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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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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 (IF 16,2)

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. (IF 3,24)

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.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Iqbal S, Quigley EM. Progress in our understanding of the gut microbiome: implications for the clinician. *Curr Gastroenterol Rep* 2016;18:49.
2 Szajewska H, Canani RB, Guarino A, *et al*. Probiotics for the prevention of antibiotic-associated diarrhea in children. *J Pediatr Gastroenterol Nutr* 2016;62:495–506.
3 Goldenberg JZ, Lytvyn L, Steurich J, *et al*. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2015:CD004827.
4 Georgieva M, Pancheva R, Rasheva N, *et al*. Use of the probiotic lactobacillus reuteri dsm 17938 in the prevention of antibiotic-associated infections in hospitallzed Bulgarian children: a randomized, controlled trial. *Journal of IMAB - Annual Proceeding* 2015;21:895–900.
5 Jirapinyo P, Densupsoontorn N, Thamonsiri N, *et al*. Prevention of antibiotic-associated diarrhea in infants by probiotics. *J Med Assoc Thai* 2002;85(Suppl 2):S739–42.
6 Turck D, Bernet JP, Marx J, *et al*. Incidence and risk factors of oral antibiotic-associated diarrhea in an outpatient pediatric population. *J Pediatr Gastroenterol Nutr* 2003;37:22–6.
7 Bartlett JG. Clinical practice. antibiotic-associated diarrhea. *N Engl J Med* 2002;346:334–9.
8 McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol* 2008;3:563–78.
9 Hill C, Guarner F, Reid G, *et al*. Expert consensus document. the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;11:506–14.
10 Hell M, Bernhofer C, Stalzer P, *et al*. Probiotics in clostridium difficile infection: reviewing the need for a multistrain probiotic. *Benef Microbes* 2013;4:39–51.
11 FAO/WHO. FAO/WHO Expert Consultation. *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Cordoba, Argentina: FAO/WHO, 2001.
12 Szajewska H, Kołodziej M. Systematic review with meta-analysis: lactobacillus rhamnosus GG in the prevention of antibiotic-associated diarrhoea in children and adults. *Aliment Pharmacol Ther* 2015;42:1149–57.
13 Szajewska H, Kołodziej M. Systematic review with meta-analysis: saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2015;42:793–801.
14 Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? *Eur J Nutr* 2011;50:1–17.
15 Koning CJ, Jonkers DM, Stobberingh EE, *et al*. The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxycillin. *Am J Gastroenterol* 2008;103:178–89.
16 Koning CJ, Jonkers D, Smidt H, *et al*. The effect of a multispecies probiotic on the composition of the faecal microbiota and bowel habits in chronic obstructive pulmonary disease patients treated with antibiotics. *Br J Nutr* 2010;103:1452–60.
17 Szajewska H, Guarino A, Hojsak I, *et al*. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN working group for probiotics and prebiotics. *J Pediatr Gastroenterol Nutr* 2014;58:531–9.
18 Castro M. Placebo versus best-available-therapy control group in clinical trials for pharmacologic therapies: which is better? *Proc Am Thorac Soc* 2007;4:570–3.
19 Hojsak I. Probiotics in children: what is the evidence? *Pediatr Gastroenterol Hepatol Nutr* 2017;20:139–46.
20 Ouwehand AC. A review of dose-responses of probiotics in human studies. *Benef Microbes* 2017;8:143–51.
21 Ghanma A, Puttemans K, Deneyer M, *et al*. Amsterdam infant stool scale is more useful for assessing children who have not been toilet trained than bristol stool scale. *Acta Paediatr* 2014;103:e91–2.
22 Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997;32:920–4.
23 Ruszczyński M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment Pharmacol Ther* 2008;28:154–61.
24 Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Aliment Pharmacol Ther* 2005;21:583–90.
25 Hazards EPoB. Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). *EFSA Journal* 2013;11.
26 van den Nieuwboer M, Claassen E, Morelli L, *et al*. Probiotic and synbiotic safety in infants under two years of age. *Benef Microbes* 2014;5:45–60.
27 Koning CJM, Jonkers D, Stobberingh E, *et al*. Effect of a multispecies probiotic on the composition of the dominant faecal flora in healthy volunteers. *Gut* 2005;54:A243.
28 Ioannidis JP, Evans SJ, Gøtzsche PC, *et al*. Better reporting of harms in randomized trials: an extension of the CONSORT statement. *Ann Intern Med* 2004;141:781–8.

JAMA Pediatrics | Original Investigation

Multispecies Probiotic for the Prevention of Antibiotic-Associated Diarrhea in Children
A Randomized Clinical Trial

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IMPORTANCE The efficacy of multispecies probiotic formulations in the prevention of antibiotic-associated diarrhea (AAD) remains unclear.

OBJECTIVE To assess the effect of a multispecies probiotic on the risk of AAD in children.

DESIGN, SETTING, AND PARTICIPANTS This randomized, quadruple-blind, placebo-controlled trial was conducted from February 2018 to May 2021 in a multicenter, mixed setting (inpatients and outpatients). Patients were followed up throughout the intervention period. Eligibility criteria included age 3 months to 18 years, recruitment within 24 hours following initiation of broad-spectrum systemic antibiotics, and signed informed consent. In total, 646 eligible patients were approached and 350 patients took part in the trial.

INTERVENTIONS A multispecies probiotic consisting of *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W37, *L acidophilus* W55, *Lactocaseibacillus paracasei* W20, *Lactoplantibacillus plantarum* W62, *Lactocaseibacillus rhamnosus* W71, and *Ligilactobacillus salivarius* W24, for a total dose of 10 billion colony-forming units daily, for the duration of antibiotic treatment and for 7 days after.

MAIN OUTCOMES AND MEASURES 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, after testing for common diarrheal pathogens. The secondary outcomes included diarrhea regardless of the etiology, diarrhea duration, and predefined diarrhea complications.

RESULTS A total of 350 children (192 boys and 158 girls; mean [range] age, 50 [3-212] months) were randomized and 313 were included in the intention-to-treat analysis. Compared with placebo (n = 155), the probiotic (n = 158) had no effect on risk of AAD (relative risk [RR], 0.81; 95% CI, 0.49-1.33). However, children in the probiotic group had a lower risk of diarrhea regardless of the etiology (RR, 0.65; 95% CI, 0.44-0.94). No differences were observed between the groups for most of the secondary outcomes, including adverse events.

CONCLUSIONS AND RELEVANCE A multispecies probiotic did not reduce the risk of AAD in children when analyzed according to the most stringent definition. However, it reduced the overall risk of diarrhea during and for 7 days after antibiotic treatment. Our study also shows that the AAD definition has a significant effect on clinical trial results and their interpretation.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT03334604

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Supplemental content

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Group Information: A complete list of the members for the Multispecies Probiotic in AAD Study Group appears in Supplement 3.

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Antibiotic-associated diarrhea (AAD) is a common complication of antibiotic treatment.[1,2] Several different definitions of AAD have been proposed, including "diarrhea that occurs in relation to antibiotic treatment with the exclusion of other etiologies."[3,4] In clinical practice and in most clinical trials, microbiological tests are not routinely performed to exclude an infectious origin of AAD, confirming its etiology.[5] AAD is considered to result from gut dysbiosis by antibiotics, which may provoke overgrowth of specific pathogens, most prominently *Clostridioides difficile*, and lead to altered function of the microbiota.[6,7]

The most thoroughly studied preventive intervention for AAD is the administration of probiotics, defined as "live microorganisms, that when administered in adequate amounts, confer a health benefit on the host."[8] According to a 2019 Cochrane review,[2] probiotics as a group have a moderate protective effect on the prevention of pediatric AAD. The results of individual studies in this review varied depending on the dose of probiotic, with higher doses of 5 billion colony-forming units (CFU) or more per day demonstrating a better effect. Among the 33 included studies, only 6 randomized clinical trials (RCTs) of limited size investigated combinations of more than 3 probiotic strains, with varied results.[9-14] Thus, the effect of multispecies probiotic supplementation on AAD incidence in children remains in question. In adult patients, one of the previously studied multispecies probiotics consisted of 9 bacterial species.[15,16] In the current study, we aimed to assess the efficacy of a comparable multispecies probiotic mixture in the prevention of AAD in a pediatric population.

Key Points

Question What is the efficacy of a multispecies probiotic in the prevention of antibiotic-associated diarrhea in children?

Findings In this placebo-controlled randomized clinical trial of 350 children, a multispecies probiotic had no significant effect on the risk of antibiotic-associated diarrhea caused by *Clostridioides difficile* or of unknown etiology, but it reduced the overall risk of diarrhea regardless of the etiology from 32% to 20%, a statistically significant difference.

Meaning The use of the studied probiotic formulation may be considered for diarrhea prevention during antibiotic treatment in children.

Methods

Study Design

A parallel-group, randomized, quadruple-blind placebo-controlled RCT (trial protocol can be found in Supplement 1) was conducted in pediatric clinical and outpatient wards of 3 Dutch and 2 Polish hospitals (eTable 1 in Supplement 2). The study was prospectively registered in ClinicalTrials.gov database (NCT03334604), and the protocol was published in a peer-reviewed journal.[17] Consolidated Standards of Reporting Trials (CONSORT) guidelines were followed for reporting trial results.[18]

Ethics

The study was approved by the Bioethics Committees of the Medical University of Warsaw (KB/198/2017) and Amsterdam UMC (2019.227). Written informed consent was obtained by the parents or the legal guardians of all participants. During the study, 2 changes in the study protocol were introduced in response to an unsatisfactory inclusion rate. First, recruitment in additional centers was started, as planned in the study protocol. Second, the lower age limit of the participants was adjusted from 6 months to 3 months.

Participants

Eligibility criteria included age from 3 months to 18 years, recruitment within 24 hours following initiation of broad-spectrum oral or intravenous antibiotic therapy, and signed informed consent. The exclusion criteria were as follows: use of antibiotics within the previous 4 weeks; use of probiotics, proton pump inhibitors, laxatives, or antidiarrheal drugs within the previous 2 weeks; severe infection or life-threatening illness at recruitment (ie, indicated or probable admission to an intensive care unit); preexisting diarrhea within the previous 4 weeks based on patient's or caregiver's report; severe chronic disease (eg, cancer, inflammatory bowel disease, short-bowel syndrome); diagnosed primary or secondary immune deficiency; required tube-feeding; exclusive breastfeeding; and known allergy or hypersensitivity to any component of the study product.

Randomization and Masking

A block randomization in blocks of 4 was performed centrally in a 1:1 ratio by Winclove Probiotics B.V. with use of a computer random-sequence generator, by a person not otherwise involved in the study. The randomization lists were stored in sealed, opaque envelopes at the study centers. The participants, caregivers, and all investigators, including data collectors and outcomes assessors, were blinded until the primary data analysis was performed. Probiotic and placebo were packed identically and had the same appearance, taste, and smell.

Procedures and Interventions

The parents were instructed to administer 2 sachets of the study product daily to their children for the duration of antibiotic treatment and for 7 days after, up to a maximum of 17 days, starting within 24 hours of the first antibiotic dose. The multispecies probiotic (Ecologic AAD 612; Winclove Probiotics B.V.) contained 8 bacterial strains: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W37, *L acidophilus* W55, *Lactocaseibacillus paracasei* W20 (formerly known as *Lactobacillus paracasei* W20), *Lactoplantibacillus plantarum* W62 (formerly known as *Lactobacillus plantarum* W62), *Lactocaseibacillus rhamnosus* W71 (formerly known as *Lactobacillus rhamnosus* W71), and *Ligilactobacillus salivarius* W24 (formerly known as *Lactobacillus salivarius* W24), for a total dose of 5 billion CFU per sachet (10 billion CFU daily).

The data on outcomes were collected using study diaries during antibiotic treatment and for 7 additional days. The consistency was reported according to the Amsterdam Infant Stool Scale (AISS)[19] or Bristol Stool Form Scale (BSFS),[20] depending

on participant's age. In case of diarrhea occurrence, the participants' caregivers were requested to provide stool samples for testing for rotavirus, adenovirus, and norovirus by immunoassay; *Campylobacter species, Salmonella species, Shigella species,* and *Yersinia species* by isolation from stool cultures; and *C difficile* in children older than 1 year by detection of glutamate dehydrogenase in conjunction with toxins A and B with immunoassay. Additionally, stool samples for microbiota and metabolomics analysis were collected from a subset of patients at 4 timepoints: at baseline, on the day of antibiotic discontinuation, at the end of the intervention period, and 1 month after the intervention period. The results of microbiota and metabolomics analysis will be reported in a separate publication.

Outcome Measures

The primary outcome measure was AAD, defined as 3 or more loose or watery stools (a score of A on the AISS or 5-7 on the BSFS) per day in a 24-hour period, caused either by *C difficile* or of otherwise unexplained etiology, after testing for common, predefined diarrheal pathogens. Secondary outcomes included diarrhea, defined as 3 or more loose or watery stools per day in a 24-hour period regardless of the etiology, mild AAD, defined as 2 or more loose or watery stools per day for a minimum of a 24-hour period caused by *C difficile* or of otherwise unexplained etiology, severe AAD defined as 3 or more loose or watery stools per day for a minimum of a 48-hour period caused by *C difficile* or of otherwise unexplained etiology, diarrhea duration, defined as the interval until normalization of stool consistency according to the BSFS (1, 2, 3, or 4) or AISS (B, C, or D) and the presence of normal stools for 48 hours, diarrhea caused by *C difficile,* discontinuation of the antibiotic treatment owing to diarrhea, hospitalization caused by diarrhea, need for intravenous rehydration owing to diarrhea, and adverse events.

Sample Size Calculation

Based on the pooled risks of AAD determined from the previous studies conducted at the Medical University of Warsaw,[21,22] as well as those reported in a Cochrane review,[2] we expected that the incidence of AAD would be 16% among children receiving placebo. To detect a difference of 11% between the arms at a 5% significance level and with 80% power, we determined that 350 participants (175 in each arm) were needed assuming potential loss to follow-up of 20%.

Statistical Analysis

Descriptive statistics were used to present the participants' characteristics. For the dichotomous outcomes, relative risk (RR) was calculated with 95% CIs, along with number needed to benefit (NNTB), if appropriate. Presented *P* values were derived from χ^2 test or Fisher exact test where appropriate. For the continuous outcome, Man Whitney *U* test was performed. All of the statistical tests were 2-tailed and performed with a 5% level of significance. The primary outcome was also analyzed by logistic regression, controlling for 5 prespecified potential risk factors for AAD (age, sex, antibiotic type, duration of antibiotic treatment, and duration of hospital stay). Intention-to-treat (ITT) analysis was performed on the available participants. Owing to the completeness of our baseline data, no imputation methods were used in ITT analysis.[23] Sensitivity analyses with plausible assumptions regarding patients lost to follow-up as described by Akl et al[24] were performed. Additionally, per-protocol analysis was performed on the participants who ingested at least 75% of the study formula based on caregivers' reports and the counting of unused sachets. For the all of the calculations, StatsDirect, version 3.3.5 (StatsDirect Ltd) was used.

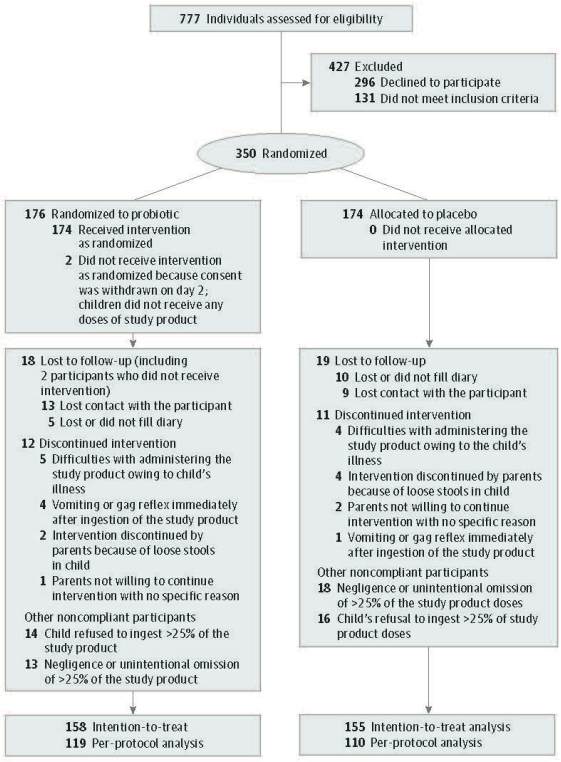
Results

Between February 2018 and May 2021, 350 participants (192 boys and 158 girls; median age: 28 months; mean [range] age, 50 [3-212] months) were consecutively enrolled. Among them, 202 participants were included in Poland and 148 in the Netherlands. Available case analysis was carried out in 313 participants and per-protocol analysis in 229 compliant participants (**Figure**). Participants' characteristics were comparable between the 2 groups (**Table 1**). Patients from the Netherlands differed from the Polish patients mainly in terms of class of used antibiotics, antibiotic administration route, and setting. Also, loss to follow-up frequency in Poland was almost 4 times higher than in the Netherlands (15.1% vs 4.1%, respectively) (eTable 2 in Supplement 2). The characteristics of the patients lost to follow-up were similar in the placebo and probiotic groups (eTable 3 in Supplement 2) and similar to characteristics of the remaining study participants (Table 1).

Among 83 patients who developed diarrhea, stools from 10 children tested positive for rotavirus, 3 for norovirus, 1 for adenovirus, and 1 for *Salmonella enterica;* 6 patients in the probiotic group and 11 patients in the placebo group did not provide a stool sample for the etiology testing. The reasons for the stool sampling failures were difficulties in communicating with patients after discharge from the hospital. All of these patients were not qualified as AAD cases for the primary outcome measure. In the ITT analysis (**Table 2**), AAD incidence was comparable between the probiotic and placebo groups (23 of 158 [14.6%] vs 28 of 155 [18.1%,] respectively; RR, 0.81; 95% CI, 0.49-1.33). The frequency of AAD according to the alternative definitions (mild, severe) was also similar between both study groups. The patients in the probiotic group had a significantly lower risk of developing diarrhea than those in the placebo group when analyzed regardless of its etiology (33 of 158 [20.9%] vs 50 of 155 [32.3%], respectively; RR, 0.65; 95% CI, 0.44-0.94; NNTB = 9; 95% CI, 5-64; *P* = .02); they were also less likely to require intravenous rehydration owing to diarrhea (0 of 158 [0%] vs 5 of 155 [3.2%], respectively; NNTB = 32; 95% CI, 14-125; *P* = .03). We found no significant difference between the groups in the other outcomes. Effect sizes in the per-protocol analysis were similar to the ones observed in the ITT analysis; however, because of a smaller sample size, they were not statistically significant (eTable 4 in Supplement 2).

To investigate whether the country-related differences might have had an effect on the results, we performed a subgroup analysis. The effect sizes for AAD, diarrhea, and diarrhea duration were similar in Poland and in the Netherlands,

Figure. CONSORT 2010 Flow Diagram



and only small differences were observed in the effect sizes for mild AAD and severe AAD outcomes between the countries. None of these differences between groups were statistically significant (eTable 5 in Supplement 2).

To examine which subgroup(s) of patients contributed to the difference between the effect sizes for AAD and diarrhea outcomes, we performed sensitivity analyses with modified outcomes: (1) patients with AAD combined with the patients with diarrhea who did not provide a stool sample, (2) infectious diarrhea with the exclusion of *C difficile* diarrhea, and (3) infectious diarrhea caused by specific pathogens (eTable 6 in Supplement 2). For all of these outcomes, the effect size was larger than that for the AAD outcome, especially for rotaviral diarrhea (RR, 0.11; 95% CI, 0.02-0.65; NNTB = 19; 95% CI, 10-63; $P = .01$). In the sensitivity analysis with plausible assumptions about missing data, the effect size for the diarrhea outcome was either no longer significant, of borderline significance, or statistically significant depending on the assumed risk of diarrhea among patients lost to follow-up (eTable 6 in Supplement 2). In the logistic regression, AAD was associated with younger age and diarrhea was associated with allocation to the placebo group, younger age, and use of amoxicillin with clavulanic acid (eTable 7 in Supplement 2).

Discussion

In this RCT, a multispecies probiotic did not significantly reduce the risk of AAD when analyzed according to the most stringent definition. However, the participants in the probiotic group had a significantly lower overall risk of diarrhea during the antibiotic treatment and 7 days after when the groups were analyzed regardless of diarrhea etiology. The studied probiotic did not demonstrate a beneficial effect on most other secondary outcomes, with the exception of the need for intravenous rehydration due to diarrhea, which was less common in the probiotic group. In the per-protocol analysis, the results were similar to those in the ITT analysis. Our results did not change after an adjustment for potential AAD risk factors.

It remains unclear why the studied probiotic had no significant effect on the AAD outcome, despite its beneficial effect in the prevention of diarrhea when analyzed regardless

Table 1. Characteristics of Participants

Characteristic	No. (%)		
	Placebo (n = 174)	Probiotic (n = 176)	Total (N = 350)
Age, median (range), mo	27 (3-204)	32 (3-212)	28 (3-212)
Sex			
Female	76 (43.7)	82 (46.6)	158 (45.1)
Male	98 (56.3)	94 (53.4)	192 (54.9)
Setting			
Inpatient	135 (77.6)	136 (77.3)	271 (77.4)
Outpatient	39 (22.4)	40 (22.7)	79 (22.6)
Reason for antibiotic treatment			
Lower respiratory tract infection	54 (31)	56 (31.8)	110 (31.4)
Upper respiratory tract infection	52 (29.9)	49 (27.8)	101 (28.9)
Urinary tract infection	35 (20.1)	24 (13.6)	59 (16.9)
Skin infection	8 (4.6)	16 (9.1)	24 (6.9)
Lymphadenitis	6 (3.4)	7 (4)	13 (3.7)
Nervous system infection	3 (1.7)	4 (2.3)	7 (2.0)
Gastrointestinal infection	5 (2.9)	5 (2.8)	10 (2.9)
Joint infection	3 (1.7)	2 (1.1)	5 (1.4)
Other	8 (4.6)	13 (7.4)	21 (6.0)
Antibiotic administration route			
Only oral	71 (40.8)	73 (41.5)	144 (41.1)
Only intravenous	25 (14.4)	28 (15.9)	53 (15.1)
Intravenous followed by oral	78 (44.8)	75 (42.6)	153 (43.7)
Antibiotic type			
Cephalosporin			
Second generation	25 (14.4)	26 (14.8)	51 (14.6)
Third generation	33 (19)	36 (20.5)	69 (19.7)
Aminopenicillin	69 (39.7)	71 (40.3)	140 (40)
Amoxicillin with clavulanic acid	67 (38.5)	55 (31.3)	122 (34.9)
Clindamycin	14 (8)	17 (9.7)	31 (8.9)
Cloxacillin/flucloxacillin	0	6 (3.4)	6 (1.7)
Gentamicin	1 (0.6)	3 (1.7)	4 (1.1)
Other	6 (3.4)	6 (3.4)	12 (3.4)
Two concomitant antibiotics	15 (8.6)	24 (13.6)	39 (11.1)
Change of antibiotic class	26 (14.9)	20 (11.4)	46 (13.1)
Treatment duration, median (range), d	10 (2-21)	10 (1-36)	10 (1-36)
Hospital stay duration, median (range), d	5 (1-35)	5 (1-45)	5 (1-45)

Table 2. Main Results of the Available Case Analysis

Outcome	Events, No. (%)		Relative risk (95% CI)	Absolute risk reduction, %	NNTB (95% CI)
	Probiotic group	Placebo group			
AAD	23 (14.6)	28 (18.1)	0.81 (0.49-1.33)	3.5	NA
Severe AAD	18 (11.4)	19 (12.3)	0.93 (0.51-1.69)	0.9	NA
Mild AAD	40 (25.3)	38 (24.5)	1.03 (0.7-1.52)	−0.8	NA
Diarrhea[b]	33 (20.9)	50 (32.3)	0.65 (0.44-0.94)[a]	11.4	9 (5-64)[a]
Clostridioides difficile diarrhea	1 (0.6)	3 (1.9)	0.33 (0.05-2.26)	1.3	NA
Hospitalization owing to diarrhea	1 (0.6)	2 (1.3)	0.49 (0.06-3.71)	0.7	NA
Antibiotic cessation owing to diarrhea	0	0	NA	0	NA
Intravenous rehydration owing to diarrhea	0	5 (3.2)	NA	3.2	32 (14-125)[a]
Adverse events[c]	16 (10.1)	10 (6.5)	1.57 (0.75-3.3)	−3.6	NA

Abbreviations: AAD, antibiotic-associated diarrhea; NA, not applicable; NNTB, number needed to benefit.

[a] $P < .05$.

[b] Diarrhea duration in days, median (IQR) for probiotic group (5 [3-7]) and placebo group (4 [3-7]).

[c] Including readmission to hospital owing to reasons other than diarrhea (5 in probiotic group; 4 in placebo group), rash (2 in probiotic group; 3 in placebo group), vomiting (3 in probiotic group; 1 in placebo group), gag reflex (2 in probiotic group), abdominal pain (3 in probiotic group; 2 in placebo group), trace of blood in the stool (1 in probiotic group).

of the etiology. One could speculate that a trial involving a larger group might have shown significant results for the primary outcome. Nevertheless, considering the satisfactory incidence of AAD in the placebo group, our study was adequately powered to detect a clinically significant difference in this outcome and even more than adequately powered for assessing the diarrhea outcome. In the sensitivity analyses, we investigated which subgroup(s) of patients contributed to this difference in outcome effect sizes to the highest extent. We found that the effect was highest for viral gastroenteritis, especially caused by rotavirus. Another significant result, ie, the number of children requiring intravenous rehydration due to diarrhea, was also related to this finding, as all of these patients received intravenous fluids owing to rotavirus infection. There is evidence supporting a role of the microbiota in rotavirus infection,[25,26] as well as for a preventive effect of certain probiotics.[27] One could speculate that our study detected a similar effect of the studied probiotic on diarrhea caused by rotavirus. However, caution is needed when interpreting this finding, as this trial was not designed to answer this specific research question. Moreover, since the participants were not tested for the presence of diarrheal pathogens at baseline, some of them might have already been within the incubation period of infectious diarrhea on hospital admission.

In our study, we used a rather stringent definition of AAD, which allowed us to differentiate between clinically relevant conditions and clinically unimportant changes in the consistency of stools. It also considered the most common etiology of diarrhea related to antibiotic administration and assumed that common nosocomial infections, such as norovirus or rotavirus gastroenteritis,[28,29] are not directly associated with antibiotic treatment. However, the definitions of AAD in published studies vary, and in many studies it was similar to the definition of diarrhea, as applied in current study. To illustrate, a 2020 review found that microbiological tests were not performed to identify AAD outcomes in 28 of 33 previous studies on probiotic supplementation during antibiotic treatment in children.[5] While this approach may pose a question as to whether the researchers really measured AAD or rather diarrhea during antibiotic treatment regardless of the etiology, it also represents a much more pragmatic point of view. Etiology testing is not routinely recommended for cases of acute diarrhea in children,[30] and for both the patient and the physician, what caused the diarrhea may not be relevant as long as the preventive intervention is effective.

Why the effect sizes in the ITT analysis were similar to those observed in the per-protocol analysis is unclear. This finding may reflect misclassification of compliance data, as it was collected only by indirect methods, ie, study diaries and counting of unused sachets. Another possible explanation is that the studied probiotic is effective even if not taken regularly. Additionally, participants deemed as overall noncompliant might have been compliant during a specific time period crucial for diarrhea, eg, during the first days of antibiotic therapy.

Strengths and Limitations

Our study had a number of strengths. To our knowledge, this is the largest trial investigating the effect of a probiotic containing more than 3 species of microorganisms on the incidence of AAD in children. The number of participants is almost 3 times higher than that in the second largest study of which we are aware.[11] It was designed with an intent to answer an unambiguous research question with a choice of clearly predefined outcomes. The study was conducted in settings of international cooperation, which enabled verification of the collected data by comparison between the different populations and recruitment centers. However, there are also some limitations. Loss to follow-up was relatively high, which is reflected by the range of uncertainty demonstrated in analyses with plausible assumptions about missing data. To search for indications of imbalances between the trial arms owing to selective missing data,[31] we investigated the number and characteristics of participants lost to follow-up in both arms. We found them to be comparable with each other, as well as with the rest of the study participants. We also compared the outcome data between the Polish and the Dutch participants, who differed greatly in terms of loss to follow-up, and we found mostly similar effect sizes. We assume that the missing data were unlikely to have introduced a significant bias to our study; nevertheless, no method of testing can rule out such a possibility completely.[32] As mentioned, there was a puzzling difference between loss to follow-up in Poland and in the Netherlands. All but 4 of the participants were recruited and followed-up by 3 researchers (J.Ł., T.D., and T.d.M.) who were in a regular contact with each other to standardize the study conduct. Therefore, this difference may be explained by country-specific attitudes of patients and overlooked differences in the researchers' practice. Another study limitation is a potential misclassification between the AAD and diarrhea outcomes, owing to the limited diagnostic accuracy of immunoassay tests,[33] the limited number of diarrheal pathogens tested, and the number of patients who failed to provide stool samples. Additionally, the limited study follow-up duration might have led to an omission of some diarrhea cases occurring later than a week after antibiotic cessation.[7]

Conclusions

The multispecies probiotic used in this trial did not reduce the risk of AAD when analyzed according to the most stringent definition. However, we found a beneficial effect of the formulation on the overall risk of diarrhea during and 7 days after antibiotic therapy (NNTB = 9). The latter outcome corresponds well with the standard approach to AAD in clinical practice. Therefore, the use of the studied probiotic may be considered for diarrhea prevention during antibiotic treatment in children. Our study also shows that the AAD outcome definition has a significant effect on clinical trial results and their interpretation.

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for the integrity of the data and the accuracy of the data analysis.
Concept and design: Łukasik, Besseling-van der Vaart, Szajewska.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Łukasik.
Critical revision of the manuscript for important intellectual content: Dierikx, Besseling-van der Vaart, de Meij, Szajewska.
Statistical analysis: Łukasik, Szajewska.
Obtained funding: de Meij, Szajewska.
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Supervision: de Meij, Szajewska.

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Group Information: A complete list of the members of the Multispecies Probiotic in AAD Study Group appears in Supplement 3.

Data Sharing Statement: See Supplement 4.

REFERENCES

1. Turck D, Bernet JP, Marx J, et al. Incidence and risk factors of oral antibiotic-associated diarrhea in an outpatient pediatric population. *J Pediatr Gastroenterol Nutr*. 2003;37(1):22-26. doi:10.1097/00005176-200307000-00004

2. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev*. 2019;4(4):CD004827. doi:10.1002/14651858.CD004827.pub5

3. Szajewska H, Canani RB, Guarino A, et al; ESPGHAN Working Group for ProbioticsPrebiotics. Probiotics for the prevention of antibiotic-associated diarrhea in children. *J Pediatr Gastroenterol Nutr*. 2016;62(3):495-506. doi:10.1097/MPG.0000000000001081

4. Liao W, Chen C, Wen T, Zhao Q. Probiotics for the prevention of antibiotic-associated diarrhea in adults: a meta-analysis of randomized placebo-controlled trials. *J Clin Gastroenterol*. 2021;55(6):469-480. doi:10.1097/MCG.0000000000001464

5. Łukasik J, Guo Q, Boulos L, Szajewska H, Johnston BC. 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. doi:10.1371/journal.pone.0228824

6. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *J Clin Microbiol*. 2004;42(3):1203-1206. doi:10.1128/JCM.42.3.1203-1206.2004

7. McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol*. 2008;3(5):563-578. doi:10.2217/17460913.3.5.563

8. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514. doi:10.1038/nrgastro.2014.66

9. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. *Iran J Pediatr*. 2013;23(1):79-84.

10. Saneeyan H, Layegh S, Rahimi H. Effectiveness of probiotic on treatment of Helicobacter pylori infection in children. *Majallah-i Danishkadah-i Pizishki-i Isfahan*. 2011;146(29):882-889.

11. Merenstein DJ, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the Measuring the Influence of Kefir (MILK) study. *Arch Pediatr Adolesc Med*. 2009;163(8):750-754. doi:10.1001/archpediatrics.2009.119

12. Conway S, Hart A, Clark A, Harvey I. Does eating yogurt prevent antibiotic-associated diarrhoea? a placebo-controlled randomised controlled trial in general practice. *Br J Gen Pract*. 2007;57(545):953-959. doi:10.3399/096016407782604811

13. Dharnai S, Nirmala P, Ramanathan R, Vanitha S. Comparative study of efficacy and safety of azithromycin alone and in combination with probiotic in the treatment of impetigo in children. *Int J Curr Pharm Res*. 2017;9(6):52-55. doi:10.22159/ijcpr.2017v9i6.23429

14. Zakordonets L, Tolstanova G, Yankovskiy D, Dyment H, Kramarev S. Different regimes of multiprobiotic for prevention of immediate and delayed side effects of antibiotic therapy In children. *Res J Pharm Biol Chem Sci*. 2016;7(3):2194-2201.

15. Koning CJ, Jonkers DM, Stobberingh EE, Mulder L, Rombouts FM, Stockbrügger RW. The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxycillin. *Am J Gastroenterol*. 2008;103(1):178-189. doi:10.1111/j.1572-0241.2007.01547.x

16. Koning CJ, Jonkers D, Smidt H, et al. The effect of a multispecies probiotic on the composition of the faecal microbiota and bowel habits in chronic obstructive pulmonary disease patients treated with antibiotics. *Br J Nutr*. 2010;103(10):1452-1460. doi:10.1017/S0007114509993497

17. Łukasik J, Szajewska H. Effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial. *BMJ Open*. 2018;8(5):e021214. doi:10.1136/bmjopen-2017-021214

18. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ*. 2010;340:c332. doi:10.1136/bmj.c332

19. Ghanma A, Puttemans K, Deneyer M, Benninga MA, Vandenplas Y. Amsterdam infant stool scale is more useful for assessing children who have not been toilet trained than Bristol Stool Scale. *Acta Paediatr*. 2014;103(2):e91-e92. doi:10.1111/apa.12422

20. Lewis SJ, Heaton KW. Stool Form Scale as a useful guide to intestinal transit time. *Scand J Gastroenterol*. 1997;32(9):920-924. doi:10.3109/00365529709011203

21. Ruszczyński M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment Pharmacol Ther*. 2008;28(1):154-161. doi:10.1111/j.1365-2036.2008.03714.x

22. Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Aliment Pharmacol Ther*. 2005;21(5):583-590. doi:10.1111/j.1365-2036.2005.02356.x

23. Jakobsen JC, Gluud C, Wetterslev J, Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials--a practical guide with flowcharts. *BMC Med Res Methodol*. 2017;17(1):162. doi:10.1186/s12874-017-0442-1

24. Akl EA, Briel M, You JJ, et al. Potential impact on estimated treatment effects of information lost to follow-up in randomised controlled trials (LOST-IT): systematic review. *BMJ*. 2012;344:e2809. doi:10.1136/bmj.e2809

25. Gozalbo-Rovira R, Rubio-Del-Campo A, Santiso-Bellón C, et al. Interaction of intestinal bacteria with human rotavirus during infection in children. *Int J Mol Sci*. 2021;22(3):1010. doi:10.3390/ijms22031010

26. Uchiyama R, Chassaing B, Zhang B, Gewirtz AT. Antibiotic treatment suppresses rotavirus infection and enhances specific humoral immunity. *J Infect Dis*. 2014;210(2):171-182. doi:10.1093/infdis/jiu037

27. Hojsak I, Szajewska H, Canani RB, et al; ESPGHAN Working Group for Probiotics/Prebiotics. Probiotics for the prevention of nosocomial diarrhea in children. *J Pediatr Gastroenterol Nutr*. 2018;66(1):3-9. doi:10.1097/MPG.0000000000001637

28. Załęski A, Banasiuk M, Karpierz K, Kuchar E, Podsiadły E. The clinical course of gastroenteritis due to nosocomial and community acquired norovirus infections in immunocompromised and immunocompetent children—single center experience. *Przegl Epidemiol*. 2020;74(1):23-31.

29. Ogilvie I, Khoury H, Goetghebeur MM, El Khoury AC, Giaquinto C. Burden of community-acquired and nosocomial rotavirus gastroenteritis in the pediatric population of Western Europe: a scoping review. *BMC Infect Dis*. 2012;12:62. doi:10.1186/1471-2334-12-62

30. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; European Society for Pediatric Infectious Diseases. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. *J Pediatr Gastroenterol Nutr*. 2014;59(1):132-152. doi:10.1097/MPG.0000000000000375

31. Groenwold RH, Moons KG, Vandenbroucke JP. Randomized trials with missing outcome data: how to analyze and what to report. *CMAJ*. 2014;186(15):1153-1157. doi:10.1503/cmaj.131353

32. Little RJ, D'Agostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. *N Engl J Med*. 2012;367(14):1355-1360. doi:10.1056/NEJMsr1203730

33. Kirby A, Iturriza-Gómara M. Norovirus diagnostics: options, applications and interpretations. *Expert Rev Anti Infect Ther*. 2012;10(4):423-433. doi:10.1586/eri.12.21

Supplemental Online Content

Łukasik J, Dierikx T, Besseling-van der Vaart I, de Meij T, Szajewska H; the Multispecies Probiotic in AAD Study Group. Multispecies probiotic for the prevention of antibiotic-associated diarrhea in children: a randomized clinical trial. *JAMA Pediatr*. Published online June 21, 2022. doi:10.1001/jamapediatrics.2022.1973

This supplemental material has been provided by the authors to give readers additional information about their work.

eTable 1. Recruitment centres.

Location	Amsterdam UMC, location VUmc De Boelelaan 1117 Amsterdam, NL	Amsterdam UMC, location AMC Meibergdreef 9, 1105 Amsterdam, NL	OLVG location East Oosterpark 9, 1092 Amsterdam, NL	OLVG location West Jan Tooropstraat 164, 1061 Amsterdam, NL	University Clinical Center of the Medical University of Warsaw, Żwirki i Wigury 63A, 02091 Warsaw, PL	St. Jadwiga Śląska Hospital Prusicka 53-55, 55100 Trzebnica, PL
Number of the included participants	14	59	31	44	198	4

eTable 2: Patient characteristics depending on the country of recruitment

Clinical values	Poland	The Netherlands
Total	202	148
Lost to follow-up, n(%)	31 (15.1)	6 (4.1)
Compliant participants, n(%)	128 (63.4)	101 (68.2)
Median age in months (range)	27 (3-212)	32 (3-204)
Sex		
Female, n(%)	100 (49.5)	58 (39.2)
Male, n(%)	102 (50.5)	90 (60.8)
Setting		
Inpatient, n(%)	200 (99)	71 (48)
Outpatient, n(%)	2 (1)	77 (52)
Reason for antibiotic treatment		
Lower respiratory tract infection, n(%)	62 (30.7)	48 (32.4)
Upper respiratory tract infection, n(%)	83 (41.1)	18 (12.2)
Urinary tract infection, n(%)	27 (13.4)	32 (21.6)
Skin infection, n(%)	3 (1.5)	21 (14.2)
Lymphadenitis, n(%)	9 (4.5)	4 (2.7)
Nervous system infection, n(%)	2 (1)	5 (3.4)
Gastrointestinal infection, n(%)	3 (1.5)	7 (4.7)
Joint infection, n(%)	1 (0.5)	4 (2.7)
Other, n(%)	12 (5.9)	9 (6.1)
Antibiotic administration route		
Only oral, n(%)	31 (15.3)	113 (76.4)
Only intravenous, n(%)	43 (21.3)	10 (6.8)
Intravenous followed by oral , n(%)	128 (63.4)	25 (16.9)
Antibiotic type		
2nd generation cephalosporin, n(%)	48 (23.8)	3 (2)
3rd generation cephalosporin, n(%)	51 (25.2)	18 (12.2)
Aminopenicillin, n(%)	90 (44.6)	50 (33.8)
Amoxicillin+clavulanic acid, n(%)	36 (17.8)	86 (58.1)
Clindamycin, n(%)	29 (14.4)	2 (1.4)
Cloxacillin/flucloxacillin, n(%)	2 (1)	4 (2.7)
Gentamicin, n(%)	0	4 (2.7)
Other, n(%)	5 (2.5)	7 (4.7)
Two concomitant antibiotics, n(%)	31 (15.3)	8 (5.4)
Change of antibiotic class n(%)	28 (13.9)	18 (12.2)
Median treatment duration days (range)	10 (1-21)	7 (2-36)
Median hospital stay duration (range)	5 (2-21)	4 (1-45)

eTable 3: Characteristics of patients lost to follow-up

Clinical values	Placebo	Probiotic
Total	19	18
Median age in months (range)	26 (3-144)	25 (6-161)
Sex		
Female, n(%)	9 (47)	9 (50)
Male, n(%)	10 (53)	9 (50)
Setting		
Inpatient, n(%)	16 (84)	17 (94)
Outpatient, n(%)	3 (16)	1 (6)
Reason for antibiotic treatment		
Lower respiratory tract infection, n(%)	10 (53)	6 (33)
Upper respiratory tract infection, n(%)	5 (26)	7 (39)
Urinary tract infection, n(%)	1(5)	2 (11)
Nervous system infection, n(%)	1 (5)	-
Lymphadenitis	-	1 (6)
Other, n(%)	2 (10)	2 (11)
Antibiotic type		
2nd generation cephalosporin, n(%)	3 (16)	5 (28)
3rd generation cephalosporin, n(%)	2 (11)	2 (11)
Aminopenicillin, n(%)	10 (53)	9 (50)
Amoxicillin+clavulanic acid, n(%)	4 (21)	2 (11)
Clindamycin, n(%)	4(21)	4 (22)
Two concomitant antibiotics, n(%)	4 (21)	4 (22)
Median treatment duration days (range)	10 (5-21)	10 (3-14)
Median hospital stay duration (range)	4 (3-14)	4 (2-9)

eTable 4. Results of the per protocol analysis including 119 patients in probiotic group and 110 patients in placebo group.

Outcome	Probiotic group no. of events (%)	Placebo group no. of events (%)	Relative Risk (95% CI)
AAD	16 (13.4)	18 (16.4)	0.82 (0.45 to 1.52)
Severe AAD	13 (10.9)	12 (10. 9)	1 (0.49 to 2.07)
Mild AAD	29 (24.4)	25 (22.7)	1.07 (0.67 to 1.71)
Diarrhea	20 (16.8)	27 (24.5)	0.68 (0.41 to 1.14)
C. difficile diarrhea	1 (0.84)	2 (1.8)	0.46 (0.06 to 3.49)
Hospitalization due to diarrhoea	0 (0)	1 (0.9)	n/a
Antibiotic cessation due to diarrhea	0 (0)	0 (0)	n/a
Intravenous rehydration due to diarrhea	0 (0)	1 (0.9)	n/a
Adverse events			
Readmission to the hospital	3 (2.5)	1 (0.9)	2.77 (0.29. 26.27)
Abdominal pain	3 (2.5)	0 (0)	n/a
Vomiting	2 (1.7)	0 (0)	n/a
Rash	1 (0.84)	0 (0)	n/a
Trace of blood in the stool	1 (0.84)	0 (0)	n/a
	Probiotic group median (IQR)	**Placebo group median (IQR)**	**Median difference (95% CI)**
Diarrhea duration in days	3 (3-5.75)	4 (3-6)	1 (-1 to 2)

eTable 5. Available case analysis by the country of recruitment.

Available case analysis - Poland (probiotic n = 84, placebo n= 87)			
Outcome	**Probiotic group no. of events**	**Placebo group no. of events**	**Relative Risk (95% CI)**
AAD	13	16	0.84 (0.44 to 1.62)
Severe AAD	8	7	1.18 (0.46 to 3.02)
Mild AAD	21	25	0.87 (0.53 to 1.42)
Diarrhoea	18	28	0.67 (0.4 to 1.1)
C. difficile diarrhea	1	2	0.52 (0.07 to 3.89)
Hospitalization	0	2	n/a
Antibiotic cessation	0	0	n/a
Intravenous rehydration	0	5	n/a
Adverse events[a]	10	5	2.07 (0.77 to 5.61)
	Probiotic group median (IQR)	**Placebo group median (IQR)**	**Median difference (95% CI)**
Diarrhea duration	3 (2 to 5,5)	4 (3 to 6)	1 (-1 to 2)
[a]Including: rash (2), readmission to the hospital (2), vomiting (1) in the placebo group and vomiting (3), rash (2), readmission to the hospital (1), gag reflex (2), trace of blood in the stool (1), abdominal pain (1) in the probiotic group.			
Available case analysis - The Netherlands (probiotic n = 74, placebo n= 68)			
Outcome	**Probiotic group no. of events**	**Placebo group no. of events**	**Relative Risk (95% CI)**
AAD	10	12	0.77 (0.36 to 1.63)
Severe AAD	10	12	0.77 (0.36 to 1.63)
Mild AAD	19	13	1.34 (0.73 to 2.5)
Diarrhoea	15	22	0.63 (0.36 to 1.09)
C. difficile diarrhea	0	1	n/a
Hospitalisation	1	0	n/a
Antibiotic cessation	0	0	n/a
Intravenous rehydration	0	0	n/a
Adverse events[a]	6	5	1.03 (0.37 to 3.28)

	Probiotic group median (IQR)	Placebo group median (IQR)	Median difference (95% CI)
Diarrhea duration	5 (3-12)	6 (4-7)	0 (-2 to 3)

[a]Including: readmission to the hospital (4), abdominal pain (2) in probiotic group and readmission to the hospital (2), abdominal pain (2), rash (1) in placebo group.

eTable 6. Sensitivity analyses

Outcome	Probiotic group no. Of events (%)	Placebo group no. of events (%)	Relative Risk (95% CI)
AAD cases + diarrhea cases where the testing for pathogens was not performed	29 (18.4)	39 (25.2)	0.73 (0.48 to 1.11)
Infectious diarrhea excluding *C. difficile* diarrhoea	4 (2.5)	11 (7.1)	0.36 (0.12 to 1.04)
Rotaviral diarrhoea	1 (0.6)	9 (5.8)	0.11 (0.2 to 0.65)[a]
Norovirus diarrhea	3 (1.9)	0 (0)	n/a
Adenovirus diarrhea	0 (0)	1 (0.6)	n/a
Salmonella diarrhea	0 (0)	1 (0.6)	n/a
Diarrhea: plausible assumption[c] 5:1	51 (29)	56 (32.2)	0.9 (0.66 to 1.23)
Diarrhea: plausible assumption[c] 2:1	41 (23.3)	56 (32.2)	0.72 (0.51 to 1.02)
Diarrhea: plausible assumption[c] 1,5:1	39 (22.2)	56 (32.2)	0.69 (0.48 to 0.97)[b]
AAD: plausible assumption[c] 5:1	36 (20.5)	31 (17.8)	1.15 (0.75 to 1.77)
AAD: plausible assumption[c] 1:1	26 (14.8)	31 (17.8)	0.83 (0.52 to 1.33)

[a]p=0.01 [b]p=0.04

[c]Explanation of plausible assumption: we performed a sensitivity analysis assuming that the incidence of events among participants lost to follow-up is equal to, or higher by a specific ratio relative to the observed event incidence among participants followed up, For example, 'plausible assumption 5:1' means that we assumed the incidence of diarrhea among missing patients in the probiotic group to be 5 times higher than that in the probiotic group patients who were followed-up, and the incidence of diarrhea among missing patients in the placebo group to be equal to the incidence of diarrhea in the placebo group patients who were followed up.

eTable 7. Results of logistic regression analysis.

A. Logistic regression – AAD outcome

Predictor	Model with covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.8	0.42 to 1.52	0.49
Age in months	0.99	0.98 to 1	0.006
Male sex	0.94	0.49 to 1.81	0.85
2nd gen. cephalosporin	0.83	0.24 to 2.91	0.78
3rd gen. cephalosporin	2.02	0.72 to 5.7	0.18
Aminopenicillin	0.76	0.24 to 2.45	0.65
Amoxicillin with clavulanic acid	2.07	0.68 to 6.31	0.2
Clindamycin	0.61	0.17 to 2.23	0.45
Other antibiotic	0.49	0.1 to 2.57	0.4
Intravenous antibiotic	1.36	0.40 to 4.62	0.62
Oral antibiotic	0.62	0.26 to 1.49	0.29
Hospital stay duration	1.04	0.97 to 1.12	0.26
Antibiotic treatment duration	1.05	0.96 to 1.14	0.28
	Model without covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.77	0.42 to 1.41	0.4

B. Logistic regression – Diarrhea outcome

Predictor	Model with covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.55	0.32 to 0.96	0.04
Age in months	0.99	0.98 to 0.99	<0.001
Male sex	1.05	0.60 to 1.82	0.86
2nd gen. cephalosporin	1.75	0.59 to 5.15	0.31
3rd gen. cephalosporin	2.44	0.98 to 6.05	0.05
Aminopenicillin	1.43	0.52 to 3.93	0.48
Amoxicillin with clavulanic acid	2.63	1 to 6.9	0.05
Clindamycin	0.72	0.23 to 2.24	0.57
Other antibiotic	1.65	0.45 to 6.02	0.45
Intravenous antibiotic	2.37	0.83 to 6.81	0.11
Oral antibiotic	0.78	0.38 to 1.61	0.5
Hospital stay duration in days	1.02	0.95 to 1.09	0.65
Antibiotic treatment duration in days	1	0.92 to 1.08	0.98
	Model without covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.55	0.33 to 0.92	0.02

PLOS ONE

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

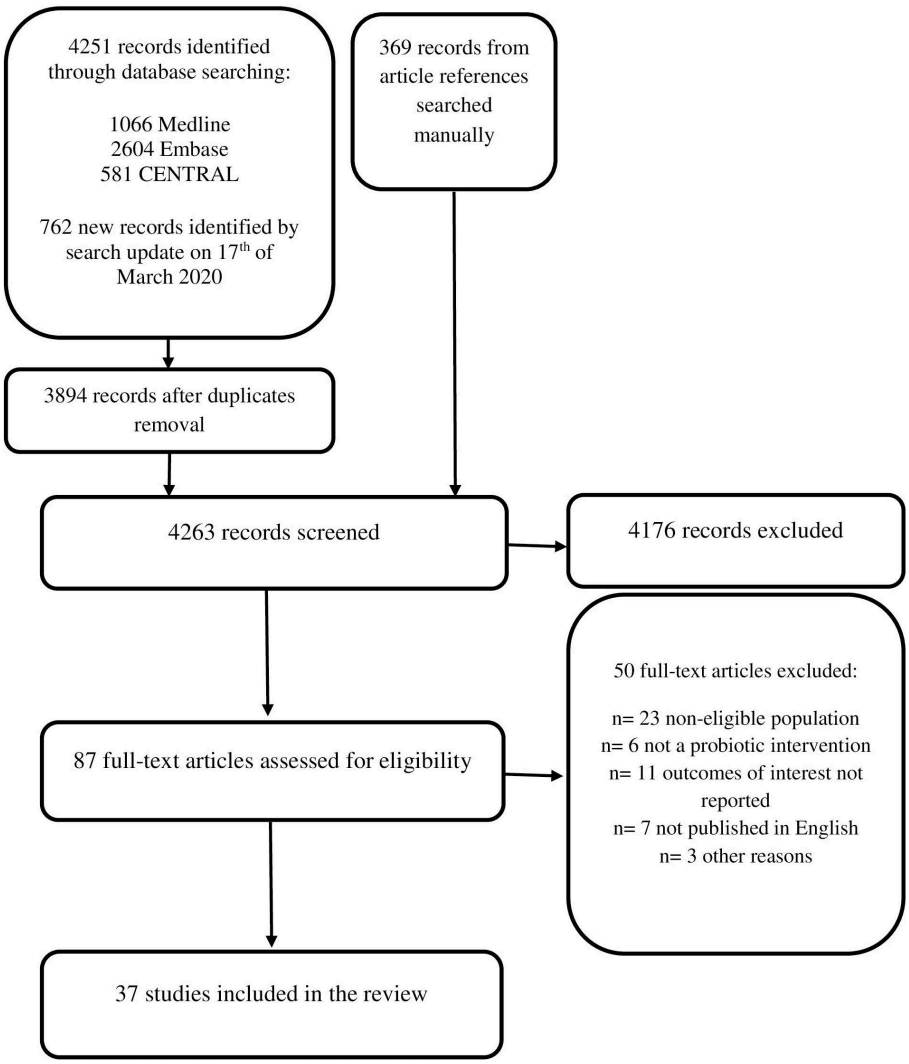

Fig 1. Flow chart diagram.

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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

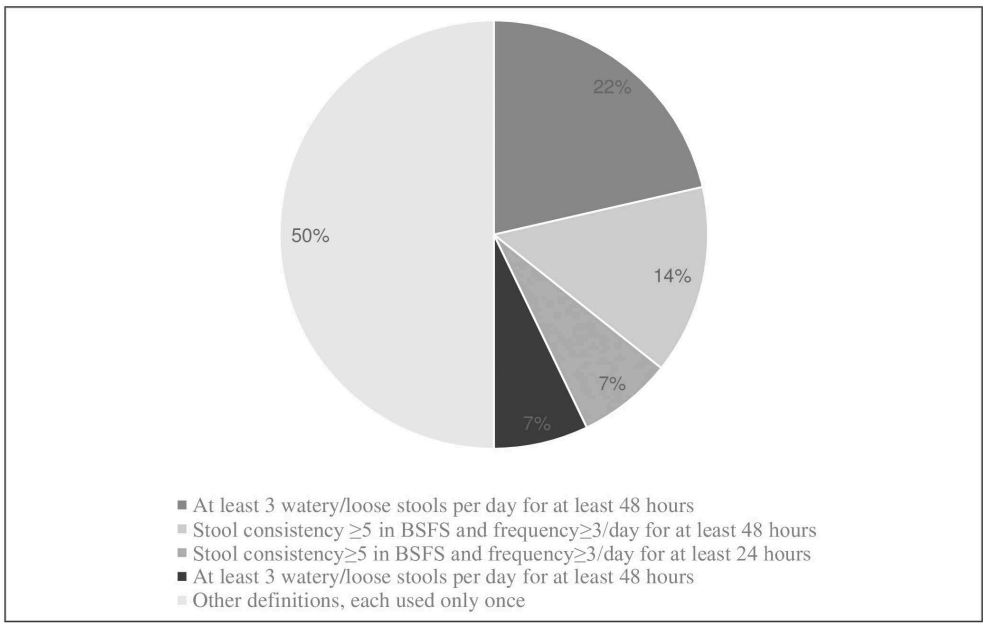

Fig 2. Most commonly used definitions of diarrhea.

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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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References

1. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet (London, England). 2003; 361 (9356):512–9. Epub 2003/02/14. https://doi.org/10.1016/s0140-6736(03)12489-0 PMID: 12583961.
2. Hugon P, Dufour JC, Colson P, Fournier PE, Sallah K, Raoult D. A comprehensive repertoire of prokaryotic species identified in human beings. Lancet Infect Dis. 2015; 15(10):1211–9. Epub 2015/08/28. https://doi.org/10.1016/s1473-3099(15)00293-5 PMID: 26311042.
3. Kramer MS, Hutchinson TA, Naimark L, Contardi R, Flegel KM, Leduc DG. Antibiotic-associated gastrointestinal symptoms in general pediatric outpatients. Pediatrics. 1985; 76(3):365–70. Epub 1985/09/01. PMID: 3875832.
4. Szajewska H, Canani RB, Guarino A, Hojsak I, Indrio F, Kolacek S, et al. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. Journal of pediatric gastroenterology and nutrition. 2016; 62(3):495–506. Epub 2016/01/13. https://doi.org/10.1097/mpg.0000000000001081 PMID: 26756877.
5. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database of Systematic Reviews. 2019;(4). https://doi.org/10.1002/14651858.CD004827.pub5 CD004827. PMID: 31039287
6. Turck D, Bernet JP, Marx J, Kempf H, Giard P, Walbaum O, et al. Incidence and risk factors of oral antibiotic-associated diarrhea in an outpatient pediatric population. Journal of pediatric gastroenterology and nutrition. 2003; 37(1):22–6. Epub 2003/06/27. https://doi.org/10.1097/00005176-200307000-00004 PMID: 12827001.
7. Georgieva M, Pancheva R, Rasheva N, Usheva N, Ivanova L, Koleva K. Use of the probiotic Lactobacillus reuteri DSM 17938 in the prevention of antibioticassociated infections in hospitalized bulgarian

children: a randomized, controlled trial. Journal of IMAB—annual proceeding (scientific papers). 2015; 21(4):895-900. https://doi.org/10.5272/jimab.2015214.895 CN-01133218.

8. Jirapinyo P, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2002; 85 Suppl 2:S739–42.
9. Zakordonets L, Tolstanova G, Yankovskiy D, Dyment H, Kramarev S. Different regimes of multiprobiotic for prevention of immediate and delayed side effects of antibiotic therapy in children. Research journal of pharmaceutical, biological and chemical sciences. 2016; 7(3):2194-201. CN-01167212.
10. Lionetti E, Miniello VL, Castellaneta SP, Magista AM, de Canio A, Maurogiovanni G, et al. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. Alimentary pharmacology & therapeutics. 2006; 24(10):1461–8.
11. Okazaki T, Asahara T, Yamataka A, Ogasawara Y, Lane GJ, Nomoto K, et al. Intestinal Microbiota in Pediatric Surgical Cases Administered Bifidobacterium Breve: a Randomized Controlled Trial. Journal of pediatric gastroenterology and nutrition. 2016; 63(1):46-50. https://doi.org/10.1097/mpg.0000000000001140 CN-01165832. PMID: 26859092
12. Rasmussen SH, Shrestha S, Bjerregaard LG, Angquist LH, Baker JL, Jess T, et al. Antibiotic exposure in early life and childhood overweight and obesity: A systematic review and meta-analysis. Diabetes, obesity & metabolism. 2018; 20(6):1508–14. Epub 2018/01/24. https://doi.org/10.1111/dom.13230 PMID: 29359849.
13. Kim DH, Han K, Kim SW. Effects of Antibiotics on the Development of Asthma and Other Allergic Diseases in Children and Adolescents. Allergy, asthma & immunology research. 2018; 10(5):457–65. Epub 2018/08/09. https://doi.org/10.4168/aair.2018.10.5.457 PMID: 30088366.
14. Kemppainen KM, Vehik K, Lynch KF, Larsson HE, Canepa RJ, Simell V, et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. JAMA pediatrics. 2017; 171(12):1217–25. Epub 2017/10/21. https://doi.org/10.1001/jamapediatrics.2017.2905 PMID: 29052687; PubMed Central PMCID: PMC5716863.
15. Atladottir HO, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics. 2012; 130(6):e1447–54. Epub 2012/11/14. https://doi.org/10.1542/peds.2012-1107 PMID: 23147969; PubMed Central PMCID: PMC4451062.
16. Lundelin K, Poussa T, Salminen S, Isolauri E. Long-term safety and efficacy of perinatal probiotic intervention: Evidence from a follow-up study of four randomized, double-blind, placebo-controlled trials. Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2017; 28(2):170–5. Epub 2016/10/26. https://doi.org/10.1111/pai.12675 PMID: 27779809.
17. Edmonson MB, Eickhoff JC. Weight Gain and Obesity in Infants and Young Children Exposed to Prolonged Antibiotic Prophylaxis. JAMA pediatrics. 2017; 171(2):150–6. Epub 2016/12/28. https://doi.org/10.1001/jamapediatrics.2016.3349 PMID: 28027334.
18. Hojsak I. Probiotics in Children: What Is the Evidence? Pediatric gastroenterology, hepatology & nutrition. 2017; 20(3):139–46. Epub 2017/10/14. https://doi.org/10.5223/pghn.2017.20.3.139 PMID: 29026729; PubMed Central PMCID: PMC5636929.
19. Johnston BC, Shamseer L, da Costa BR, Tsuyuki RT, Vohra S. Measurement issues in trials of pediatric acute diarrheal diseases: a systematic review. Pediatrics. 2010; 126(1):e222–31. Epub 2010/06/23. https://doi.org/10.1542/peds.2009-3667 PMID: 20566617.
20. Williamson PR, Altman DG, Bagley H, Barnes KL, Blazeby JM, Brookes ST, et al. The COMET Handbook: version 1.0. Trials. 2017; 18(Suppl 3):280. Epub 2017/07/07. https://doi.org/10.1186/s13063-017-1978-4 PMID: 28681707; PubMed Central PMCID: PMC5499094.
21. Karas J, Ashkenazi S, Guarino A, Lo Vecchio A, Shamir R, Vandenplas Y, et al. Developing a core outcome measurement set for clinical trials in acute diarrhoea. Acta Paediatr. 2016; 105(4):e176–80. Epub 2016/01/30. https://doi.org/10.1111/apa.13349 PMID: 26821646.
22. Armstrong R, Hall BJ, Doyle J, Waters E. 'Scoping the scope' of a cochrane review. Journal of Public Health. 2011; 33(1):147–50. https://doi.org/10.1093/pubmed/fdr015 PMID: 21345890
23. Boers M, Kirwan JR, Wells G, Beaton D, Gossec L, d'Agostino M-A, et al. Developing Core Outcome Measurement Sets for Clinical Trials: OMERACT Filter 2.0. Journal of Clinical Epidemiology. 2014; 67(7):745–53. https://doi.org/10.1016/j.jclinepi.2013.11.013 PMID: 24582946
24. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions: Wiley; 2011.
25. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses 2011. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

26. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iranian journal of pediatrics. 2013; 23(1):79–84.

27. Akcam M, Koca T, Salman H, Karahan N. The effects of probiotics on treatment of Helicobacter pylori eradication in children. Saudi medical journal. 2015; 36(3):286–90. https://doi.org/10.15537/smj.2015.3.10124 PMID: 25737169

28. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics. 1999; 104(5):e64.

29. Basnet S, Gauchan E, Adhikari S, Sathian B. Probiotics in the prevention of antibiotic associated diarrhoea in a tertiary teaching hospital in pokhara: A prospective study. Journal of Clinical and Diagnostic Research. 2017; 11(10):SC11–SC3. https://doi.org/10.7860/JCDR/2017/25936.10777

30. Bin Z, Ya-Zheng X, Zhao-Hui D, Bo C, Li-Rong J, Vandenplas Y. The Efficacy of Saccharomyces boulardii CNCM I-745 in Addition to Standard Helicobacter pylori Eradication Treatment in Children. Pediatric gastroenterology, hepatology & nutrition. 2015; 18(1):17–22. https://doi.org/10.5223/pghn.2015.18.1.17 PMID: 25866729

31. Corrêa NB, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. Journal of clinical gastroenterology. 2005; 39(5):385-9. CN-00521370.

32. Erdeve O, Tiras U, Dallar Y. The probiotic effect of Saccharomyces boulardii in a pediatric age group. Journal of tropical pediatrics. 2004; 50(4):234–6. https://doi.org/10.1093/tropej/50.4.234 PMID: 15357564

33. Esposito C, Roberti A, Turra F, Cerulo M, Severino G, Settimi A, et al. Frequency of Antibiotic-Associated Diarrhea and Related Complications in Pediatric Patients Who Underwent Hypospadias Repair: a Comparative Study Using Probiotics vs Placebo. Probiotics and antimicrobial proteins. 2018; 10(2):323–8. https://doi.org/10.1007/s12602-017-9324-4 PMID: 28871492

34. Fox MJ, Ahuja KD, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. BMJ open. 2015; 5(1):e006474. https://doi.org/10.1136/bmjopen-2014-006474 CN-01111087. PMID: 25588782

35. Hurduc V, Plesca D, Dragomir D, Sajin M, Vandenplas Y. A randomized, open trial evaluating the effect of Saccharomyces boulardii on the eradication rate of Helicobacter pylori infection in children. Acta paediatrica (Oslo, Norway: 1992). 2009; 98(1):127–31. https://doi.org/10.1111/j.1651-2227.2008.00977.x PMID: 18681892

36. Jindal M, Goyal Y, Lata S, Sharma RK. Preventive role of probiotic in antibiotic associated diarrhoea in children. Indian Journal of Public Health Research and Development. 2017; 8(3):66–9. https://doi.org/10.5958/0976-5506.2017.00162.0

37. Kolodziej M, Szajewska H. Lactobacillus reuteri DSM 17938 in the prevention of antibiotic-associated diarrhoea in children: a randomized clinical trial. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018. https://doi.org/10.1016/j.cmi.2018.08.017 PMID: 30149135

38. Korpela K, Salonen A, Virta LJ, Kumpu M, Kekkonen RA, de Vos WM. Lactobacillus rhamnosus GG Intake Modifies Preschool Children's Intestinal Microbiota, Alleviates Penicillin-Associated Changes, and Reduces Antibiotic Use. PloS one. 2016; 11(4):e0154012. https://doi.org/10.1371/journal.pone.0154012 PMID: 27111772

39. Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. Alimentary pharmacology & therapeutics. 2005; 21(5):583–90.

40. Merenstein DJ, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the measuring the influence of Kefir (MILK) Study. Archives of pediatrics & adolescent medicine. 2009; 163(8):750–4. https://doi.org/10.1001/archpediatrics.2009.119 PMID: 19652108

41. Olek A, Woynarowski M, Ahren IL, Kierkus J, Socha P, Larsson N, et al. Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children-Randomized, Double-Blind, Placebo-Controlled Study. The Journal of pediatrics. 2017; 186:82–6. https://doi.org/10.1016/j.jpeds.2017.03.047 PMID: 28438377

42. Plewinska EM, Planeta-Malecka I, Bak-Romaniszyn L, Czkwianlanc E, Malecka-Panas E. Probiotics in the treatment of Helicobacter pylori infection in children. Gastroenterologia polska. 2006; 13(4):315-9. CN-00623178.

43. Ranasinghe J, Gamlath G, Samitha S, Abeygunawardena A. Prophylactic use of yoghurt reduces antibiotic induced diarrhoea in children. Sri Lanka Journal of Child Health. 2008; 36(2):53–6. https://doi.org/10.4038/sljch.v36i2.50

44. Ruszczynski M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. Alimentary pharmacology & therapeutics. 2008; 28(1):154–61. https://doi.org/10.1111/j.1365-2036.2008.03714.x PMID: 18410562

45. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, et al. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. Pediatr Int. 2003; 45(1):86–90. Epub 2003/03/26. https://doi.org/10.1046/j.1442-200x.2003.01671.x PMID: 12654076.

46. Shahraki T, Shahraki M, Shahri ES, Mohammadi M. No significant impact of Lactobacillus reuteri on eradication of Helicobacter pylori in children (double-blind randomized clinical trial). Iranian red crescent medical journal. 2017; 19(3) (no pagination). https://doi.org/10.5812/ircmj.42101 CN-01366602.

47. Shan LS, Hou P, Wang ZJ, Liu FR, Chen N, Shu LH, et al. Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. Beneficial microbes. 2013; 4(4):329-34. https://doi.org/10.3920/bm2013.0008 CN-00959577. PMID: 24311316

48. Sykora J, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. Journal of clinical gastroenterology. 2005; 39(8):692–8.

49. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. Journal of pediatric gastroenterology and nutrition. 2009; 48(4):431–6.

50. Szymanski H, Armanska M, Kowalska-Duplaga K, Szajewska H. Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the prevention of antibiotic-associated diarrhea in children: a randomized controlled pilot trial. Digestion. 2008; 78(1):13–7. https://doi.org/10.1159/000151300 PMID: 18701826

51. Tankanow RM, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP: the annals of pharmacotherapy. 1990; 24(4):382–4.

52. Tolone S, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. Italian journal of pediatrics. 2012; 38:63. https://doi.org/10.1186/1824-7288-38-63 PMID: 23114016

53. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. The Journal of pediatrics. 1999; 135(5):564–8.

54. Wang YH, Huang Y. Effect of Lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora. World journal of microbiology & biotechnology. 2014; 30(3):847-53. https://doi.org/10.1007/s11274-013-1490-2 CN-01014256. PMID: 24233772

55. Zoppi G, Cinquetti M, Benini A, Bonamini E, Bertazzoni E. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Current Therapeutic Research-clinical and Experimental—CURR THER RES. 2001; 62:418–35. https://doi.org/10.1016/S0011-393X(01)89006-8

56. Dharani Sudha G, Nirmala P, Ramanathan R, Samuel V. Comparative study of efficacy and safety of azithromycin alone and in combination with probiotic in the treatment of impetigo in children. International Journal of Current Pharmaceutical Research. 2017; 9(6):52–5. https://doi.org/10.22159/ijcpr.2017v9i6.23429

57. Baù M, Moretti A, Bertoni E, Vazzoler V, Luini C, Agosti M. Risk and Protective Factors for Gastrointestinal Symptoms associated with Antibiotic Treatment in Children: A Population Study. Pediatric Gastroenterology, Hepatology & Nutrition. 2020; 23:35. https://doi.org/10.5223/pghn.2020.23.1.35 PMID: 31988874

58. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997; 32(9):920–4. Epub 1997/09/23. https://doi.org/10.3109/00365529709011203 PMID: 9299672.

59. Svedlund J, Sjodin I, Dotevall G. GSRS—a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci. 1988; 33(2):129–34. Epub 1988/02/01. https://doi.org/10.1007/bf01535722 PMID: 3123181.

60. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, et al. Best practices for analysing microbiomes. Nature reviews Microbiology. 2018; 16(7):410–22. Epub 2018/05/26. https://doi.org/10.1038/s41579-018-0029-9 PMID: 29795328.

61. Zheng M, Zhang R, Tian X, Zhou X, Pan X, Wong A. Assessing the Risk of Probiotic Dietary Supplements in the Context of Antibiotic Resistance. Front Microbiol. 2017; 8:908. Epub 2017/06/06. https://doi.org/10.3389/fmicb.2017.00908 PMID: 28579981; PubMed Central PMCID: PMC5437161.

62. Chan AW, Hrobjartsson A, Haahr MT, Gotzsche PC, Altman DG. Empirical evidence for selective reporting of outcomes in randomized trials: comparison of protocols to published articles. Jama. 2004; 291(20):2457–65. Epub 2004/05/27. https://doi.org/10.1001/jama.291.20.2457 PMID: 15161896.

63. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krleza-Jeric K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013; 158(3):200–7. Epub 2013/01/09. https://doi.org/10.7326/0003-4819-158-3-201302050-00583 PMID: 23295957; PubMed Central PMCID: PMC5114123.

64. Williamson P, Altman D, Blazeby J, Clarke M, Gargon E. Driving up the quality and relevance of research through the use of agreed core outcomes. Journal of health services research & policy. 2012; 17(1):1–2. Epub 2012/02/02. https://doi.org/10.1258/jhsrp.2011.011131 PMID: 22294719.

65. Hojsak I, Szajewska H, Canani RB, Guarino A, Indrio F, Kolacek S, et al. Probiotics for the Prevention of Nosocomial Diarrhea in Children. J Pediatr Gastroenterol Nutr. 2018; 66(1):3–9. Epub 2017/06/03. https://doi.org/10.1097/mpg.0000000000001637 PMID: 28574970.

66. Desselberger U. Rotaviruses. Virus research. 2014; 190:75–96. Epub 2014/07/13. https://doi.org/10.1016/j.virusres.2014.06.016 PMID: 25016036.

67. Robilotti E, Deresinski S, Pinsky BA. Norovirus. Clin Microbiol Rev. 2015; 28(1):134–64. Epub 2015/01/09. https://doi.org/10.1128/cmr.00075-14 PMID: 25567225; PubMed Central PMCID: PMC4284304.

68. Damrongmanee A, Ukarapol N. Incidence of antibiotic-associated diarrhea in a pediatric ambulatory care setting. J Med Assoc Thai. 2007; 90(3):513–7. Epub 2007/04/13. PMID: 17427529.

69. Johnston BC, Donen R, Pooni A, Pond J, Xie F, Giglia L, et al. Conceptual framework for health-related quality of life assessment in acute gastroenteritis. J Pediatr Gastroenterol Nutr. 2013; 56(3):280–9. Epub 2012/11/09. https://doi.org/10.1097/MPG.0b013e3182736f49 PMID: 23135341.

70. Paterson C, Britten N. In pursuit of patient-centred outcomes: a qualitative evaluation of the 'Measure Yourself Medical Outcome Profile'. Journal of health services research & policy. 2000; 5(1):27–36. Epub 2000/05/02. https://doi.org/10.1177/135581960000500108 PMID: 10787584.

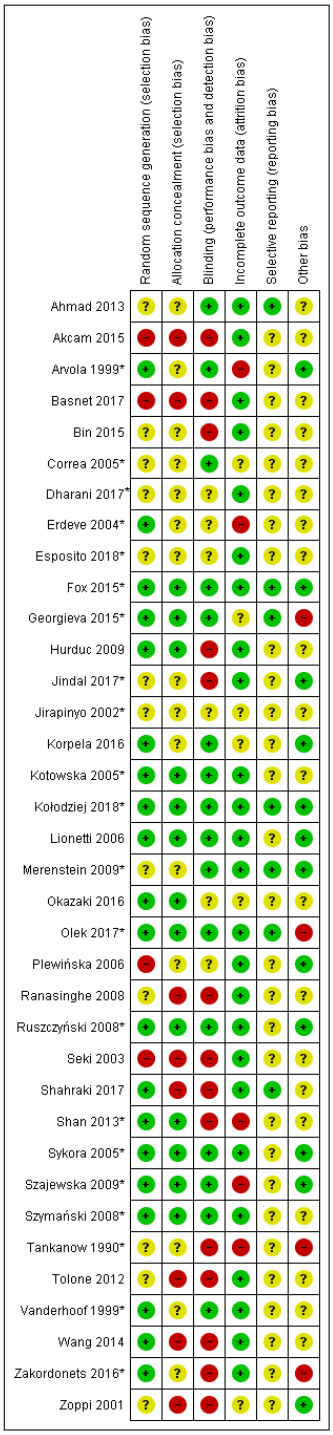
71. McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. Future Microbiol. 2008; 3(5):563–78. Epub 2008/09/25. https://doi.org/10.2217/17460913.3.5.563 PMID: 18811240.

72. van den Nieuwboer M, Claassen E, Morelli L, Guarner F, Brummer RJ. Probiotic and synbiotic safety in infants under two years of age. Benef Microbes. 2014; 5(1):45–60. Epub 2014/01/28. https://doi.org/10.3920/bm2013.0046 PMID: 24463207.

73. Ioannidis JP, Evans SJ, Gotzsche PC, O'Neill RT, Altman DG, Schulz K, et al. Better reporting of harms in randomized trials: an extension of the CONSORT statement. Ann Intern Med. 2004; 141(10):781–8. Epub 2004/11/17. https://doi.org/10.7326/0003-4819-141-10-200411160-00009 PMID: 15545678.

74. Bafeta A, Koh M, Riveros C, Ravaud P. Harms Reporting in Randomized Controlled Trials of Interventions Aimed at Modifying Microbiota: A Systematic Review. Ann Intern Med. 2018; 169(4):240–7. Epub 2018/07/18. https://doi.org/10.7326/m18-0343 PMID: 30014150.

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

1. Ameen AM, Abdulridha MK, Najeeb AA. Comparative effectiveness of probiotics timing regimen in helicobacter pylori-induced peptic ulcer disease patients. Journal of Pharmaceutical Sciences and Research. 2019;11(1):75-83.
2. Andaloro C, Santagati M, Stefani S, La Mantia I. Bacteriotherapy with Streptococcus salivarius 24SMB and Streptococcus oralis 89a oral spray for children with recurrent streptococcal pharyngotonsillitis: a randomized placebo-controlled clinical study. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery. 2019;276(3):879-87. doi: https://dx.doi.org/10.1007/s00405-019-05346-3.
3. Awasthi S. Lactobacillus GG reduced diarrhoea incidence in children treated with antibiotics. Evidence-Based Medicine. 2000;5(4):113. doi: 10.1136/ebm.5.4.113.
4. Cherian S, Sibyjoseph, Anitha S. Study of the prescribing pattern of probiotics in paediatric patients of a teritiary care teaching hospital, South India. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(1):505-8.
5. Conway S, Hart A, Clark A, Harvey I. Does eating yogurt prevent antibiotic-associated diarrhoea? A placebo-controlled randomised controlled trial in general practice. The British journal of general practice : the journal of the Royal College of General Practitioners. 2007;57(545):953-9. doi: https://dx.doi.org/10.3399/096016407782604811.
6. Czerwionka-Szaflarska M, Kuczynska R, Mierzwa G, Bala G, Murawska S. Effect of probiotic bacteria supplementation on the tolerance of Helicobacter pylori eradication therapy in children and youth. Pediatria polska. 2006;81(5):334-41. PubMed PMID: CN-00623129.
7. Dajani A, Hammour AA, Nounou ME, Zakaria M. Treatment of helicobacter pylori: Role of probiotics, an experience from the UAE. Journal of Gastroenterology and Hepatology. 2013;28:330-1. doi: 10.1111/jgh.12363_2.
8. Dajani AI, Abu Hammour AM, Yang DH, Chung PC, Nounou MA, Yuan KY, et al. Do probiotics improve eradication response to Helicobacter pylori on standard triple or sequential therapy? Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association. 2013;19(3):113-20. doi: https://dx.doi.org/10.4103/1319-3767.111953.
9. De Bortoli N, Leonardi G, Ciancia E, Merlo A, Bellini M, Costa F, et al. Helicobacter pylori eradication: A randomized prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics. American Journal of Gastroenterology. 2007;102(5):951-6. doi: 10.1111/j.1572-0241.2007.01085.x.
10. Doyle H, Pierse N, Tiatia R, Williamson D, Baker M, Crane J. Effect of Oral Probiotic Streptococcus salivarius K12 on Group A Streptococcus Pharyngitis: A Pragmatic Trial in Schools. The Pediatric infectious disease journal. 2018;37(7):619-23. doi: https://dx.doi.org/10.1097/INF.0000000000001847.
11. Duman DG, Bor S, Ozütemiz O, Sahin T, Oğuz D, Iştan F, et al. Efficacy and safety of Saccharomyces boulardii in prevention of antibiotic-associated diarrhoea due to Helicobacterpylori eradication. European journal of gastroenterology & hepatology. 2005;17(12):1357-61. PubMed PMID: CN-00561517.
12. Francavilla R, Lionetti E, Castellaneta SP, Magistà AM, Maurogiovanni G, Bucci N, et al. Inhibition of Helicobacter pylori infection in humans by Lactobacillus reuteri ATCC 55730 and effect on eradication therapy: A pilot study. Helicobacter. 2008;13(2):127-34. doi: 10.1111/j.1523-5378.2008.00593.x.
13. Francavilla R, Polimeno L, Demichina A, Maurogiovanni G, Principi B, Scaccianoce G, et al. Lactobacillus reuteri strain combination in Helicobacter pylori infection: a randomized, double-blind, placebo-controlled study. Journal of clinical gastroenterology. 2014;48(5):407-13. doi: https://dx.doi.org/10.1097/MCG.0000000000000007.
14. Goldman CG, Barrado DA, Balcarce N, Rua EC, Oshiro M, Calcagno ML, et al. Effect of a probiotic food as an adjuvant to triple therapy for eradication of Helicobacter pylori infection in children. Nutrition (burbank, los angeles county, calif). 2006;22(10):984-8. doi: 10.1016/j.nut.2006.06.008. PubMed PMID: CN-00572240.
15. Huang Y, Wang YH, Leung YK. Combination of Lactobacillus acidophilus and bifidobacillus with standard triple therapy (PPI plus amoxicillin and clarithromycin, STT) in the management of H. pylori infection: Therapeutic efficacy and changes in intestinal bacterial flora. Journal of Gastroenterology and Hepatology. 2011;26:256. doi: 10.1111/j.1440-1746.2011.06898.x.
16. Islek A, Sayar E, Yilmaz A, Artan R. Bifidobacterium lactis B94 plus inulin for Treatment of Helicobacter pylori infection in children: does it increase eradication rate and patient compliance? Acta gastro-enterologica Belgica. 2015;78(3):282-6.
17. Kim MN, Kim N, Lee SH, Park YS, Hwang JH, Kim JW, et al. The effects of probiotics on PPI-triple therapy for Helicobacter pylori eradication. Helicobacter. 2008;13(4):261-8. doi: 10.1111/j.1523-5378.2008.00601.x. PubMed PMID: CN-00650099.
18. Kitz R, Martens U, Ziesenß E, Enck P, Rose MA. Probiotic E.faecalis - Adjuvant therapy in children with recurrent rhinosinusitis. Central European Journal of Medicine. 2012;7(3):362-5. doi: 10.2478/s11536-011-0160-8.
19. Korpela K, Salonen A, Vepsalainen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. Microbiome. 2018;6(1):182. doi: https://dx.doi.org/10.1186/s40168-018-0567-4.
20. Kumar S, Bansal A, Chakrabarti A, Singhi S. Evaluation of efficacy of probiotics in prevention of candida colonization in a PICU-a randomized controlled trial. Critical care medicine. 2013;41(2):565-72. doi: https://dx.doi.org/10.1097/CCM.0b013e31826a409c.
21. Li N, Zheng B, Cai HF, Chen YH, Qiu MQ, Liu MB. Cost-effectiveness analysis of oral probiotics for the prevention of Clostridium difficile-associated diarrhoea in children and adolescents. Journal of Hospital Infection. 2018;99(4):469-74. doi: 10.1016/j.jhin.2018.04.013.
22. Li B, Zheng J, Zhang X, Hong S. Probiotic Lactobacillus casei Shirota improves efficacy of amoxicillin-sulbactam against childhood fast breathing pneumonia in a randomized placebo-controlled double blind clinical study. Journal of clinical biochemistry and nutrition. 2018;63(3):233-7. doi: https://dx.doi.org/10.3164/jcbn.17-117.
23. Li KL, Wang BZ, Li ZP, Li YL, Liang JJ. Alterations of intestinal flora and the effects of probiotics in children with recurrent respiratory tract infection. World journal of pediatrics : WJP. 2019;15(3):255-61. doi: https://dx.doi.org/10.1007/s12519-019-00248-0.

24. Lukasik J, Szajewska H. Effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial. BMJ open. 2018;8(5):e021214. doi: https://dx.doi.org/10.1136/bmjopen-2017-021214.
25. Madden-Fuentes RJ, Arshad M, Ross SS, Seed PC. Efficacy of Fluoroquinolone/Probiotic Combination Therapy for Recurrent Urinary Tract Infection in Children: A Retrospective Analysis. Clinical therapeutics. 2015;37(9):2143-7. doi: https://dx.doi.org/10.1016/j.clinthera.2015.06.018.
26. Maziade PJ, Andriessen JA, Pereira P, Currie B, Goldstein EJC. Impact of adding prophylactic probiotics to a bundle of standard preventative measures for Clostridium difficile infections: enhanced and sustained decrease in the incidence and severity of infection at a community hospital. Current medical research and opinion. 2013;29(10):1341-7. doi: https://dx.doi.org/10.1185/03007995.2013.833501.
27. Mohseni MJ, Aryan Z, Emamzadeh-Fard S, Paydary K, Mofid V, Joudaki H, et al. Combination of probiotics and antibiotics in the prevention of recurrent urinary tract infection in children. Iranian journal of pediatrics. 2013;23(4):430-8. PubMed PMID: CN-00918907.
28. Murphy JL, Fenn N, Pyle L, Heizer H, Hughes S, Nomura Y, et al. Adverse events in pediatric patients receiving long-term oral and intravenous antibiotics. Hospital Pediatrics. 2016;6(6):330-8. doi: 10.1542/hpeds.2015-0069.
29. Namkin K, Zardast M, Basirinejad F. Saccharomyces Boulardii in Helicobacter Pylori Eradication in Children: A Randomized Trial From Iran. Iranian journal of pediatrics. 2016;26(1):e3768. doi: https://dx.doi.org/10.5812/ijp.3768.
30. Nista EC, Candelli M, Cremonini F, Cazzato IA, Zocco MA, Franceschi F, et al. Bacillus clausii therapy to reduce side-effects of anti-Helicobacter pylori treatment: randomized, double-blind, placebo controlled trial. Alimentary pharmacology & therapeutics. 2004;20(10):1181-8.
31. Pantoflickova D, Corthesy-Theulaz I, Dorta G, Stolte M, Isler P, Rochat F, et al. Favourable effect of regular intake of fermented milk containing Lactobacillus johnsonii on Helicobacter pylori associated gastritis. Alimentary pharmacology & therapeutics. 2003;18(8):805-13.
32. Prado V, Agüero ME, Ernst Y, Marín P, Díaz MC. Effects of administration of lactobacilli on intestinal flora in infants treated with broad spectrum antibiotics. Rev Chil Pediatr. 1980;51(1):9-12. PubMed PMID: CN-01623988.
33. Röhrenbach J, Matthess A, Maier R, Von Bünau R. Treatment of children with E. coli strain Nissle 1917. Results of a prospective data collection with 668 patients. Padiatrische Praxis. 2009;73(4):645-52.
34. Saneeyan H, Layegh S, Rahimi H. Effectivness of probiotic on treatment of Helicobacter pylori infection in children. Journal of isfahan medical school. 2011;29(146):882-9. PubMed PMID: CN-00893921.
35. Schrezenmeir J, Heller K, McCue M, Llamas C, Lam W, Burow H, et al. Benefits of oral supplementation with and without synbiotics in young children with acute bacterial infections. Clinical pediatrics. 2004;43(3):239-49.
36. N Sirvan B, K Usta M, U Kizilkan N, Urganci N. Are Synbiotics added to the Standard Therapy to eradicate Helicobacter pylori in Children Beneficial? A Randomized Controlled Study. Euroasian journal of hepato-gastroenterology. 2017;7(1):17-22. doi: https://dx.doi.org/10.5005/jp-journals-10018-1205.
37. Song MJ, Park DI, Park JH, Kim HJ, Cho YK, Sohn CI, et al. The effect of probiotics and mucoprotective agents on PPI-based triple therapy for eradication of helicobacter pylori. Helicobacter. 2010;15(3):206-13. doi: 10.1111/j.1523-5378.2010.00751.x.
38. Srinivasan R, Meyer R, Padmanabhan R, Britto J. Clinical safety of Lactobacillus casei shirota as a probiotic in critically ill children. Journal of pediatric gastroenterology and nutrition. 2006;42(2):171-3.
39. Tamma PD, Avdic E, Li DX, Dzintars K, Cosgrove SE. Association of adverse events with antibiotic use in hospitalized patients. JAMA Internal Medicine. 2017;177(9):1308-15. doi: 10.1001/jamainternmed.2017.1938.
40. Tongtawee T, Dechsukhum C, Leeanansaksiri W, Kaewpitoon S, Kaewpitoon N, Loyd RA, et al. Effect of Pretreatment with Lactobacillus delbrueckii and Streptococcus thermophillus on Tailored Triple Therapy for Helicobacter pylori Eradication: A Prospective Randomized Controlled Clinical Trial. Asian Pacific journal of cancer prevention : APJCP. 2015;16(12):4885-90.
41. Uitz E, Tonninger-Bahadori K, Nekrep K, Bahadori B. The effect of lactobacillus casei rhamnosus (lcr35) supplementation on the adherence, tolerance and efficacy of helicobacter pylori eradication therapy: An open-label, observational, non-interventional, multicentre pilot study. International Journal of Probiotics and Prebiotics. 2017;12(4):159-66.
42. Ustundag GH, Altuntas H, Soysal YD, Kokturk F. The Effects of Synbiotic "Bifidobacterium lactis B94 plus Inulin" Addition on Standard Triple Therapy of Helicobacter pylori Eradication in Children. Canadian journal of gastroenterology & hepatology. 2017;2017:8130596. doi: https://dx.doi.org/10.1155/2017/8130596.
43. Valsecchi C, Marseglia A, Montagna L, Tagliacarne SC, Elli M, Licari A, et al. Evaluation of the effects of a probiotic supplementation with respect to placebo on intestinal microflora and secretory IgA production, during antibiotic therapy, in children affected by recurrent airway infections and skin symptoms. Journal of biological regulators and homeostatic agents. 2014;28(1):117-24.
44. Wan CM, Yu H, Liu G, Xu HM, Mao ZQ, Xu Y, et al. A multicenter randomized controlled study of Saccharomyces boulardii in the prevention of antibiotic-associated diarrhea in infants and young children. Zhonghua er ke za zhi = Chinese journal of pediatrics. 2017;55(5):349-54. doi: https://dx.doi.org/10.3760/cma.j.issn.0578-1310.2017.05.008.
45. Wang ZJ, Chen XF, Zhang ZX, Li YC, Deng J, Tu J, et al. Effects of anti-Helicobacter pylori concomitant therapy and probiotic supplementation on the throat and gut microbiota in humans. Microbial pathogenesis. 2017;109:156-61. doi: 10.1016/j.micpath.2017.05.035. PubMed PMID: CN-01454307.
46. Witsell DL, Garrett CG, Yarbrough WG, Dorrestein SP, Drake AF, Weissler MC. Effect of Lactobacillus acidophilus on antibiotic-associated gastrointestinal morbidity: a prospective randomized trial. The Journal of otolaryngology. 1995;24(4):230-3.
47. Xiang R, Tang Q, Chen XQ, Li MY, Yang MX, Yun X, et al. Effects of Zinc Combined with Probiotics on Antibiotic-associated Diarrhea Secondary to Childhood Pneumonia. Journal of tropical pediatrics. 2019;65(5):421-6. doi: https://dx.doi.org/10.1093/tropej/fmy069.
48. Zhao HM, Ou-Yang HJ, Duan BP, Xu B, Chen ZY, Tang J, et al. Clinical effect of triple therapy combined with Saccharomyces boulardii in the treatment of Helicobacter pylori infection in children. Zhongguo dang dai er ke za zhi [Chinese journal of contemporary pediatrics]. 2014;16(3):230-3. PubMed PMID: CN-01117599.
49. Investigating Group for Prevention of AADiCwPbCB, Bifidobacterium. Multicenter, randomized, controlled clinical trial on preventing antibiotic-associated diarrhea in children with pneumonia using the live Clostridium butyricum and Bifidobacterium combined Powder. Zhonghua er ke za zhi = Chinese journal of pediatrics. 2012;50(10):732-6.
50. Ziemniak W. Efficacy of Helicobacter pylori eradication taking into account its resistance to antibiotics. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2006;57 Suppl 3:123-41.

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

1. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iranian journal of pediatrics. 2013;23(1):79-84.
2. Akcam M, Koca T, Salman H, Karahan N. The effects of probiotics on treatment of Helicobacter pylori eradication in children. Saudi medical journal. 2015;36(3):286-90. doi: https://dx.doi.org/10.15537/smj.2015.3.10124.
3. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics. 1999;104(5):e64.
4. Basnet S, Gauchan E, Adhikari S, Sathian B. Probiotics in the prevention of antibiotic associated diarrhoea in a tertiary teaching hospital in pokhara: A prospective study. Journal of Clinical and Diagnostic Research. 2017;11(10):SC11-SC3. doi: 10.7860/JCDR/2017/25936.10777.
5. Baù M, Moretti A, Bertoni E, Vazzoler V, Luini C, Agosti M. Risk and Protective Factors for Gastrointestinal Symptoms associated with Antibiotic Treatment in Children: A Population Study. Pediatric Gastroenterology, Hepatology & Nutrition. 2020;23:35. doi: 10.5223/pghn.2020.23.1.35.
6. Bin Z, Ya-Zheng X, Zhao-Hui D, Bo C, Li-Rong J, Vandenplas Y. The Efficacy of Saccharomyces boulardii CNCM I-745 in Addition to Standard Helicobacter pylori Eradication Treatment in Children. Pediatric gastroenterology, hepatology & nutrition. 2015;18(1):17-22. doi: https://dx.doi.org/10.5223/pghn.2015.18.1.17.
7. Corrêa NB, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. Journal of clinical gastroenterology. 2005;39(5):385-9. PubMed PMID: CN-00521370.
8. Dharani Sudha G, Nirmala P, Ramanathan R, Samuel V. Comparative study of efficacy and safety of azithromycin alone and in combination with probiotic in the treatment of impetigo in children. International Journal of Current Pharmaceutical Research. 2017;9(6):52-5. doi: 10.22159/ijcpr.2017v9i6.23429.
9. Erdeve O, Tiras U, Dallar Y. The probiotic effect of Saccharomyces boulardii in a pediatric age group. Journal of tropical pediatrics. 2004;50(4):234-6. doi: https://dx.doi.org/10.1093/tropej/50.4.234.
10. Esposito C, Roberti A, Turra F, Cerulo M, Severino G, Settimi A, et al. Frequency of Antibiotic-Associated Diarrhea and Related Complications in Pediatric Patients Who Underwent Hypospadias Repair: a Comparative Study Using Probiotics vs Placebo. Probiotics and antimicrobial proteins. 2018;10(2):323-8. doi: https://dx.doi.org/10.1007/s12602-017-9324-4.
11. Fox MJ, Ahuja KD, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. BMJ open. 2015;5(1):e006474. doi: 10.1136/bmjopen-2014-006474. PubMed PMID: CN-01111087.

12. Georgieva M, Pancheva R, Rasheva N, Usheva N, Ivanova L, Koleva K. Use of the probiotic Lactobacillus reuteri DSM 17938 in the prevention of antibioticassociated infections in hospitalized bulgarian children: a randomized, controlled trial. Journal of IMAB - annual proceeding (scientific papers). 2015;21(4):895-900. doi: 10.5272/jimab.2015214.895. PubMed PMID: CN-01133218.
13. Hurduc V, Plesca D, Dragomir D, Sajin M, Vandenplas Y. A randomized, open trial evaluating the effect of Saccharomyces boulardii on the eradication rate of Helicobacter pylori infection in children. Acta paediatrica (Oslo, Norway : 1992). 2009;98(1):127-31. doi: https://dx.doi.org/10.1111/j.1651-2227.2008.00977.x.
14. Jindal M, Goyal Y, Lata S, Sharma RK. Preventive role of probiotic in antibiotic associated diarrhoea in children. Indian Journal of Public Health Research and Development. 2017;8(3):66-9. doi: 10.5958/0976-5506.2017.00162.0.
15. Jirapinyo P, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2002;85 Suppl 2:S739-42.
16. Kolodziej M, Szajewska H. Lactobacillus reuteri DSM 17938 in the prevention of antibiotic-associated diarrhoea in children: a randomized clinical trial. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018. doi: https://dx.doi.org/10.1016/j.cmi.2018.08.017.
17. Korpela K, Salonen A, Virta LJ, Kumpu M, Kekkonen RA, de Vos WM. Lactobacillus rhamnosus GG Intake Modifies Preschool Children's Intestinal Microbiota, Alleviates Penicillin-Associated Changes, and Reduces Antibiotic Use. PloS one. 2016;11(4):e0154012. doi: https://dx.doi.org/10.1371/journal.pone.0154012.
18. Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. Alimentary pharmacology & therapeutics. 2005;21(5):583-90.
19. Lionetti E, Miniello VL, Castellaneta SP, Magista AM, de Canio A, Maurogiovanni G, et al. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. Alimentary pharmacology & therapeutics. 2006;24(10):1461-8.
20. Merenstein DJ, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the measuring the influence of Kefir (MILK) Study. Archives of pediatrics & adolescent medicine. 2009;163(8):750-4. doi: https://dx.doi.org/10.1001/archpediatrics.2009.119.
21. Okazaki T, Asahara T, Yamataka A, Ogasawara Y, Lane GJ, Nomoto K, et al. Intestinal Microbiota in Pediatric Surgical Cases Administered Bifidobacterium Breve: a Randomized Controlled Trial. Journal of pediatric gastroenterology and nutrition. 2016;63(1):46-50. doi: 10.1097/mpg.0000000000001140. PubMed PMID: CN-01165832.
22. Olek A, Woynarowski M, Ahren IL, Kierkus J, Socha P, Larsson N, et al. Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children-Randomized, Double-Blind, Placebo-Controlled Study. The Journal of pediatrics. 2017;186:82-6. doi: https://dx.doi.org/10.1016/j.jpeds.2017.03.047.
23. Plewinska EM, Planeta-Malecka I, Bak-Romaniszyn L, Czkwianlanc E, Malecka-Panas E. Probiotics in the treatment of Helicobacter pylori infection in children. Gastroenterologia polska. 2006;13(4):315-9. PubMed PMID: CN-00623178.
24. Ranasinghe J, Gamlath G, Samitha S, Abeygunawardena A. Prophylactic use of yoghurt reduces antibiotic induced diarrhoea in children. Sri Lanka Journal of Child Health. 2008;36(2):53-6. doi: http://doi.org/10.4038/sljch.v36i2.50.
25. Ruszczynski M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. Alimentary pharmacology & therapeutics. 2008;28(1):154-61. doi: https://dx.doi.org/10.1111/j.1365-2036.2008.03714.x.
26. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, et al. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. Pediatr Int. 2003;45(1):86-90. Epub 2003/03/26. PubMed PMID: 12654076.
27. Shahraki T, Shahraki M, Shahri ES, Mohammadi M. No significant impact of Lactobacillus reuteri on eradication of Helicobacter pylori in children (double-blind randomized clinical trial). Iranian red crescent medical journal. 2017;19(3) (no pagination). doi: 10.5812/ircmj.42101. PubMed PMID: CN-01366602.
28. Shan LS, Hou P, Wang ZJ, Liu FR, Chen N, Shu LH, et al. Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. Beneficial microbes. 2013;4(4):329-34. doi: 10.3920/bm2013.0008. PubMed PMID: CN-00959577.
29. Sykora J, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. Journal of clinical gastroenterology. 2005;39(8):692-8.
30. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. Journal of pediatric gastroenterology and nutrition. 2009;48(4):431-6.
31. Szymanski H, Armanska M, Kowalska-Duplaga K, Szajewska H. Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the prevention of antibiotic-associated diarrhea in children: a randomized controlled pilot trial. Digestion. 2008;78(1):13-7. doi: https://dx.doi.org/10.1159/000151300.
32. Tankanow RM, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP : the annals of pharmacotherapy. 1990;24(4):382-4.
33. Tolone S, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. Italian journal of pediatrics. 2012;38:63. doi: https://dx.doi.org/10.1186/1824-7288-38-63.
34. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. The Journal of pediatrics. 1999;135(5):564-8.
35. Wang YH, Huang Y. Effect of Lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora. World journal of microbiology & biotechnology. 2014;30(3):847-53. doi: 10.1007/s11274-013-1490-2. PubMed PMID: CN-01014256.
36. Zakordonets L, Tolstanova G, Yankovskiy D, Dyment H, Kramarev S. Different regimes of multiprobiotic for prevention of immediate and delayed side effects of antibiotic therapy in children. Research journal of pharmaceutical, biological and chemical sciences. 2016;7(3):2194-201. PubMed PMID: CN-01167212.
37. Zoppi G, Cinquetti M, Benini A, Bonamini E, Bertazzoni E. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Current Therapeutic Research-clinical and Experimental - CURR THER RES. 2001;62:418-35. doi: 10.1016/S0011-393X(01)89006-8.

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

1. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iranian journal of pediatrics. 2013;23(1):79-84.
2. Akcam M, Koca T, Salman H, Karahan N. The effects of probiotics on treatment of Helicobacter pylori eradication in children. Saudi medical journal. 2015;36(3):286-90. doi: https://dx.doi.org/10.15537/smj.2015.3.10124.
3. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics. 1999;104(5):e64.
4. Baù M, Moretti A, Bertoni E, Vazzoler V, Luini C, Agosti M. Risk and Protective Factors for Gastrointestinal Symptoms associated with Antibiotic Treatment in Children: A Population Study. Pediatric Gastroenterology, Hepatology & Nutrition. 2020;23:35. doi: 10.5223/pghn.2020.23.1.35.
5. Basnet S, Gauchan E, Adhikari S, Sathian B. Probiotics in the prevention of antibiotic associated diarrhoea in a tertiary teaching hospital in pokhara: A prospective study. Journal of Clinical and Diagnostic Research. 2017;11(10):SC11-SC3. doi: 10.7860/JCDR/2017/25936.10777.
6. Bin Z, Ya-Zheng X, Zhao-Hui D, Bo C, Li-Rong J, Vandenplas Y. The Efficacy of Saccharomyces boulardii CNCM I-745 in Addition to Standard Helicobacter pylori Eradication Treatment in Children. Pediatric gastroenterology, hepatology & nutrition. 2015;18(1):17-22. doi: https://dx.doi.org/10.5223/pghn.2015.18.1.17.
7. Corrêa NB, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. Journal of clinical gastroenterology. 2005;39(5):385-9. PubMed PMID: CN-00521370.
8. Dharani Sudha G, Nirmala P, Ramanathan R, Samuel V. Comparative study of efficacy and safety of azithromycin alone and in combination with probiotic in the treatment of impetigo in children. International Journal of Current Pharmaceutical Research. 2017;9(6):52-5. doi: 10.22159/ijcpr.2017v9i6.23429.
9. Erdeve O, Tiras U, Dallar Y. The probiotic effect of Saccharomyces boulardii in a pediatric age group. Journal of tropical pediatrics. 2004;50(4):234-6. doi: https://dx.doi.org/10.1093/tropej/50.4.234.
10. Esposito C, Roberti A, Turra F, Cerulo M, Severino G, Settimi A, et al. Frequency of Antibiotic-Associated Diarrhea and Related Complications in Pediatric Patients Who Underwent Hypospadias Repair: a Comparative Study Using Probiotics vs Placebo. Probiotics and antimicrobial proteins. 2018;10(2):323-8. doi: https://dx.doi.org/10.1007/s12602-017-9324-4.
11. Fox MJ, Ahuja KD, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. BMJ open. 2015;5(1):e006474. doi: 10.1136/bmjopen-2014-006474. PubMed PMID: CN-01111087.
12. Georgieva M, Pancheva R, Rasheva N, Usheva N, Ivanova L, Koleva K. Use of the probiotic Lactobacillus reuteri DSM 17938 in the prevention of antibioticassociated infections in hospitalized bulgarian children: a randomized, controlled trial. Journal of IMAB - annual proceeding (scientific papers). 2015;21(4):895-900. doi: 10.5272/jimab.2015214.895. PubMed PMID: CN-01133218.
13. Hurduc V, Plesca D, Dragomir D, Sajin M, Vandenplas Y. A randomized, open trial evaluating the effect of Saccharomyces boulardii on the eradication rate of Helicobacter pylori infection in children. Acta paediatrica (Oslo, Norway : 1992). 2009;98(1):127-31. doi: https://dx.doi.org/10.1111/j.1651-2227.2008.00977.x.
14. Jindal M, Goyal Y, Lata S, Sharma RK. Preventive role of probiotic in antibiotic associated diarrhoea in children. Indian Journal of Public Health Research and Development. 2017;8(3):66-9. doi: 10.5958/0976-5506.2017.00162.0.
15. Jirapinyo P, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2002;85 Suppl 2:S739-42.
16. Kolodziej M, Szajewska H. Lactobacillus reuteri DSM 17938 in the prevention of antibiotic-associated diarrhoea in children: a randomized clinical trial. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018. doi: https://dx.doi.org/10.1016/j.cmi.2018.08.017.
17. Korpela K, Salonen A, Virta LJ, Kumpu M, Kekkonen RA, de Vos WM. Lactobacillus rhamnosus GG Intake Modifies Preschool Children's Intestinal Microbiota, Alleviates Penicillin-Associated Changes, and Reduces Antibiotic Use. PloS one. 2016;11(4):e0154012. doi: https://dx.doi.org/10.1371/journal.pone.0154012.
18. Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. Alimentary pharmacology & therapeutics. 2005;21(5):583-90.
19. Lionetti E, Miniello VL, Castellaneta SP, Magista AM, de Canio A, Maurogiovanni G, et al. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. Alimentary pharmacology & therapeutics. 2006;24(10):1461-8.
20. Merenstein DJ, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the measuring the influence of Kefir (MILK) Study. Archives of pediatrics & adolescent medicine. 2009;163(8):750-4. doi: https://dx.doi.org/10.1001/archpediatrics.2009.119.
21. Okazaki T, Asahara T, Yamataka A, Ogasawara Y, Lane GJ, Nomoto K, et al. Intestinal Microbiota in Pediatric Surgical Cases Administered Bifidobacterium Breve: a Randomized Controlled Trial. Journal of pediatric gastroenterology and nutrition. 2016;63(1):46-50. doi: 10.1097/mpg.0000000000001140. PubMed PMID: CN-01165832.
22. Olek A, Woynarowski M, Ahren IL, Kierkus J, Socha P, Larsson N, et al. Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children-Randomized, Double-Blind, Placebo-Controlled Study. The Journal of pediatrics. 2017;186:82-6. doi: https://dx.doi.org/10.1016/j.jpeds.2017.03.047.
23. Plewinska EM, Planeta-Malecka I, Bak-Romaniszyn L, Czkwianlanc E, Malecka-Panas E. Probiotics in the treatment of Helicobacter pylori infection in children. Gastroenterologia polska. 2006;13(4):315-9. PubMed PMID: CN-00623178.
24. Ranasinghe J, Gamlath G, Samitha S, Abeygunawardena A. Prophylactic use of yoghurt reduces antibiotic induced diarrhoea in children. Sri Lanka Journal of Child Health. 2008;36(2):53-6. doi: http://doi.org/10.4038/sljch.v36i2.50.
25. Ruszczynski M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. Alimentary pharmacology & therapeutics. 2008;28(1):154-61. doi: https://dx.doi.org/10.1111/j.1365-2036.2008.03714.x.
26. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, et al. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. Pediatr Int. 2003;45(1):86-90. Epub 2003/03/26. PubMed PMID: 12654076.
27. Shahraki T, Shahraki M, Shahri ES, Mohammadi M. No significant impact of Lactobacillus reuteri on eradication of Helicobacter pylori in children (double-blind randomized clinical trial). Iranian red crescent medical journal. 2017;19(3) (no pagination). doi: 10.5812/ircmj.42101. PubMed PMID: CN-01366602.
28. Shan LS, Hou P, Wang ZJ, Liu FR, Chen N, Shu LH, et al. Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. Beneficial microbes. 2013;4(4):329-34. doi: 10.3920/bm2013.0008. PubMed PMID: CN-00959577.
29. Sykora J, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. Journal of clinical gastroenterology. 2005;39(8):692-8.
30. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. Journal of pediatric gastroenterology and nutrition. 2009;48(4):431-6.
31. Szymanski H, Armanska M, Kowalska-Duplaga K, Szajewska H. Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the prevention of antibiotic-associated diarrhea in children: a randomized controlled pilot trial. Digestion. 2008;78(1):13-7. doi: https://dx.doi.org/10.1159/000151300.
32. Tankanow RM, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP : the annals of pharmacotherapy. 1990;24(4):382-4.
33. Tolone S, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. Italian journal of pediatrics. 2012;38:63. doi: https://dx.doi.org/10.1186/1824-7288-38-63.
34. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. The Journal of pediatrics. 1999;135(5):564-8.
35. Wang YH, Huang Y. Effect of Lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora. World journal of microbiology & biotechnology. 2014;30(3):847-53. doi: 10.1007/s11274-013-1490-2. PubMed PMID: CN-01014256.
36. Zakordonets L, Tolstanova G, Yankovskiy D, Dyment H, Kramarev S. Different regimes of multiprobiotic for prevention of immediate and delayed side effects of antibiotic therapy in children. Research journal of pharmaceutical, biological and chemical sciences. 2016;7(3):2194-201. PubMed PMID: CN-01167212.
37. Zoppi G, Cinquetti M, Benini A, Bonamini E, Bertazzoni E. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Current Therapeutic Research-clinical and Experimental - CURR THER RES. 2001;62:418-35. doi: 10.1016/S0011-393X(01)89006-8.

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

References

1. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iranian journal of pediatrics. 2013;23(1):79-84.
2. Akcam M, Koca T, Salman H, Karahan N. The effects of probiotics on treatment of Helicobacter pylori eradication in children. Saudi medical journal. 2015;36(3):286-90. doi: https://dx.doi.org/10.15537/smj.2015.3.10124.
3. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics. 1999;104(5):e64.
4. Basnet S, Gauchan E, Adhikari S, Sathian B. Probiotics in the prevention of antibiotic associated diarrhoea in a tertiary teaching hospital in pokhara: A prospective study. Journal of Clinical and Diagnostic Research. 2017;11(10):SC11-SC3. doi: 10.7860/JCDR/2017/25936.10777.
5. Baù M, Moretti A, Bertoni E, Vazzoler V, Luini C, Agosti M. Risk and Protective Factors for Gastrointestinal Symptoms associated with Antibiotic Treatment in Children: A Population Study. Pediatric Gastroenterology, Hepatology & Nutrition. 2020;23:35. doi: 10.5223/pghn.2020.23.1.35.
6. Bin Z, Ya-Zheng X, Zhao-Hui D, Bo C, Li-Rong J, Vandenplas Y. The Efficacy of Saccharomyces boulardii CNCM I-745 in Addition to Standard Helicobacter pylori Eradication Treatment in Children. Pediatric gastroenterology, hepatology & nutrition. 2015;18(1):17-22. doi: https://dx.doi.org/10.5223/pghn.2015.18.1.17.
7. Corrêa NB, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. Journal of clinical gastroenterology. 2005;39(5):385-9. PubMed PMID: CN-00521370.
8. Dharani Sudha G, Nirmala P, Ramanathan R, Samuel V. Comparative study of efficacy and safety of azithromycin alone and in combination with probiotic in the treatment of impetigo in children. International Journal of Current Pharmaceutical Research. 2017;9(6):52-5. doi: 10.22159/ijcpr.2017v9i6.23429.
9. Erdeve O, Tiras U, Dallar Y. The probiotic effect of Saccharomyces boulardii in a pediatric age group. Journal of tropical pediatrics. 2004;50(4):234-6. doi: https://dx.doi.org/10.1093/tropej/50.4.234.
10. Esposito C, Roberti A, Turra F, Cerulo M, Severino G, Settimi A, et al. Frequency of Antibiotic-Associated Diarrhea and Related Complications in Pediatric Patients Who Underwent Hypospadias Repair: a Comparative Study Using Probiotics vs Placebo. Probiotics and antimicrobial proteins. 2018;10(2):323-8. doi: https://dx.doi.org/10.1007/s12602-017-9324-4.
11. Fox MJ, Ahuja KD, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. BMJ open. 2015;5(1):e006474. doi: 10.1136/bmjopen-2014-006474. PubMed PMID: CN-01111087.
12. Georgieva M, Pancheva R, Rasheva N, Usheva N, Ivanova L, Koleva K. Use of the probiotic Lactobacillus reuteri DSM 17938 in the prevention of antibioticassociated infections in hospitalized bulgarian children: a randomized, controlled trial. Journal of IMAB - annual proceeding (scientific papers). 2015;21(4):895-900. doi: 10.5272/jimab.2015214.895. PubMed PMID: CN-01133218.
13. Hurduc V, Plesca D, Dragomir D, Sajin M, Vandenplas Y. A randomized, open trial evaluating the effect of Saccharomyces boulardii on the eradication rate of Helicobacter pylori infection in children. Acta paediatrica (Oslo, Norway : 1992). 2009;98(1):127-31. doi: https://dx.doi.org/10.1111/j.1651-2227.2008.00977.x.
14. Jindal M, Goyal Y, Lata S, Sharma RK. Preventive role of probiotic in antibiotic associated diarrhoea in children. Indian Journal of Public Health Research and Development. 2017;8(3):66-9. doi: 10.5958/0976-5506.2017.00162.0.
15. Jirapinyo P, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2002;85 Suppl 2:S739-42.
16. Kolodziej M, Szajewska H. Lactobacillus reuteri DSM 17938 in the prevention of antibiotic-associated diarrhoea in children: a randomized clinical trial. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018. doi: https://dx.doi.org/10.1016/j.cmi.2018.08.017.
17. Korpela K, Salonen A, Virta LJ, Kumpu M, Kekkonen RA, de Vos WM. Lactobacillus rhamnosus GG Intake Modifies Preschool Children's Intestinal Microbiota, Alleviates Penicillin-Associated Changes, and Reduces Antibiotic Use. PloS one. 2016;11(4):e0154012. doi: https://dx.doi.org/10.1371/journal.pone.0154012.
18. Kotowska M, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. Alimentary pharmacology & therapeutics. 2005;21(5):583-90.

19. Lionetti E, Miniello VL, Castellaneta SP, Magista AM, de Canio A, Maurogiovanni G, et al. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. Alimentary pharmacology & therapeutics. 2006;24(10):1461-8.
20. Merenstein DJ, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the measuring the influence of Kefir (MILK) Study. Archives of pediatrics & adolescent medicine. 2009;163(8):750-4. doi: https://dx.doi.org/10.1001/archpediatrics.2009.119.
21. Okazaki T, Asahara T, Yamataka A, Ogasawara Y, Lane GJ, Nomoto K, et al. Intestinal Microbiota in Pediatric Surgical Cases Administered Bifidobacterium Breve: a Randomized Controlled Trial. Journal of pediatric gastroenterology and nutrition. 2016;63(1):46-50. doi: 10.1097/mpg.0000000000001140. PubMed PMID: CN-01165832.
22. Olek A, Woynarowski M, Ahren IL, Kierkus J, Socha P, Larsson N, et al. Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children-Randomized, Double-Blind, Placebo-Controlled Study. The Journal of pediatrics. 2017;186:82-6. doi: https://dx.doi.org/10.1016/j.jpeds.2017.03.047.
23. Plewinska EM, Planeta-Malecka I, Bak-Romaniszyn L, Czkwianlanc E, Malecka-Panas E. Probiotics in the treatment of Helicobacter pylori infection in children. Gastroenterologia polska. 2006;13(4):315-9. PubMed PMID: CN-00623178.
24. Ranasinghe J, Gamlath G, Samitha S, Abeygunawardena A. Prophylactic use of yoghurt reduces antibiotic induced diarrhoea in children. Sri Lanka Journal of Child Health. 2008;36(2):53-6. doi: http://doi.org/10.4038/sljch.v36i2.50.
25. Ruszczynski M, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. Alimentary pharmacology & therapeutics. 2008;28(1):154-61. doi: https://dx.doi.org/10.1111/j.1365-2036.2008.03714.x.
26. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, et al. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. Pediatr Int. 2003;45(1):86-90. Epub 2003/03/26. PubMed PMID: 12654076.
27. Shahraki T, Shahraki M, Shahri ES, Mohammadi M. No significant impact of Lactobacillus reuteri on eradication of Helicobacter pylori in children (double-blind randomized clinical trial). Iranian red crescent medical journal. 2017;19(3) (no pagination). doi: 10.5812/ircmj.42101. PubMed PMID: CN-01366602.
28. Shan LS, Hou P, Wang ZJ, Liu FR, Chen N, Shu LH, et al. Prevention and treatment of diarrhoea with Saccharomyces boulardii in children with acute lower respiratory tract infections. Beneficial microbes. 2013;4(4):329-34. doi: 10.3920/bm2013.0008. PubMed PMID: CN-00959577.
29. Sykora J, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. Journal of clinical gastroenterology. 2005;39(8):692-8.
30. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. Journal of pediatric gastroenterology and nutrition. 2009;48(4):431-6.
31. Szymanski H, Armanska M, Kowalska-Duplaga K, Szajewska H. Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the prevention of antibiotic-associated diarrhea in children: a randomized controlled pilot trial. Digestion. 2008;78(1):13-7. doi: https://dx.doi.org/10.1159/000151300.
32. Tankanow RM, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP : the annals of pharmacotherapy. 1990;24(4):382-4.
33. Tolone S, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. Italian journal of pediatrics. 2012;38:63. doi: https://dx.doi.org/10.1186/1824-7288-38-63.
34. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. The Journal of pediatrics. 1999;135(5):564-8.
35. Wang YH, Huang Y. Effect of Lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora. World journal of microbiology & biotechnology. 2014;30(3):847-53. doi: 10.1007/s11274-013-1490-2. PubMed PMID: CN-01014256.
36. Zakordonets L, Tolstanova G, Yankovskiy D, Dyment H, Kramarev S. Different regimes of multiprobiotic for prevention of immediate and delayed side effects of antibiotic therapy in children. Research journal of pharmaceutical, biological and chemical sciences. 2016;7(3):2194-201. PubMed PMID: CN-01167212.
37. Zoppi G, Cinquetti M, Benini A, Bonamini E, Bertazzoni E. Modulation of the intestinal ecosystem by probiotics and lactulose in children during treatment with ceftriaxone. Current Therapeutic Research-clinical and Experimental - CURR THER RES. 2001;62:418-35. doi: 10.1016/S0011-393X(01)89006-8.

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.

Opinia Komisji Bioetycznej

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KB/[illegible]/2017

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

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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:

Łukasik J., Szajewska H.: Effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial. BMJ Open, 2018; 8(5): 1-7

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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Piśmiennictwo

1. Youngster I, Avorn J, Belleudi V, et al. Antibiotic Use in Children - A Cross-National Analysis of 6 Countries. *J Pediatr*. 2017;182:239-244.e1.

2. Anderson R, Rhodes A, Cranswick N, et al. A nationwide parent survey of antibiotic use in Australian children. *J Antimicrob Chemother*. 2020;75(5):1347-1351.

3. Guo S, Sun Q, Zhao X, Shen L, Zhen X. Prevalence and risk factors for antibiotic utilization in Chinese children. *BMC Pediatrics*. 2021;21(1):255.

4. Klein EY, Boeckel TPV, Martinez EM, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. 2018;115(15):E3463-E3470.

5. Allwell-Brown G, Hussain-Alkhateeb L, Kitutu FE, Strömdahl S, Mårtensson A, Johansson EW. Trends in reported antibiotic use among children under 5 years of age with fever, diarrhoea, or cough with fast or difficult breathing across low-income and middle-income countries in 2005-17: a systematic analysis of 132 national surveys from 73 countries. *Lancet Glob Health*. 2020;8(6):e799-e807.

6. ECDC. *Antimicrobial consumption in the EU/EEA (ESAC-Net) – Annual Epidemiological Report 2020*. 2021.

7. English surveillance programme for antimicrobial utilisation and resistance (ESPAUR). Report 2020 to 2021. UK Health Security Agency. Updated November 17, 2021. Accessed May 28th, 2022. https://www.gov.uk/government/publications/english-surveillance-programme-antimicrobial-utilisation-and-resistance-espaur-report

8. Polcwiartek LB, Polcwiartek C, Andersen MP, et al. Consequences of coronavirus disease-2019 (COVID-19) lockdown on infection-related hospitalizations among the pediatric population in Denmark. *Eur J Pediatr*. 2021;180(6):1955-1963.

9. Pierantoni L, Lo Vecchio A, Lenzi J, et al. Parents' Perspective of Antibiotic Usage in Children: A Nationwide Survey in Italy. *Pediatr Infect Dis J*. 2021;40(10):906-911.

10. Butler AM, Brown DS, Durkin MJ, et al. Association of Inappropriate Outpatient Pediatric Antibiotic Prescriptions With Adverse Drug Events and Health Care Expenditures. *JAMA Network Open*. 2022;5(5):e2214153-e2214153.

11. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P t*. 2015;40(4):277-283.

12. WHO. Ten threats to global health in 2019. Accessed 15th May, 2022. https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

13. Diallo OO, Baron SA, Abat C, Colson P, Chaudet H, Rolain JM. Antibiotic resistance surveillance systems: A review. *J Glob Antimicrob Resist*. 2020;23:430-438.

14. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome*. 2015;3:31.

15. Berg G, Rybakova D, Fischer D, et al. Microbiome definition re-visited: old concepts and new challenges. *Microbiome*. 2020;8(1):103.

16. Hugon P, Dufour JC, Colson P, Fournier PE, Sallah K, Raoult D. A comprehensive repertoire of prokaryotic species identified in human beings. *Lancet Infect Dis*. 2015;15(10):1211-1219.

17. Almeida A, Mitchell AL, Boland M, et al. A new genomic blueprint of the human gut microbiota. *Nature*. 2019;568(7753):499-504.

18. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol*. 2016;14(8):e1002533.

19. Stewart CJ, Ajami NJ, O'Brien JL, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*. 2018;562(7728):583-588.

20. Radjabzadeh D, Boer CG, Beth SA, et al. Diversity, compositional and functional differences between gut microbiota of children and adults. *Scientific Reports*. 2020;10(1):1040.

21. Reyman M, van Houten MA, van Baarle D, et al. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. *Nat Commun*. 2019;10(1):4997.

22. Ihekweazu FD, Versalovic J. Development of the Pediatric Gut Microbiome: Impact on Health and Disease. *Am J Med Sci*. 2018;356(5):413-423.

23. Korpela K, Blakstad EW, Moltu SJ, et al. Intestinal microbiota development and gestational age in preterm neonates. *Sci Rep*. 2018;8(1):2453.

24. McDonnell L, Gilkes A, Ashworth M, et al. Association between antibiotics and gut microbiome dysbiosis in children: systematic review and meta-analysis. *Gut Microbes*. 2021;13(1):1-18.

25. Levy EI, Hoang DM, Vandenplas Y. The effects of proton pump inhibitors on the microbiome in young children. *Acta Paediatr*. 2020;109(8):1531-1538.

26. Proctor L. Priorities for the next 10 years of human microbiome research. *Nature*. 2019;569(7758):623-625.

27. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol*. 2014;16(7):1024-1033.

28. Zhuang L, Chen H, Zhang S, Zhuang J, Li Q, Feng Z. Intestinal Microbiota in Early Life and Its Implications on Childhood Health. *Genomics Proteomics Bioinformatics*. 2019;17(1):13-25.

29. Shin A, Preidis GA, Shulman R, Kashyap PC. The Gut Microbiome in Adult and Pediatric Functional Gastrointestinal Disorders. *Clin Gastroenterol Hepatol*. 2019;17(2):256-274.

30. Hooks KB, O'Malley MA. Dysbiosis and Its Discontents. *mBio*. 2017;8(5).

31. Wei S, Bahl MI, Baunwall SMD, Hvas CL, Licht TR. Determining Gut Microbial Dysbiosis: a Review of Applied Indexes for Assessment of Intestinal Microbiota Imbalances. *Appl Environ Microbiol*. 2021;87(11).

32. Shanahan F, Ghosh TS, O'Toole PW. The Healthy Microbiome – What Is the Definition of a Healthy Gut Microbiome? *Gastroenterology*. 2021;160(2):483-494.

33. McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol*. 2008;3(5):563-578.

34. Szajewska H, Canani RB, Guarino A, et al. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. *J Pediatr Gastroenterol Nutr*. 2016;62(3):495-506.

35. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. *Immunol Rev*. 2017;279(1):90-105.

36. McFarland LV, Ozen M, Dinleyici EC, Goh S. Comparison of pediatric and adult antibiotic-associated diarrhea and Clostridium difficile infections. *World J Gastroenterol*. 2016;22(11):3078-3104.

37. Lo TS, Borchardt SM. Antibiotic-associated diarrhea due to methicillin-resistant Staphylococcus aureus. *Diagnostic Microbiology and Infectious Disease*. 2009;63(4):388-389.

38. Azimirad M, Gholami F, Yadegar A, et al. Prevalence and characterization of Clostridium perfringens toxinotypes among patients with antibiotic-associated diarrhea in Iran. *Scientific Reports*. 2019;9(1):7792.

39. Larcombe S, Hutton ML, Lyras D. Involvement of Bacteria Other Than Clostridium difficile in Antibiotic-Associated Diarrhoea. *Trends Microbiol*. 2016;24(6):463-476.

40. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev*. 2001;81(3):1031-1064.

41. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *J Clin Microbiol*. 2004;42(3):1203-1206.

42. Liao W, Chen C, Wen T, Zhao Q. Probiotics for the Prevention of Antibiotic-associated Diarrhea in Adults: A Meta-Analysis of Randomized Placebo-Controlled Trials. *J Clin Gastroenterol*. 2021;55(6):469-480.

43. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of clostridium difficile infection in the molecular test era. Article. *JAMA Internal Medicine*. 2015;175(11):1792-1801.

44. Broad J, Sanger GJ. The antibiotic azithromycin is a motilin receptor agonist in human stomach: comparison with erythromycin. *Br J Pharmacol*. 2013;168(8):1859-1867.

45. Högenauer C, Hammer HF, Krejs GJ, Reisinger EC. Mechanisms and management of antibiotic-associated diarrhea. *Clin Infect Dis*. 1998;27(4):702-710.

46. Kramer MS, Hutchinson TA, Naimark L, Contardi R, Flegel KM, Leduc DG. Antibiotic-associated gastrointestinal symptoms in general pediatric outpatients. *Pediatrics*. 1985;76(3):365-370.

47. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database of Systematic Reviews*. 2019;(4).

48. Georgieva M, Pancheva R, Rasheva N, Usheva N, Ivanova L, Koleva K. Use of the probiotic Lactobacillus reuteri DSM 17938 in the prevention of antibioticassociated infections in hospitalized bulgarian children: a randomized, controlled trial. *Journal of IMAB – annual proceeding (scientific papers)*. 2015;21(4):895-900.

49. Jirapinyo P, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*. 2002;85 Suppl 2:S739-742.

50. Turck D, Bernet JP, Marx J, et al. Incidence and risk factors of oral antibiotic-associated diarrhea in an outpatient pediatric population. *J Pediatr Gastroenterol Nutr*. 2003;37(1):22-26.

51. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*. 2012;13(10):701-712.

52. Strandwitz P. Neurotransmitter modulation by the gut microbiota. *Brain Res*. 2018;1693(Pt B):128-133.

53. de Theije CG, Wu J, da Silva SL, et al. Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. *Eur J Pharmacol*. 2011;668 Suppl 1:S70-80.

54. Rosenfeld CS. Microbiome Disturbances and Autism Spectrum Disorders. *Drug Metab Dispos*. 2015;43(10):1557-1571.

55. Prehn-Kristensen A, Zimmermann A, Tittmann L, et al. Reduced microbiome alpha diversity in young patients with ADHD. *PLoS One*. 2018;13(7):e0200728.

56. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression – A systematic review. *Clin Psychol Rev*. 2021;83:101943.

57. Yu HY, Zhou YY, Pan LY, Zhang X, Jiang HY. Early Life Antibiotic Exposure and the Subsequent Risk of Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder: A Systematic Review and Meta-Analysis. *J Autism Dev Disord*. 2022;52(5):2236-2246.

58. Hamad AF, Alessi-Severini S, Mahmud SM, Brownell M, Kuo IF. Antibiotic Exposure in the First Year of Life and the Risk of Attention-Deficit/Hyperactivity Disorder: A Population-Based Cohort Study. *Am J Epidemiol*. 2019;188(11):1923-1931.

59. Axelsson PB, Clausen TD, Petersen AH, et al. Relation Between Infant Microbiota and Autism?: Results from a National Cohort Sibling Design Study. *Epidemiology*. 2019;30(1):52-60.

60. Slob EMA, Brew BK, Vijverberg SJH, et al. Early-life antibiotic use and risk of attention-deficit hyperactivity disorder and autism spectrum disorder: results of a discordant twin study. *Int J Epidemiol*. 2021;50(2):475-484.

61. Atladóttir H, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics*. 2012;130(6):e1447-1454.

62. Wimberley T, Agerbo E, Pedersen CB, et al. Otitis media, antibiotics, and risk of autism spectrum disorder. *Autism Res*. 2018;11(10):1432-1440.

63. Łukasik J, Patro-Gołąb B, Horvath A, Baron R, Szajewska H. Early Life Exposure to Antibiotics and Autism Spectrum Disorders: A Systematic Review. *J Autism Dev Disord*. 2019;49(9):3866-3876.

64. Solans M, Barceló MA, Morales-Suárez-Varela M, Moya A, Saez M. Prenatal exposure to antibiotics and risk of childhood overweight or obesity: A systematic review and meta-analysis. 2022;23(S1):e13382.

65. Miller SA, Wu RKS, Oremus M. The association between antibiotic use in infancy and childhood overweight or obesity: a systematic review and meta-analysis. *Obes Rev*. 2018;19(11):1463-1475.

66. Aghaali M, Hashemi-Nazari SS. Association between early antibiotic exposure and risk of childhood weight gain and obesity: a systematic review and meta-analysis. *J Pediatr Endocrinol Metab*. 2019;32(5):439-445.

67. Srivastava A, Chau K, Kwon H, Guo Q, Johnston BC. Early and frequent exposure to antibiotics in children and the risk of obesity: systematic review and meta-analysis of observational studies. *F1000Res*. 2020;9:711.

68. Baron R, Taye M, Besseling-van der Vaart I, et al. The relationship of prenatal and infant antibiotic exposure with childhood overweight and obesity: a systematic review. *J Dev Orig Health Dis*. 2020;11(4):335-349.

69. Kimura I, Ozawa K, Inoue D, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun*. 2013;4(1):1829.

70. Martinez-Guryn K, Hubert N, Frazier K, et al. Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe*. 2018;23(4):458-469.e5.

71. Wang Y, Kuang Z, Yu X, Ruhn KA, Kubo M, Hooper LV. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science*. 2017;357(6354):912-916.

72. Agustí A, García-Pardo MP, López-Almela I, et al. Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. *Front Neurosci*. 2018;12:155.

73. Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature*. 2016;535(7610):65-74.

74. Li D, Wu M. Pattern recognition receptors in health and diseases. *Signal Transduction and Targeted Therapy*. 2021;6(1):291.

75. Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN. Potential Role of Gut Microbiota in Induction and Regulation of Innate Immune Memory. *Front Immunol*. 2019;10:2441.

76. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9(5):313-323.

77. Zhang Z, Li J, Zheng W, et al. Peripheral Lymphoid Volume Expansion and Maintenance Are Controlled by Gut Microbiota via RALDH+ Dendritic Cells. *Immunity*. 2016;44(2):330-342.

78. Piovani D, Danese S, Peyrin-Biroulet L, Nikolopoulos GK, Lytras T, Bonovas S. Environmental Risk Factors for Inflammatory Bowel Diseases: An Umbrella Review of Meta-analyses. *Gastroenterology*. 2019;157(3):647-659.e4.

79. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. *Scand J Gastroenterol*. 2018;53(1):1-7.

80. Horton DB, Scott FI, Haynes K, et al. Antibiotic Exposure and Juvenile Idiopathic Arthritis: A Case-Control Study. *Pediatrics*. 2015;136(2):e333-343.

81. Arvonen M, Virta LJ, Pokka T, Kröger L, Vähäsalo P. Repeated exposure to antibiotics in infancy: a predisposing factor for juvenile idiopathic arthritis or a sign of this group's greater susceptibility to infections? *J Rheumatol*. 2015;42(3):521-526.

82. Kindgren E, Ludvigsson J. Infections and antibiotics during fetal life and childhood and their relationship to juvenile idiopathic arthritis: a prospective cohort study. *Pediatric Rheumatology*. 2021;19(1):145.

83. Livanos AE, Greiner TU, Vangay P, et al. Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nat Microbiol*. 2016;1(11):16140.

84. Duong QA, Pittet LF, Curtis N, Zimmermann P. Antibiotic exposure and adverse long-term health outcomes in children: A systematic review and meta-analysis. J Infect. 2022;Jan 10;S0163-4453(22)00004-4.

85. Kołodziej M, Patro-Gołąb B, Gieruszczak-Białek D, et al. Association between early life (prenatal and postnatal) antibiotic administration and coeliac disease: a systematic review. *Arch Dis Child*. 2019;104(11):1083-1089.

86. Dydensborg Sander S, Nybo Andersen AM, Murray JA, Karlstad Ø, Husby S, Størdal K. Association Between Antibiotics in the First Year of Life and Celiac Disease. *Gastroenterology*. 2019;156(8):2217-2229.

87. Zhang Z, Wang J, Wang H, et al. Association of infant antibiotic exposure and risk of childhood asthma: A meta-analysis. *World Allergy Organ J*. 2021;14(11):100607.

88. Zhong Y, Zhang Y, Wang Y, Huang R. Maternal antibiotic exposure during pregnancy and the risk of allergic diseases in childhood: A meta-analysis. *Pediatr Allergy Immunol*. 2021;32(3):445-456.

89. Gianicolo EAL, Eichler M, Muensterer O, Strauch K, Blettner M. Methods for Evaluating Causality in Observational Studies. *Dtsch Arztebl Int*. 2020;116(7):101-107.

90. Joseph KS, Mehrabadi A, Lisonkova S. Confounding by Indication and Related Concepts. *Current Epidemiology Reports*. 2014;1(1):1-8.

91. Faillie JL. Indication bias or protopathic bias? *Br J Clin Pharmacol*. 2015;80(4):779-780.

92. Frost I, Van Boeckel TP, Pires J, Craig J, Laxminarayan R. Global geographic trends in antimicrobial resistance: the role of international travel. *J Travel Med*. 2019;26(8).

93. ECDC/WHO. *Antimicrobial resistance surveillance in Europe 2022 – 2020 data*. 2022.

94. Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet Health*. 2018;2(9):e398-e405.

95. KOROUN. Projekt Aleksander/RESPI-Net. Accessed May 27th, 2022. https://koroun.nil.gov.pl/koroun/projekt-aleksander/

96. Bryce A, Costelloe C, Wootton M, Butler CC, Hay AD. Comparison of risk factors for, and prevalence of, antibiotic resistance in contaminating and pathogenic urinary Escherichia coli in children in primary care: prospective cohort study. *J Antimicrob Chemother*. 2018;73(5):1359-1367.

97. Rodríguez-Lozano J, de Malet A, Cano ME, et al. Antimicrobial susceptibility of microorganisms that cause urinary tract infections in pediatric patients. *Enferm Infecc Microbiol Clin (Engl Ed)*. 2018;36(7):417-422.

98. Willerton L, Lucidarme J, Walker A, et al. Increase in penicillin-resistant invasive meningococcal serogroup W ST-11 complex isolates in England. *Vaccine*. 2021;39(19):2719-2729.

99. Alonso A, Sánchez P, Martínez JL. Environmental selection of antibiotic resistance genes. *Environ Microbiol*. 2001;3(1):1-9.

100. Pettigrew MM, Johnson JK, Harris AD. The human microbiota: novel targets for hospital-acquired infections and antibiotic resistance. *Ann Epidemiol*. 2016;26(5):342-347.

101. Chung A, Perera R, Brueggemann AB, et al. Effect of antibiotic prescribing on antibiotic resistance in individual children in primary care: prospective cohort study. *BMJ*. 2007;335(7617):429.

102. Williams G, Craig JC. Long-term antibiotics for preventing recurrent urinary tract infection in children. *Cochrane Database of Systematic Reviews*. 2019;4(4):CD001534.

103. Same RG, Hsu AJ, Cosgrove SE, et al. Antibiotic-Associated Adverse Events in Hospitalized Children. *J Pediatric Infect Dis Soc*. 2021;10(5):622-628.

104. CDC. Core Elements of Antibiotic Stewardship. Updated April 7, 2021. Accessed May 28th, 2022. https://www.cdc.gov/antibiotic-use/core-elements/index.html

105. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514.

106. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14(8):491-502.

107. Swanson KS, Gibson GR, Hutkins R, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol*. 2020;17(11):687-701.

108. Salminen S, Collado MC, Endo A, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol*. 2021;18(9):649-667.

109. Han S, Lu Y, Xie J, et al. Probiotic Gastrointestinal Transit and Colonization After Oral Administration: A Long Journey. *Front Cell Infect Microbiol*. 2021;11:609722.

110. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of Action of Probiotics. *Adv Nutr*. 2019;10(suppl 1):S49-66.

111. ISAPP. Probiotics: Dispelling Myths. International Society for Probiotics and Prebiotics; 2018.

112. Zmora N, Zilberman-Schapira G, Suez J, et al. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell*. 2018;174(6):1388-1405.e21.

113. Maldonado-Gómez MX, Martínez I, Bottacini F, et al. Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. *Cell Host Microbe*. 2016;20(4):515-526.

114. Mokoena MP. Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. *Molecules*. 2017;22(8)

115. Tuomola EM, Ouwehand AC, Salminen SJ. The effect of probiotic bacteria on the adhesion of pathogens to human intestinal mucus. *FEMS Immunol Med Microbiol*. 1999;26(2):137-142.

116. Darbandi A, Asadi A, Mahdizade Ari M, et al. Bacteriocins: Properties and potential use as antimicrobials. *J Clin Lab Anal*. 2022;36(1):e24093.

117. Olivares M, Díaz-Ropero MA, Gómez N, et al. Oral administration of two probiotic strains, Lactobacillus gasseri CECT5714 and Lactobacillus coryniformis CECT5711, enhances the intestinal function of healthy adults. *Int J Food Microbiol*. 2006;107(2):104-111.

118. Pavlović N, Stankov K, Mikov M. Probiotics--interactions with bile acids and impact on cholesterol metabolism. *Appl Biochem Biotechnol*. 2012;168(7):1880-1895.

119. van der Hee B, Wells JM. Microbial Regulation of Host Physiology by Short-chain Fatty Acids. *Trends Microbiol*. 2021;29(8):700-712.

120. Ferrario C, Taverniti V, Milani C, et al. Modulation of fecal Clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. *J Nutr*. 2014;144(11):1787-1796.

121. Gómez-Llorente C, Muñoz S, Gil A. Role of Toll-like receptors in the development of immunotolerance mediated by probiotics. *Proc Nutr Soc*. 2010;69(3):381-389.

122. Tillisch K, Labus J, Kilpatrick L, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*. 2013;144(7):1394-1401, 1401.e1-4.

123. McFarland LV, Evans CT, Goldstein EJC. Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. *Front Med (Lausanne)*. 2018;5:124.

124. Bertazzoni E, Donelli G, Midtvedt T, Nicoli J, Sanz Y. Probiotics and clinical effects: is the number what counts? *J Chemother*. 2013;25(4):193-212.

125. Kolaček S, Hojsak I, Berni Canani R, et al. Commercial Probiotic Products: A Call for Improved Quality Control. A Position Paper by the ESPGHAN Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr*. 2017;65(1):117-124.

126. Lewis ZT, Shani G, Masarweh CF, et al. Validating bifidobacterial species and subspecies identity in commercial probiotic products. *Pediatr Res*. 2016;79(3):445-452.

127. Drago L, Rodighiero V, Celeste T, Rovetto L, De Vecchi E. Microbiological evaluation of commercial probiotic products available in the USA in 2009. *J Chemother*. 2010;22(6):373-377.

128. Vallabhaneni S, Walker TA, Lockhart SR, et al. Notes from the field: Fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated dietary supplement--Connecticut, 2014. *MMWR Morb Mortal Wkly Rep*. 2015;64(6):155-156.

129. Hojsak I. Probiotics in Children: What Is the Evidence? *Pediatr Gastroenterol Hepatol Nutr*. 2017;20(3):139-146.

130. Goldenberg JZ, Yap C, Lytvyn L, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Database of Systematic Reviews*. 2017;(12).

131. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. *J Pediatr Gastroenterol Nutr*. 2014;59(1):132-152.

132. Collinson S, Deans A, Padua-Zamora A, et al. Probiotics for treating acute infectious diarrhoea. *Cochrane Database of Systematic Reviews*. 2020;(12).

133. Szajewska H, Guarino A, Hojsak I, et al. Use of Probiotics for the Management of Acute Gastroenteritis in Children: An Update. *J Pediatr Gastroenterol Nutr*. 2020;71(2):261-269.

134. Murphy JL, Fenn N, Pyle L, et al. Adverse Events in Pediatric Patients Receiving Long-term Oral and Intravenous Antibiotics. *Hosp Pediatr*. 2016;6(6):330-338.

135. Ogilvie I, Khoury H, Goetghebeur MM, El Khoury AC, Giaquinto C. Burden of community-acquired and nosocomial rotavirus gastroenteritis in the pediatric population of Western Europe: a scoping review. *BMC Infect Dis*. 2012;12:62.

136. Hojsak I, Szajewska H, Canani RB, et al. Probiotics for the Prevention of Nosocomial Diarrhea in Children. *J Pediatr Gastroenterol Nutr*. 2018;66(1):3-9.

137. Ong TG, Gordon M, Banks SS, Thomas MR, Akobeng AK. Probiotics to prevent infantile colic. *Cochrane Database Syst Rev*. 2019;3(3):Cd012473.

138. Thapar N, Benninga MA, Crowell MD, et al. Paediatric functional abdominal pain disorders. *Nature Reviews Disease Primers*. 2020;6(1):89.

139. Foster JP, Dahlen HG, Fijan S, et al. Probiotics for preventing and treating infant regurgitation: A systematic review and meta-analysis. *Matern Child Nutr*. 2022;18(1):e13290.

140. Rosen R, Vandenplas Y, Singendonk M, et al. Pediatric Gastroesophageal Reflux Clinical Practice Guidelines: Joint Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr*. 2018;66(3):516-554.

141. Wojtyniak K, Szajewska H. Systematic review: probiotics for functional constipation in children. *Eur J Pediatr*. 2017;176(9):1155-1162.

142. Caruso R, Lo BC, Núñez G. Host–microbiota interactions in inflammatory bowel disease. *Nature Reviews Immunology*. 2020;20(7):411-426.

143. Miele E, Shamir R, Aloi M, et al. Nutrition in Pediatric Inflammatory Bowel Disease: A Position Paper on Behalf of the Porto Inflammatory Bowel Disease Group of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2018;66(4):687-708.

144. Iheozor-Ejiofor Z, Kaur L, Gordon M, Baines PA, Sinopoulou V, Akobeng AK. Probiotics for maintenance of remission in ulcerative colitis. *Cochrane Database of Systematic Reviews*. 2020;(3).

145. Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B. Probiotics Reduce Mortality and Morbidity in Preterm, Low-Birth-Weight Infants: A Systematic Review and Network Meta-analysis of Randomized Trials. *Gastroenterology*. 2020;159(2):467-480.

146. Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database Syst Rev*. 2020;10(10):Cd005496.

147. van den Akker CHP, van Goudoever JB, Shamir R, et al. Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr*. 2020;70(5):664-680.

148. Chiang MC, Chen CL, Feng Y, Chen CC, Lien R, Chiu CH. Lactobacillus rhamnosus sepsis associated with probiotic therapy in an extremely preterm infant: Pathogenesis and a review for clinicians. *J Microbiol Immunol Infect*. 2021;54(4):575-580.

149. Łukasik J, Salminen S, Szajewska H. Rapid review shows that probiotics and fermented infant formulas do not cause d-lactic acidosis in healthy children. *Acta Paediatr*. 2018;107(8):1322-1326.

150. Depoorter L, Vandenplas Y. Probiotics in Pediatrics. A Review and Practical Guide. *Nutrients*. 2021;13(7).

151. Halken S, Muraro A, de Silva D, et al. EAACI guideline: Preventing the development of food allergy in infants and young children (2020 update). 2021;32(5):843-858.

152. Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. EAACI Food Allergy and Anaphylaxis Guidelines: diagnosis and management of food allergy. 2014;69(8):1008-1025.

153. Timmerman HM, Koning CJ, Mulder L, Rombouts FM, Beynen AC. Monostrain, multistrain and multispecies probiotics--A comparison of functionality and efficacy. *Int J Food Microbiol*. 2004;96(3):219-33.

154. Sniffen JC, McFarland LV, Evans CT, Goldstein EJC. Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. *PLoS One*. 2018;13(12):e0209205.

155. Kwoji ID, Aiyegoro OA, Okpeku M, Adeleke MA. Multi-Strain Probiotics: Synergy among Isolates Enhances Biological Activities. *Biology (Basel)*. 2021;10(4).

156. Ouwehand AC, Isolauri E, Kirjavainen PV, Tölkko S, Salminen SJ. The mucus binding of Bifidobacterium lactis Bb12 is enhanced in the presence of Lactobacillus GG and Lact. delbrueckii subsp. bulgaricus. *Lett Appl Microbiol*. 2000;30(1):10-13.

157. Fredua-Agyeman M, Stapleton P, Basit AW, Gaisford S. Microcalorimetric evaluation of a multi-strain probiotic: Interspecies inhibition between probiotic strains. *Journal of Functional Foods*. 2017;36:357-361.

158. McFarland LV. Efficacy of Single-Strain Probiotics Versus Multi-Strain Mixtures: Systematic Review of Strain and Disease Specificity. *Dig Dis Sci*. 2021;66(3):694-704.

159. Viljanen M, Savilahti E, Haahtela T, et al. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy*. 2005;60(4):494-500.

160. Hays S, Jacquot A, Gauthier H, et al. Probiotics and growth in preterm infants: A randomized controlled trial, PREMAPRO study. *Clin Nutr*. 2016;35(4):802-811.

161. Gajewski P, Jaeschke R, Brożek J. *Podstawy EBM czyli medycyny opartej na danych naukowych dla lekarzy i studentów*. Medycyna Praktyczna; 2008.

162. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials*. 2010;11:32.

163. Care AGSEPoP-C. Person-Centered Care: A Definition and Essential Elements. *J Am Geriatr Soc*. 2016;64(1):15-18.

164. Black N. Patient reported outcome measures could help transform healthcare. *BMJ*. 2013;346:f167.

165. de Wit M, Abma T, Koelewijn-van Loon M, Collins S, Kirwan J. Involving patient research partners has a significant impact on outcomes research: a responsive evaluation of the international OMERACT conferences. *BMJ Open*. 2013;3(5).

166. Higgins J, Thomas J, Chandler J, et al. *Cochrane Handbook for Systematic Reviews of Interventions*. Cochrane; 2022. Accessed May 2022. www.training.cochrane.org/handbook

167. Miyar J, Adams CE. Content and quality of 10,000 controlled trials in schizophrenia over 60 years. *Schizophr Bull*. 2013;39(1):226-229.

168. Joober R, Schmitz N, Annable L, Boksa P. Publication bias: what are the challenges and can they be overcome? *J Psychiatry Neurosci*. 2012;37(3):149-152.

169. Chan AW, Song F, Vickers A, et al. Increasing value and reducing waste: addressing inaccessible research. *Lancet*. 2014;383(9913):257-266.

170. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. *Lancet*. 2009;374(9683):86-89.

171. Webbe J, Sinha I, Gale C. Core Outcome Sets. 2018;103(3):163-166.

172. COMET Initiative. Accessed April 2022, 2022. https://www.comet-initiative.org/

173. Williamson PR, Altman DG, Bagley H, et al. The COMET Handbook: version 1.0. *Trials*. 2017;18(Suppl 3):280.