The role of healthcare professionals in addressing drug-microbiome interactions

How will emerging drug-microbiome relationships change drug development and clinical practice?

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Abstract

Growing evidence is highlighting the potentially significant impact of drug-microbiome interactions on patient care. It is possible that hundreds of drugs alter the composition of the microbiome, including many drugs with non-microbial targets. Drug-induced alteration of the microbiome could increase patients' risk of dysbiosis, a state of microbiome unbalance that increases the chance of disease. Further, drugs' pharmacokinetics and pharmacodynamics can be altered by the microbiome via direct (e.g., biotransformation, bioaccumulation) and indirect processes. Though these interactions are potentially important for patients' health and therapeutic success, they are rarely considered in the clinic or during drug development. Healthcare professionals working in numerous roles have the opportunity to consider drug-microbiome interactions for the ultimate improvement of patient outcomes. This review provides a timely update on recent evidence relating to drug-microbiome interactions, and describes how professionals working in clinical settings, academia, policy, and drug development can immediately begin to address drug-microbiome interactions in their roles.

Keywords

Microbiota; drug-microbiome relationship; biotechnology and biopharmaceutics; antimicrobial stewardship; future pharmacist roles; drug discovery and development; pharmaceutical industry.

Introduction

Emerging evidence revealing important interactions between the microbiome, drugs, and human health could significantly alter the way that medicines are developed and prescribed in coming years. It is now recognised that drugs and the microbiome can have bidirectional relationships: drugs may affect microbiome composition, possibly leading to changes in host health, and the microbiome can correspondingly affect drugs, possibly promoting pharmacokinetic (PK) and/or pharmacodynamic (PD) variability between and within individuals [1]. As early as 2010, the role of the microbiome in modulating PK and PD has been defined as pharmacomicrobiomics, which represents a growing field of research working to characterise and even predict drug-microbiome interactions [2,3]. Similarly, the fields of pharmacometabolomics and pharmacometabonomics describe the measurement and analysis, respectively, of how metabolites (in this case, microbial metabolites) affect drug response [4]. The activities of the microbiota across the human body, from its metabolic functions to effects on the host immune system and transcriptome, can have significant impacts on patients' dose requirements and even experience of toxicity [5,6]. Moreover, microbiome functioning may be altered by medications in a patient-specific manner due to the uniqueness of the microbiome composition [7,8]. Based on increasing evidence that drug-microbiome interactions can have clinically relevant effects on patient outcomes, it is important that healthcare professionals are aware of the findings, and where possible account for them in their work.

Drug-microbiome relationships are relevant to all roles within the development and administration of medicines, including patient-facing clinicians, industry professionals, policy advisers, and academic researchers. That said, assessment of such interactions does not typically feature in formal training or clinical guidelines. This may be due to the recentness of many findings and the large body of research remaining to be elucidated. Despite this, there are numerous ways in which professionals can immediately begin to consider the microbiome in their roles. The second part of this review will focus on how professionals can ensure optimal management of drug-microbiome interactions when interacting with patients, managing the prescription of medicines, conducting research, educating others, and developing new treatments (Figure 1). As a background, an overview of the microbiome will first be provided with respect to its relationships with human health and interactions with medicines. Key topics will include an up-to-date outline of documented pharmacomicrobiomic interactions; the effects of drugs on microbiome composition; the feasibility of basing clinical decisions on emerging evidence, and the benefits of screening for interactions in the pre-clinical phases of drug development.

Figure 1. Current ways healthcare professionals can consider drug-microbiome relationships in their work.

The microbiome and its relationships with human health

The human microbiome is composed of trillions of microorganisms, including the living microbiota (such as bacteria, fungi, and archaea) and non-living elements (such as eukaryotic viruses, phages, and plasmids) [9-11]. Microorganisms can be found on nearly all surfaces of the human body, such as the skin, lungs, oral cavity, and reproductive organs (Figure 2). The densest and most varied populations of microbes are found in the intestines, particularly the colon [12]. In the colon, there are up to 1 billion bacteria alone per mL content, each a member of up to a thousand possible species [13-15]. Within these species, there is also strain-level variation whereby microorganisms classed as belonging to the same species still differ genetically, often leading to functional differences; these differences can now be identified and investigated due to advances in 'omics technologies, such as shotgun metagenomic sequencing [16]. In the intestines, the gut microbiota plays vital roles in the digestion of macronutrients (such as fibre); the production of vitamins and hormones; and the regulation of immune function and gut epithelium integrity [17-19]. Elsewhere in the body, symbiotic microorganisms have a plethora of functions, including defence against sexually-transmitted infections [20], skin regeneration [21], and protection against dental caries [22].

Over a lifetime, the microbiome composition changes in response to ageing and various external factors such as diet, lifestyle, geography, cohabitation, and medication use [9,23,24]. Such changes

can occur slowly, over years, or much more rapidly, over hours to days [25]. To a lesser extent, host genetics can also play a role in microbiome composition, as shown for the gut microbiome in twin studies [26]. Due to the complexity of the microbiome, and the many factors that shape it, the microorganisms that inhabit individuals are recognised to be as unique as a fingerprint [27].

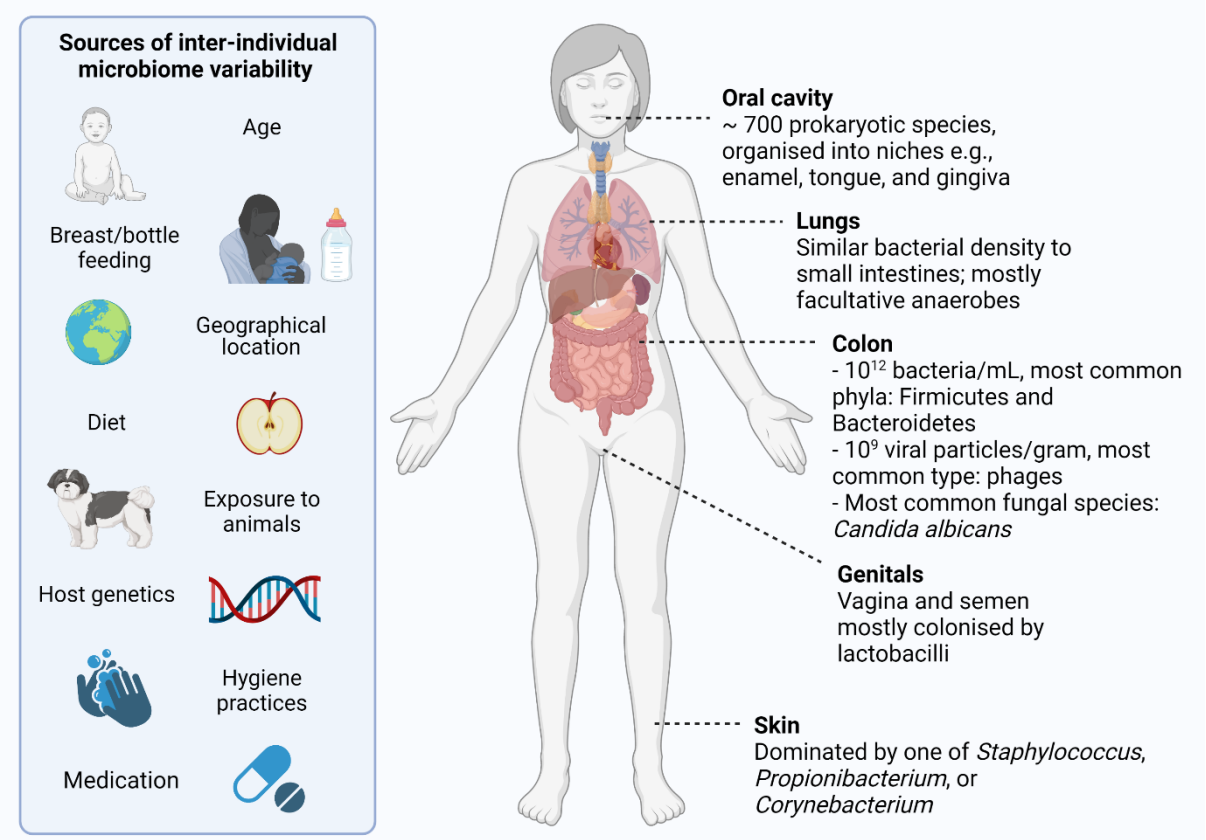

Figure 2. Significant elements of the microbiome at various body sites and sources of inter-individual microbiome variability [9,11,28-31].

Gut bacteria alone may encode for up to 150 times more genes than their human hosts [14]. Driven by this genomic power, the microbiome is increasingly recognised as playing an intrinsic role in health [32,33]. Imbalance in the gastrointestinal microbiome, whereby species with pathogenic traits multiply to unhealthy concentrations, is a well-known source of disease. *Helicobacter pylori* colonisation of the stomach and duodenum increases individuals' risk of ulcers and cancer due to chronic mucosal inflammation [34]. In addition, *Clostridioides difficile* (formerly *Clostridium difficile*), a bacterium that inhabits the colons of up to 90% of patients, can multiply to dangerous levels and produce toxins, often causing debilitating diarrhoea that can lead to colitis, sepsis, and death [35]. The term 'dysbiosis' describes a state in which microbiome composition is imbalanced. Due to imbalance in the number and types of microorganisms present at any particular body site, the microbiome can cease to perform its symbiotic functions and disease may ensue. Well documented

examples of diseases that may be triggered or worsened by dysbiosis include metabolic syndrome; autoimmune dysfunction; Parkinson's disease; and autism spectrum disorder [36-38]. Medication use can also be a significant trigger for dysbiosis [39,40]. Whilst alteration of microbiome composition may form part of a drug's therapeutic action, in other cases, medications can negatively impact the growth or functions of the gut microbiota. *C. difficile* infection is a prominent example of drug-induced dysbiosis, as the disease is often instigated by the administration of broad-spectrum antibiotics [41]. In such cases, antibiotics substantially reduce intestinal bacterial diversity allowing the overgrowth of *C. difficile*. Whilst antibiotic-induced *C. difficile* infection is a widely recognised condition, there are numerous other examples of microbiome modulating drugs that are far less acknowledged, several of which will be discussed in subsequent sections [39].

The effect of drugs on the microbiome

Antimicrobials

Understandably, drugs aimed at reducing microbial growth can exert ruinous effects on the microbiome. Though intended to eradicate pathogenic microorganisms, antimicrobials administered via all routes are typically indiscriminate: killing or preventing the growth of both commensal and pathogenic species [42]. Antimicrobials of all types (antibiotics, antifungals, and antivirals) have the propensity to disrupt microbiome composition [39]. Most research to date has focused on the effects of antibiotics on the gut microbiome [43,44]. The extent to which an antimicrobial will affect the microbiome will depend on the drug, dosing, and patient characteristics [45]. Antibiotic-induced gut microbiome changes can vary widely between and within individuals, depending on their age, sex, microbiome stability, and previous exposure to antimicrobials [25,46]. A study examining 12,422 full-term neonates found an association between antibiotic exposure during the first days after birth and higher body mass index (both sexes) and impaired weight and height gain (boys only), during the first 6 years of life [47]. These effects were attributed to significant alterations in the stool microbiome composition of a patient subset followed for 24 months after birth. Though these observations cannot amount to proof of causation, transfer of neonates' faeces to germ-free mice did produce similar results, i.e., male mice administered faeces from antibiotic treated neonates had reduced weight gain compared to non-antibiotic treated controls. In adults, antibiotics can exert many effects on the gut microbiome, leading to depletion of vitamin-producing bacteria, reduced bacterial diversity, and possibly increased risk of infection, inappropriate immune activity, or altered digestion [44]. Antibiotic-induced dysbiosis can be longstanding, persisting years after treatment: gut bacterial diversity may be lowered for up to 4 years following prescription of macrolides and lincosamides, and a year for beta-lactams and quinolones [40]. Additionally, these antimicrobial-

induced changes may alter patients' response to other pharmaceuticals. For example, antibiotics with considerable activity against intestinal *Bacteroides fragilis* have been found to increase bleeding risk in patients coadministered warfarin [48]. The bioavailability of the antipsychotic olanzapine may also be increased by antibiotic administration [49]. Additionally, the use of broad-spectrum antibiotics may alter immune response to vaccines; patients with low pre-existing antibodies against the H1N1 virus have exhibited impaired vaccine responses following a course of multiple oral antibiotics [50].

Human-targeted drugs

It may be surprising that drugs with human targets, i.e., with no intended antimicrobial action, can have multifarious effects on the microbiome. Many human targeted medicines, including oral and parenteral formulations, may alter microbiome composition and could impart wide-ranging consequences for patients. The possible scale of these effects can be appreciated from the results obtained in a 2018 study by Maier et al., which incubated over 1,000 drugs with 40 strains of gut bacteria and measured drug effects on microbial growth. The *in vitro* experiment found that 24% of human-targeted drugs significantly impaired the growth of at least one of the strains, with calcium channel blockers, antipsychotics, and antineoplastics producing the highest anti-bacterial activity across all tested species. The findings from this work could point towards widespread and unexpected effects of medicines on patients' gut microbiome composition, however they should be taken in context of the experimental limitations. Firstly, bacteria were grown as monocultures, i.e., not in the presence of other microorganisms usually found in the intestines. It is known that bacteria function differently when growing within microbial communities compared to when alone, for example due to cross-feeding and competition from other microbes [51]. Therefore, the results produced by Maier et al. may not be a totally accurate description of species' response to drug exposure in an intestinal community. Secondly, drug-bacteria incubations were measured for 16 - 24 hours. In the intestinal environment, it is likely that most drugs would be absorbed into systemic circulation long before the study endpoint, therefore they may not have time to exert the same anti-bacterial effects observed in the study.

Several other researchers have now investigated the effects of drugs on the gut microbiome *in vivo*, providing data that are perhaps more clinically relevant than the high throughput *in vitro* screen conducted by Maier et al. [52]. For example, administration of atypical antipsychotics has been shown to significantly alter abundance of bacterial species associated with health (i.e., *Akkermansia muciniphila*) in adults of both sexes, and decrease bacterial diversity in only females, possibly indicating why females are more at risk of antipsychotic-induced weight gain [53]. Interestingly, whilst drug-microbiome effects could lead to dysbiosis, they could also form part of a drug's

mechanism of action. For instance, oral metformin administration has been found to increase the abundance of *A. muciniphila* and other beneficial species, possibly accounting for a portion of the drug's positive effects on host metabolism [54]. In addition, statins seem to be protective against a gut microbiome composition that has been associated with systemic inflammation and obesity [55]. Further examples of drugs revealed to alter the gut microbiome in human or animal studies are fluoxetine, proton pump inhibitors, methotrexate, paracetamol, opioids, and inhaled anticholinergics [56-59]. Whilst these findings are interesting and uncover real alterations in microbiome structure, it is important to recognise that most current work reveals correlations between drugs and the microbiome rather than delineated mechanisms and causation. As such, to fully appreciate drugs' effects on the microbiome it is necessary to explore the *in vivo* interactions taking place, and their clinical impact on patients. Technologies that may be useful for such investigations are causal inference networks, transcriptomics, proteomics, and metabolomics [60].

The effect of the microbiome on drugs: pharmacomicrobiomics

Direct drug depletion by the microbiota

Humans have evolved alongside their microbiomes, thus have come to rely on resident microorganisms to perform a myriad of activities they cannot, from the digestion and absorption of food components to the detoxification of ingested poisons [17,61]. As such, the microbiota possesses considerable metabolic power. Most researched are the metabolic activities of gut bacteria, whose reactive potential has been compared to that of the liver [62]. Bacteria residing within the intestines produce enzymes that can chemically transform drugs, potentially altering PK and PD. In addition, certain strains of gut bacteria have recently been shown to accumulate drugs, in a process whereby the drug molecule is stored within the bacterial cell but not chemically modified [63,64]. Similar to biotransformation, bioaccumulation may decrease drug bioavailability and thus result in changes to PK. Together, drug transformation and accumulation represent the two methods by which the microbiota can deplete drug concentration within the intestinal lumen (Figure 3).

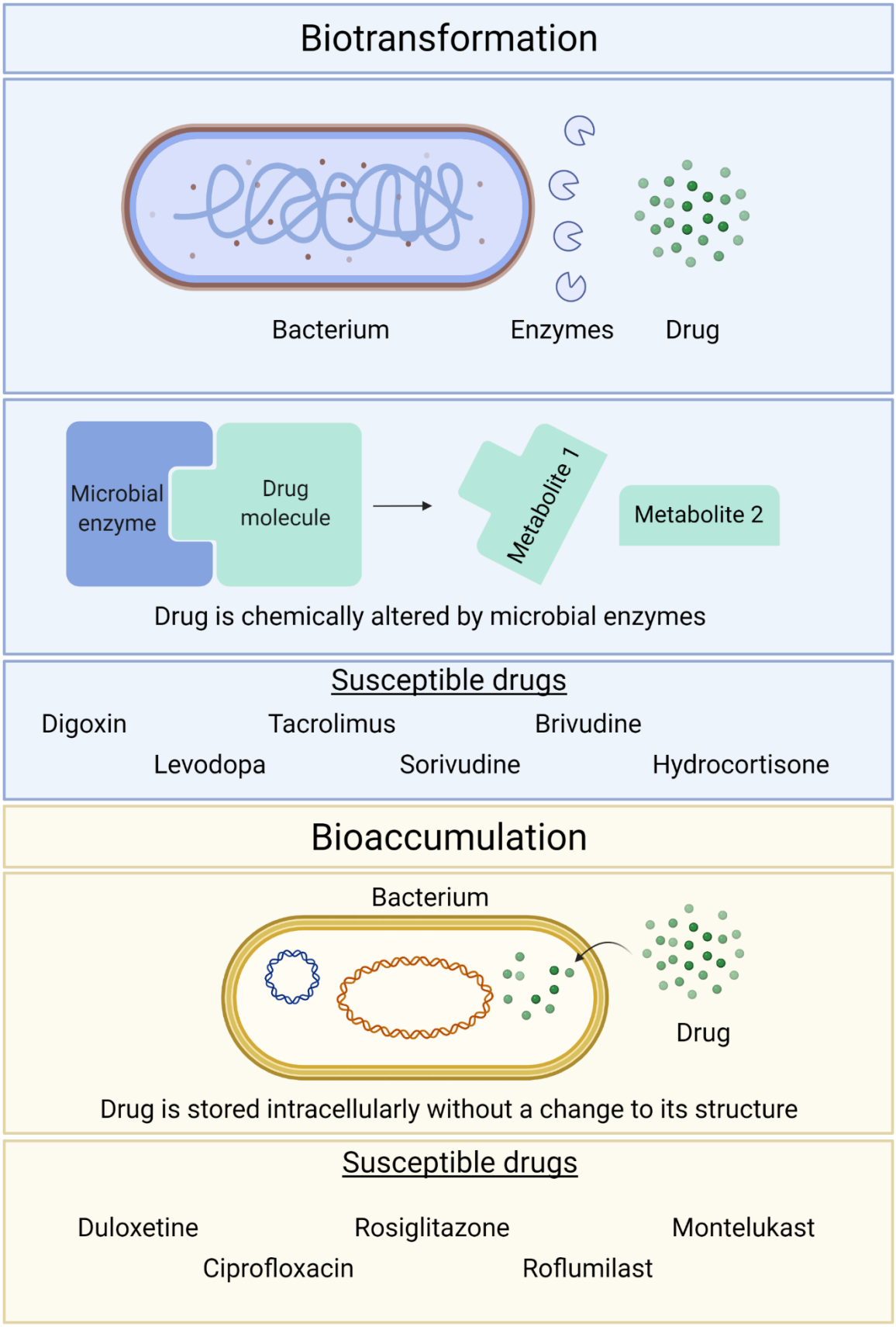

Figure 3. A summary of biotransformation and bioaccumulation: the two methods by which the microbiota can directly deplete drug concentration within the intestinal lumen. Examples of drugs

susceptible to both types of interactions are given; this is not an exhaustive list but an indication of the different types of drugs that can be affected [63-70].

Biotransformation has been reported as early as the 1930s, with the discovery that an early sulphonamide antibiotic (prontosil) was structurally activated by gut bacteria [71]. However, most biotransformation reactions have only been revealed in the last two decades [70,72-76]. Two recent high throughput *in vitro* screens have found over 150 drugs to be significantly transformed by the gut microbiota [70,76]. Drugs containing urea, azo, nitro, and lactone groups were demonstrated to be particularly susceptible [76]. Further, analysis of inter-individual variability found several drugs (ketoprofen, levonorgestrel, lovastatin, hydrocortisone, and nicardipine) to be variably metabolised by the gut microbiota of 20 individuals [70]. Though not all these in vitro findings will translate to measurements in humans, a few drugs do now have validated microbial metabolism in human studies. For example, jejunal *Enterococcus faecalis* producing tyrosine decarboxylases can convert levodopa to dopamine, which can be subsequently converted to m-tyramine by dehydroxylase-producing *Eggerthella lenta* [66]. The presence of these bacterial enzymes predicts levodopa metabolism in Parkinson's disease patients, leading to increased dosing requirements [77]. Similarly, kidney transplant patients with high stool abundances of *Faecalibacterium prausnitzii* have been observed to require higher doses of tacrolimus, due to the drug's biotransformation into a metabolite with 15-fold lower immunosuppressant activity [5,67]. In some cases biotransformation may not only alter a drug's PK, but could alter PD, as microbial drug metabolites could exert unexpected physiological activities. An infamous example of such an occurrence is the antiviral sorivudine, which was found to undergo microbial hydrolysis to bromovinyluracil and subsequent host metabolism to an inhibitor of dihydropyrimidine dehydrogenase, a hepatic enzyme [68]. Tragically, this reaction was only identified after the death of 18 oncology patients in 1993, who were coadministered sorivudine and 5-fluorouracil (5-FU). Here, the inhibition of dihydropyrimidine dehydrogenase led to the accumulation of 5-FU in patients, resulting in diarrhoea, pancytopenia, and eventual death. This tragedy highlights the importance of screening for drug-microbiome interactions well before drugs are administered to patients.

In comparison to biotransformation, there is much less evidence regarding the microbial accumulation of drugs. A study by Klünemann et al. recently published 17 cases of bioaccumulation, which were measured by incubating 12 small molecule drugs with 25 strains of gut bacteria (resulting in 375 bacteria-drug combinations) [63]. Here, duloxetine, montelukast, rosiglitazone, and roflumilast were identified as accumulation-susceptible drugs. Elsewhere, the accumulation of 100 antibiotics by a strain of *Escherichia coli* was measured, revealing that antibiotics with low globularity, high amphiphilicity and rigidity, and containing an amine group were most likely to be

accumulated [64]. This work also identified that porin channels represented key routes for drugs' traversal of the bacterial outer membrane.

Indirect pharmacokinetic effects

Direct metabolism by the microbiota is not the only way that the microbiome can alter drugs' PK. The microbiome can affect drugs' absorption, metabolism, and excretion via a plethora of indirect mechanisms. Firstly, intestinal drug absorption may be altered by changes to epithelial permeability, gut motility, intestinal drug transporters, and bile acids (Figure 4).

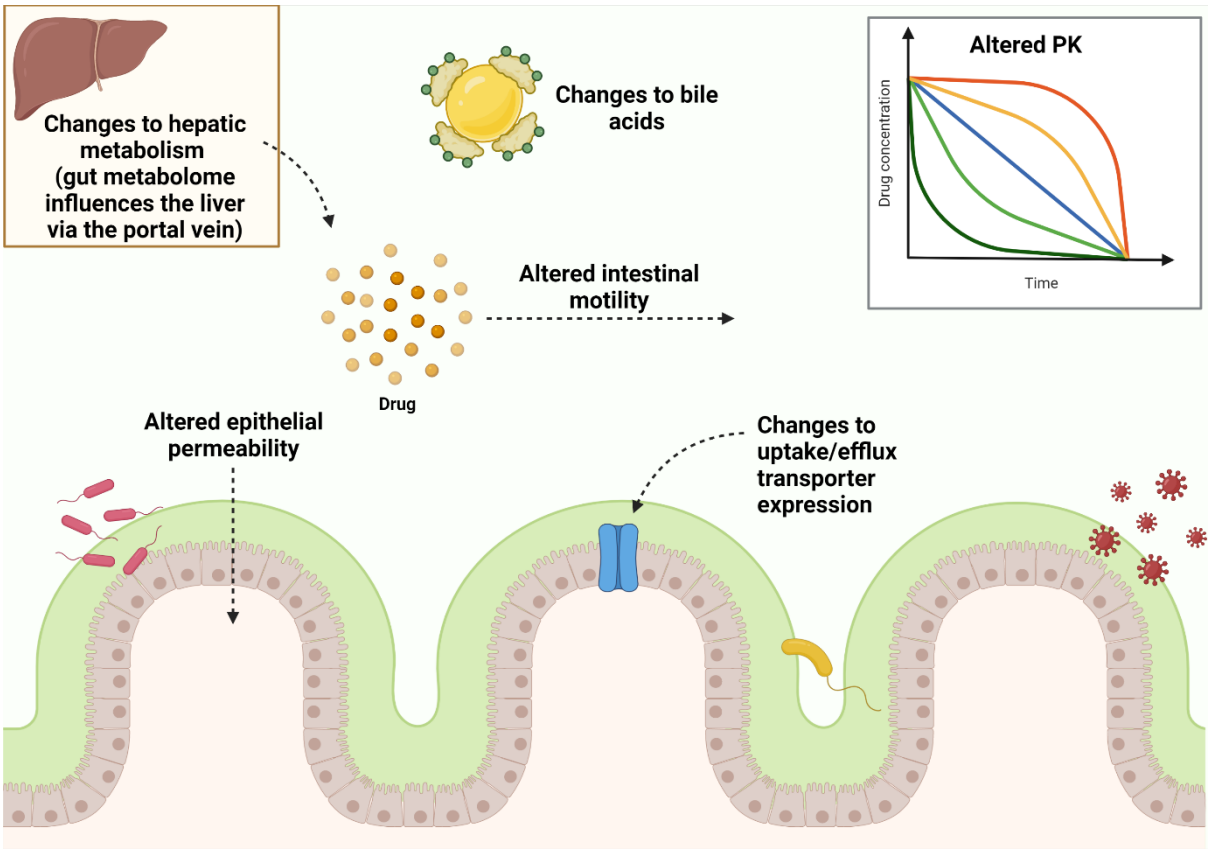

Figure 4. Mechanisms by which the gut microbiome can indirectly affect pharmacokinetics (PK).

The gut microbiome is an important modulator of epithelial tight junction integrity, acting via numerous immunoregulatory pathways [78-81]. This is also true at other body sites, such as the skin [82]. In dysbiotic states, an unbalanced microbiota can cease to maintain barrier integrity, leading to potentially increased drug absorption [83-85]. The expression of epithelial drug efflux transporters can also be affected by microbiome composition, altering the transport of drugs over the gut epithelium [86]. In addition, drug absorption may be affected by microbiome effects on intestinal motility. The richness and diversity of colonic bacteria is positively correlated with colonic transit time [87]. Thus, patients with more diverse colonic microbiomes may absorb drugs in delayed-

release formulations to a greater extent than patients with less microbial diversity, due to longer colonic transits.

Modifications to bile acids, which play a role in lipophilic drug solubilisation, can also be triggered by the metabolic activities of the microbiota in the distal gut [88,89]. Such changes have been found to impact the solubility capacity of nine oral drugs, including phenytoin, which raises concerns due to the drug's critical indications and narrow therapeutic index [90]. Hepatic drug metabolites excreted in bile can be 'reactivated' by colonic bacteria, extending their systemic half-lives, in a process known as enterohepatic reabsorption [91]. Further, drug metabolism in the liver may be significantly altered by changes to gut microbiome composition, due to the transfer of microbial metabolites from the intestine to the liver via the hepatic portal vein, and subsequent effects on the hepatic transcriptome. A study found more than 4,000 genes to be differentially expressed in the livers of germ-free and colonised mice, several of which were implicated in drug metabolism, such as *Cyp2b10* and *Cyp3a11* [92]. It is key to recognise that these results may not be fully transferable to humans, however the work does point to a potentially important relationship between the gut microbiota and hepatic metabolism. Certain diseases of the liver, such as alcoholic hepatitis, have been associated with substantial changes to the gut metagenome and metabolome, representing that microbiome-liver relationships are likely present in human patients [93].

Patient education

The public are developing an increasing interest in microbiome health. Scientific development describing new disease-microbiome associations and microbiome-targeted medicines are reaching mainstream news, direct-to-consumer microbiome sequencing kits have been developed, and sales of probiotics and fermented foods are experiencing a surge in popularity [94-97]. As such, it is increasingly likely that patient-facing professionals will be asked questions relating to interactions between medicines and the microbiome. For example, the risk of a medicine causing dysbiosis, the best probiotic product to take for a given indication, or how drug-microbiome interactions could affect a treatment's therapeutic efficacy or toxicity (Figure 5).

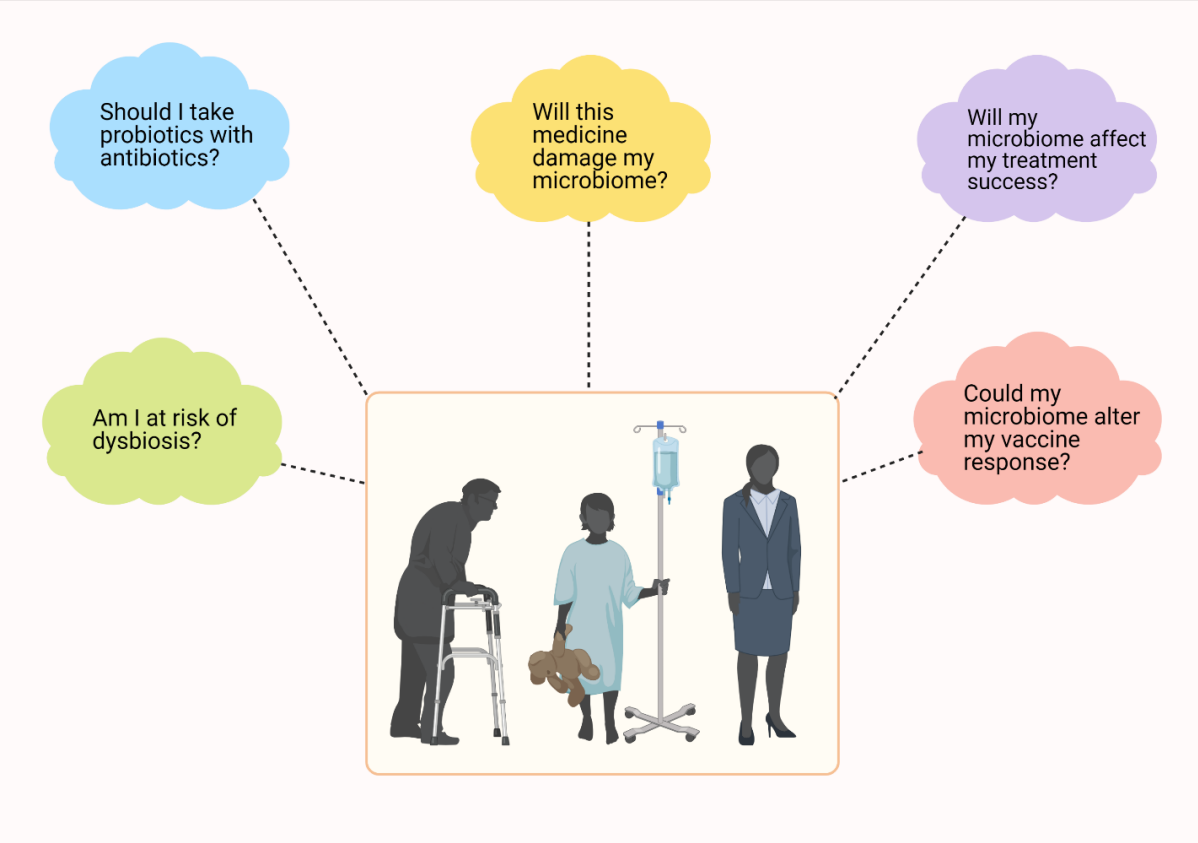

Figure 5. Potential questions that patients may ask healthcare professionals relating to drug-microbiome interactions [50,52,98,99].

When speaking to patients about drugs' effects on the microbiome it is important to convey the current infancy of the field. Whilst several drugs have been implicated in altering microbiome composition, it is often not clear how likely alterations are to impact specific patients' health. In the cases where evidence points to medicines having positive effects on the microbiome, professionals may wish to communicate this evidence as part of wider patient counselling, to promote medication adherence. If, based on clear evidence, professionals suspect medicines may impact a patient's microbiome negatively, they may wish to discuss this risk with the wider healthcare team and the patient, to manage the risk of dysbiosis in tandem with the clinical picture.

Some patients may also enquire as to the efficacy and/or suitability of probiotic formulations for a given indication. As over-the-counter probiotic products are currently not subject to the same stringent regulations as drug products, this can be a difficult query to answer, as the efficacy of many probiotics has not been validated. Several factors can influence the ability of a probiotic product to effectively exert a therapeutic benefit, including the identify and concentration of microbial strains included, the formulation type, the level of quality control during manufacturing, and the product stability [100]. Formulations should enable the safe passage of probiotic strains through the stomach,

as exposure to gastric acid can substantially reduce microbial viability. A study measuring the *in vitro* acid tolerance of 8 commercial probiotic formulations found that liquid-based products promoted probiotic viability to a greater extent than freeze-dried products [101]. Solid oral dosage forms may be protected through the use of coatings designed to resist degradation in the upper gastrointestinal tract [102]. A common reason for patients to seek a probiotic product is the recent administration of antibiotics, to limit the chance of ensuing dysbiosis. Though this practice is frequent, it is not necessarily evidence based. A study examining human gut microbiome recovery after broad spectrum antibiotic exposure found that an enteric coated probiotic (containing at least 25 billion bacteria from several strains, mainly lactobacilli and bifidobacteria) actually impaired microbiome reconstitution [99]. On the other hand, a large meta-analysis found that administration of probiotics (strains varied between studies) significantly reduced patients' risk of antibiotic-associated diarrhoea: a common symptom of intestinal dysbiosis [103]. Because antimicrobial-associated dysbiosis is highly variable, it is likely that there is no 'perfect' probiotic that can prevent or treat dysbiosis in all patients. It is likely that precision probiotics, composed of microbial strains personalised to individual cases, will produce more consistent benefits [104]. Rather than prescribing a generic mix of probiotics to prevent dysbiosis, it is probable that carefully selected species with characterised functionalities will be more efficacious [105]. Whilst precision probiotics are not currently a reality, advances in microbiome science mean that they may soon reach clinical practice [98]. Identification of the most suitable probiotic strains for specific patients will likely require metagenomic sequencing, to identify the compositional and/or functional deficits in individual microbiomes.

Communicating the impact of pharmacomicrobiomics to patients can be challenging. For one, there is a stark lack of evidence concerning interactions involving microorganisms outside of the intestines. For example, the effect of the skin microbiome on the PK and PD of topical drugs is underexplored. Furthermore, with a few exceptions, the impact of drug-gut microbiome interactions on patients' dose requirements is largely unknown. Therefore, the clinical relevance of reactions measured using *in vitro* and animal models can be difficult to interpret. It is expected that the clinical impact of pharmacomicrobiomics will be increasingly characterised as more human studies are conducted. With this research, healthcare professionals may be given the tools to predict drug-microbiome interactions on an individual basis. This could involve the use of point-of-care tests that measure microbiome composition via sequencing and then infer how an individual's results could influence their dose requirements. For example, determination of a patient's stool *F. prausnitzii* abundance could guide their starting dose of tacrolimus [5]. If this becomes a reality, then professionals should be comfortable in explaining the drug-microbiome interactions underpinning the tests to patients. In

addition, professionals using such tests should be confident that underlying evidence is applicable to their patient. For instance, dose-microbiome correlations gathered from certain patient groups may not always be relevant to other patient groups.

Managing the prescription of medicines

As evidence continues to emerge, it is likely that professionals will be required to increasingly incorporate drug-microbiome interactions into prescribing guidelines. At present, guidelines already exist around preventing antibiotic-induced *C. difficile* infection, which include measures such as avoiding repeat prescriptions of antimicrobials; considering non-antimicrobial interventions; identifying sources of infection; considering local resistance patterns; and prescribing the shortest effective course via the most appropriate dose and route when necessary [106]. As *C. difficile* infection is an outcome of gut dysbiosis, these existing guidelines provide good advice for protection against dysbiosis in general. It is perhaps due to the overt and immediately life-threatening nature of *C. difficile* infection that this consequence of dysbiosis has received substantial attention whereas others have not [41]. As associated evidence is hopefully strengthened over coming years, the effects of antibiotic-induced dysbiosis on other pharmaceuticals (such as warfarin, olanzapine, and vaccines) may be integrated into prescribing guidance [48-50]. Further, the impact of non-antimicrobial drugs on the microbiome may be more considered. For instance, the prescription of proton pump inhibitors is now cautioned in patients at risk of gastrointestinal infections (including *C. difficile*), following reports that their pH-raising effects promotes microbial growth in the stomach [107].

Microbiome sequencing is becoming progressively cheaper, faster, and more accurate. Due to this, the characterisation of patients' microbiomes may soon influence the prescribing process (Figure 6). Just as it is second nature for clinicians to check a patient's creatinine clearance, liver function tests, or blood pressure before initiating a new medicine, this may become the case for microbiome descriptors. Information on patients' microbiome composition may allow professionals to predict individual risk of drug-microbiome interactions. This could facilitate the identification of patients at risk of drug-induced dysbiosis or microbial drug depletion. Such knowledge could prompt healthcare professionals to adjust the doses of drugs or even switch to alternative therapeutics. To enable this, software that can relate raw sequencing reads to probability of drug-microbiome interactions will be necessary. To date, substantial efforts have been made to realise this outcome, with enabling technologies such as machine learning (ML) receiving significant attention. In 2018, Mallory et al. used unsupervised ML to predict drugs' metabolism by bacterial enzymes using reaction vectors [108]. Here, vectors were composed of known small molecule-bacterial enzyme reactions, and untested drugs' probability of reaction was computed using ML clustering. Zimmerman et al. also

utilised clustering after their drug-bacteria screening study to identify phyla-specific metabolic activities and the functional groups that increased drugs' susceptibility to depletion [76]. Elsewhere, supervised ML has been employed to predict drugs' effects on the gut microbiota's growth and whether drugs are at risk of biotransformation or bioaccumulation [109,110]. These studies represent progress within the field, as they have successfully related drug structure to probability of interactions with the gut microbiome. To advance further, research could consider both drug structures and the genetic sequence of microorganisms, to characterise how strain-level characteristics affect interactions with drugs' chemical features. Moreover, more work is needed to understand the mechanisms underlying the bioaccumulation of drugs, and the occurrence of drug-microbiome interactions outside of the gut. Finally, these capabilities should be combined to predict the likelihood *and* clinical impact of drug-microbiome interactions in real patients, a task that will require analysis of large patient cohorts. In the field of nutrition, the metagenomic sequences of 1,908 individuals' faecal microbiomes were used to predict postprandial metabolic response, demonstrating the promise of similar methodologies for the field of pharmacomicrobiomics [23].

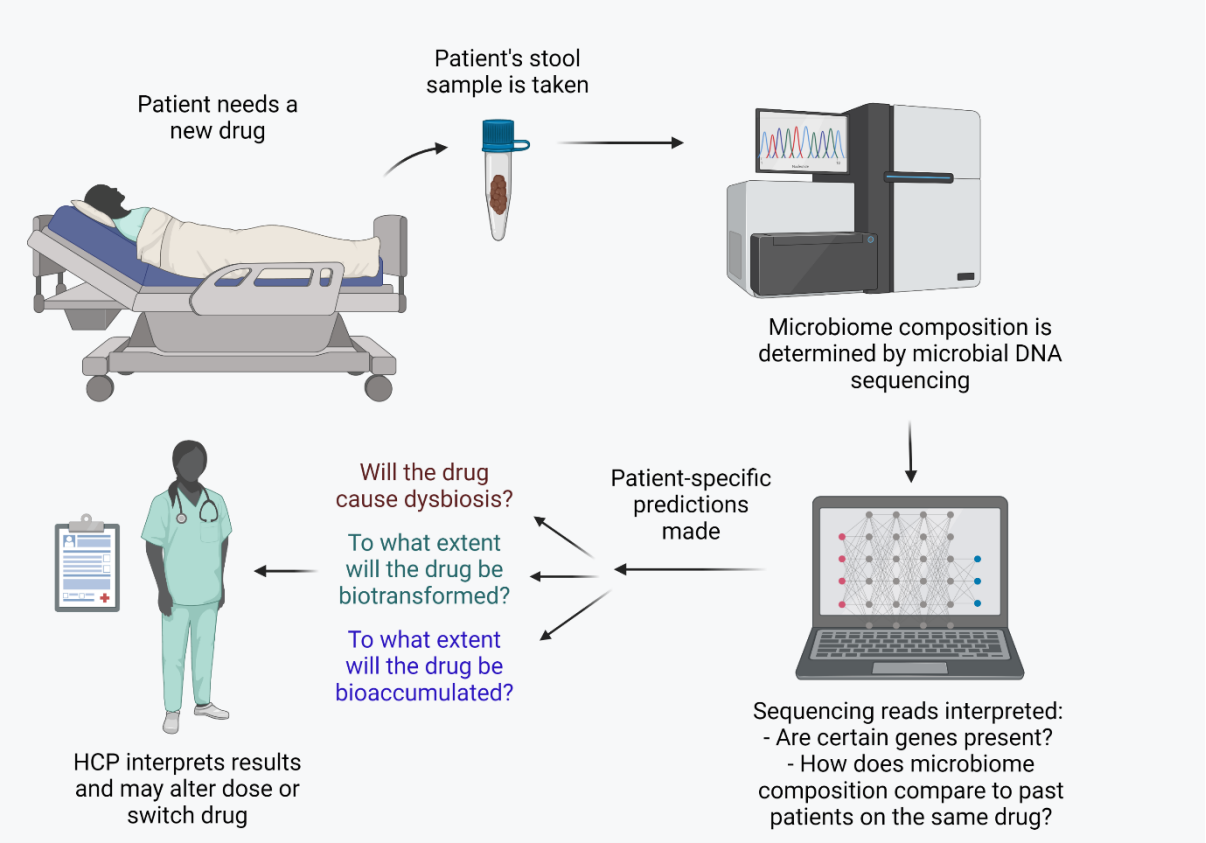

Figure 6. An example of a future workflow in which a patient's stool microbiome composition is sequenced and interpreted using dedicated software. This software could output personalised predictions of possible drug-microbiome interactions. Based on this, the patient's healthcare professional (HCP) may decide to alter the starting dose of a drug or switch to a new drug.

Academic research and education

There is great opportunity for university researchers to contribute towards emerging drug-microbiome evidence. At present, there remains a significant amount of work to be done in characterising the drug-microbiome axis. Key areas in need of greater research include the mechanistic description of interactions, measurement of interactions' clinical impact, prediction of interactions' occurrence in individuals, and analysis of interactions outside of the gut. Projects exploring these areas will likely require a multidisciplinary approach, involving researchers with skills such as microbiology, pharmacometabolomics, genomics, *in silico* modelling, and the organisation of studies involving patients. Whilst researchers working within academic settings are expected to conduct most of the fundamental science, there will be increasing opportunity for those working within clinical settings to collaborate with academics on projects involving patients. Professionals within healthcare settings could also contribute to evidence through audits and data analysis projects, whereby possible interactions between drugs and the microbiome could be identified from medical records. For example, medical records have been utilised to identify an association between use of antibiotics with high *B. fragilis* activity and extended prothrombin time in patients taking warfarin [48]. Such work could provide important indicators of clinically relevant drug-microbiome interactions that could subsequently be mechanistically explored in laboratories.

To fully spread awareness of drug-microbiome relationships, it is important to educate current and emerging healthcare professionals. Teaching can be incorporated into existing training modules, such as those focusing on PK, PD, and toxicology. Inspiration may be taken from other specialities, such as public health, where microbiome science is being taught through both dedicated courses and as part of more general modules [111].

Developing new treatments

Upon discovery, a novel drug candidate will undergo stringent preclinical and clinical testing prior to market approval. Due to the risk and investment involved, only 1 in 10,000 candidate molecules are typically advanced from preclinical to clinical trials; compounds must perform well across all testing categories [112]. Despite the risk that drug-microbiome interactions pose to a compound's PK variability and toxicity profile, they are not routinely tested for during drug development [113]. As demonstrated by the sorivudine tragedy, early screening for drug-microbiome interactions is highly advisable [68]. Though documented cases of microbiome-mediated drug toxicity are rare, microbial metabolism of drugs has been implicated in promoting PK variability between patients [5,77]. This PK variability could cause problems for companies during clinical trials, as treatments may not achieve their clinical endpoints if patients do not respond to the treatment uniformly. The primary benefit of

identifying a new drug's interactions with the microbiome is that risks to patients can be assessed well before the clinical phase begins. In addition, companies could save significant funds by ending the progression of unsuitable drugs.

Screening for drug-microbiome interactions could involve the use of the human faecal microbiota, animal models, *in silico* methods, or high throughput *in vitro* analyses [74,75,114]. Key questions would be 'is this drug's PK or PD affected by the microbiome?' and 'could this drug alter microbiome composition?'. Results showing PK and PD effects may highlight potential dosing challenges in clinical phases, due to the wide variability between patients' microbiomes. Further, if investigational drugs are found to alter microbiome composition, then investigations into the risk of dysbiosis, or even repurposing opportunities, could be conducted. For example, a drug may have unexpected beneficial effects on microbiome composition that could be transferred to other indications [115]. To save laboratory resources, *in silico* prediction of new drugs' interactions with the microbiome could be incorporated into the drug discovery phase [52]. Here, existing ML algorithms with reliable performances could begin to be used immediately [108-110]. The chemical features that promote microbial metabolism could also be considered in the design of new drugs, through awareness of functional groups that increase risk of biotransformation or bioaccumulation [64,76]. Prediction of a drug's stability in the presence of the microbiota could also inform formulation strategies. For example, work conducted by Astra Zeneca found a strong correlation (R^2 = 0.90) between drugs' permeability through the human colonic epithelium and their stability in the presence of the faecal microbiota [116]. If a new active compound is identified as being depleted by the microbiota at a particular site within the intestines, then targeted delivery of the compound to an alternative site could protect its bioavailability. Site specific delivery of oral drugs can be achieved using coatings designed to selectively release drugs in defined regions of the gastrointestinal tract [117]. As examples, two polysaccharide-based coating technologies, OPTICORE™ and Phloral®, have recently been launched onto the market for colonic drug delivery [118,119]. Both systems combine resistant starch and a pH sensitive polymer to achieve fail-safe delivery of any drug to the colon, by exploiting intestinal changes in microbial concentrations and pH [120].

Conclusions

Research over the last twenty years has revealed the importance of the human microbiome for health and uncovered many ways it can interact with medicines. Drug-microbiome relationships can be bidirectional: drugs can affect the microbiome, and the microbiome can affect drugs' PK and PD, both directly and indirectly. Because microbiome composition is unique to individuals and changes in response to numerous factors, it is currently difficult to predict drug-microbiome interactions for

specific patients. Despite this, it is likely that healthcare professionals will be increasingly called upon to incorporate the drug-microbiome relationship in their work. Patients may ask whether they should take probiotics with their antibiotics; prescribers may query which drugs are least likely to cause dysbiosis in their patients; and drafting of new prescribing guidelines may require consideration of drug-microbiome interactions. Whilst personalised predictions of drug-microbiome interactions are not yet a clinical reality, those writing and screening prescriptions are well placed to advise on the current best evidence. Professionals working in research and drug development can contribute towards emerging evidence by identifying and characterising new drug-microbiome interactions; information which can eventually inform prescribing guidelines in the clinic. In addition, as healthcare professionals are trained, it is essential that they are educated on the importance of drug-microbiome relationships. In coming years, it is a distinct possibility that microbiome profiling will become a common clinical investigation in healthcare settings. If so, software enabling clinicians to generate personalised predictions will likely be developed, allowing them to consider drug-microbiome interactions at the individual patient level.

Key points box

- The human microbiome is composed of trillions of microorganisms, including bacteria, fungi, archaea, and viruses. Gut bacteria alone may encode up to 150 times more genes than their human hosts.
- Drugs can affect microbiome composition, including those with intended and unintended antimicrobial actions. These effects can form part of drugs' therapeutic activity or may promote disease.
- The broad metabolic capacity of the gut microbiome can alter drugs' pharmacokinetics directly and indirectly. Over 150 drugs are now known to be susceptible to chemical transformation or accumulation by intestinal bacteria.
- Healthcare professionals working in clinical settings may be increasingly required to consider drug-microbiome interactions, for example when answering patients' questions or managing the prescription of medicines.
- In the future, new technology may enable professionals to personalise patients' dosing regimens based on their predicted drug-microbiome interactions.
- Professionals working in drug development should consider screening for drug-microbiome interactions at pre-clinical phases, this may identify potential challenges or opportunities at an early stage.

Financial disclosure/conflict of interest statement

A.W.B. has granted patents relating to the OPTICORE™ and Phloral® technologies.

Author contributions

L.E.M researched and wrote the first draft of the review. A.W.B provided supervision and advice on structure, content, and presentation. L.E.M and A.W.B both contributed towards the production of the final draft.

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