapy in children remains a subject of discussion. To facilitate synthesis of individual study results and guideline formulation, it is important to assess predefined, similar, and clinically important outcomes. Core outcome sets are a proposed solution for this issue. The aim of this review was to document choice, design, and heterogeneity of outcomes in studies that assessed the effects of probiotics used for the prevention of antibiotic-associated adverse events in children.

Methods

A scoping literature search covering three major databases was performed. Studies that evaluated oral probiotics' use concomitant with antibiotic therapy in children were included. Data on outcome definitions, measurement instruments, and follow-up were extracted. The outcomes were assigned to predefined core areas and domains. Data were analyzed descriptively.

Results

Thirty-seven studies were included in this review. Diarrhea, the most commonly reported outcome, had diagnostic criteria clearly defined only in 21 studies. In total, 16 different definitions of diarrhea were identified. Diarrhea duration, severity, and etiology were reported in 9, 4, and 7 studies, respectively. Twenty studies assessed gastrointestinal symptoms other than diarrhea. Seven studies reported outcomes related to resource use or the economic impact of the intervention. Only 2 studies assessed outcomes related to life impact. None of the studies predefined adverse events of probiotic use.

Conclusions

Identified outcomes were characterized by substantial heterogeneity. The majority of outcomes were not designed to evaluate endpoints of real-life relevance. Results from this review suggest the need for a new core outcome set consisting of outcomes important for decision-making.

Introduction

The human gastrointestinal tract is colonized by hundreds of different microorganisms, which together form the gut microbiota [1, 2]. Use of antibiotics is one of the factors known to alter the microbiota composition, which in turn may have an effect on an individual's health. Typical adverse events associated with antibiotic use include various gastrointestinal symptoms such as diarrhea, nausea, vomiting, and abdominal pain [3]. Among them, antibiotic-associated diarrhea (AAD), often defined as 'diarrhea that occurs in relation to antibiotic treatment with the exclusion of other etiologies' [4], is the best documented.

Over 30 randomized controlled trials (RCTs), mostly with probiotics as an intervention, have been performed to assess the prophylactic strategies for AAD in children [5]. In the largest observational study of 650 children published in 2003, the estimated AAD incidence in the pediatric outpatient population was 11% [6]. On the other hand, in a recent (2019) Cochrane review [5], the incidence of AAD varied greatly from study to study, ranging from 2% [7] to 80% [8]. In addition to estimates sometimes being derived from very small underpowered studies [8–11], one of the factors responsible for this heterogeneity in reported incidences could be the definition of AAD adopted by authors of different RCTs and the methods used for measurement of this outcome. Among others, AAD diagnostic criteria vary between the studies in the terms of stool frequency, time from the start of antibiotic therapy, and microbiological methods, if any, used to exclude other etiologies of diarrhea.

Other potential effects of early-life microbiota alterations include later-life consequences such as obesity [12], allergies [13], autoimmune disorders [14], and neurodevelopmental abnormalities [15]. The long-term health impact of probiotics and antibiotics administered during infancy has been evaluated in some RCTs [16, 17], but this outcome is not a part of a routine trial design.

According to the 2016 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines, some probiotic strains may be effective in AAD prevention [4]. Consistent with this, a 2019 Cochrane systematic review of 33 studies concluded that there is a moderate protective effect of probiotics for preventing AAD [5]. Still, this use of probiotics is the subject of a lasting discussion due to their cost, and the fact that AAD is usually a mild and self-limiting disease [18]. To draw practical conclusions from RCTs, it is important to assess AAD severity and its impact on the patient's everyday life, including global assessment and health-related quality of life, with agreed-upon definitions and outcomes. However, a 2010 systematic review of outcomes used in trials of pediatric acute diarrhea revealed substantial heterogeneity in both the definitions of and the measurement methods for diarrhea [19]. Similarly, in the 2019 Cochrane systematic review, the criteria for defining the incidence of diarrhea according to each primary investigator's definition varied widely among the studies [5]. Differences in reported definitions, outcomes, and their measurement methods between studies may lead to difficulties in synthesizing results and hinder the process of guideline formulation. Standard definitions for main outcomes are a possible solution to these issues, and reviews addressing the choice of outcomes in already performed studies are one of the first

steps in the process of designing a core outcome set (COS) [20]. In 2016, a document by the Consensus Group on Outcome Measures Made in Pediatric Enteral Nutrition Clinical Trials (COMMENT) was published, proposing core outcomes for future use in RCTs evaluating therapeutic and preventive strategies for acute gastroenteritis [21]. However, authors of this document did not include any statements regarding outcomes specific for AAD. Also, no core outcome set to date has been proposed for use in trials in which probiotics are administered concurrently with antibiotic therapy.

Our primary aim was to document the definitions of AAD, as well as all of the methods used to measure and describe this outcome, in studies that assessed the effect(s) of probiotics used for AAD prevention. Additionally, we aimed to document any other outcomes reported in studies on probiotic use during antibiotic therapy, provided that they were used to examine probiotics' effect(s) in the prevention of antibiotic-associated adverse events. Due to the broad research question and its focus on methodology, we decided that a 'scoping review' would be the optimal approach for this study [22].

Methods

Inclusion/exclusion criteria for the review

Studies that evaluated oral probiotics' potential to prevent adverse events associated with antibiotic therapy were eligible for inclusion in this review. Eligible studies could be RCTs, non-randomized trials (NRTs), or observational studies (e.g., cohort studies, case-control studies) and had to be conducted in a population of children up to 18 years of age. Among the studies conducted in mixed populations of children and adults, only those that reported separate data for a subgroup of children were included. Furthermore, only studies published in English were included.

Studies that reported only laboratory outcomes (e.g., only stool microbiota composition) were not included in this review. Since the main focus of this review was the prevention of AAD, studies on probiotics used concurrently with antibiotics in the treatment of *Clostridium difficile*-associated diarrhea or other types of diarrhea were excluded. Additionally, studies conducted exclusively in premature infants and in critically ill children hospitalized in intensive care units were also not included, because the characteristics of these populations and the goals of probiotic use differ greatly from those in the general population.

Search methods

A systematic search was performed from inception to October 23, 2018 in three major databases (MEDLINE, Embase, and CENTRAL). Subsequently, a search update was performed on March 17, 2020. The search strategy was developed by an information specialist and included controlled vocabulary and keywords related to 'antibiotic' and 'probiotic' terms. The full search strategy for the MEDLINE database is available in S1 Table. Additionally, references of relevant review articles were manually searched.

Selection of studies

JŁ screened titles and abstracts of the entries identified by the search strategy. After screening, full texts of potentially eligible studies were acquired. The data appropriate for eligibility assessment (i.e., population, intervention, outcomes, language, and type of study) were independently extracted by JŁ and QG and then compared. Any disagreements concerning eligibility were resolved by discussion between the authors and, if needed, resolved by a senior researcher (BCJ or HS).

Data extraction

The data from the included studies were extracted using an abstraction form developed specifically for this review. Extracted data included standard characteristics of studies (author, publication year, country, study type and setting, age and number of participants, indication for antibiotic treatment, type of antibiotics, investigated probiotic, and type of control group) and data specific to the outcomes. Each identified outcome was assigned to one of 4 core areas: "life impact", "resource use", "pathophysiological manifestations" or "death", in accordance with the OMERACT Filter 2.0 [23]. Specific outcomes were also assigned to one of the predefined outcome domains included within the core areas. In case of identification of an outcome not falling into any of the predefined domains, a new domain was created. An explanation of the outcome-related taxonomy used in the article is presented in Table 1. The data extraction and assignment of the outcomes to the core areas and domains were done independently by JŁ and QG, and any differences in opinion were resolved by discussion. The data extracted for each identified outcome included: outcome name in accordance with the terminology used in the original publication, outcome characteristics (e.g., incidence, duration, severity, primary/secondary outcome), outcome definition, outcome measurement instruments, and follow-up. The outcome was considered as primary if either: 1) the authors of the original study declared it as such, or 2) a sample size calculation was performed for this specific outcome. The data for purely biochemical or microbiological outcomes (e.g., microbiota composition) were not extracted, because their documentation and evaluation would require an entirely different methodological approach.

Assessment of risk of bias in the included studies

Risk of bias (RoB) assessment is not a mandatory part of reviews of outcomes [20]; however, we decided to present it for informative purposes. The Cochrane Collaboration's Tool for Assessing Risk of Bias [24] was used for RCTs and non-randomized trials and Newcastle-Ottawa Scale [25] was used for one identified cohort study. Wherever possible, we present the RoB assessment derived from the recent Cochrane review [5]. For the remaining studies, the RoB assessment was performed by JŁ.

Synthesis of results

Data on the identified outcomes are presented in numbers and percentages and analyzed descriptively. Since this review aims to document the methods of outcome measurement and reporting, no analysis of the treatment effects was performed.

Table 1. Definitions of the terminology used in the article, in accordance with OMERACT definitions [23].

Term	Definition	Examples
Core area	An aspect of health or a health condition that needs to be measured to appropriately assess the effects of a health intervention. Core Areas are broad concepts consisting of a number of more specific concepts called domains.	Pathophysiological manifestations, life impact, resource use/economic impact
Outcome domain	An aspect of the effect of illness, categorized within the core area, but still relatively broad.	Diarrhea, gastrointestinal symptoms, absenteeism, need for additional medical procedures.
Outcome	Any identified result in a domain arising from exposure to a causal factor or a health intervention.	Diarrhea incidence, number of school absence days, need for intravenous rehydration.
Outcome measurement instrument	A tool chosen to assess the outcome.	Visual stool form scale, symptom questionnaire, immunoassay tests for rotavirus detection.

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Protocol and reporting

The protocol for this review was not registered. Data included in this review were reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist, available in the S1 File.

Results

Search results and overall characteristics

In total, we identified 4251 records by the initial database search, 762 records by the search update on 17th of March 2020 and an additional 369 records from the review articles' references. After exclusion of duplicates and title and abstract screening, full texts of 87 articles were assessed for eligibility. After full-text assessment, 37 articles ultimately met the inclusion criteria for this review [7–11, 26–57]. The flow diagram of the study selection process is presented in Fig 1. Reasons for exclusion of the specific studies are presented in S2 Table.

Among the included studies, 32 (86%) were RCTs, 4 were NRTs, and 1 study was a cohort study. The total number of participants was 5842, ranging from 18 to 653 children. Ten studies were conducted in an inpatient setting, 14 in an outpatient setting, 6 in a mixed setting, and 1 in an unclear setting. Additionally, in 6 trials on *Helicobacter pylori* treatment, the setting was not clearly defined; however, we assumed it to be 'probably outpatient', as *H. pylori* eradication usually takes place at home. The most common indications for antibiotic therapy were various childhood infections (12 studies, 32%), *H. pylori* treatment (11 studies, 30%), and respiratory tract infections (7 studies, 19%). Various beta-lactams were most often used (31 studies, 84%), followed by macrolides (22 studies, 59%). The majority of the trials (19 studies, 51%) used single-strain probiotics as an intervention and were placebo-controlled (21 studies, 57%). A summary of the included studies' characteristics is presented in S3 Table. All of the identified outcomes and their characteristics are presented in S4 and S5 Tables.

The RoB in the included trials varied. Most of the studies were characterized by substantial RoB. A summary of the RoB assessment is presented in S1 Fig and S6 Table.

Outcome domain: Diarrhea

The occurrence/incidence of diarrhea was reported as an outcome in 33 (89%) of the included studies, and 20 (61%) of these studies reported it as a primary outcome. In only 21 (64%) of these 33 studies were the criteria for diarrhea diagnosis clearly defined. In the remaining studies, the occurrence of diarrhea was reported by parents or patients during interviews or in study diaries, and diagnosed based on the participants' or investigators' judgment, with unclear diagnostic criteria. In 9 (27%) of the studies which assessed this outcome, various stool form scales were used, most commonly (7 studies) the Bristol Stool Form Scale (BSFS) [58].

Based on the frequency and minimal duration of loose stools occurrence, 8 different definitions of diarrhea were used by the authors of the original studies. Most commonly (11 studies, 33%), diarrhea was diagnosed when at least 3 stools of abnormally loose consistency occurred during 48 hours. However, when different definitions of "abnormal stool consistency" were taken into an account, as many as 16 different definitions of diarrhea were identified. The most commonly used definitions of diarrhea are presented in Fig 2.

Surprisingly, among the 33 studies that reported data on diarrhea occurrence, the authors referred to their outcome as 'antibiotic-associated diarrhea' or 'treatment-associated diarrhea' in only 14 articles (42%). Among them, only 7 of the 33 studies (21%) investigated a potentially infectious origin of diarrhea. Moreover, in 2 of them, the authors did not utilize this information to support or exclude a diagnosis of AAD [9, 31]. Authors of 4 studies diagnosed AAD as

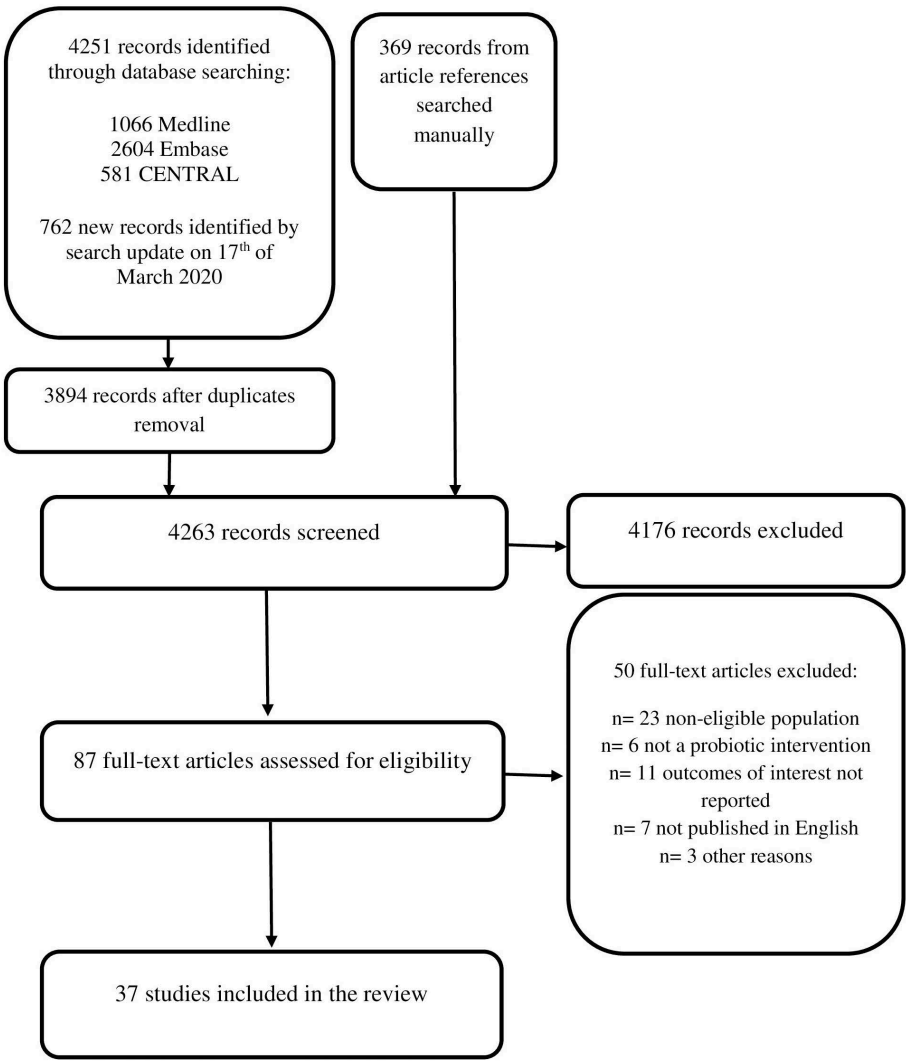

Fig 1. Flow chart diagram.

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"diarrhea caused by *C. difficile* or of otherwise unknown origin" and performed enzyme immunoassay tests for rota- and adenoviruses detection and stool cultures for bacterial pathogens [37, 39, 44, 47]. A single study additionally tested for norovirus infection using enzyme

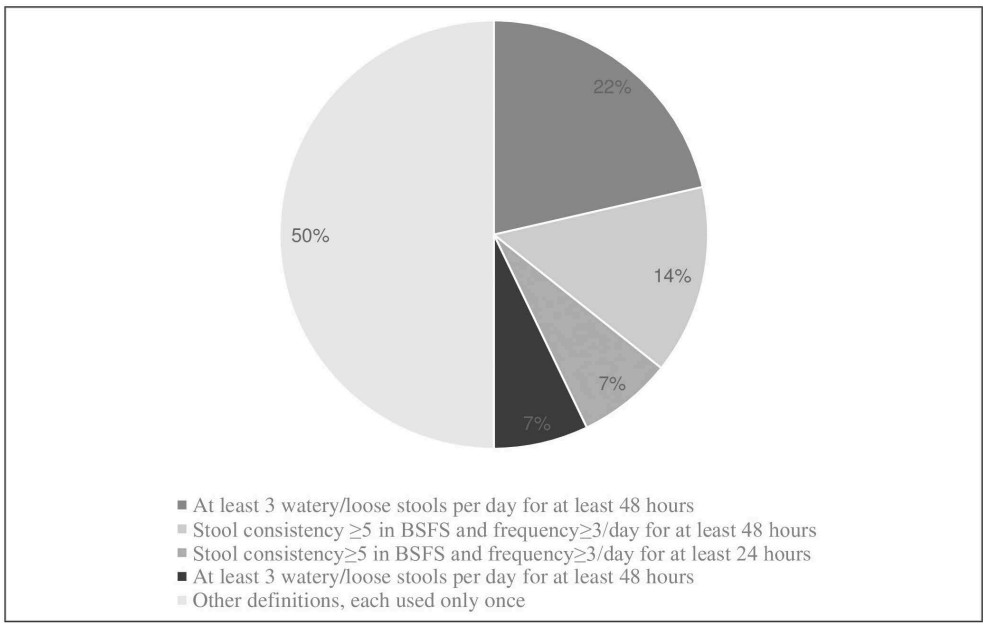

Fig 2. Most commonly used definitions of diarrhea.

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immunoassay [37]. In one study, testing for rota- and noroviruses was performed, but a specific method was not reported [57].

Included studies varied with respect to follow-up duration. In 22 (67%) of the 33 trials that assessed diarrhea as an outcome, the incidence of diarrhea was assessed during antibiotic treatment and an additional follow-up period, which varied from 1 week after the end of antibiotic therapy [34, 41] to up to 7 months after its beginning [38]. Seven studies (21%) assessed diarrhea only during antibiotic treatment [30, 32, 33, 37, 52, 53, 56], and 3 studies (9%), only during the first 3 to 6 days of antibiotic therapy [29, 43, 45].

Among other characteristics of the diarrhea, its duration was reported in only 9 out of 33 studies, which corresponds to 27% of the studies with diarrhea as an outcome. In 6 of these studies, the duration was not defined [8, 28, 29, 31, 53, 57], whereas in each of the 3 remaining studies its definition varied [30, 33, 47]. Diarrhea severity was reported as an outcome in only 4 of the studies (12%), and it was defined differently in every one of them, usually on the basis of discharge frequency and stool consistency [7, 28, 30, 34]. Diarrhea duration and severity were reported as co-primary outcomes in one study each [34, 47], while in the other studies they were either secondary or unspecified outcomes. Where provided, the definitions of diarrhea duration and severity can be found in S5 Table.

Other outcomes regarding diarrhea included occurrence of infectious diarrhea—5 studies [28, 31, 37, 39, 44], stool consistency regardless of diarrhea occurrence—5 studies [33, 40, 41, 53, 54], bowel movement frequency—3 studies [50, 53, 55], and time to diarrhea onset from the start of antibiotic therapy—5 studies [8, 30, 31, 34, 57]. Additionally, the efficacy of

diarrhea treatment, diarrhea-associated dehydration and time to first occurrence of loose stool were reported in one study each [30, 31, 34].

Outcome domain: *Clostridium difficile* infection

In 6 studies, patients were investigated for the *C. difficile* infection. In 1 study, the tests for toxin A and B were performed regardless of whether or not diarrhea occurred (i.e., asymptomatic carrier) [7], while in the other 5 they were performed only in case of diarrhea [28, 37, 39, 44, 47]. One study used both the immunoassay for *C. difficile* toxin A detection and stool culture [28], whereas the others utilized only the toxin A and B immunoassays.

Outcome domain: Other gastrointestinal symptoms

The most commonly reported gastrointestinal outcomes other than diarrhea in the 37 included studies were the following: abdominal pain (16 studies, 43%), vomiting (16 studies, 43%), nausea (11 studies, 30%), lack of appetite (7 studies, 19%), constipation (10 studies, 27%), bloating (7 studies, 19%), taste problems (5 studies, 14%), and flatulence (7 studies, 19%). Other less commonly assessed outcomes included belching, abdominal discomfort, symptoms included in the Gastrointestinal Symptom Rating Score (GSRS) [59] (heartburn, acid regurgitation, sucking sensations in the stomach, borborygmus, abdominal distension, eructation, passage of stools, loose stools, hard stool, urgent need for defecation and feeling of incomplete defecation), and undefined 'gastrointestinal complications'.

In 2 studies [7, 10], the GSRS was used to assess the gastrointestinal symptoms [59]. Additionally, a visual analog scale for abdominal pain intensity was used in one study [53], and a 3-point GI symptom rating scale was used in another [46]. In the remaining studies, the gastrointestinal symptoms were reported by parents and/or children during interviews or in study diaries.

Other outcomes from "pathophysiological manifestations" core area

None of the included studies assessed long-term adverse events associated with antibiotic use. Among the included studies, 18 (49%) reported data on adverse events potentially associated with probiotic use. In none of those studies were the adverse events predefined by the authors.

Outcomes from other core areas

Seven studies (19%) reported outcomes from the "resource use/economical impact" core area [29, 33, 37, 39, 44, 49, 50]. The most common outcomes from this area were need for antibiotic discontinuation due to diarrhea (6 studies), need for intravenous rehydration (5 studies), and need for hospitalization due to diarrhea (5 studies).

Only 2 studies assessed outcomes from "life impact" core area. A single study reported data on absence from school/day care, missed parental days at work, and overall health [40], and another study reported the data on duration of hospital stay [33].

Discussion

In this review of outcomes used in studies assessing probiotic prophylactic interventions during antibiotic therapy in children, 32 RCTs, 4 NRTs, and 1 cohort study were included. The incidence (occurrence) of diarrhea was the most commonly reported outcome. However, diagnostic criteria for diarrhea were clearly defined in only 21 (64%) of the 33 studies reporting this outcome. The majority of those studies did not utilize a validated instrument to assess stool frequency and consistency, did not report data on diarrhea duration and/or severity, and

did not perform any microbiological tests to rule out its infectious origin. Sixteen different definitions of diarrhea were identified ranging from 1 or more abnormally loose stools per day [51] to 3 abnormally loose or liquid stools per 48 hours [9, 28, 31, 39, 44, 49, 50]. The follow-up duration in the included studies also varied. Diarrhea duration and severity were often not reported, and their definitions, if provided, were different in each study. Less than half of the included studies reported data on other GI symptoms, such as abdominal pain or vomiting, and in most of them authors did not report use of any assessment instruments aside from study diaries. Finally, studies rarely included outcomes from 'pragmatic' core areas, i.e., 'life impact' and 'resource use and economical impact'.

To our knowledge, this is the first review documenting the outcome measurement and reporting methods used in studies on this particular subject. Its methodology adhered both to the Cochrane Collaboration's guidelines for systematic reviews [24] and to the recommendations of COMET (Core Outcome Measures in Effectiveness Trials) Initiative [20]. Authors of this review have previous experience in probiotic and AAD research as well as in the field of systematic reviews. The potential limitations of this review result from the possibility of not including all relevant studies, since the search was limited to the articles published in English and only a basic search of the grey literature was performed (i.e., manual search within the article references). However, this review aims to document the outcomes and their definitions rather than the effectiveness of interventions. Not including all of the available studies is unlikely to influence the overall conclusions, particularly given our study team also has expertise in general pediatrics, including ongoing commitments to patient care. The other limitation of this review is lack of microbiota composition-related outcomes. The authors recognize microbiome analysis as an important element of studies on probiotics and antibiotics alike, however documentation and comparative assessment of the analysis methods requires a wholly different approach compared to clinical outcomes [60]. Another important group of microbiological outcomes which is absent in this review is the antibiotic resistance [61], as none of the otherwise eligible studies reported this outcome.

Results of this review reveal substantial heterogeneity in the definitions of reported diarrhea-related outcomes. In 12 (36%) of the 33 included studies that reported the incidence of diarrhea as an outcome, the authors did not define criteria for diarrhea diagnosis, which increases the risk of reporting bias [62, 63]. In the remaining studies, including the papers published subsequent to the core outcome set for use in clinical trials of pediatric acute diarrhea [21], multiple definitions of diarrhea were identified. The definitions of diarrhea duration and severity also varied. This heterogeneity may theoretically lead to difficulty in combining data from different studies for the purpose of meta-analysis [64]. In the recent Cochrane review on pediatric AAD, substantial heterogeneity (I^2 = 57%) was found in the analysis of diarrhea incidence (5). When subgroup analysis was based on only one definition of diarrhea (i.e., 3 or more loose/water/liquid stools per day for at least 2 consecutive days), the heterogeneity was significantly reduced (I^2 = 15%). On the other hand, a test for interaction by diarrhea definition was not statistically significant, which suggests that different definitions of diarrhea were not the main reason for the overall heterogeneity of the result in the aforementioned review [5].

The other finding of our review concerns the criteria for AAD diagnosis. Even though the included studies investigated symptoms related to antibiotic use, authors referred to their outcome as 'antibiotic-associated diarrhea' in only 14 (42%) of the 33 articles that reported the incidence of diarrhea. Moreover, infectious origin of diarrhea was investigated by microbiological methods in only 7 (19%) of 37 included studies. Considering the fact that most of the studies' participants were either inpatients or visited healthcare facilities at the beginning of trial, they were at risk of nosocomial diarrhea [65]. Not ruling out the possibility of infectious gastroenteritis in this group of patients introduces a risk of outcome misclassification. Even in

studies that utilized microbiological methods to identify diarrhea etiology, it is impossible to completely rule out its infectious origin, due to the limited diagnostic accuracy of enzyme immunoassay methods [66, 67]. Diarrhea reported as an outcome in the few studies which performed the microbiological testing is much more likely to be an actual AAD.

The most commonly assessed outcome from the 'diarrhea' domain was incidence data. Surprisingly, other outcomes that are arguably more patient-important, such as diarrhea duration or severity, were rarely reported. Furthermore, even the most anticipatory criterion for diarrhea diagnosis was 'at least 3 loose or watery stools per day for at least 48 hours'. This constitutes a relatively mild course of illness, especially assuming that the symptoms are likely to resolve on the third day after occurrence [68]. Based only on the data for diarrhea incidence, it is difficult to assess whether the reported effect of any intervention was of actual importance to the patients. Other GI outcomes that could contribute to drawing clinically significant conclusions such as abdominal pain or vomiting, were only assessed in a small portion of the studies, even though they are likely to occur during antibiotic treatment [3]. When they were reported, authors typically assessed incidence rather than duration or severity, again focusing on outcomes they may be less patient-important. Outcomes from 'resource use' and 'life impact' core areas, which reflect the pragmatic approach to clinical trial design, were rarely reported. The lack of available outcomes on life impact, particularly quality of life, is concerning. Although quality of life measures are not often an outcome employed in clinical trials assessing acute outcomes, there are examples in acute gastroenteritis [69]. Although we did not find validated disease specific quality of life outcomes used in our target population, individualized quality of life instruments such as Measure Yourself Medical Outcome Profile (MYMOP) should be considered as a part of core outcomes [70].

The included studies also varied in the terms of follow-up duration with the majority of the studies following patients during the entire duration of antibiotic therapy and for at least one week after antibiotic cessation. Considering the usually short incubation time of AAD [71], these lengths of follow-up should be sufficient to identify most of the cases.

None of the included studies predefined outcomes from the domain 'adverse events of the probiotic use'. This may result from the fact that the probiotics are unlikely to cause adverse events in immunocompetent children [72]. Nevertheless, a clear and carefully planned documentation of adverse events is still important [73], as claims of harmful effects of probiotic use, particularly in immunocompromised patients, are being occasionally published [74].

Conclusions

Outcomes reported in studies on probiotic use in children receiving antibiotic therapy are characterized by substantial heterogeneity. In the majority of trials, the outcomes and outcome measures are not designed to evaluate outcomes of real-life relevance such as patient and parent reported quality of life. Results from this review suggest the need for a new core outcome set with endpoints that cover the span of domains and outcomes important to patients, families and clinicians for decision-making.

Supporting information

S1 Fig. Risk of bias summary for the included trials.
(PDF)

S1 Table. MEDLINE search strategy (Ovid MEDLINE(R) and epub ahead of print, in-process & other non-indexed citations, daily and versions(R)).
(DOCX)

S2 Table. Excluded studies with reasons of exclusion.
(DOCX)

S3 Table. Characteristics of the included studies.
(DOCX)

S4 Table. Outcomes identified in the included studies.
(DOCX)

S5 Table. Characteristics of the identified outcomes.
(DOCX)

S6 Table. Risk of bias assessment of the included cohort study.
(DOCX)

S1 File. PRISMA-ScR checklist.
(DOCX)

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Validation: Qin Guo.

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Writing – review & editing: Jan Łukasik, Hania Szajewska, Bradley C. Johnston.

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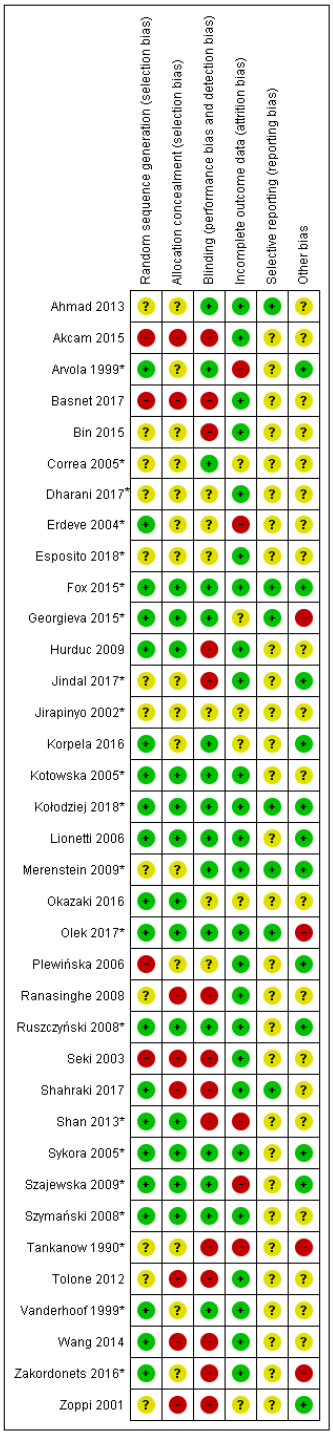
Supplementary Figure 1. Risk of bias summary for the included studies.



*studies with the risk of bias assessment derived from the recent Cochrane review

S1 Table. MEDLINE Search Strategy – Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R)

1	exp probiotics/ or probiotic*.mp.
2	exp lactobacillus/ or (lactobacill* or "l acidophilus" or "l casei").mp.
3	exp bifidobacterium/ or (bifidobacter* or "b infantis" or "b bifidum" or "b longum").mp.
4	exp saccharomyces/ or (saccaromyce* or "s boulardii").mp.
5	clostridium butyricum/ or clostridium difficile/ or (clostridium butyricum or clostridium difficile).mp.
6	streptococcus thermophilus/ or streptococcus thermophilus.mp.
7	enterococcus faecium/ or enterococcus faecium.mp.
8	or/1-7
9	exp anti-bacterial agents/
10	exp beta-lactams/ or exp macrolides/ or exp fluoroquinolones/ or exp tetracyclines/ or exp lincosamides/ or exp aminoglycosides/ or exp trimethoprim/
11	(antibiotic* or anti biotic* or antimicrobial* or anti microbial* or antimycobial* or anti mycobial* or antimycobacteri* or anti mycobacteri* or antibacteri* or anti bacteri* or bacteriocid* or antiinfective* or anti infective*).mp.
12	(penicillin* or flucloxacillin* or amoxicillin* or clavula* or macrolide* or fluoroquinolone* or tetracycline* or lincosamid* or aminoglycosid* or trimethoprim*).mp.
13	or/9-12
14	pediatrics/
15	(infan* or newborn* or new-born* or perinat* or neonat* or baby or baby* or babies or toddler* or minors or minors* or boy or boys or boyfriend or boyhood or girl* or kid or kids or child or child* or children* or schoolchild* or schoolchild or adolescen* or juvenil* or youth* or teen* or underage* or pubescen* or pediatric* or paediatric* or peadiatric* or prematur* or preterm*).mp.
16	school*.ti,ab.
17	or/14-16[1]
18	randomized controlled trial.pt.
19	controlled clinical trial.pt.
20	randomized.ab.
21	placebo.ab.
22	clinical trials as topic.sh.
23	randomly.ab.
24	trial.ti.[2]
25	Epidemiologic studies/
26	exp case control studies/
27	exp cohort studies/
28	Case control.tw.
29	(cohort adj (study or studies)).tw.
30	Cohort analy$.tw.
31	(Follow up adj (study or studies)).tw.
32	(observational adj (study or studies)).tw.
33	Longitudinal.tw.
34	Retrospective.tw.
35	Cross sectional.tw.
36	Cross-sectional studies/
37	or/18-36[3]
38	exp animals/ not humans.sh.
39	37 not 38
40	8 and 13 and 17 and 39

[1] Pediatric search filter adapted from: Leclercq E, Leeflang MM, van Dalen EC, Kremer LC. Validation of search filters for identifying pediatric studies in PubMed. J Pediatr. 2013 Mar;162(3):629-634.e2.

[2]Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximizing version (2008 revision); Ovid format (Box 6.4.c). In Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.handbook.cochrane.org.

[3] Observational studies filter, Scottish Intercollegiate Guidelines Network. Available from https://www.sign.ac.uk/search-filters.html.

S2 Table. Excluded studies with reasons of exclusion

Study ID	Reason for exclusion
Ameen 2019 [1]	Non-eligible population
Andaloro 2019 [2]	Outcomes of interest not reported
Awasthi 2000 [3]	Commentary on another study
Cherian 2012 [4]	Non-eligible population Outcomes of interest not reported
Conway 2007 [5]	Non-eligible population
Czerwionka-Szaflarska 2006 [6]	Article not in English
Dajani 2013 [7]	Non-eligible population
Dajani 2013 [8]	Non-eligible population
De Bortoli 2007 [9]	Non-eligible population
Doyle 2018 [10]	Outcomes of interest not reported
Duman 2005 [11]	Non-eligible population
Francavilla 2008 [12]	Non-eligible population
Francavilla 2014 [13]	Non-eligible population
Goldman 2006 [14]	Outcomes of interest not reported
Huang 2011 [15]	Conference abstract
Islek 2015 [16]	Not a probiotic intervention
Kim 2008 [17]	Non-eligible population
Kitz 2012 [18]	Outcomes of interest not reported
Korpela 2018 [19]	Outcomes of interest not reported
Kumar 2013 [20]	Non-eligible population
Li 2008 [21]	Secondary study
Li 2018 [22]	Outcomes of interest not reported
Li 2019 [23]	Outcomes of interest not reported
Lukasik 2018 [24]	Study protocol
Madden Fuentes 2015 [25]	Outcomes of interest not reported
Maziade 2013 [26]	Non-eligible population
Mohseni 2013 [27]	Outcomes of interest not reported
Murphy 2016 [28]	Not a probiotic intervention
Namkin 2016 [29]	Non-eligible population
Nista 2004 [30]	Non-eligible population
Pantoflickova 2003 [31]	Non-eligible population
Prado 1980 [32]	Article not in English
Rohrenbach 2009 [33]	Article not in English
Saneeyan 2011 [34]	Article not in English
Schrezenmeir 2004 [35]	Not a probiotic intervention
Sirvan 2017 [36]	Not a probiotic intervention
Song 2010 [37]	Non-eligible population
Srinivasan 2006 [38]	Non-eligible population
Tamma 2017 [39]	Non-eligible population
Tongtawee 2016 [40]	Non-eligible population
Uitz 2017 [41]	Non-eligible population

Ustundag 2017 [42]	Not a probiotic intervention
Valsecchi 2014 [43]	Outcomes of interest not reported
Wan 2017 [44]	Article not in English
Wang 2017 [45]	Non-eligible population
Witsell 1995 [46]	Non-eligible population
Xiang 2019 [47]	Non-eligible population and intervention
Zhao 2014 [48]	Article not in English
Zheng 2012 [49]	Article not in English
Ziemniak 2006 [50]	Non-eligible population

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S3 Table. Characteristics of the included studies

Study ID	Study type	Setting	Number and age of participants	Indication for antibiotic therapy	Types of antibiotics	Intervention	Control
Ahmad 2013 [1]	RCT	Outpatient	N = 66 Age: 3-14 y	H. pylori infection	Amoxicillin + furazolidone	*L. acidophilus, L. rhamnosus, L. bulgaricus, L. casei, S. thermophilus, B. infantis and B. breve*	Placebo
Akcam 2015 [2]	RCT	Outpatient	N = 61 Age: 7-18 y	H. pylori infection	Amoxicillin + clarithromycin	*L. casei 2401, L. acidophilus 2027 and B. lactis 2211*	No treatment
Arvola 1999 [3]	RCT	Mixed setting	N = 119 Age: 2 – 11,8 y	Acute respiratory infections	Oral penicillin, amoxicillin, cephalosporins, erythromycin or trimethoprim-sulphamethoxazole	*Lactobacillus GG*	Placebo
Basnet 2017 [4]	NRT	Outpatient	N = 174 Age: 6 m-16 y	Respiratory tract infections	Amoxicillin + clavulanic acid	*L. sporogenes, St. faecalis, C. butyricum, Ba. mesentericus TO-A 1*	No treatment
Bau 2020 [5]	Cohort study	Mixed setting	N = 336 Age: 1m – 17 y	Any indication	Any antibiotics	Any probiotics, according to patient choice (observational study), most commonly: *L. rhamnosus GG, L. reuteri DSM 17938*	No treatment
Bin 2015 [6]	RCT	Probably outpatient	N = 194 Age: 22 m – 16 y	H. pylori infection	Amoxicillin+clarithromycin, metronidazole	*S. boulardii CNCM I-745*	No treatment
Correa 2005 [7]	RCT	Inpatient	N = 169 Age: 6-36 m	Not described	Penicillin, ampicillin, oxacillin, amoxicillin, cephalosporin, amoxicillin+clavulanic acid, others	*B. lactis and St. thermophilus*	Placebo
Dharani 2017 [8]	RCT	Outpatient	N=100 Age: 1-15 y	Impetigo	Azithromycin	*L. sporegens, S. faecalis, Clostridium butyricum and Bacillus mesentericus*	No treatment
Erdeve 2004 [9]	NRT	Setting unclear	N = 653 Age: 1-15 y	Not described	Sulbactam-ampicillin or azithromycin	*S. boulardii*	No treatment
Esposito 2018 [10]	RCT	Inpatient	N = 90 Age: 11-36 m	UTI prophylaxis after hypospadias repair	Amoxicillin – clavulanate or macrolide	*L. rhamnosus GG*	Placebo
Fox 2015 [11]	RCT	Outpatient	N = 72 Age: 1-12 y	Any indication other than prophylaxis	Beta-lactams, macrolides, tetracyclines,	*L. rhamnosus GG, B. lactis BB-12, L. acidophilus La-5*	Placebo
Georgieva 2015 [12]	RCT	Inpatient	N = 100 Age: 3-12 y	Any infection	Aminoglycosides, fluoroquinolones, beta-lactams, metronidazole	*L. reuteri 17938*	Placebo
Hurduc 2009 [13]	RCT	Probably outpatient	N = 90 Age: 3-18 y	H. pylori infection	Amoxicillin + clarithromycin	*S. boulardii*	No treatment
Jindal 2017 [14]	RCT	Outpatient	N = 600 Age: 6 m – 12 y	UTI, otitis media, tonsillitis	Beta-lactams	*S. boulardii*	No treatment
Jirapinyo 2002 [15]	RCT	Inpatient	N = 18 Age: 1-36 m	Sepsis, meningitis	Broad-spectrum antibiotics	*L. acidophilus, B. infantis*	Placebo
Kołodziej 2018 [16]	RCT	Inpatient	N = 250	Any infection	Any antibiotics	*L. reuteri 17938*	Placebo

			Age: 0-18 y				
Korpela 2016 [17]	RCT	Outpatient	N = 231 Age: 2-6 y	Infections	Macrolides, penicillins, cephalosporins, trimethoprim-sulphamethoxazole	*L. rhamnosus GG*	Placebo
Kotowska 2005 [18]	RCT	Mixed setting	N = 269 Age: 6 – 14 y	Otitis media, respiratory tract infection	Any antibiotics	*S. boulardii*	Placebo
Lionetti 2006 [19]	RCT	Probably outpatient	N =40 Age: 3,3-18 y	H. pylori infection	Amoxicillin + clarithromycin	*L. reuteri ATCC 55730*	Placebo
Merenstein 2009 [20]	RCT	Outpatient	N = 125 Age: 1-5 y	Upper respiratory infections	Not described	*Kefir containing various strains of bacteria.*	Placebo
Okazaki 2016 [21]	RCT	Inpatient	N = 30 Age: <15 y	Postoperative prophylaxis	Not described	*B. Breve strain Yakult*	Placebo
Olek 2017 [22]	RCT	Outpatient	N = 447 Age: 1-11 y	Common infections	Beta-lactams, trimethoprim-sulphamethoxazole, macrolides	*L. plantarum DSM9843*	Placebo
Plewińska 2006 [23]	NRT	Outpatient	N = 60 Age: 8,8-18,3 y	H. pylori infection	Amoxicillin, clarithromycin	*L. acidophilus R0052, L. rhamnosus R0011*	Placebo
Ranasinghe 2008 [24]	RCT	Inpatient	N = 76 Age: 6 m – 5 y	Any indication other than diarrhea	Amoxicillin, amoxiclav	*Yogurt containing bifidobacteria*	No treatment
Ruszczyński 2008 [25]	RCT	Mixed setting	N = 240 Age: 3m-14 y	Common infections	Penicillins, broad-spectrum penicillins (ampicillin, amoxicillin, amoxicillin plus clavulanate), cephalosporins, macrolides, clindamycin	*L. rhamnosus E/N, oxy, pen*	Placebo
Seki 2003 [26]	NRT	Mixed setting	N = 110 Age: 1 m - 15 y	Upper respiratory infections, gastroenteritis	Penicillin, cephalosporin, tetracycline, fosfomycin	*C. butyricum Miyairi*	No treatment
Shahraki 2017 [27]	RCT	Outpatient	N = 50 Age: 5-18 y	H. pylori infection	Amoxicillin + clarithromycin	*L. reuteri*	No treatment
Shan 2013 [28]	RCT	Inpatient	N = 333 Age: 6-14 y	Lower respiratory tract infection	Cefepime, Cefoperazone +sulbactam, Cefuroxime, Amoxicillin+clavulanic acid, Erythromycin, others	*S. boulardii*	No treatment
Sykora 2005 [29]	RCT	Probably outpatient	N = 86 Mean age: 12.6y	H. pylori infection	Amoxicillin, clarithromycin	*L .casei DN-114 001*	Placebo
Szajewska 2009 [30]	RCT	Outpatient	N = 66 Age: 5-17 y	H. pylori infection	Clarithromycin	*L. rhamnosus GG*	Placebo
Szymański 2008 [31]	RCT	Mixed setting	N = 78 Age: 5 m – 16 y	Otitis media, and/or respiratory tract infections, and/or	Amoxicillin, amoxicillin+clavulanate, cephalosporins, penicillin, macrolides,	*B. longum PL03, L. rhamnosus KL53A and L. plantarum PL02*	Placebo

				urinary tract infections	aminoglycosides		
Tankanow 1990 [32]	RCT	Outpatient	N = 60 Age: 5m-6y	Not described precisely, mostly otitis media and pharyngitis	Amoxicillin	*L. acidophilus and L. bulgaricus*	Placebo
Tolone 2012 [33]	RCT	Probably outpatient	N = 68 Age mean: 8.3 y	H. pylori infection	Amoxicillin + clarithromycin	*L. plantarum, L. reuteri, L. casei subsp. rhamnosus, B. infantis and B. longum, L. salivarius, L. acidophilus, S. thermophilus, L. sporogenes.*	No treatment
Vanderhoof 1999 [34]	RCT	Outpatient	N = 202 Age: 6 m – 10 y	Upper to lower respiratory tract; urinary tract; soft tissues infection; skin infection	Oral antibiotics	*L. rhamnosus GG*	Placebo
Wang 2014 [35]	RCT	Probably outpatient	N = 88 Age mean: 7.8 y	H. pylori infection	Clarithromycin + amoxicillin/metronidazole	*L. acidophilus-5 and B. bifidum-12*	No treatment
Zakordonets 2016 [36]	RCT	Inpatient	N = 40 Age: 3-17 y	Moderate or severe acute bacterial infection diseases	Ceftriaxone	*Lactobacilli and Lactococci, Bifidobacteria, propionate-oxidising bacteria, acetic acid bacteria*	No treatment
Zoppi 2001 [37]	RCT	Inpatient	N = 51 Age: mean 5,1 y	Febrile respiratory tract infections	Ceftriaxone	*7 experimental arms: S. boulardii (1); E. species (2); lactulose (3); L. casei GG (4) L. rhamnosus, L. bifidus, and L. acidophilus (5); B. bifidum and L. acidophilus (6); or a mixture of various lactobacilli and bifidobacteria at high concentrations (7).*	No treatment

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S4 Table. Outcomes identified in the included studies.

	Core area	Pathophysiological manifestations																						Resource use/economic impact				Life impact			
	Domain	Diarrhoea								Clostridium difficile infection		Gastrointestinal (GI) symptoms										AE of probiotic	Other clinical outcomes		Need for additional medical procedures				Absenteeism			Quality of life
	Outcome	Occurrence	Duration	Severity	Infectious diarrhoea	Stool consistency	Frequency of stools	Incubation	Other†	C. difficile diarrhoea	Asymptomatic carriage	Vomiting	Bloating	Nausea	Abdominal pain	Constipation	Taste problems	Lack of appetite	Flatulence	Belching	Symptom scale score	Other ‡	Adverse events of probiotic	Post-surgery complications	Other§	Need for i.v. rehydration	Need for antibiotic discontinuation	Need for hospitalization due to diarrhoea	Other****	School/day care absence	Hospital stay duration	Missed parental work	Overall health
Study ID																																
Ahmad 2013 [1]		2										2	2																			
Akcam 2015 [2]		2										2		2	2	2	2	2		2												
Arvola 1999 [3]		1	2	2	2					2																						
Bau 2020 [4]		**3**	3					3							3	3																
Basnet 2017 [5]		**1**	2																			2			2	2	2					
Bin 2015 [6]		1	2	2				2	2															2								
Correa 2005 [7]		**1**	2		2			2	2													2										
Dharani 2017 [8]		2										2							2			2	2									

Erdeve 2004 [9]	**1**																															
Esposito 2018 [10]	**1**	2			2																	2					2		2			
Fox 2015 [11]	1		1				2	2			2		2	2			2					2		2								
Georgiev a 2015 [12]	1		2					2		2	2	2	2	2	2			2			2											
Hurduc 2009 [13]	2											2	2	2	2	2	2							2								
Jindal 2017 [14]	**1**																					2										
Jirapinyo 2002 [15]	1	2					2																									
Kołodziej 2018 [16]	**1**			2					2													2			2	2	2					
Korpela 2016 [17]	2											2		2	2			2														
Kotowsk a 2005 [18]	**1**			2					2													2			2	2	2					
Lionetti 2006 [19]											2	2	2	2	2	2	2	2		1		2										
Merenste in 2009 [20]	**1**				2						2			2	2							2		2					2		2	2
Okazaki 2016 [21]																					1	2	2									
Olek 2017 [22]	1 & **2**				2						2			2				2				2										
Plewińsk a 2006 [23]	2										2		2	2		2																
Ranasing he 2008 [24]	1																															
Ruszczy ński 2008 [25]	**1**			2					2													2			2	2	2					

Seki 2003 [26]	2																															
Shahraki 2017 [27]											2			2				2														
Shan 2013 [28]	1 & **2**	1							2													2										
Sykora 2005 [29]	2										2		2	2								2		2								
Szajewska 2009 [30]	2										2		2	2	2	2	2	2				2				2						
Szymański 2008 [31]	1					2																2			2	2	2					
Tankanow 1990 [32]	1										2	2			2				2			2										
Tolone 2012 [33]	2										2		2	2	2																	
Vanderhoof 1999 [34]	2	2			2	2					2	2	2	2			2															
Wang 2014 [35]	2				2						2		2	2			2															
Zakordonets 2016 [36]	**1**																					2										
Zoppi 2001 [37]						2															2											

Table legend: 1 = primary outcome; 2 = secondary outcome or undefined; 3 = outcome in an observational study; numbers in bold – outcomes declared by the authors as "antibiotic-associated diarrhoea".

†Including: efficacy of diarrhoea treatment (Bin 2015), diarrhoea-associated dehydration (Correa 2005), time to first occurrence of loose stool (Fox 2015), and mild diarrhoea (Georgieva 2015).

‡Including: "abdominal discomfort" (Dharani 2017), symptoms from Gastrointestinal Symptom Rating Scale (Georgieva 2015), "gastrointestinal complications" (Okazaki 2016), "intestinal complaints" (Zoppi 2001).

§Including: compliance with antibiotic treatment (Bin 2015), headache (Fox 2015, Hurduc 2009, Sykora 2005), fatigue (Hurduc 2009), runny nose, cough, earaches, fever, irritability, and lethargy (Merenstein 2009).

¶Including: duration of hospital stay, number of needed postoperative wound dressings (Esposito 2018).

AE = adverse events

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S5 Table. Characteristics of the identified outcomes.

Study ID	Main diarrhea - related outcome measure	Diarrhea definition	Measurement instrument	Outcome assessment timeline	Other outcome measures
Ahmad 2013 [1]	Diarrhea	Not provided.	No measurement instruments used, diarrhea was reported by patient or parent during routine phone calls.	Outcome assessed once in a week, during the treatment (one week of antibiotic therapy + 3 weeks of omeprazole) and follow-up. Duration of follow-up unclear.	Other GI symptoms patient- or parent-reported, not defined.
Akcam 2015 [2]	Diarrhea	Not provided.	No measurement instruments used, patient-reported outcome during routine check-ups.	Outcome assessed on days 0, 7, 14, and 28 of the study (during antibiotic treatment + 2 week follow-up).	Other GI symptoms patient- or parent-reported, not defined.
Arvola 1999 [3]	Diarrhea	At least three watery or loose stools per day for a minimum of 2 consecutive days.	Symptom diary with three levels of stool consistency (solid, loose, watery).	First 2 weeks after the beginning of the antimicrobial treatment.	Definition of diarrhea severity: comparison of the stool frequency and stool consistency (solid, loose, watery) between groups. Diarrhea duration not defined. In case of diarrhea, microbiological tests were performed: immune assay for adenovirus, rotavirus and C. difficile toxin A, fecal cultures for Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile, Staphylococcus aureus, and yeasts, PCR for Norwalk-like (genogroup I and II) caliciviruses and astroviruses. AAD diagnosis not dependent on the results of those tests.
Basnet 2017 [4]	AAD	At least three loose/liquid stools per day.	Parents asked to record the stool frequency and consistency. No specific instrument used.	5 days after initiation of therapy.	Diarrhea duration not defined. Adverse events: Any other side effects or complaints the parents might have observed during the course of treatment.
Bau 2020 [5]	AAD	Presence of 3 or more loose or liquid bowel movements per day during antibiotic treatment or within 14 days from the antibiotics course excluding other etiologies.	No specific stool form assessment tool used. Some participants were tested for rota- and norovirus infection.	During antibiotic treatment and for additional 14 days.	Antibiotic-associated abdominal pain: presence of abdominal pain not reduced by defecation and not related to other recognizable conditions/fussiness and persisting crying without any other obvious causes in infants and younger children. Antibiotic-associated constipation: presence of at least 2 of the following: difficult or painful evacuation, hard or voluminous stools, and need to use a laxative or enema. Duration and onset of: diarrhea, abdominal pain and constipation not defined.
Bin 2015 [6]	Diarrhea	An increase in the frequency of bowel movements (>3/day) or decrease in stool consistency (BSFS score 5 or 6).	BSFS	During antibiotic treatment.	Diarrhea onset: time between the inclusion of the patient and the onset of diarrhoea Diarrhea duration: number of days until normalization of stool consistency (BSF score ＜4) and frequency (＜3 stools/day). Efficacy of diarrhea treatment: 3 categories – “significantly effective” (diarrhea cessation within 72 hours of treatment, and systemic symptoms

					disappear); “effective” (appearance and frequency of the stool markedly improve within 72 hours of treatment, and systemic symptoms markedly improve), and “ineffective” (appearance and frequency of the stool and systemic symptoms do not improve, or even worse within 72 hours of treatment). Diarrhea severity: based on occurrence of differently severe types of diarrhea – “diarrhea without dehydration or toxic symptoms”; “diarrhea with moderate to severe dehydration, or with obvious toxic symptoms and signs”.
Correa 2005 [7]	AAD	A change in bowel habits with the passage of three or more liquid stools per day for at least 2 consecutive days.	Tools and other definitions not described.	Stools recorded daily for 30 days.	Fecal samples from all patients with diarrhea and one among 3 patients without diarrhea were tested for rotavirus and enteric strains of adenovirus by enzyme immunoassay. AAD diagnosis not dependent on the results of those tests. Definitions of duration, incubation and diarrhea-associated dehydration were not provided.
Dharani 2017 [8]	Diarrhea	Not provided	No measurement instruments used, patient-reported outcome during routine check-up.	During antibiotic treatment.	Abdominal discomfort, flatulence, vomiting
Erdeve 2004 [9]	AAD	Watery stools more than twice a day.	Patients were re-evaluated and were questioned about watery stools three or more times on any day of the treatment.	Duration of antibiotic treatment.	
Esposito 2018 [10]	AAD	3 or more liquid stools (BSFS type 7) in 24 h.	BSFS	Evaluated the patients on each day of hospitalization.	Diarrhea duration: “number of continuous days of diarrhea”. Consistency: comparison between groups regarding number of stools of BSFS type: <3, 3-5 and >5 respectively. Other outcomes: duration of hospital stay, postoperative complications, number of needed postoperative wound dressings.
Fox 2015 [11]	Diarrhea	Various definitions of diarrhea. (A) stool consistency≥5 and frequency≥2/day for more than 2 days; (B) stool consistency≥5 and frequency≥3/day for more than 2 days; (C) stool consistency≥6 and stool frequency≥2/day for more than 2 days; and (D) stool consistency≥6 and stool frequency≥3/day for more than 2 days.	Study diary. BSFS.	Duration of antibiotic treatment + 1 week.	Diarrhea incubation – various definitions: 1) time to occurrence of >=2 stools per day; 2) time to occurrence of >=3 stools per day; 3) time to first occurrence of stool consistency >=6; 4) time to first occurrence of stool consistency >=5 Diarrhea severity assessment based on comparison between different types of diarrhea (definitions provided in the column “definitions of diarrhea”).

Georgieva 2015 [12]	Diarrhea	Three or more soft and not formed or watery bowel movements per day for at least 48 hours.	BSFS. Study diary.	During and up to 21 days post antibiotic treatment.	Mild diarrhea: Any soft and not formed or watery bowel movements. Diarrhea severity: total number of soft and not formed or watery bowel movements during an episode of diarrhea and the presence of blood and mucus in feces. Frequency of stool samples positive for C. difficile toxin A and B. Frequencies of other gastrointestinal symptoms during the study period according to GSRS.
Hurduc 2009 [13]	Diarrhea	Self-reported diarrhea, not defined beyond that.	No measurement instruments described.	Assessment on day 28 after the start of the treatment.	Other GI symptoms (abdominal pain, constipation, bloating, taste disturbance, nausea) reported, but not defined. No measurement instruments used.
Jindal 2017 [14]	AAD	Self-reported diarrhea, not defined beyond that.	"The frequency and consistency of stool was enquired and noted at each visit", no measurement instruments described.	Until 14 day after the start of antibiotic therapy.	
Jirapinyo 2002 [15]	Diarrhea	Diarrhea not defined.	"Characteristics and frequency of stools were recorded".	Not described.	Incubation and duration of diarrhea – not defined.
Kołodziej 2018 [16]	AAD & diarrhea	Various definitions: 1) three or more loose or watery stools per day for a minimum of 48 h 2) three or more loose or watery stools per day for a minimum of 24h 3) two or more loose or watery stools per day for a minimum of 24 h. AAD was diagnosed in cases of diarrhea, defined clinically as above, caused by C. difficile or for otherwise unexplained origin (i.e., negative laboratory stool tests for infectious agents).	BSFS. Amsterdam infant stool scale. Rapid, qualitative, chromatographic immunoassay that simultaneously detects rotaviruses, adenoviruses and noroviruses. Stool culture to identify bacterial pathogens (Salmonella spp., Shigella spp., Campylobacter spp., Yersinia spp.). Clostridium difficile toxins A and B were identified by standard enzyme immunoassay.	Throughout antibiotic treatment.	Additionally: need for i.v. rehydration, need for antibiotic discontinuation, need for hospitalization to manage diarrhea.
Korpela 2016 [17]	Diarrhea	Not defined.	Daily symptom diaries.	7 months in total.	Various gastrointestinal and respiratory complaints were marked down in diaries. The frequency of gastrointestinal complaints (pain, bloating, diarrhea, constipation, flatulence) was documented and reported.
Kotowska 2005 [18]	AAD & diarrhea	>=3 loose or watery stools per day for a minimum of 48 h. AAD was diagnosed in cases of diarrhea, defined clinically as	Study diaries. The presence of rotavirus-antigen was investigated in all diarrheal stool samples using a commercial latex agglutination	During and up to two weeks after antibiotic therapy.	Need for i.v. rehydration, need for antibiotic discontinuation, need for hospitalization to manage diarrhea.

		above, caused by C. difficile or for otherwise unexplained diarrhea.	test with a rotavirus-specific monoclonal antibody. Standard stool cultures were used to screen for bacteria (Salmonella, Shigella), and C. difficile toxins A and B were identified by enzyme immunoassay.		
Lionetti 2006 [19]	Not assessed			During therapy and for 10 additional days (20 days in total)	GI symptoms according to GSRS – both total score and individual symptoms.
Merenstein 2009 [20]	AAD	No definition, parent-reported.	Reported by parents. Study diary.	5, 10 and 15th day of the study.	Vomiting, stomach pain, constipation, loose stools, runny nose, cough, earaches, fever, irritability, lethargy. Absences from day care or school owing to illness. Missed parental work owing to the child being ill. Overall health: Likert scale. Adverse events: adverse events were defined either by the parent or healthcare provider as any event that could possibly be related to the study drink. Serious adverse events were defined as any incidence of death, a life-threatening event, hospitalization, prolonged hospital stay, or an event resulting in permanent disability.
Okazaki 2016 [21]	Not assessed			Until 3 weeks after surgery.	Gastrointestinal complications – not defined. Postoperative infections – not defined.
Olek 2017 [22]	AAD	>= 3 loose/watery stools/24 h	BSFS	Up to one week after probiotic/placebo cessation.	Incidence of loose/watery stools and mean number of loose/watery stools according to BSFS. Pain, vomiting, flatulence, distension – no measurement tools reported.
Plewińska 2006 [23]	Diarrhea	Not defined.	Not described.	Not clear, presumably during antibiotic therapy and for 20 next days.	Abdominal pain, taste disturbances, nausea, vomiting – not defined.
Ranasinghe 2008 [24]	Diarrhea	a change from the patient's normal bowel habit, with two or more loose or watery stools for at least two days.	Not described	3 first days of antibiotic therapy	
Ruszczyński 2008 [25]	AAD & diarrhea	>=3 loose or watery stools per day for a minimum of 48 h. AAD: Diarrhea defined as above, caused by C. difficile or otherwise unexplained diarrhea.	Study diary. Immunoassay that simultaneously detects rotaviruses and adenoviruses Stool culture for Salmonella spp., Shigella spp., Escherichia coli, Campylobacter spp. Clostridium difficile toxins A and B were identified by enzyme immunoassay.	Until 2 weeks after the end of the antibiotic therapy.	Additionally: need for i.v. rehydration, need for antibiotic discontinuation, need for hospitalization to manage diarrhea.
Seki 2003 [26]	Diarrhea	Muddy or watery stool at over threefold the normal daily frequency.	Not described.	Until sixth day of antibiotic therapy.	

Shahraki 2017 [27]	Not assessed			Day 0 and 4 weeks after completion of the treatment.	Vomiting, abdominal pain, flatulence and halitosis: questionnaire with symptom rating scale (0 = no symptom, 1 = mild, 2=moderate, 3=severe).
Shan 2013 [28]	AAD & diarrhea	>=3 loose or watery stools (5,6 or 7 on the BSFS) during 2 consecutive days caused by C. difficile or of unknown aetiology.	BSFS Immunoassay for rotavirus antigen and C. difficile toxin A and B. Cultures for Salmonella, Shigella, Campylobacter, Yersinia, Escherichia.	Up to 2 weeks after the end of antibiotic therapy.	Diarrhea duration: From first diarrheic stool to first normal (BSFS score 4 or less) stool.
Sykora 2005 [29]	Diarrhea	Not defined.	Reported at follow-up visit, no instruments specified.	A follow-up visit after the treatment period and 4 weeks after stopping treatment.	Nausea, abdominal pain, vomiting, headache – not defined.
Szajewska 2009 [30]	Treatment - associated diarrhea	3 or more loose or watery stools per day for a minimum of 48 hours occurring during and/or up to 2 weeks after the end of the therapy.	Study diary. Microbiological tests to exclude infectious origin – specific tests not described.	Up to 2 weeks after the end of the therapy.	Abdominal pain, nausea, vomiting, constipation, flatulence, taste disturbance, or loss of appetite – patient-reported, not defined, noted in study diary. Need for antibiotic therapy discontinuation.
Szymański 2008 [31]	Diarrhea	3 or more loose or watery stools per day for a minimum of 48 h.	Study diary.	during and/or up to 2 weeks after the end of the antibiotic therapy.	Number of stools per day. Need for i.v. rehydration, need for antibiotic discontinuation, need for hospitalization to manage diarrhea.
Tankanow 1990 [32]	Diarrhea	Abnormal frequency and liquidity of fecal discharges. One or more abnormally loose bowel movements throughout study period.	Parent-reported on routine telephone contact.	Diarrhea occurrence through days 1 to 10. Days 2-3 and 10-12 day of study – telephone contact.	
Tolone 2012 [33]	Diarrhea	Not defined.	Parent-reported, study diary.	During treatment period.	Constipation, epigastric pain, nausea, vomiting – not defined, reported in study diary.
Vanderhoof 1999 [34]	Diarrhea	2 liquid stools per day on at least 2 observation periods during the course of this study.	The Stool Consistency Continuum. Investigation of diarrhoea causes was to be pursued if clinical presentation suggested an infectious cause (vomiting, abdominal cramping, and loose, bloody frequent stools).	Parents were contacted every 3 days until antibiotic completion or cessation of diarrhoea	Pain intensity: Intensity score based on a visual analogue scale. Diarrhea duration: not defined. Occurrence of loose stools: occurrence of stools, which scored < 4 on the consistency continuum. Stool frequency: determined by counting the number of stools passed during a 24-hour period. Visible blood in the stool. Abdominal pain: according to intensity score. Nausea, vomiting, bloating, appetite suppression: parent-reported.
Wang 2014 [35]	Diarrhea	Not defined.	Not specified.	Up to 6 weeks after treatment.	Deformed excrement, nausea, vomiting, abdominal pain and loss of appetite – not defined.
Zakordonets 2016 [36]	AAD	At least 3 soft or liquid stools for at least 2 consecutive days.	Microscopic examination of faecal smears, pathogenic microflora examination. Pathogenic microflora examination not	Following 4 weeks after AB cessation.	

			described in detail. Stool form measurement instrument not described.		
Zoppi 2001 [37]	Not assessed			Up to 7 days after discharge.	Bowel movement frequency, intestinal complaints – measurement instruments not specified.

Table legend: AAD – Antibiotic-associated diarrhea, BSFS –Bristol stool form scale, GSRS – gastrointestinal symptom rating score

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S6 Table. Risk of bias assessment for the singe included cohort study

Quality Assessment Criteria	Criterion to be Fulfilled to Award Asterix (*)	Bau 2020
		Selection (4*maximum)
Representativeness of the exposed cohort	a) truly representative of the average children in the community * b) somewhat representative of the average children in the community * c) selected group of users eg nurses, volunteers d) no description of the derivation of the cohort	*
Selection of the non exposed cohort	a) drawn from the same community as the exposed cohort * b) drawn from a different source c) no description of the derivation of the non exposed cohort	*
Ascertainment of exposure	a) secure record (eg surgical records) * b) structured interview * c) written self report d) no description	-
Demonstration that outcome of interest was not present at start of study	a) yes * b) no	*
		Comparability (2*maximum)
Comparability of cohorts on the basis of the design or analysis	a) The study controls for clearly described confounding factors** b) No control for, or no adequate description of confounding factors,	-
		Outcome (3* maximum)
Assessment of outcome	a) independent blind assessment * b) record linkage* c) self report d) no description	-
Was follow-up long enough for outcomes to occur?	a) yes * b) no	*
Adequacy of follow-up of cohorts	a) complete follow up - all subjects accounted for * b) subjects lost to follow up unlikely to introduce bias - small number lost - <20% follow up, or description provided of those lost * c) follow up rate < 20% and no description of those lost d) no statement	*

Adapted from Newcastle-Ottawa scale (Retrieved from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

4. Podsumowanie i wnioski

Antybiotykoterapia we współczesnej praktyce pediatrycznej ma niepodważalną i w pełni zasłużoną pozycję. Ze względu na liczne niekorzystne skutki wynikające ze stosowania tej grupy leków konieczne jest prowadzenie racjonalnej polityki antybiotykowej, polegającej między innymi na ograniczaniu terapii przeciwbakteryjnej tylko do popartych dowodami wskazań, z uwzględnieniem najkrótszego bezpiecznego czasu leczenia i najmniejszych skutecznych dawek. Dobrze udokumentowanym działaniem niepożądanym jest biegunka związana z antybiotykoterapią. W celu jej uniknięcia najczęściej zaleca się stosowanie probiotyków, jednak nieliczne preparaty mają udowodnioną naukowo skuteczność.

Punkty końcowe oceniane w badaniach klinicznych, w tym w badaniach na temat skutków antybiotykoterapii u dzieci, powinny być przejrzyście zdefiniowane i raportowane, istotne klinicznie i możliwie homogenne, aby stać się podstawą do podejmowania właściwych decyzji klinicznych.

W pierwszej części niniejszej rozprawy przedstawiono wyniki badania z randomizacją przeprowadzonego metodą poczwórnie ślepej próby, oceniającego skuteczność probiotyku wielogatunkowego zawierającego 8 szczepów bakterii (*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W37, *Lactobacillus acidophilus* W55, *Lacticaseibacillus paracasei* W20, *Lactiplantibacillus plantarum* W62*, Lacticaseibacillus rhamnosus* W71 i *Ligilactobacillus salivarius* W24) w zapobieganiu biegunce związanej z antybiotykoterapią u dzieci. Badanie przeprowadzono zgodnie z opublikowanym wcześniej protokołem. Do badania zakwalifikowano 350 dzieci w wieku od 3 miesięcy do 18 lat (mediana wieku 28 miesięcy), otrzymujących antybiotyki o szerokim spektrum działania. Uczestnicy byli losowo przydzielani do grupy otrzymującej ww. probiotyk (w dawce 10 miliardów jednostek tworzących kolonie na dobę) lub placebo o identycznym smaku, zapachu i wyglądzie. Interwencja trwała przez cały okres antybiotykoterapii oraz przez 7 kolejnych dni. Pierwotnym punktem końcowym była biegunka związana z antybiotykoterapią, zdefiniowana jako co najmniej 3 luźne lub wodniste stolce na dobę w okresie 24 godzin, wywołane przez *C. difficile* lub o nieustalonej etiologii. Przeprowadzono analizę w grupach wyodrębnionych zgodnie z zaplanowanym leczeniem (ang. *intention to treat analysis*). W grupie otrzymującej badany probiotyk (n = 158) w porównaniu z grupą otrzymująca placebo (n = 155) stwierdzono

podobne ryzyko biegunki związanej z antybiotykoterapią (ryzyko względne [*relative risk,* RR] 0,81, 95% przedział ufności [*confidence interval*, CI] 0,49–1,33) ocenianej według najbardziej konserwatywnej definicji, czyli opartej na wykluczeniu etiologii zakaźnej innej niż *C. difficile* (rotawirusów, norowirusów i adenowirusów oraz *Salmonella spp., Campylobacter spp.* i *Yersinia spp.*). Jednocześnie, w grupie otrzymującej badany probiotyk stwierdzono istotnie mniejsze całkowite ryzyko biegunki ocenianej niezależnie od etiologii (RR 0,65, 95% CI 0,44–0,94). Dzieci w grupie otrzymującej probiotyk istotnie rzadziej wymagały również nawodnienia dożylnego. Nie stwierdzono istotnych różnic w odniesieniu do pozostałych wtórnych punktów końcowych. Zdarzenia niepożądane występowały w obu grupach z podobną częstością.

W drugiej części przedstawiono wyniki przeglądu systematycznego dokumentującego punkty końcowe raportowane w badaniach na temat stosowania probiotyków w trakcie antybiotykoterapii u dzieci. Kryteria rozpoznania AAD nie były jasno zdefiniowane w 12 spośród 33 badań oceniających biegunkę jako punkt końcowy. W pozostałych 21 badaniach zidentyfikowano aż 16 różnych definicji biegunki. Wykluczenie typowej zakaźnej etiologii traktowano jako warunek diagnozy AAD jedynie w 7 badaniach. Inne istotne klinicznie punkty końcowe, takie jak czas trwania AAD i jej ciężkość, były rzadko oceniane. Jedynie w dwóch badaniach raportowano punkty końcowe związane z jakością życia pacjentów.

Wnioski

- W przeprowadzonym badaniu z randomizacją metodą poczwórnie ślepej próby z placebo wykazano skuteczność probiotyku wielogatunkowego w zmniejszaniu całkowitego ryzyka biegunki, niezależnie od jej etiologii, w trakcie antybiotykoterapii i przez 7 kolejnych dni. Tak zdefiniowany punkt końcowy lepiej oddaje pragmatyczne podejście przyjęte w praktyce klinicznej niż konserwatywna definicja AAD. Na podstawie wyników badania można rozważać zastosowanie badanego probiotyku w trakcie antybiotykoterapii u dzieci.
- Punkty końcowe w badaniach dotyczących stosowania probiotyków w trakcie antybiotykoterapii u dzieci są heterogenne oraz rzadko uwzględniają perspektywę pacjenta. Wyniki przeglądu systematycznego uzasadniają potrzebę stworzenia zestawu podstawowych punktów końcowych dedykowanego tej tematyce.

- Oceniane łącznie wnioski obu badań demonstrują istotność definicji punktu końcowego dla interpretacji wyników badań klinicznych dotyczących stosowania probiotyków w trakcie antybiotykoterapii.

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Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
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dotyczącym: wyrażenia opinii w sprawie badania pt :„ Probiotyk wielogatunkowy w zapobieganiu biegunce związanej z antybiotykoterapią u dzieci –badanie z randomizacją i podwójnie ślepą próbą "

wyraża następującą
o p i n i ę

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .
W załączeniu: skład komisji oraz lista obecności

Przewodniczący Komisji Bioetycznej

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Oświadczenie współautorów publikacji

WARSZAWSKI UNIWERSYTET MEDYCZNY
MEDICAL UNIVERSITY OF WARSAW
Klinika Pediatrii

Warszawa, 7 czerwca 2022

Potwierdzam kluczową rolę i wkład merytoryczny lek. Jana Łukasika, jako głównego badacza oraz pierwszego autora, na wszystkich etapach powstawania publikacji stanowiących rozprawę doktorską:

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Łukasik J., Dierikx T., Besseling-van der Vaart I., de Meij T., Szajewska H., on behalf of the Multispecies Probiotic in AAD Study Group: Multispecies probiotic for the prevention of antibiotic-associated diarrhea in children. JAMA Pediatrics, 2022; accepted

Łukasik J., Guo Q., Boulos L., Szajewska H., Johnston B.C.: Probiotics for the prevention of antibiotic-associated adverse events in children – a scoping review to inform development of a core outcome set. PLoS One, 2020; 15(5): e0228824.

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