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Review article: the future of microbiome-based therapeutics

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Summary

Background: From consumption of fermented foods and probiotics to emerging applications of faecal microbiota transplantation, the health benefit of manipulating the human microbiota has been exploited for millennia. Despite this history, recent technological advances are unlocking the capacity for targeted microbial manipulation as a novel therapeutic.

Aim: This review summarises the current developments in microbiome-based medicines and provides insight into the next steps required for therapeutic development.

Methods: Here we review current and emerging approaches and assess the capabilities and weaknesses of these technologies to provide safe and effective clinical interventions. Key literature was identified through Pubmed searches with the following key words, 'microbiome', 'microbiome biomarkers', 'probiotics', 'prebiotics', 'synbiotics', 'faecal microbiota transplant', 'live biotherapeutics', 'microbiome mimetics' and 'postbiotics'.

Results: Improved understanding of the human microbiome and recent technological advances provide an opportunity to develop a new generation of therapies. These therapies will range from dietary interventions, prebiotic supplementations, single probiotic bacterial strains, human donor-derived faecal microbiota transplants, rationally selected combinations of bacterial strains as live biotherapeutics, and the beneficial products or effects produced by bacterial strains, termed microbiome mimetics.

Conclusions: Although methods to identify and refine these therapeutics are continually advancing, the rapid emergence of these new approaches necessitates accepted technological and ethical frameworks for measurement, testing, laboratory practices and clinical translation.

[Correction added on June 04, 2022, after first online publication: Author Claire L. O'Brien affiliation has been updated]

The Handling Editor for this article was Professor Mike Burkitt, and this uncommissioned review was accepted for publication after full peer-review.

1 | INTRODUCTION

The gastrointestinal (GI) microbiome is known to play an integral role in overall homeostasis; however, alterations can lead to the development and progression of disease. These complex communities contain between 100 and 1000 bacterial species all of which have the ability to interact with the host in different ways. The concept of altering the GI microbiome to improve health outcomes is now well established in modern medicine. Microbiome-based medicines can fall into two categories, microbiome-based biomarkers, and therapeutics (Figure 1). Although some dietary interventions, prebiotics, probiotics, antibiotics and faecal microbiota transplant (FMT) are well-established therapeutics, recent work has raised the possibility of live biotherapeutics, and phage therapies for managing and treating a large array of diseases[1–6] (Figure 1). With the expansion of diverse microbially targeted therapies, coupled with an increasing availability of cost-effective gut metagenomic profiling, it is timely to critically evaluate current capabilities and determine fundamental areas on which to focus future research.

2 | BIOMARKERS FOR DIAGNOSIS AND TREATMENT

The microbiome, particularly the gut microbiome, shares an expansive interface with the host immune system, rendering it an excellent candidate for biomarker development (Figure 2). Advances in metagenomic sequencing technologies have improved characterisation of microbial communities and provided associations with disease phenotypes.[1,7,8] This has facilitated the identification of potential microbial disease biomarkers in type 2 diabetes,[1] colorectal cancer,[2] liver cirrhosis[9] and hepatocellular carcinoma.[3] For example, a decrease in the abundance of butyrate producing bacteria is indicative of type 2 diabetes,[1] and an increase in *Fusobacterium* and *Porphyromonas* is a biomarker for colorectal cancer.[2] In addition to the identification of biomarkers for diagnosis of disease, microbial biomarkers are being developed to stratify patient cohorts prior to treatment to ensure patients receive the best treatment for them. Furthermore, biomarkers can be used to monitor patients following treatment to ensure treatment efficacy (Figure 2). Currently, the causal relationship between the microbiome and these disease states is unknown and microbial strain level granularity is largely lacking. Given the complexity of these microbiome–disease interactions future biomarkers may require microbial signatures comprised of multiple bacteria or bacterial functions as disease biomarkers. Furthermore, identifying and validating key species or functions may complement or reduce the need for expensive scans and invasive biopsies for patients.

Understanding interactions between the microbiome and therapeutic response provides the opportunity for tailored interventions to achieve optimal outcomes or avoid adverse reactions.[10,11] In this context, microbiome-based patient stratification to appropriately target existing therapies to specific patients[12–16] and to define responses to vaccines and other therapies,[17–20] represent two emerging areas for the application of microbiome-based technologies.

The importance of microbiome diversity on vaccine response is exemplified by studies in paediatric cohorts. These studies suggest greater bacterial diversity in the GI tract correlates with an increased immune response to a variety of vaccines, including the oral rotavirus and polio vaccines, the intramuscular hepatitis B vaccine and the intradermal BCG vaccines.[10,18,19] Differences in the microbiome have been associated with lower efficacy of these vaccines in lower-income nations.[17–20] Specifically, studies have demonstrated that an increase in the abundance of bacteria from the Firmicutes or Actinobacteria phyla is associated with a greater production of antibodies in response to vaccines.[18] Furthermore, patients with a higher relative abundance of Proteobacteria and Bacteroidetes had a lower antibody titre following immunisation.[17–20] This is consistent with previous studies that link the GI microbiome with immune development, tolerance and priming.[21] In this context, the microbiome has been linked to a decrease in the development of allergies, through anti-inflammatory effects of microbial metabolites such as

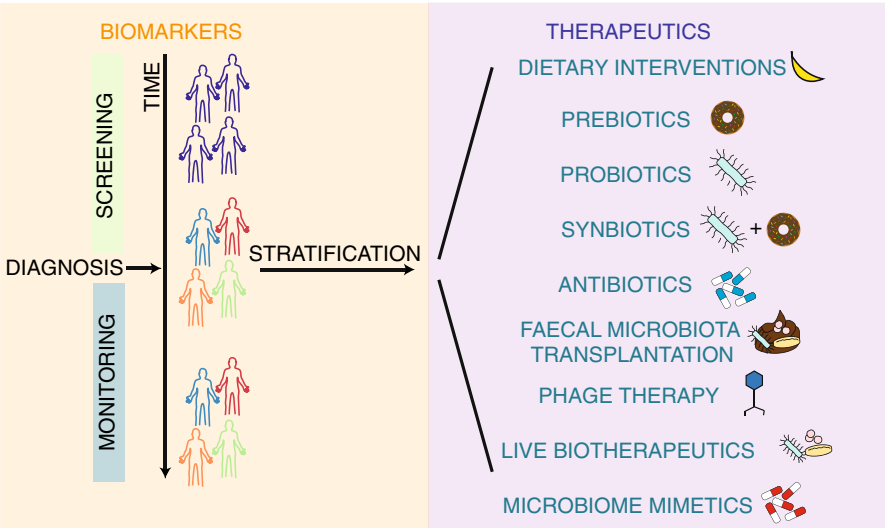

FIGURE 1 Overview of the different uses of the microbiome for medicine. Microbiota uses include biomarkers (orange box), where patients are screened monitored and stratified, and therapeutics (purple box), where there are currently nine forms of therapeutics: Dietary interventions, prebiotics, probiotics, synbiotics, antibiotics, faecal microbiota transplantation, phage therapy, live biotherapeutics and microbiome mimetics.

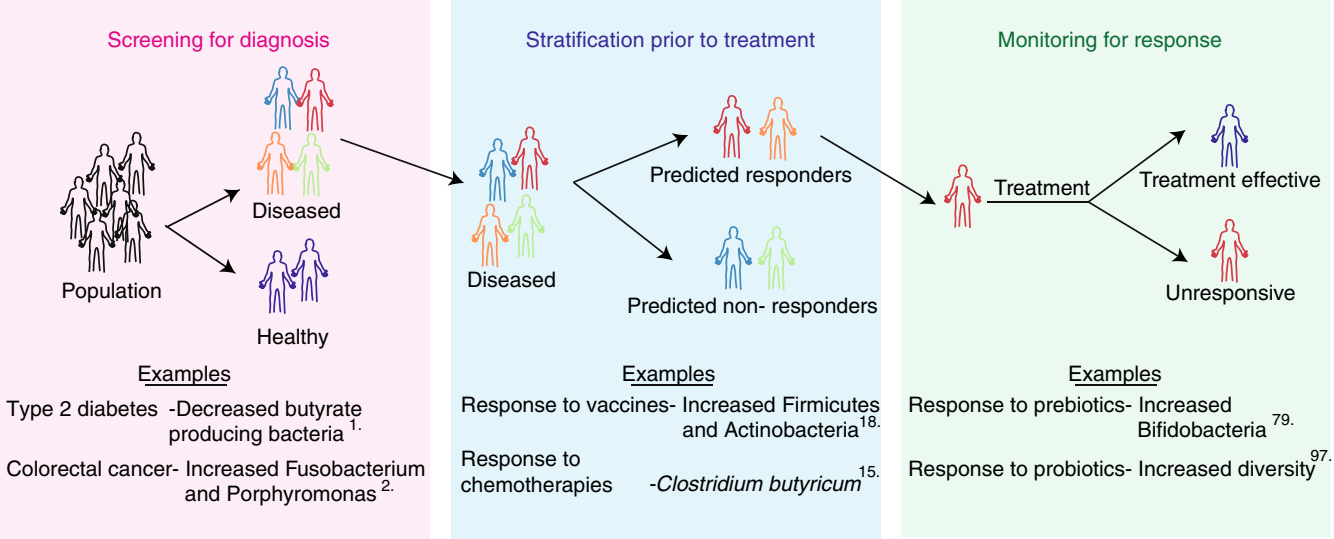

FIGURE 2 Categorisation of microbiome-based biomarkers for disease. Microbiome-based biomarkers can be classed as tools for screening for diagnosis (pink box), stratification prior to treatment (blue box) and monitoring for response to treatment (green box).

long and short-chain fatty acids (SCFAs).[22–24] Although understanding the proportions of certain bacterial phyla within the microbiome may be the first step in predicting a patient's response to a vaccine, a higher-resolution taxonomic classification may be important given species and strain-level functional differences.[25] Determining species and strain-level variation could allow for determination of causation, leading to personalised microbial therapeutic options with increased efficacy.

Improved patient responses to chemotherapies, radiation and immunotherapies have been associated with a more diverse GI microbiome and key bacterial species.

Reducing microbiome diversity through an antibiotic cocktail, prior to chemotherapy with oxaliplatin or cisplatin for subcutaneous lymphoma in a T-cell lymphoma–induced mouse model, reduced the efficacy of both treatments.[26] Furthermore, the depletion of microbiome diversity through antibiotic treatment has been observed to decrease patient response to immune checkpoint inhibitors, such as anti-programmed cell death protein 1 (PD-1) immune checkpoint inhibitor (anti-PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).[27,28] Although the relationship between general loss of diversity and adverse outcomes is clear, identification of key microbes as biomarkers requires a more detailed analysis.

Emerging evidence has identified key relationships between Ruminococcaceae/*Faecalibacterium* strains and *Akkermansia muciniphila* with some immunotherapies,[23] *Clostridium butyricum* with chemotherapies[15] and *Lactobacillus rhamnosus* GG with radiation therapy.[15,16,29] The association of Ruminococcaceae/*Faecalibacterium* strains with improved metastatic melanoma patient outcomes was identified following treatment with anti-PD-1.[14] Furthermore, when *A. muciniphila*, is administered to mice through five oral gavages, treatment efficacy of anti-PD-1 increased, and was associated with increased microbiota diversity with a specific increase in *A. muciniphila*.[28] Similarly, administration of *C. butyricum*, to patients undergoing chemotherapy for lung cancer,[15] and *L. rhamnosus* GG to patients undergoing radiation therapy,[16] have been shown to decrease diarrhoeal incidents and intestinal mucosal disruption. This correlates with a reduction in adverse event related cessation of treatment.[15,16] These results are consistent with murine studies showing administration of *L. rhamnosus* GG is radioprotective through Toll-like Receptor 2 and cyclooxygenase-2 mediated secretion of radioprotective prostaglandin E2, which mitigates intestinal cell damage.[30]

As most of the evidence for the use of microbiota as biomarkers has been identified in murine studies, it important to note, that these biomarkers may not be applicable to humans.[31] Indeed, recent studies suggest that only 2.58% of bacterial species are found in both human and mouse GI microbiomes.[31] Therefore, more research is required to ensure functionally equivalent biomarkers are identified in humans. Despite these challenges, in the near future microbiome-based screening prior to the initiation of some cancer therapies may provide the opportunity to supplement the microbial communities in patients to improve potential responses and outcomes. Similarly, many therapeutic interventions can alter the patient's microbiome composition. This provides the opportunity to develop biomarkers to monitor treatment progression and success. Following treatments for diseases such as irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBDs), the microbiome could be monitored for changes, including increases in diversity or abundance of key species. Thus, biomarkers could be used preceding treatment to determine potential efficacy and post treatment to monitor outcomes.

3 | THERAPEUTICS

Unlike prognostic biomarkers, that may be or show a causal relationship, therapeutic intervention requires a causal relationship between the microbes and disease states. There is increasing evidence

that changes to microbiome composition, specifically diversity loss and a decrease in bacterial load within the GI system, may be associated with a deleterious effect on the host.[32] This phenomenon is commonly referred to as dysbiosis and is correlated with many GI and metabolic diseases, including diarrhoea, *Clostridioides difficile* infection (CDI), IBD and type 2 diabetes.[33–37] Microbiome-based therapies include dietary interventions, prebiotics, probiotics, antibiotics, phage therapy, FMT, live biotherapeutics and microbiome mimetics (Figure 3; Table 1), each aiming to modify the microbiome to treat diseases.

3.1 | Dietary interventions

Diet plays an essential role in health and disease, with intake of dietary fibres from foods, such as whole grains, resistant starch and fruits, being clearly beneficial for the development of a diverse microbiome.[38–40] Alteration of diet has shown to be important for patients with IBS, where it is recommended for some patients to reduce consumption of fermentable oligo-, di-, monosaccharides and polyols (FODMAPs).[41] FODMAPs are poorly digested but readily fermented by the microbiome,[42] thus a low FODMAP diet (LFD) provides the opportunity to leverage nutrient availability to shift the microbiome community. This is achieved by decreasing the amount of gas produced by fermentation, therefore decreasing the associated bloating symptoms.[43–45] LFDs have also been linked to a reduction in the amount of Bifidobacteria present in patients with IBS.[43,46] As such, a long-term LFD is not recommended in patients with IBS, given the potential for long-term health consequences of generating and perpetuating dysbiosis.

Dietary interventions have been extensively explored for the treatment of IBD, with a large variety diets being investigated for uses in either Crohn's disease (CD) or ulcerative colitis (UC).[47] These diets include, exclusive enteral nutrition (EEN),[48,49] Crohn's disease exclusion diet (CDED),[50–52] specific carbohydrate diet,[53] LFD,[54] Mediterranean diet (MD),[55] Crohn's Disease TReatment with EATing diet (CD-TREAT)[56] and partial enteral nutrition.[47] Only two of these diets have proven clinical efficacy in CD treatment and evidence to show their impact on the GI microbiome, EEN[48,49,57] and CDED.[50–52]

Exclusive enteral nutrition involves the replace of all food and beverages with a liquid meal replacement, which can induce remission in 80%–85% of patients with CD.[48] The proposed mechanism of action suggests that inflammatory dietary factors are interacting with the microbiome, host immune system and GI environment.[58] Therefore, removal of these products will decrease inflammation and the associated symptoms of CD.[59] Following the success of EEN, CDED was developed to allow patients to eat whole foods, while restricting the intake of inflammatory dietary factors such as processed foods, gluten, dairy and food additives. Patients on CDED have shown a 70%–75% remission rate,[48,50,51,60] and changes to their microbiome composition have been identified, similar to those seen with EEN.[50,51] Both EEN and CDED broadly identified increases in the abundance of bacteria from the Firmicutes phylum, and a decrease in those from the Proteobacteria and Actinobacteria phyla.[48,50,51,60] More specifically, EEN treatment led to an increase in the abundance of bacteria from the Veillonellaceae family[51] and a decrease the amount of *Faecalibacterium prausnitzii*.[53] Changes at the family or species level have yet to be identified following CDED; however, abundance of bacteria from the Clostridiales class were increased, while those from the Gammaproteobacteria were decreased.[50,51] Currently, the causal relationship between these interventions and microbiota changes are unclear; however, it is clear that EEN and CDED are effective diets for the treatment of CD. Furthermore, CDED exemplifies the ability to produce more targeted therapeutics, which may alter microbiome structure and functions.

3.2 | Prebiotics

Many dietary fibres act as prebiotics, components in food that are used by the microbiome, confer a health benefit, are easily administered, and support numbers of beneficial bacteria.[42,61] Currently, there are five main classes of prebiotics: (1) readily fermentable dietary fibre,[62] (2) phenolics and phytochemicals,[63] (3) human milk oligosaccharides,[64] (4) other oligosaccharides (i.e. fructooligosaccharides

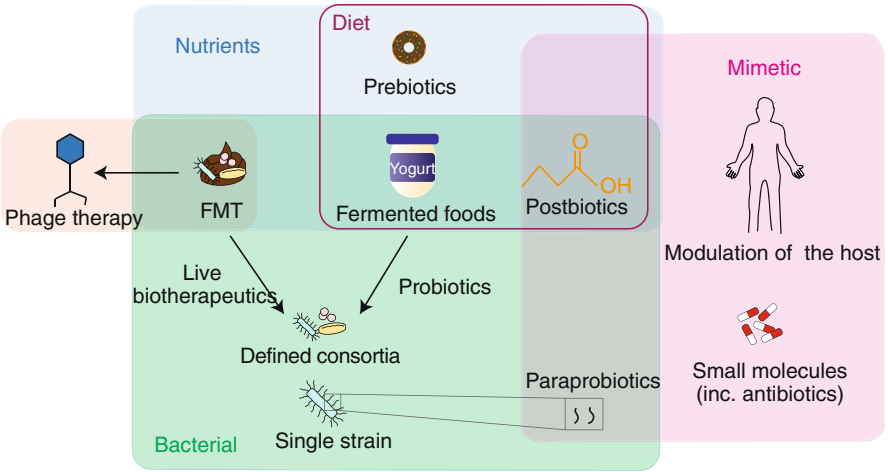

FIGURE 3 Categorisation of microbiome-based therapeutics. Microbiome-based therapeutics can be categorised as nutrients (blue box), bacterial (green box), or microbiome mimetics (pink box). Many therapeutics can be found within the diet (purple box) but are composed of components that are nutrient, bacterial and mimetics.

TABLE 1 The advantages, disadvantages and future direction/ implementations for microbiome-based therapeutics

Therapeutic	Advantages	Disadvantages	Future directions/implications
Dietary interventions	• Safe • Easily manipulated	• Variable components in each food item • Insufficient dose for therapeutic benefit • Temporary therapeutic response	Further work required to identify key components of diet that can be altered to allow for a therapeutic response
Prebiotics	• Safe • Components of food • Easily administered	• Dependent on specific microbe colonisation • Dependent on gut microenvironment • Therapeutic response temporary • Potential adverse responses (e.g. bloating)	Potential in prevention of paediatric immune diseases (e.g. respiratory disease and allergy). Prebiotics should be examined for their treatment of other conditions
Probiotics	• Relatively safe • Readily available as standardised mix	• Not targeted to a disease or patient • Dependent on specific microbe colonisation • Dependent on gut microenvironment • Therapeutic response temporary • Viability not requirement of regulator	Efficacious following antibiotics and in the prevention of NEC. Potential as non-specific treatments to increase bacterial diversity
Synbiotics	• Relatively safe • Includes all components for efficacy	• Therapeutic response temporary • Require a specific gut microenvironment • Potential adverse responses (e.g. post antibiotics)	Efficacious in the treatment of metabolic diseases. Further combinations should be explored for the treatment of other diseases
Antibiotics	• Safe • Cheap • Approved medication • Existing regulatory framework	• Potential off-target effects (antibiotic resistance, disruption of colonisation resistance) • Limited to disruption of the microbiota	Examination for use in targeted microbiome manipulation; however, caution is required to avoid off-target, adverse effects
Phage therapy	• Highly specific	• Limited to disruption of the microbiota • Targets require specific development • Emerging therapy	Examination for use in altering microbiome structure due to their highly specific nature
FMT	• Contains all microbes and nutrients • Proven efficacious for *Clostridioides difficile* treatment	• Donor variability • Requires rigorous pre-screening • Efficacy only seen for some conditions • Some administration costly • Inability to standardise composition	Further work is required to determine causality in FMT treatment. This will allow for FMT to be considered for the treatment of other diseases
Live biotherapeutics	• Approved for specific indications	• Requires maintenance of bacterial viability • Potential adverse long-term health effects • Difficulty determining causal relationship	Determination of causality required to allow for development
Microbiome mimetics	• Not reliant on current microbiome state	• Limited research to develop mimetics	More research required to identify candidates as mimetics and mechanisms of delivery, including diet should be explored

Abbreviations: FMT, faecal microbiota transplant; NEC, necrotising enterocolitis.

[FOS],[65] galactooligosaccharides [GOS][66] and inulin[67]) and (5) Conjugated linoleic acid and polyunsaturated fatty acid.[68–70] Although all the aforementioned prebiotics have been used in disease management, the most well studied are FOS and GOS, which have been associated with an increase in Bifidobacteria within the gut.[42] FOS and GOS have shown efficacy in the management of metabolic diseases, such as prediabetes and obesity[66,71]; and GI diseases, including IBS.[72] However, it has been noted that even small doses of prebiotics can cause side effects such as diarrhoea, bloating and flatulence, which may exacerbate some of the conditions being treated. These side-effects have been attributed to prebiotic-induced osmotic changes in the GI tract or gasses produced from rapid prebiotic fermentation in the small bowel.[73] Thus, highlighting the patient and disease-specific efficacy of prebiotics.

Currently, the prebiotics GOS and fructans, are being routinely administered to newborns through infant formula.[74,75] These prebiotics increase the abundance of bifidobacteria and lactobacilli within the GI microbiome,[74] and this is correlated with a decreased chance of respiratory infections[75,76] and allergic responses.[77] Therefore, GOS and fructans can be used to improve infant outcomes through increasing microbiota diversity; however, many other prebiotics are not as successful.

As observed with other treatments, a subgroup of patients treated with prebiotics will be 'non-responders'. One driver of

patients being 'non-responders' could be the reliance on the patient being colonised with bacteria that are able to metabolise the treatment.[78,79] This observed with the prebiotic fructan, where it is able to increase the amount of bifidobacteria in the GI tract, but this increase is proportional to the amount of bifidobacteria present before treatment.[79] Thus, if a patient only had a small amount of bifidobacteria present before treatment, the increase in abundance would be smaller or non-existent compared with that of a patient who started with a larger amount. Additionally, other factors such as differences in the host and gut microenvironments can also lead to similar variability in treatment response. In this context, the state of the patients' microbiome and gut before treatment has the capacity to impact the efficacy of prebiotics. Therefore, complementing provision of prebiotics to feed the current microbiota population, with therapies that directly provide beneficial bacteria to the gut may improve patient outcomes.

3.3 | Probiotics and synbiotics

The Food and Agriculture Organisation of the United Nations and the World Health Organisation defines probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'.[80] Probiotics have been consumed for at least 10,000 years,[81] where historically they have been components of foods such as yogurt and fermented milk.[82] It was identified that these fermented foods usually contained a mix of lactobacilli, bifidobacteria or other lactic acid producing bacterial strains, which in specific circumstances, can be beneficial.[83,84] This discovery has led to the development of modern probiotics that can be administered in controlled doses of purified, live bacteria.[83,85] Probiotics function by either colonising or being transiently present at a given body site, where they confer a health benefit. These benefits include increasing colonisation resistance by inhibiting the growth and colonisation of pathogens through competition for nutrients[86] and direct killing by antimicrobials such as bacteriocins.[87–89] Bacteria within the lumen are more likely to interact with probiotics than mucosa-associated bacteria; therefore, their use as a treatment in diseases associated with mucosal bacteria such as IBD may be limited.[90] Probiotics can also regulate host innate and adaptive immune functions through interactions with epithelial[91]or dendritic cells.[92,93] These interactions lead to anti-inflammatory immune responses from macrophages and T and B lymphocytes.[91–93] Additionally, they can increase mucin production, thereby improving the integrity of the mucosal barrier in the gut, through the production of SCFAs.[94]

Generally, most probiotics do not colonise the gut.[95] Therefore, where efficacy is dependent on bacterial presence, sustained or repeated dosing may be required for the benefit to be maintained.[96] This has been observed in elderly, human, subjects where those that consumed probiotics for 13.5 years showed greater changes in their abundance of the beneficial bacterial genera *Bifidobacterium* than those limited to 3 years of treatment.[96] However, although a probiotic may not colonise the GI system directly, their transient presence appears to allow for colonisation by other beneficial bacteria, such as *Lachnoclostridium*, *Blautia* and *Clostridium* strains.[97] Similarly, in mice where the microbiota has been disrupted by antibiotics, and probiotics were able to restore microbiome diversity to 99.8% of what was observed pre-treatment, compared with the 80% restoration observed without probiotics.[97] Importantly, the probiotic did not colonise these mice,[97] and it is speculated that these beneficial effects were observed as either the probiotics interacted with the intestinal barrier to increase its integrity, or they interacted with the intestinal epithelium to exert an anti-inflammatory response.[97] Currently, probiotics are considered food products, in most jurisdictions. As such standardisation of viability or efficacy is limited. To combat this, the American Gastroenterological Association[98] has developed guidelines[99] for the use of probiotics in humans. These do not currently support the use of probiotics for the treatment or prevention of CDI, CD, UC or IBD.[98,99] However, preliminary evidence supports the use of probiotics for patients following antibiotic treatment or with pouchitis; however, their use in pouchitis remains controversial.[98,99] Additionally, there is strong evidence for probiotic use in preterm babies with low-birth-weight to prevent necrotising enterocolitis (NEC).[98,99] Conversely, there is moderate evidence against probiotic use for children with acute gastroenteritis.[99] Given the large knowledge gap for the use of probiotics, it is clear that more trials are required to determine if probiotics should be used for each of the above indications.

Probiotics can also be synergistically combined with selected prebiotics and these treatments are called synbiotics.[84] Within synbiotics, there are two sub-groups: synergistic synbiotics, in which the prebiotic acts by providing nutrients for the probiotic and complementary synbiotics, in which the prebiotic component supports the growth and survival of other bacteria already present within the microbiota, known as autochthonous bacteria.[100] For example, within the synergistic synbiotic mix of FOS with *Bifidobacterium longum*, *B. breve* or *B. bifidum*,[101–103] the bifidobacteria preferentially metabolise FOS, thereby increasing the bacterial numbers.[104] Higher amounts of bifidobacteria within the microbiota have been associated with reduced chances of developing antibiotic-associated diarrhoea (AAD),[105] CDI,[106] NEC[107] and a reduction of symptoms associated with IBS.[108] Synbiotic combinations also allow for the development of beneficial cross-feeding networks.[109] This occurs as the synbiotic is metabolised by the probiotic strains and the by-products can be used to cross-feed beneficial bacteria.[109] Cross-feeding has been observed in probiotic strains of *Lactobacillus* where *L. salivarius* W57 cannot fully use inulin-type fructans; however, when co-cultured with *L. paracasei* subsp. *paracasei* W20, an extracellular enzyme from *L. paracasei* allows for the breakdown of the fructan so that it can be used by the *L. salivarius*.[110]

Synbiotic combinations have shown efficacy in humans for the treatment of non-alcoholic fatty liver disease using a combination of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* strains and FOS[111]; IBS using *Bacillus coagulans* and FOS[112]; diarrhoea using *B. lactis* B94 and inulin[113] and type 2 diabetes with *L. sporogenes* and inulin.[114] Type 2 diabetes is a precursor for many other diseases including

polycystic ovary syndrome and cardiovascular disease, therefore synbiotic treatments successfully targeting type 2 diabetes may also be delaying the progression of other metabolic diseases.

As seen with prebiotics, recipients of probiotics and synbiotics include cohorts of 'non-responders', due to factors such as the timing of treatment, disease progression and composition of the microbiome before treatment.[115] Although the bacterial strains found in probiotics and synbiotics can be effective in the management of some diseases, these strains are unable to treat all diseases associated with microbiome composition and can be harmful in some conditions, including following antibiotic treatment.[116] Finally, the transient nature of probiotic treatment may be insufficient in diseases that require a more permanent and substantial restructuring of the bacterial community.

3.4 | Antibiotics

Since the discovery of penicillin in 1928[117] and its approval for clinical use in 1945, many antibiotics have been discovered and successfully used to treat bacterial infections. Antibiotics are also commonly administered prophylactically to decrease the risk of post-operative infections.[118] More recently, the administration of antibiotics prior to delivering microbial therapies has been shown to enhance the efficacy of the treatment in some settings, possibly by opening ecological niches and enabling colonisation.[119,120] Although these approaches offer potential, the antibiotic-associated decrease in bacterial number and diversity may also reduce colonisation resistance[121] or induce other pathologies.[122] Pathogenic strains, including multi-drug-resistant *C. difficile*,[123] *Escherichia coli*, *Enterococcus feacium* and *Klebsiella pneumoniae*[124] can exploit this niche and infect the gut causing AAD. These diseases can be exacerbated by further antibiotic use, which can lead to the development of resistance. In particular, antibiotic-resistant 'super-bugs' can develop, leading to untreatable infections in patients.[125] Although largely unexplored, microbiota modification through selective administration of antibiotics represents a potential mechanism for targeted community change. However, such interventions will require careful design to minimise off-target or adverse effects but may form a critical component of future microbiome-based therapies.

3.5 | Phage therapy

Bacteriophages (phages), viruses that exclusively infect bacterial cells, have been used as antibacterial monotherapy for the treatment of bacterial infections for over 100 years.[126] Clinical trials of phage cocktails targeting difficult to treat *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections[127] and multi-drug-resistant bacterial infections including methicillin-resistant *S. aureus*[128] and *Acinetobacter baumannii*[129] have shown substantial promise. Within faecal transplants, phage have been shown to colonise the host following treatment[130] and may be responsible for a proportion of the beneficial effects achieved. It has been postulated that the efficacy of the sterile filtrate of FMT demonstrated in a small cohort of patients with CDI may have been partly attributed to phage.[131] The narrow host range typical of phage[132] provides an opportunity for targeted bacterial depletion and microbiota remodelling as a therapeutic intervention. Coupled with broad-range antibiotic treatment, these therapies allow for highly controlled microbiome disruption.

3.6 | Faecal microbiota transplantation

In contrast to antibiotics and phage therapies, FMT can restore bacterial diversity and health-associated functions such as colonisation resistance by introducing a faecal-associated microbiota from a healthy individual.[133] FMT also provides a diversity of bioactive compounds, and other microbes, such as phage. Together these components are a functioning, synbiotic community, which allows for better colonisation within the GI tract.[134]

Traditionally, FMT had medicinal use in ancient China and Indigenous Australian culture,[135] however, its potential as a modern medicine was only identified within the last 20–30 years.[136–138] For the treatment of recurrent CDI, FMT has proved highly effective[136,139,140] with efficacy of 85%–95% reported.[141,142] It is thought that the increased diversity and abundance of bacteria that the FMT provides to the GI tract outcompetes *C. difficile* and prevents reinfection. As FMT has been successful in the treatment of CDI, it is now being examined for efficacy in other GI diseases.

Faecal microbiota transplant has shown promise in the treatment of non-GI diseases, including insulin resistance, liver disease and autism spectrum disorders,[143–145] and has been used successfully to induce remission in UC.[146–148] Meta-analysis has shown that multiple forms of FMT administration can induce remission in UC[149]; however, this is not the case for all diseases, which has led to a call for standardised methods of preparation or administration.[150] Until recently most faecal transplants were prepared in aerobic conditions, leading to the loss of many obligate anaerobic bacteria.[151–153] Studies have now shown that many of the bacteria correlated with a positive treatment outcome, particularly in patients with UC, are obligate anaerobes such as *F. prausnitzi*, which can be preserved if the treatment is prepared anaerobically.[146,154] It is unclear whether anaerobic stool processing confers a clinical benefit relative to aerobic processing; however, there are advantages for microbial drug discovery using anaerobic stool processing in FMT clinical trials. Currently, FMT can be administered to the lower GI tract, by colonoscopy or enema, or the upper GI tract, through gastroscope, or nasogastric, nasojejunal or gastrostomy tube, with each method using varied doses, and frequencies of administration.[155–157] Faecal microbiota transplant delivery via colonoscopy in the lower GI tract has been the most effective (86% success rate, compared with 74% success rate for upper GI tract delivery),[158] particularly in the treatment of CDI.[159] Unfortunately, colonoscopies are relatively invasive procedures and cannot be performed on all patients due to risks such as bowel perforation in groups such as the elderly or critically ill.[160]

Administration through the upper GI tract has shown higher rates of adverse events with multiple reports of aspiration pneumonia.[161] To overcome these issues, substantial effort developing encapsulation methods for oral delivery has been undertaken. Specialised acid-resistant hypromellose capsules have been used, which allow for colonic release of the bacteria and protection from the gastric environment.[162,163] However, is it possible that exposure to the GI environment could enhance colonisation efficiency. In addition, lyophilisation of donor stool allows greater stability of the FMT within the capsule. These capsule-based approaches have shown high rates of clinical success.[164,165] Despite this success, a small number of serious complications, including bacteraemia and transient UC flares, have been observed and one death due to infection with an extended spectrum beta-lactamase (ESBL)-producing *E. coli* has been noted with capsule delivered FMT.[166] Therefore, further screening of capsule FMT preparation may be necessary prior to large scale adoption.

Faecal microbiota transplant is remarkably safe. Effective donor and sample screening to prevent transfer of detrimental bacteria including, multi-drug-resistant pathogens or other detrimental species is performed. While relatively harmless within the donor, these species may be harmful for an immunocompromised or otherwise susceptible recipient. Introduction of multi-drug-resistant bacteria, such as ESBL-producing *E. coli*, has resulted in fatal, untreatable sepsis.[167] While screening for pathogens is routine, and should effectively eliminate the risk, identifying patient-specific detrimental bacteria is substantially more challenging.

Screening samples prior to delivery highlights the substantial donor-specific variability, which can alter efficacy, allowing for higher rates of treatment success by some donors termed 'super donors'.[168] This phenomenon was first postulated in a clinical trial testing the efficacy of FMT in inducing UC remission, where 7/9 patients who entered remission received FMT from the same donor.[152] It has been suggested that some donors may be associated with higher efficacy due to having higher bacterial diversity or specific bacteria that are therapeutic for a given disease.[168] It should be noted, studies of 1999 FMTs used to treat CDI have failed to identify evidence of 'super donors',[169] highlighting the disease specificity of this relationship. Conditions where 'super donors' have been identified, highlight the possibility of donor–patient compatibility and the opportunity for refining microbial therapeutic treatments to contain only the bacterial strains required to induce health benefits.[170]

3.7 | Live biotherapeutics

To provide more targeted intervention and overcome the risks associated with pathogen or pathobiont transfer, significant research and development effort has been focused on determining bacterial strains that could be used as therapeutics.[171] Termed, live biotherapeutics, these therapies have been defined by the FDA as 'a biological product that (1) contains live organisms, such as bacteria; (2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings and (3) is not a vaccine'.[172] These are distinct from probiotics as they are microbes that may colonise the gut and have an established clinical benefit for the treatment of a specific disease.[173] Live biotherapeutics may be comprised of a single bacterial species or selected combinations that act synergistically.

To date, very few studies have determined direct causation between bacterial species and disease,[174] due to the complexity of microbiome interactions and limitations of existing experimental models. In the case of CDIs, through both murine and clinical studies in humans, researchers have identified *C. scindens* as being inversely correlated with the establishment of CDI.[175] It was consequently found that the administration of *C. scindens* can reduce *C. difficile* bacterial load in mice[174,175] through dehydroxylation of bile acid, which produces a toxic by-product to *C. difficile*.[174] Although *C. scindens* reduced *C. difficile* levels, colonisation resistance was not restored.[174] However, two studies in mice have identified consortiums of four[175] and six[176] bacterial strains that were able to increase resistance to CDI, with the consortium of six bacterial isolates able to prevent recurrent infection.[176] These studies demonstrate the potential for specifically chosen live biotherapeutics to colonise the gut and provide beneficial health outcomes.

Researchers began developing a more specific treatment for UC using Firmicutes spores derived from ethanol shocked human donor stool, termed SER-287.[120] This treatment can induce remission in patients and is thought to be superior to FMT as there is less risk of introducing harmful bacteria into patients.[120] It is proposed that SER-287 is effective because the ratio of metabolites within the gut is observed to change following treatment; however, a direct causation has not been identified.[120] Unfortunately, SER-287 failed to meet its primary endpoint in a phase 2b clinical trial, and the product is no longer in development with Seres Therapeutics.

As most biotherapeutic development is in its early stages, very little is known about whether long-term persistent colonisation with these therapies may impact health or chronic conditions. In particular, live biotherapeutics are being developed to treat paediatric conditions, such as paediatric IBD,[177] so it is important to evaluate not only the effect of the bacteria but the dosage, treatment frequency and delivery mode to ensure the safest treatment that will have the least adverse health outcomes later in life. These concerns are one of the reasons that researchers are now also considering microbiome mimetics.

3.8 | Microbiome mimetics

Microbiome mimetics describes any intervention that replicates the interaction between the microbiome and the host, that yields a therapeutically beneficial outcome. This can include bacterial derived products, small molecules, conventional therapeutics or host derived products. The majority of research has focused on postbiotics, which are molecules or components of bacteria that confer a health benefit.[178] Within postbiotics there are two main classes: paraprobiotics and fermented infant formulas (FIFs). Paraprobiotics

are non-viable components of bacteria, including bacterial proteins and polysaccharides.[179] Meanwhile, FIFs are the purified products produced after infant formula is fermented by bacteria.[180] Research into these products is currently aimed at determining which bacterial molecules provide health benefits. This can be achieved through mass spectrometry of bacterial supernatants and targeted purification of these metabolites for use. The most prominent candidate for use as postbiotics are SCFAs.[181] Short-chain fatty acids are compounds such as butyrate, propionate and acetate, which are produced by bacterial fermentation of prebiotic or dietary fibre, and resistant starches and are the primary energy source of colonocytes.[182] Additionally, SCFAs have beneficial effects on the mucosal immune system,[183,184] including anti-inflammatory effects through blocking inflammatory cytokine production, increasing mucus production, promoting immune tolerance through regulatory T (T_{reg}) cells, and promoting tissue repair.[185–187] While patients with UC have decreased SCFA levels,[188] trials of butyrate enemas have been unsuccessful, likely due to the dysfunction of colonocytes and their reduced ability metabolise butyrate as an energy source in this disease.[189] SCFAs also increase gut mucin production, decreasing the 'leaky gut' syndrome in human patients with type 1 diabetes mellitus.[190] Consequently, SCFAs have shown efficacy in delaying the onset of type 1 diabetes mellitus in mice.[191] Therefore, although postbiotics may be used to manage and treat disease without requiring patients to have particular bacterial species present in their microbiome, they may still require their immune system to be primed for an effective response. Interestingly, postbiotics such as SCFAs can also be found in fermented foods such as cheese and yogurt, and beverages such as beer and kombucha. Postbiotics occur at lower doses in these foods; therefore, it is hypothesised that the higher doses used in postbiotics would likely be required for therapeutic efficacy.[192–194] Therefore, future microbiome mimetic treatments may incorporate dietary interventions targeted to replicate the beneficial effects provided by the microbiota.

4 | FROM THE LABORATORY TO THE CLINIC

Advances in sequencing technology have revolutionised the study of the microbiome; however, identifying key bacterial species involved in health and disease remains a challenge. Despite the prevalence of microbiome sampling and evidence suggesting that storage time, temperature and storage medium can affect the bacterial strains,[195] there remains no standardised method for sample collection, storage, data analysis or dosage calculation.[196–198]

For many microbiome-based medicines, preclinical safety testing may not be required as the therapeutic may already be approved for use in humans by the FDA or other stringent regulatory authorities. These include commonly consumed foods that may contain prebiotics, probiotics or postbiotics and other medicines that have been repurposed as microbiome mimetics. Importantly, disease-specific testing of therapeutic efficacy is still required. Preclinical testing and safety profiles of FMT are now well established, although the classification and regulation of the treatment varies from a stringently regulated biological agent in some countries (USA, Canada, Australia), to a medicinal product or treatment with variable regulation (UK, France, Germany, Switzerland), to no regulation (Austria, Denmark, Sweden, Finland).[199,200]

To improve selection of therapeutic candidates it is necessary to establish acceptable methods for candidate prioritisation and preclinical safety and validation testing. Although animal models can play an important role in preliminary safety testing, limitations of mouse models in replicating human microbiome interactions introduce a unique difficulty in validating microbiome-based medicines.[201] To address these concerns, gnotobiotic mice with defined microbiome have been used.[202–204] The use of gnotobiotic mice, with a human microbiome for example, have enabled the identification of a T-cell response integral to the success of FMT, that is associated with increased bacterial abundance in the gut.[205] Many groups have also used human immortalised cell-culture methods for safety or validation testing,[206] but these methods do not allow replication of the complex physical and mechanistic interactions between the microbiome and host. As a result, cell-based models, such as organoids and organ-on-a-chip technologies, are emerging as an important component for developing novel microbiome-based therapeutics.

Organoids are enclosed, three-dimensional (3D) cultures that mimic the multicellular structure from the corresponding tissue.[207] Methods now exist to establish and maintain patient-derived GI organoids, which partially recapitulate the environment that the microbiota normally inhabits and provide the opportunity for a 'personalised' microbiome cell culture model.[208,209] Microinjection technology allows for bacteria to be introduced into the lumen of gut organoids to investigate microbiome–host GI epithelial interactions.[210,211] Unfortunately, organoids still contain no stroma or vasculature, limiting the capacity to infer microbiome–host immune interactions beyond those in the epithelium. To overcome this, some studies have used induced pluripotent stem cells to generate organoids containing mesenchymal stem cells,[212] and others have cultured organoids with supporting mesenchymal and/or immune cells.[213,214] However, as organoids are enclosed 3D structures, the bacteria within the system are trapped, which leads to the build-up of detrimental metabolites and other cellular debris that may impact bacterial replication or modify host cell responses.[215] Therefore, when using this technology to investigate novel intervention strategies such as live biotherapeutics, determining bacterial colonisation or transience, host physiology and immune responses following treatment is not possible. These limitations have been addressed with the development of organ-on-a-chip technology.

Organ-on-a-chip is a method that combines microfluidics and cell culture to generate mini human organs on a chip. To date, researchers have modelled brain,[216] lung,[217] heart,[218] skin[219] and the GI tract or gut-on-a-chip systems.[220] Microfluidic channels in these systems enable optimal fluid flow and cyclic mechanical strain on cells to mimic peristalsis.[221] These systems replicate the GI tract as they generate villi-like structures and exist in two compartments

with media perfusion and an oxygen gradient. The upper epithelial layer can be maintained anaerobically, whereas the endothelial layer containing immune cells can be cultured in an aerobic environment. Gut-on-a-chip can include complex cell types such as immortalised cell lines and primary tissues, similar to organoids.[220] Through the use of gut-on-a-chip, microbiome researchers have demonstrated the interactions of bacterial cells, including pathogenic *Shigella*[222] and *E. coli* strains,[220] and probiotic *Lactobacillus* strains,[220] with not only the epithelium, but also immune cells.[220] Other work has examined specific bacterial consortia and the whole microbiome of an individual in this context.[220,222–225] Although some immune cells can be added and the innate immune response to a therapeutic can be examined, gut-on-a-chip cannot replicate complex, adaptive immune responses, which are generated systemically and over longer time periods in response to a stimulus. Further work is required to refine this emerging platform to comprehensively assess host-microbe responses and test developing medicines. With this collection of current technologies, preclinical testing of microbiome-based medicines remains dependent on a combination of animal models and cell-based systems to demonstrate efficacy and safety in the preclinical setting. Following this pre-clinical testing, each therapeutic will need to be examined under specific clinical settings, to confirm these findings and determine efficacy in humans.

5 | CONCLUSION

Microbiome-based medicines have advanced dramatically over the last decade, from prebiotics and probiotics, to live biotherapeutics and microbiome mimetics. The ability to culture the GI bacteria and new applications of metagenomic sequencing have overcome many of the previous technical hurdles in this area. The primary challenge now faced is identifying clinical diseases amenable to intervention with microbiome-based medicines and developing appropriate methods to identify, refine and test candidate therapies. Although much progress has been made, further work to optimise methods to identify candidate microbes, develop appropriate preclinical validation models, and progress to personalised targeting of microbiome-based medicines are the essential next steps.

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DATA AVAILABILITY STATEMENT

No datasets were generated or analysed as part of this study.

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