Manipulating the infant respiratory microbiomes to improve clinical outcomes

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Manipulating the infant respiratory microbiomes to improve clinical outcomes: a review of the literature

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Abstract:	Background: The association between infant respiratory microbiota and disease (including respiratory tract infections and asthma) is increasingly recognised, although the mechanism remains unclear. Respiratory infections and asthma account for a large proportion of infant morbidity and mortality, so the possibility of preventing disease or modifying clinical outcomes by manipulating microbiome development warrants investigation. Objectives and methods: We identified studies that investigated the efficacy of live bacteria (probiotics or human challenge) or their substrates to modify respiratory colonisation or clinical outcomes in infants. Eligibility criteria: Interventional studies involving infants under one year of age, administration of live bacteria or their substrates, and outcome measures including bacterial colonisation, microbiome profile, or respiratory disease phenotypes. Results and limitations: Some bacterial interventions can reduce infant respiratory infections, although none have been shown to reduce asthma incidence. The literature is heterogeneous in design and quality, precluding meaningful meta-analysis. Conclusions: Upper respiratory tract infant microbiome manipulation may alter outcomes in respiratory tract infection, but further well-conducted research is needed to confirm this. Improved regulation of proprietary bacterial products is essential for further progress.

12/03/2021

Dear Editor,

Many thanks for the thoughtful review and helpful comments from yourself and Reviewer 1. I have taken these on board, and changed the title to avoid the term “systematic review”. I have also removed the details pertaining to adult human challenge with *N. lactamica*.

Please find attached the updated manuscript and title page.

Many thanks again for your consideration,

Best wishes,

Anastasia

Title

Manipulating the infant respiratory microbiomes to improve clinical outcomes: a review of the literature

Running title

Manipulating the infant respiratory microbiomes

Authors

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- Infant respiratory microbiome profiles are associated with disease (including respiratory tract infections and asthma), although the mechanism for this remains unclear.
- There have been increasing attempts since the 1960s to alter infant respiratory pathogen carriage and clinical outcomes using live bacteria and their substrates.
- However, the evidence for these interventions is heterogeneous, and there is insufficient high-quality evidence to recommend their widespread use.
- Controlled human challenge studies offer an avenue for characterising the clinical, immunological and microbiome effects of such live bacterial interventions in infants.

Abstract / Summary

Background: The association between infant respiratory microbiota and disease (including respiratory tract infections and asthma) is increasingly recognised, although the mechanism remains unclear. Respiratory infections and asthma account for a large proportion of infant morbidity and mortality, so the possibility of preventing disease or modifying clinical outcomes by manipulating microbiome development warrants investigation.
Objectives and methods: We identified studies that investigated the efficacy of live bacteria (probiotics or human challenge) or their substrates to modify respiratory colonisation or clinical outcomes in infants.
Eligibility criteria: Interventional studies involving infants under one year of age, administration of live bacteria or their substrates, and outcome measures including bacterial colonisation, microbiome profile, or respiratory disease phenotypes.
Results and limitations: Some bacterial interventions can reduce infant respiratory infections, although none have been shown to reduce asthma incidence. The literature is heterogeneous in design and quality, precluding meaningful meta-analysis.
Conclusions: Upper respiratory tract infant microbiome manipulation may alter outcomes in respiratory tract infection, but further well-conducted research is needed to confirm this. Improved regulation of proprietary bacterial products is essential for further progress.

Keywords

Probiotic; microbiome; human challenge; respiratory; infant

Introduction

The infant upper respiratory tract (URT) is home to complex and evolving communities of bacteria, including clinically significant pathobionts. Pathobionts are commensal bacteria that, although harmless in many hosts, are capable of causing severe disease. Such bacteria include *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae*, which can cause respiratory tract infections (RTI), meningitis and septicaemia in infants.

An ever-growing body of evidence has identified associations between infant clinical outcomes and the developing URT microbiome (the site-specific total microbial community). This research includes large longitudinal birth cohorts, such as the Dutch Microbiome Utrecht Infant Study (MUIS)[1] and the Danish Copenhagen Prospective Studies on Asthma in Childhood (COPSAC)[2]. Distinct microbiome profiles are seen during, and even preceding, acute RTI, including increased relative abundance of *Haemophilus* and *Streptococcus* spp., and loss of topographic distinction between adjacent microbial niches[1,3]. Further, favourable 'keystone organisms' (including *Corynebacterium* spp. and *Dolosigranulum* spp.) are predictors of microbiome stability and respiratory health[4]. Longer-term outcomes, including recurrent RTI and asthma, are also associated with both the composition and rate of change of the URT microbiome, with a faster progression to a more adult-like microbiome seen in infants with recurrent RTI[2,5].

There is overall consensus that the neonatal URT becomes rapidly colonised at birth with a highly diverse 'pioneer' microbiome, with significant differences appearing between adjacent anatomical niches (e.g. mouth, nasal cavity and nasopharynx) within the first few days of life, and certainly by one week old[4,6]. Maternal microbiota account at least in part for infant microbiome profiles, and there are associations between infant URT microbial evolution and external factors (e.g. vaginal versus caesarean delivery, breast versus formula feeding, cohabiting siblings, and antibiotic exposure)[5,6]. However, in the absence of interventional research, it is difficult to draw firm conclusions on causality between these factors.

Taken together, these research findings raise the interesting question of whether perinatal and neonatal interventional microbiome studies are desirable in order to: better characterise the relationship between infant URT flora, external factors, and clinical outcomes; and ultimately to manipulate neonatal flora to improve childhood health. This review summarises the published literature on interventional research involving infant URT bacteria: from the early attempts to alter pathobiont carriage using commensal inoculation, to the use of perinatal probiotics to prevent RTI and asthma; and including both traditional culture-based microbiological methods and more recent sequencing-based microbiome research. Ongoing challenges and limitations are highlighted, and scope for further work is evaluated.

Materials and methods

One author searched the PubMed database from (inception to December 2020) using the MESH-based search: '(probiotics OR ((microbiota OR bacteria) AND therapeutics)) AND (infant OR newborn OR pregnancy OR maternal-fetal relations) AND (respiratory system OR pharynx OR nasopharynx OR oropharynx OR hypopharynx OR mouth OR nasal cavity)'. Relevant articles were used to identify further articles and additional (non-MESH) search terms: '(bacterial interference OR controlled human infection OR human challenge OR symbiotic OR prebiotic OR postbiotic OR microbiota transplant OR bacteriotherapy) AND (infant OR newborn OR pregnancy OR maternal-fetal relations) AND (respiratory system OR pharynx OR nasopharynx OR oropharynx OR hypopharynx OR mouth OR nasal cavity)'. Given the very broad remit of this review, we focus here on the upper respiratory bacteriome and pathobiont colonisation status (by traditional microbiological culture) in infants under one year of age. We have excluded studies reporting solely on the lower respiratory tract or lung, on the virome or mycobiome. Furthermore, we included studies on the therapeutic applications of bacteria and their substrates, but excluded studies on bacteriophage (viral) therapy. Where relevant data are lacking, we signpost to studies of other microbial niches or older children and adults, but details of these are beyond the scope of this review. Where possible, we use terminology for which consensus definitions exist (Table 1). To assess risk of bias in the studies included, we comment on randomisation, blinding, sample size, intervention reporting (dose and schedule), outcome measures, conflicts of interest, and analysis strategy (per-protocol or intention-to-treat).

Probiotic[7]	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
Prebiotic[7]	A substrate that is selectively utilized by host microorganisms conferring a health benefit
Synbiotic[7]	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host
Human challenge[8]	Trials in which participants are intentionally challenged (whether or not they have been vaccinated) with an infectious disease organism

Table 1. Consensus definitions used in this review.

Results

Bacterial inoculation to alter URT pathobiont carriage

In 1963, Shinefield *et al.* reported on neonatal nasal inoculation with low-pathogenicity *Staphylococcus aureus* (strain 502A), in response to outbreaks of high-pathogenicity *S. aureus* (phage complex 80/81) infections in three neonatal units (Georgia, Ohio and Louisiana, USA)[9,10]. Neonates were inoculated with >500 colony-forming units (CFU) *S. aureus* 502A in 0.5 microlitres solution applied directly to the nasal mucosa by sterile microburette. Inoculation was associated with colonisation in 70/79 (88%) of infants, and reduction of non-502A *S. aureus* carriage from 41% (45/111 uninoculated control infants) to 5% (4/76 infants colonised with 502A). This effect was maintained at follow-up 2-4 weeks later (non-502A *S. aureus* carriage 54% [56/104] in uninoculated infants versus 6% [4/69] in 502A-colonised infants). Nasal inoculation was also associated with reduced manifestations of staphylococcal disease in infants and their household contacts (including impetigo, conjunctivitis, and maternal mastitis), from 73% (22/30 individuals colonised with only 80/81) to 9% (9/96 individuals colonised with only 502A).

A follow-up study using higher inoculum doses (2,000-50,000 CFU) demonstrated 502A colonisation in 95% (446/470) infants, and also led to spontaneous horizontal acquisition of 502A by 67% (159/236) of uninoculated infants being cared for in the same neonatal unit[11]. However, subsequent inoculations with even higher inoculum doses (up to 10 million CFU) were associated with development of pustular skin lesions in 502A-colonised infants[12,13], and even one fatality associated with *S. aureus* 502A septicaemia and meningitis[14].

A group in New York (USA) have reported on nasal inoculation with alpha-haemolytic streptococcus strain 215 in neonatal intensive care unit patients, resulting in colonisation in 16/22[15] and 31/42[16] inoculated babies. This intervention was associated with reduced pharyngeal pathobiont carriage, including *Escherichia coli* and *Klebsiella pneumoniae*, although no control group was included. Further, these studies did not report clinical outcomes or inoculum dose.

A small number of studies have reported on pathobiont carriage following upper respiratory bacterial inoculation in older children and adults. These include attempts to displace *S. aureus* (including methicillin-resistant *S. aureus*) using *Corynebacterium* spp., *S. epidermidis,* or lactobacilli (Table 2). However, these studies did not consider inoculation or sampling of infants.

Study	Sample size: intervention(s); control	Intervention(s); control	*S. aureus* eradication: intervention(s); control
Uehara 2000[17]	17; 10	10^9 CFU/d *Corynebacterium* spp. Co304 by swab to nares for 12-26d; 0.9% sodium chloride by swab to nares for 12d	12/17 (70.6%); 0/10 (0%)
Iwase 2010[18]	19; 0	10^9 CFU *S. epidermidis* JK16 (7/19) OR 10^9 CFU Esp-deficient *S. epidermidis* JK16 (6/19) OR 10^9 CFU *S. epidermidis* JK11 (3/19) OR 500pmol purified Esp (3/19) by nasal swab for 5d	6/7 (85.7%); 0/6 (0%); 0/3 (0%); data not shown
Roos 2011[19]	7; 0	10^9 CFU/d nasal spray AND 3ml/d oral suspension (dose not given) lactobacilli (*L. paracasei* AND *L. rhamnosus* AND *L. plantarum*) for 3-11m	5/7 (71.5%)
Kiryukhina 2013[20]	4; 0	$0.9x10^8$ CFU/d *C. pseudodiphtheriticum* 090104 by nasal spray for 2-3w	3/4 (75%)

Table 2. Summary of studies investigating the impact of topical bacterial application in adults and children older than one year. CFU: colony-forming units; *C*: *Corynebacterium*; d: days; Esp: serine protease; *L: Lactobacillus;* ml: millilitres; m: months; pmol: picomoles; spp: species; *S: Staphylococcus*

Bacterial inoculation to alter URT microbiome profile

To date, there are no published respiratory microbiome analyses in infants receiving topical (nose or throat) bacterial inoculation. One interventional study has reported on infant oral (as well as anal and skin, but not respiratory) microbiota, following exposure of neonates to their mothers' vaginal fluid after caesarean section[21]. This proof-of-concept study was performed in response to observational research showing distinct microbiome profiles associated with vaginal and caesarean delivery, and a higher risk of later adverse outcomes (including obesity, asthma and allergies) in caesarean-delivered infants[22]. Four caesarean-delivered babies were inoculated at birth on the mouth, face and body with gauze that had been incubated within the mother's vagina. Over the first month of life, neonatal microbiome profiles appeared more similar to those of seven vaginally-delivered neonates than to those of seven uninoculated caesarean-delivered control infants. This study was limited by small sample size and use of antibiotics (in all eleven caesarean deliveries but only one of the seven vaginal deliveries). However, further trials exploring the efficacy and safety of this technique are underway[22], at least one of which includes infant oral and nasal microbiome sampling (ClinicalTrials.gov Identifier NCT03567707). Critiques of this 'vaginal seeding' or 'baptism' approach include the unclear role of possible confounding factors (e.g. antibiotic use, the absence of labour or ruptured membranes, formula feeding, maternal obesity, and younger gestational age at delivery, all of which are associated with caesarean section), as well as the potential for pathogen transfer from the mother's vagina to the neonate, such as group B streptococcus and herpes simplex[23].

There is one published study examining airway microbiota in 695 infants following maternal administration of high-dose vitamin D3, n-3 long-chain fatty acids, both or placebo[24]. These non-bacterial interventions were associated with altered airway microbiota in the volunteers' one-month-old infants, including reduced *S. pneumoniae* relative abundance following maternal vitamin D3 treatment, although no clinical outcomes were reported.

Topical bacterial inoculation to alter respiratory clinical outcomes

A small number of studies have reported on clinical outcomes in infants following use of topical (oral or nasal sprays, drops or lozenges) bacterial application. In a double-blinded randomised study, 34 1-month old infants who received a proprietary probiotic tablet via a slow-release pacifier ($5x10^9$ CFU *Bifidobacterium animalis* subsp. *lactis* BB-12 twice daily until 8 months old) had a reduced incidence of respiratory infections compared with 35 infants receiving placebo (risk ratio [RR] 0.69; 95% CI 0.5 to 0.89)[25]. The probiotic strain was recovered from faecal samples in 62% of probiotic-treated infants, compared with only 17% of controls. However, there was no association between probiotic use and acute otitis media or antibiotic use, and this study was limited by the use of subjective (parent-reported) endpoints, and per-protocol (rather than intention-to-treat) analysis. A potential conflict of

interest was noted, as the probiotic manufacturer donated the tablets and contributed to faecal analysis.

The potential of a streptococcal nasal spray to prevent otitis media has been explored. Two double-blind, placebo-controlled, randomised studies conducted by a Swedish research group[26,27] reported on the use of streptococcal nasal spray (a mixture of two *S. sanguinis* strains, two *S. mitis* strains, and one *S. oralis* strain) in infants and young children with a history of otitis media. Roos *et al.*[26] demonstrated a reduction in otitis media recurrence after two ten-day courses compared with placebo (21/53 children receiving streptococcal nasal spray versus 28/55 children receiving placebo, p=0.02), with both groups receiving a ten-day course of antibiotics at baseline (during an acute episode of otitis media). In contrast, a four-month course of the same streptococcal spray in a smaller group of children was not associated with a reduction in otitis media relapse rate or total number of URT infections[27]. However, in the latter study, neither group received antibiotics at baseline, and the authors speculated that antibiotics may facilitate colonisation by the nasal spray streptococci by eradicating pre-existing flora. Both of these studies were limited by per-protocol (rather than intention-to-treat) analysis, which may be a source of bias. Further, although both studies included infants (minimum age 6 months and 4 months, respectively), the mean age was over 1 year (23 months and 21 months, respectively), which may limit the relevance of the findings to infants under one year old.

Likewise, the use of topical bacterial application to reduce respiratory infections has been investigated in older children and adults, including: *S. sanguinis* or *L. rhamnosus* nasal spray[28], a nasal spray containing 13 strains of lactobacilli and bifidobacteria[29], a nasal spray containing *S. salivarius* 24SMB and *S. oralis* 89a[30,31], and S*. salivarius* strain K12 in a slow-release lozenge[32,33]. These studies did not focus on infants, and it is worth noting that they each had significant limitations, including retrospective, uncontrolled or open-label design, unclear dosing schedule, per-protocol analysis, and conflicts of interest (including authors with commercial interest in the probiotic tested). Thus, a detailed account of these studies is of little value in this review on infant microbiome manipulation.

Ingested bacterial products to alter respiratory clinical outcomes

The majority of probiotic research to date involves ingested (rather than topically-applied) bacteria, and there is far more published interventional research involving gastrointestinal than respiratory microbiota, including characterisation of faecal microbiota following oral probiotic administration to neonates (or their mothers during or after pregnancy)[34,35]. Further, only a minority of studies report on respiratory outcomes (including infection, wheeze or asthma), with even fewer involving infants (rather than older children and adults).

The literature includes seven randomised controlled double-blind studies investigating the effects of probiotics on RTI in infants (Table 3). The most robust of

these studies involved randomisation of 4,556 infants in rural India to *L. plantarum* (ATCC strain 202195) or placebo (each administered for 7 days)[41]. This probiotic was associated with a reduction in LRTI requiring antibiotic therapy (RR 0.66, 95% CI 0.51 to 0.88), culture-positive septicaemia (RR 0.22, 95% CI 0.09 to 0.53), and culture-negative sepsis requiring hospitalisation and intravenous antibiotics (RR 0.53, 95% CI 0.30 to 0.92). However, it is not clear how generalisable these findings are to other probiotic choices or study populations, as this study has not been replicated in other settings to date.

The association between oral probiotics, prebiotics and infant allergic disease has also been investigated, with several systematic reviews collating current evidence[43-45]. In terms of respiratory disease, there is no convincing evidence that probiotics given to infants or their mothers (during or after pregnancy) are associated with a reduction in infant wheeze, childhood asthma, or allergic rhinoconjunctivitis. However, a reduction in eczema has been reported by several investigators, including large randomised controlled trials with up to 11 years follow-up[46,47]. Of note, the World Allergy Organization suggests that probiotics should be used in pregnant women and infants at high risk of developing allergy, but that this is supported by very low quality evidence, and is a conditional recommendation (i.e. recognition that many patients would not want the suggested course of action, and that policymakers would require substantial debate before adopting it)[48].

Study	Sample size: intervention(s); control	Infant age at randomisation (gestational age at birth in w+d)	Intervention (dose); control	Intervention duration	RTI risk ratio (intervention: control) [95% CI]	Potential sources of bias
Weizman 2005[36]	133 (73 BB12, 68 *L. reuteri*); 60	4-10m (38.9-39.6 mean)	BB12 or *L. reuteri* (10^7CFU/g IF) in IF; placebo	12w	Risk ratio not reported; no significant difference in respiratory illness incidence (p=0.46) or duration (p=0.17 between either intervention & placebo	Volume of IF not controlled; probiotics donated by manufacturer; per-protocol analysis
Rautava 2009[37]	32; 40	<2m (35.1 to 42.3)	LGG and Bb12 (each $1x10^{10}$CFU/d) in IF; placebo	10m	Respiratory infection: **0.51 [0.27, 0.95] at 12m**, 0.89 [0.6,1.18] at 7m; AOM: **0.44 [0.21, 0.90] at 7m**, 0.50 [0.17,1.45] at 12m	IF & probiotics donated by manufacturer
Maldonado 2012[38]	97; 91	6m	GOS (0.4g/100ml IF) and *L. fermentum* ($2x10^8$CFU/d), 582-887ml IF/d; GOS only	6m	**URTI: 0.73 [0.56, 0.95];** LRTI: 0.87 [0.4, 1.9]; Otitis: 0.55 [0.22, 1.32]	Study funded by IF manufacturer; per-protocol analysis
Cohen 2013[39]	112; 112	7-13m	*S. thermophilus* (10^7CFU/g IF) and *S. salivarius* ($2.5x10^7$CFU/g IF) and *L. rhamnosus* (10^7CFU/g IF), 300-630ml IF/d; placebo	12m	Risk ratio not reported; no significant difference in AOM incidence (p=0.80) or LRTI incidence (p=0.63) between intervention & placebo	1 author employed by funder; study funded by IF manufacturer; URTI incidence (2ary outcome) not published
Luoto 2013[40]	62 (31 LGG, 31 GOS); 32	0-3d (32+0 to 36+6)	LGG ($1-2x10^9$CFU/d) or GOS (1-2x600mg/d) in IF or breastmilk; placebo	60d	**Prebiotic vs placebo: 0.24 [0.12, 0.49]; Probiotic vs placebo: 0.50 [0.28, 0.90]**	Excipient (IF or breastmilk) not controlled; 1 author employed by funder; Probiotic, prebiotic & study funding provided by manufacturer
Panigrahi 2017[41]	2278; 2278	2-4d (>35)	*L. plantarum* (10^9CFU/d) and FOS (150mg/d) in dextrose saline; placebo	7d	**0.66 [0.51, 0.88]**	Extensive exclusion criteria (2506 of 7089 participants excluded before randomisation)
Szajewska 2017[42]	90; 92	28d	*L. paracasei* (10^9CFU/L IF) and FOS (0.061g/100ml IF) and GOS (0.54g/100ml IF); FOS and GOS only	5m	**LRTI: 0.34 [0.13, 0.85] at 12m**, 0.6 [0.2, 1.6] at 6m; URTI: 2.0 [0.9, 4.5] at 12m, 1.6 [0.6, 4.6] at 6m	Volume of IF not controlled; IF manufacturer donated IF, & contributed to study funding & design

Table 3. Summary of studies investigating the impact of oral probiotics (with or without prebiotics) on respiratory tract infections in infants.
BB12: *Bifidobacterium lactis* Bb12; L.: *Lactobacillus*; LGG: *Lactobacillus rhamnosus* GG: GOS: galacto-oligosaccharide; m: months; d: days; w: weeks; IF: infant formula; CFU: colony-forming units; mg: milligrams; g: grams; FOS: fructo-oligosaccharide; ml: millilitres; RTI: respiratory tract infection; AOM: acute otitis media; CI: confidence interval; LRTI: lower respiratory tract infection; URTI: upper respiratory tract infection; **bold** = significant difference between intervention & control ($p<0.05$).

Discussion

Infections caused by URT pathobionts are a significant cause of infant morbidity and mortality, globally accounting for 492,000 deaths due to RTI and 89,000 deaths due to meningitis per year[49]. The question of whether infant respiratory microbiota may be manipulated to prevent such infections, or even chronic diseases like asthma, is complex and nuanced. In general, there is a paucity (rather than an absence) of evidence in favour of microbiome manipulation, encompassing a wide range of interventions (topical and ingested live bacteria and their substrates) and research techniques (traditional culture-based microbiology and genomics-based microbiome analysis). Even the terminology used varies widely over time and disciplines, with attempts to alter pathobiont carriage or clinical outcomes termed 'bacterial interference' in the 1960s to 1980s[9-16], before being overtaken by terms such as 'probiotics' and 'human challenge' (Table 1). As such, meta-analysis is limited by significant heterogeneity between studies, including: bacterial strain (and even different proprietary formulations of the same strain), dose, duration, and excipient (infant formula, dextrose saline, or expressed breast milk); choice of control (placebo or prebiotic); gestational age at birth and infant age at randomisation; sample size; outcome measures (parent-, investigator-, or doctor-reported RTI, or microbiological evidence of RTI); and statistical analysis (per-protocol or intention to treat). Furthermore, several of the studies presented in this review involved proprietary probiotics and infant formula, as well as funding and product donation by their manufacturers, representing potential conflicts of interest.

Although improvements in the throughput, availability and cost of sequencing have dramatically enhanced our understanding of the infant respiratory microbiomes, certain challenges and limitations remain[6]. For example, probiotic-associated microbiome changes are often expressed as altered relative abundance, rather than absolute abundance; given the exogenous application of live bacteria, this may result in spurious reports of reduced abundance of other taxa[50]. Further, bioinformatic analysis (especially post-hoc) of the resulting very large datasets can result in identification of spurious associations between probiotics and microbiota.

Even for studies demonstrating a convincing effect of microbial products on infant respiratory pathobiont colonisation, microbiome profile, or clinical outcome, the underlying mechanism of action is often unclear, and may vary with probiotic strain. Diverse effector mechanisms, including host immunomodulation, direct competition with pathogens, and improved mucosal barrier, have been studied using mouse and *in vitro* models, although many unknowns remain[50]. In particular, it is not clear how ingested bacteria influence distant respiratory ecology and health, although both systemic immune effects and bacterial migration from the gut to the URT have been suggested[51].

Many probiotics are classed as food supplements rather than drugs, and are therefore not subject to pharmaceutical regulation. One consequence of this is that

there is no legal requirement to demonstrate product efficacy prior to sale. Further, a lack of enforceable standards on labelling proprietary probiotics means that crucial information, such as strain identity and dose, is often missing, negatively impacting consumer (let alone scientific) trust in the quality of these products[52]. Given all these issues, it has been suggested that efforts to manipulate microbiota are premature, and may even pose health risks, such as transfer of pathogens by probiotic contamination or donor-derived bacteria (e.g. 'vaginal seeding')[53]. Potential risks remain unclear, but adverse outcomes such as obesity, mood disorders, and malignancy have been reported in association with other related microbial therapies, such as faecal transplant.

Despite these valid considerations, it is important not to draw rigid conclusions from meta-analyses of heterogeneous studies, including studies of widely varying quality. Rather, the relative merits of each intervention (microbial strain and mode of delivery) should be considered in their own right, and recommended if supported by robust evidence. To improve quality and transparency of such research, there have been calls to harmonise global regulatory approaches for probiotics, including terminology, labelling and third-party evaluation[52,54]. Such harmonised advice already exists for probiotic safety, including the European Food Safety Authority's Qualified Presumption of Safety status, which designates bacterial species as safe for human consumption[55].

Looking ahead, there remains a need for high quality basic science research to complement clinical studies of microbial interventions. Rather than selecting interventions based on availability of proprietary products, a more nuanced understanding of evolving microbiota and immunity may help identify candidate microbial interventions for study. For example, human challenge models with controlled doses of well-characterised commensals provide a valuable template for URT microbial interventional research rooted in robust basic science. To date, however, such research has only been performed in healthy adults, rather than in infants or pregnant women[56,57].

Conclusions

As sequencing technologies continue to improve in throughput, resolution, cost and accessibility, research goals have shifted towards more in-depth characterisation of longitudinal cohorts and data synthesis. Improved characterisation of the human microbiome across the life course has raised the prospect of manipulating microbiota to benefit human health. Although the majority of such work pertains to the adult gastrointestinal microbiome, increasing attention is being paid to the infant URT microbiota, and their association with major causes of childhood morbidity and mortality (RTI, meningitis and asthma). To date, there is insufficient high-quality evidence to recommend widespread use of probiotics and other microbial interventions in the perinatal or infant period. However, there are a small number of studies that hint at a possible benefit, and further high-quality basic science and clinical research as well as a move towards more transparent and consistent regulation of microbial products may enable a future public health impact.

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