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Expert Review:

The Human Microbiome Project, Personalized Medicine, and the Birth of Pharmacomicrobiomics

Mariam Reyad Rizkallah[1], Rama Saad[2,3], and Ramy Karam Aziz[4*]

[1]Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt

[2]Paleogenetics Research Laboratory, The Egyptian Museum, 11557 Cairo, Egypt

[3]Ancient DNA Laboratory, Faculty of Medicine, Cairo University, 11562 Cairo, Egypt

[4]Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt

Running title: HMP and personalized medicine

* Corresponding author: Ramy Karam Aziz

Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt; Email: ramy.aziz@salmonella.org

ABSTRACT

After the completion of the human genome sequence, international efforts have been directed to the characterization of the genomes of human-associated resident microbes. The Human Microbiome Project was launched in the United States in 2007 with the aim of sequencing the resident microbiota from different sites of the human body. In this review article, we briefly introduce the Human Microbiome Project, the role of the human microbiome in health and disease, and the implications of the microbiome variations in personalized medicine and in pharmacomicrobiomics, which we define as the effect of microbiome variations on drug disposition, action, and toxicity.

Keywords: metagenomics, microbiome, microbiota, next-generation sequencing, pharmacogenomics, personalized medicine

1. INTRODUCTION

The Human Genome Project (HGP) opened the way to personalized medicine by providing the first blueprint for the human species, which consequently allowed high-resolution mapping of human variations to the full genome to generate a human variome, a combined set of polymorphic genetic loci responsible for interindividual variations [1-3].

Cataloguing the human variome allows not only the prediction of predisposition to or protection from genetic diseases, but also the prediction of the effects of genetic variations on disease prevention and therapy. Pharmacogenomics was born, as an expansion of pharmacogenetics, to study the combined effects of genetic variations on drug action (pharmacodynamics) and fate (pharmacokinetics) within an individual [4].

The HGP was indisputably a landmark in the history of science; however, one of the most surprising outcomes of this project is the much lower-than-expected number of human genes (currently estimated to be slightly above 20,000 protein-coding genes) which makes the number of human genes slightly larger than that of the fruit fly [5], equivalent to that of the roundworm *Caenorhabditis elegans* [6], but smaller than the number of genes of rice grains [7, 8]! Even more stunning was the finding that the human genetic variations represent a minute proportion of the sequence of coding genes and that this little variation does not fully account for the vast phenotypic variations observed between humans. What then drives these variations? Epigenetic factors represent one player; variations in regulatory and intergenic sequences represent another player; but what else?

It then became evident that the resident biota, organisms that use the human body as their primary or transient ecological niche, are large contributors to and modulators of human phenotypes. The vast majority of these organisms are microscopic (microbiota) and they not only constitute more cells than their host's (10^{14} microbial cells vs. 10^{13}) [9, 10], but also harbor many more genes than those contributed by the human genome [11, 12] (given the high diversity of microbial cells in comparison to the isogenic human cells). Consequently, the range of variation of microbial genes is more ample than the human variome, at least theoretically. In addition to cellular microbes, even more numerous viruses reside in the human body, and sometimes integrate into the human genome.

The aim of this review article is to introduce the human microbiome and the Human Microbiome Project (HMP), and to provide an overview of the contribution of the human microbiome (i.e., the human microbial metagenome or the combined genomes of the human-associated microbes) to human health and disease. In addition, the article provides examples of the effects of the human microbiota on the pharmacodynamics, pharmacokinetics, and toxicity of drugs—including antimicrobial agents and antibiotics.

The article is divided into four sections, in addition to this introductory section and a final concluding paragraph. First, we present a historical perspective and a current update on the initiation and progress of the HMP. Second, we provide an overview of the role of human-associated microbiota in health and disease, with specific examples on how resident bacteria contribute to particular diseases or add beneficial roles to their human host. Third, we link the HMP and its expected outcomes to diagnostic, therapeutic, and pharmacological applications. Finally, we expand the scope of the human microbiome by listing eventual projects that may follow the HMP, and we predict some of the applications that may come up in the near future.

2. THE HUMAN MICROBIOME PROJECT (HMP)

2.1. From HGP to HMP

Immediately after the completion of the draft human genome [13, 14], it was obvious that understanding human-associated phenotypes cannot be possible or complete unless the genomes of human-associated microbes are also sequenced [15, 16]. To be achieved in a reasonable time, however, such endeavor would require a radically different approach from that used in finishing the human genome project, mostly sequenced by cloning-based, low-throughput methods.

During the fourteen years of the HGP (1990-2003 [17]), microbiology has advanced in several directions, and DNA sequencing has almost been reinvented. A combination of factors made the sequencing of billions of human-associated microbes feasible. First, the field of microbial ecology has established culture-independent strategies to assess biodiversity, and computational, statistical, and mathematical methods for estimating diversity, optimizing sampling size, and modeling ecosystems dynamics. Additionally, DNA sequencing has been revolutionized by the development of cloning-independent, high-throughput, low cost methods, dubbed as "next-generation sequencing." Pyrosequencing [18], sequencing by ligation [19], and sequencing by synthesis [20, 21] methods have brought the cost down, the speed up, and magnified the output of sequencing machines.

Concomitant with next-generation sequencing technologies, the field of random community genomics, or metagenomics, has emerged and quickly become the method of choice for assessing biodiversity in different environments, using both Sanger and next-generation sequencing methods. Metagenomics allows the estimation of the biodiversity and biochemical potential in a microbial or viral community with neither culture nor complete cataloguing of taxonomic units [22-24].

The convergence of the need to expand the human genome, the advances in microbial ecology, and the reinvention of DNA sequencing has set the stage for international efforts for studying the human microbiome. After an international meeting in Paris (November 2005), an International Human Microbiome Consortium, IHMC [25], was established and officially launched in September 2008 to coordinate the different international microbiome projects, ensure data sharing and release, and maintain high quality and standards (see: [26] and [27]). The membership of this international consortium is open to any group working on the human microbiome that is willing to meet the consortium's standards and abide by its policies [28]. In practice, however, large-scale funding initiatives and multicenter projects, rather than individual laboratories, were needed for such a project to succeed.

In the United States, initial microbiome sequencing efforts gained momentum when the roadmap initiative, introduced by then National Institutes of Health (NIH) director Elias Zerhouni, called for proposals for sequencing the human microbiome [10, 12]. Simultaneously, a four-year European project was launched in 2008 specifically for large-scale, high-quality metagenomic analysis of the human intestinal microbiota (MetaHIT, Metagenomics of the Human Intestinal Tract: [29]).

2.2. First phase of the HMP

Pilot studies of the NIH-funded HMP started as early as 2007. According to the HMP working group, the initial "Jumpstart" phase is almost over and has achieved most of its goals of creating a reference map for microbes, collecting samples and standardizing sample collection protocols, and creating 16S libraries from collected samples (Table 1). An integral part of the funding is directed to the study of ethical, legal, and social implications (ELSI) of such an unprecedented project [10]. As the HMP enters its second phase, it is expected to focus on health-related issues, including, of course, the effect of microbiome variations on health, disease, and therapeutics [10, 12].

In addition to the U.S. laboratories funded by the HMP, the European MetaHIT published the most comprehensive catalog of gut microbial genes yet [30], and other laboratories in U.S.A. have also been working on the characterization of skin [31], gut [32, 33], and nares [34] microbiomes.

It is noteworthy that the IHMC, HMP, and other such modern human microbiome initiatives are taking advantage of novel technologies to solve microbiological puzzles that are as old as the field of microbiology itself. For examples, some issues such as the effect of diet on the human microbiome [35] or the analysis of microbial metabolites for diagnostic purposes [36, 37] were discussed 100 years ago [38, 39].

Table 1. What has been Achieved in the First Phase of the HMP

Goal	Achievements	References
Sequencing 500 reference bacterial genomes	More than 500 bacterial genomes are being sequenced, of which 375 are "in draft sequencing pipelines" [10]. As of May 2010, the Integrated Microbial Genomes-HMP website lists 666 HMP genomes that are more or less complete, of which 270 have been directly funded by the HMP.	[40]
Creating and standardizing protocols for sample collection and sequencing	Standard operating procedures were created and tested, and rigorous standards and quality control guidelines were developed. Mock microbial communities were reconstructed and tested for consistency and reliability of data analysis.	[41]
Recruiting and sampling volunteers	The desired number of normal volunteers (250 women and men from diverse ethnic backgrounds within the United States) were recruited and sampled at least once.	
16S rDNA sequencing from different body sites	Pilot studies were performed with 16S rDNA-based determination of microbial diversity in different body sites.	[42]

Information in this table was synthesized from the literature [10]

3. HUMAN MICROBIOTA IN HEALTH AND DISEASE

The human body hosts, on average, about 10^{14} microbial cells (i.e., bacterial and archaeal cells) in addition to an estimated 10 times more viruses. The combined microbial genomes residing in a human body likely harbor 100 times more genes than those in the human genome. While a comprehensive description of the human microbiota and its impact on human phenotypes is beyond the scope of this review article, we provide specific examples representing different characterized sub-ecosystems of the human body (Table 2).

Generally speaking, resident microbes affect human health and disease through various mechanisms, listed below.

3.1. Beneficial roles of the human microbiota (See specific examples in Table 2)

A. Protective effects:

- Passive competition with exogenous infectious microbes: Human-associated microbes, being well established in their natural habitats, represent a mechanical barrier that prevents exogenous microbes, including pathogens, from colonizing human tissues. This implies that removing microbial communities colonizing certain skin areas or internal tissues (for example, as a side effect of antibiotic therapy) may lead to the colonization of these by pathogenic species.

- Active competition: Resident microbes play also active roles in fighting exogenous microbes that are likely to compete with them for the limited resources within the human tissues. To do so, they have developed several mechanisms, including the secretion of antimicrobial products (bacteriocins, lantibiotics, and other antimicrobial peptides), or iron-sequestering molecules that may deprive other bacteria from the well-needed iron.

- Immunogenic effects: Resident microbes elicit non-specific immune responses that prepare the human body for invading microbes.

B. Productive effects:

- Biosynthetic functions: Human-associated microbes are known, in particular, for their ability to expand the limited human metabolic landscape by synthesizing several primary and secondary metabolic products, some of which are essential for humans.

C. Cooperative effects/ catabolic or digestive roles:

In addition to biosynthetic functions whereby microbes produce essential molecules on which humans depend, microbes may also produce enzymes that help their hosts:

- digest some sugars (trehalose [43] and oligosaccharides) or polysaccharides: A recent example is the ability of Japanese individuals to digest seaweed because some of their gut microbes had acquired the necessary enzyme-encoding genes from marine bacteria [44].

- process xenobiotics: Microbial catabolic abilities can detoxify xenobiotics, including toxins and drugs [45, 46].

- harvest energy from nutrients [47, 48].

3.2. Pathogenic roles of the human microbiota (See specific examples in Table 2)

Under particular conditions, human-associated microbes may also cause diseases by one of the following mechanisms:

A. Imbalance of microbiota composition:

Imbalance in the population structure of resident microbes may lead to metabolic patterns that interfere with the normal functioning of the human systems (e.g., bacterial vaginosis [49], obesity [48, 50], diarrhea [51], and gum diseases [52, 53]).

B. Breaking barriers and colonizing otherwise sterile areas:

Wounds are examples of conditions that allow skin microbes to reach sterile body tissues causing infections, some of which are life threatening. Moreover, medical and prosthetic devices can be often colonized by human resident microbes, causing infections (e.g., catheters contaminated with gut microbes can cause serious urinary tract infections; cannulae contaminated with skin microbes often cause septicemia; respiratory intubation is a major cause of pneumonia and death in hospitalized patients [54]).

C. Genetic conversion of commensal or opportunistic microbes to pathogens:

Random mutations occur continuously, and their outcomes are—by definition—unpredictable. Spontaneous mutations can inactivate a bacterial essential gene, altering the nutritional requirements of the mutated cells, sometimes at the expense of the host's tissues (e.g., antivirulence genes [55, 56]). Gene acquisition via lateral gene transfer is another mechanism by which human-associated microbes become virulent or make exogenous organisms more virulent. An exogenous microbe can transfer a virulence gene to a member of the microbiota transforming it from a commensal to an *insider pathogen*, which is already preadapted to survive the immune system but also possesses (a) newly acquired gene(s) whose product(s) may harm the host. The most classic examples include the lysogenic conversion of resident corynebacteria and streptococci into diphtheria-causing and scarlet fever-causing agents, respectively. In addition, resident microbes may possess antibiotic-resistance cassettes that they can transfer to an exogenous organism (via transformation, conjugation, or phage transduction) potentiating its pathogenesis [57-60].

D. Immune deficiency:

The host's immune deficiency (which may be congenital or acquired, for example as a consequence of HIV infection or immunosuppressive therapy) may lead unexpected harmless or opportunistic microbial species to become fiercely pathogenic. A classic example is the pathogen *Mycobacterium tuberculosis* that colonizes one third of humans but causes infection mostly in those with suboptimal immune systems. Other mycobacteria that are normally ubiquitous in the environment, and possibly resident in humans, (e.g., *Mycobacterium avium-intracellulare*) often

cause infections in immunocompromised patients [61-63]. Other examples are fungal infections that are common in AIDS patients or infections involving multiple organisms in transplantation patients.

Table 2. Resident Microbiota of Five Sites in the Human Body and Examples of Their Effects on Health and Disease

Body site	Major microbial taxa	Role in health	Role in disease
Skin Acidic pH, lower temperature than the human body, various degrees of humidity	- Proteobacteria (about 90%), mainly genera *Pseudomonas* and, to a lesser extent, *Janthinobacterium* - Actinobacteria (5.6%), including genera *Corynebacterium*, *Kocuria*, *Propionibacterium*, *Microbacterium*, and *Micrococcus* - Firmicutes (4.3%), including species of *Staphylococcus* and *Clostridium* - Bacteroidetes (<1%), including *Sphingobacterium* and *Chryseobacterium* [31, 64]	- Resident skin microbes compete with pathogens over the limited resources in that habitat. For example, *Staphylococcus epidermidis* outcompetes pathogenic staphylococci, and *Corynebacterium jeikeium* produces siderophores to acquire iron, which also deprive other organisms from iron and prevent their colonization of the skin [65]. - Skin microbes produce molecules that inhibit the growth of pathogens or even kill them (e.g., lantibiotics or bacteriocins).	- The potential role of skin bacteria in psoriasis and atopic dermatitis or eczema is under investigation [64, 66, 67]. - The skin microbe, *Propionibacterium acnes*, has classically been associated with acne vulgaris [65], although its role may be secondary [68]. - Skin microbes are a continuous threat for any medical devices such as injections, catheters, and cannulae. - Skin microbes pose a special risk to immunocompromised individuals or immunodeficient patients (e.g., device-related bacteremia due to dissemination of *S. epidermidis* [69]). - Some skin bacterial metabolites reportedly attract the malaria vector *Anopheles gambiae* [70].

Oral cavity Aerobic and anaerobic environments, nutrient rich	Predominant bacterial members inhabiting a healthy individual's oral cavity include species from the following divisions [52]: - Firmicutes (*Streptococcus*, *Granulicatella*, and members of family Veillonellaceae) - Proteobacteria (*Neisseria* and *Haemophilus*), - Actinobacteria (*Corynebacterium*, *Rothia*, and *Actinomyces*), - Bacteroidetes (*Prevotella*, *Capnocytophaga*, and *Porphyromonas*), and - Fusobacteria (*Fusobacterium*).	- *Streptococcus gordonii* secretes hydrogen peroxide, which inhibits the growth of other microbial species (e.g., *Actinomyces naeslundii*), and thus minimizes dental plaque formation [71]. - *Streptococcus mutans* inhibits the growth of other microbial species through secretion of the antimicrobial peptide, bacteriocin [72]. - *Streptococcus* spp., *Actinomyces* spp., and *Lactobacillus* spp. inhibit the growth of other microbial species by lowering oral pH [72].	- Imbalance in the composition of oral microbes can lead to multiple conditions ranging from bad breath [53] to dental caries and periodontal diseases. - Alterations in biofilm composition or volume may cause diseases, e.g., inflammatory periodontal diseases [73]. - Biofilms can serve as an agent of sustained-release of respiratory pathogens, e.g., lung infections in patients in intensive care units [72]. - *Candida albicans*, a member of the oral microbiota of healthy individuals [74], can cause invasive diseases, and its biofilms are resistant to antifungal agents [72]. - Viridans streptococci, *Candida*, and *Neisseria* spp. may contribute to oral cancer associated with alcohol consumption, by producing acetaldehyde from alcohol [75, 76].
Nasal cavity Aerobic environment, continuously exposed to the outside air	**Dominant taxa:** - Actinobacteria, mostly *Propionibacterium*, *Corynebacterium*, and *Mycobacterium* spp. - Firmicutes, mostly *S. epidermidis* and other coagulase-negative staphylococci - Proteobacteria, e.g., *Neisseria* - Bacteroidetes [34, 77] Kirtsreesakul *et al*. suggested that the nasal microbial community consists of aerobes and facultative anaerobes, but no anaerobes [78]. Chen *et al*. found no age effect on nasal microbiota composition [79].	- Colonization of the nares by *S. epidermidis* and other coagulase-negative staphylococci is negatively associated with carriage and *Staphylococcus aureus* diseases [34].	- Ventilator-associated pneumonia (VAP) is caused by the colonization of the lower respiratory tract by nasopharyngeal microbes facilitated by the insertion of endotracheal tube (ETT) [80]. This may suggest a targeted antibiotic therapy approach and may affect the method of disinfection according to the suspected bacterial strain [54]. - *S. aureus* bacteremia was three times more frequent in *S. aureus* carriers than in non-carriers [81]

Gut Largely anaerobic, mixture of digested and indigested food products	The majority (90%) of gut bacteria belong to the divisions Bacteroidetes and Firmicutes [82]. The most predominant bacterial genera are: - *Clostridia* - *Bacteroides* - *Bifidobacterium* - *Peptostreptococcus* - *Fusobacterium* - *Eubacterium* - *Escherichia* - *Lactobacillus*	**1- Protective effects:** - Competitive functions: For example, prolonged and excessive use of antibiotics predispose to *Clostridium difficile* pseudomembranous colitis [51]. - Immunogenic effect: Gut microbiota provokes the production of antibodies from the lymphoid tissue and stimulates the expression of Toll-Like Receptors (TLRs) in the intestine [83]. Furthermore, Bacteroides alter their surface receptors like that of the host to provoke an immune response [51]. - A negative correlation between colonization by the gut pathogen, *Helicobacter pylori* and childhood asthma was suggested [84-86] **2- Productive effects:** *Escherichia coli* plays a significant role in the production of vitamin K2 that is released upon rupture of dead bacterial cells [51]. **3- Digestive effects:** *E. coli* and other bacterial species, such as *Methanobrevibacter smithii*, are responsible for the digestion of certain types of carbohydrates as starches, fibers, lactose and oligosaccharides. Through saccharolytic fermentation, gut microbes are able to metabolize carbohydrates to short-chained fatty acids (SCFAs) and, accordingly, render them a readily utilizable energy source by host cells [87].	Members of the gut microbiota are associated with multiple diseases that range from diarrhea to peptic ulcer to cancer. Diseases caused can be attributed to several reasons, such as inflammation, exaggerated immune response, DNA cross-linking, *etc*. **Examples are:** - Crohn's disease and ulcerative colitis: Evidence suggests that the reduced numbers of lactobacilli and bifidobacteria results in increased numbers of intestinal microorganisms and abnormal immune response to gut microbiota. This has been confirmed by the positive effects of treatment with probiotics and antibiotics [88-90]. - Colon cancer: some members of gut flora have been associated with the prevalence of colon cancers through conversion of dietary procarcinogens into DNA-damaging agents [91-93]. - Obesity: obesity has been found to be associated with changes in the relative abundance between Bacteroidetes and Firmicutes that have been suggested to affect the energy production [32, 48].

Urogenital tract (female) Environment varies depending on hormonal state and sexual activity	- *Lactobacillus* spp. - *Atopobium vaginae* - *Megasphaera* spp. - *Leptotrichia* spp. [94]	- Lactobacilli produce lactic acid, which has broad-spectrum protective effects against pathogens owing to its low pH. - Lactobacilli and other vaginal resident microbes produce various bacteriocins that inhibit and kill other pathogenic organisms.	- Bacterial vaginosis is caused mainly by an alteration of the composition of the vaginal microbiota. As *Lactobacillus* spp. are reduced, the subsequent rise in the vaginal pH favors the predomination of the vaginal anaerobes, e.g., *Gardnerella vaginalis, Bacteroides* spp., *Actinobacteria* spp., and *Mobiluncus* spp. [49]. - Intrauterine devices may carry *C. albicans* biofilms, enhancing their virulence potential [95].

4. HMP, PERSONALIZED MEDICINE, AND PHARMACOMICROBIOMICS

So far, we have discussed the general roles of human-associated microbes in human health and disease. To study the potential implication of these microbes in personalized medicine, one has to focus on the impact of variations in the microbiome composition (variations in species composition and gene content), or less radically, phenotypic variations in the combined metabolic state of these microbial communities.

It has to be noted that the extent of interindividual microbiome variations is still debated. While earlier studies expected larger number of gut-associated genes [96] and higher variations among gut microbiomes [32, 50], a more recent study that used deeper metagenomic sequencing suggested that humans share a larger common core metagenome than previously estimated [30]. On the other hand, another recent study demonstrated that there is enough interindividual variability in skin microbiomes to provide forensic identification of individuals based on their bacterial traces [97].

Besides interindividual variations, the HMP is also expected to investigate other sources of variation of an individual's microbiome. These intraindividual variations include topographical diversity [31], temporal or seasonal variations [31, 98], diet-related changes [35, 44, 99], developmental [100], and hormonal changes.

In this section, we present examples from different body sites demonstrating how microbiome variations differentially influence the pathogenesis of diseases, their prevention, and therapy. As a corollary, cataloguing and understanding these variations—through HMP, IHMC, and other human microbiome research programs—may lead to a revolution in personalized medicine on many levels, including the diagnosis, dose determination, and control of adverse effects of many drugs.

Being the most numerically abundant, the gut microbiota has been the best studied as well, and several good examples of microbial effects on pharmacokinetics come from gut microbes. However, a few examples from other body sites also suggest that each microbiota can have its impact on drugs, and that this branch of pharmacology remains largely unexplored.

4.1. Predictive/Diagnostic value of microbiome variations

It is a common practice to perform microbial cultures and sensitivity tests before determining the suitable antibiotics. This simple form of personalized medicine can be further extrapolated to the diagnosis of more complex diseases. Instead of culturing bacteria isolated from infection sites (in the case of antibiotic therapy), physicians will soon be able to determine the entire microbial diversity in the body site studied, even in the absence of an infectious disease, to predict/diagnose another disease, such as obesity for example.

Recently, a link has been found between the composition of gut microbiota and obesity in mice [48] and humans [32]. Moreover, a link between microbiota and metabolic syndrome [33] and even chocolate craving [101] was established as well. While it is not fully understood whether the change in microbiota is a cause or effect of these metabolic disorders, there is evidence that transfer of obesity-associated or metabolic disorder-associated microbes into gnotobiotic mice can cause these conditions. Regardless of the causality, these studies suggest that determining the microbiome can be diagnostic for an individual's predisposition to metabolic syndrome or obesity.

Another study of the nasal microbiome has recently demonstrated a negative association between *S. aureus* and *S. epidermidis* in the nares, suggesting a potential diagnostic value (and perhaps a potential for intervention via microbiota alteration) [34].

In the microbe-rich oral cavity, the healthy status of the mouth can be microbiologically assessed as the absence of *Porphyromonas gingivalis, Treponema denticola, S. mutans,* and *Lactobacillus* spp. In case of dental caries and cavitations, carcinogenic members of plaque (e.g., *S. mutans, S. oralis, Lactobacillus* spp., *Actinomyces* spp. and *C. albicans*) lower the pH and favor teeth demineralization. In case of periodontitis, on the other hand, the pH is rather higher, and predominant microbes are *P. gingivalis, Treponema denticola, Prevotella intermedia, Aggregatibacter actinomycetemcomitans,* and *Fusobacterium nucleatum* [73].

4.2. Therapeutic values

The use of antibiotics in therapy has always been more or less "personalized." The selection of the appropriate antibiotic and its optimal dose are determined based on the composition and antibiotic-sensitivity of the microbial communities isolated from the site of infection. This principle in antibiotic therapy can be expanded to a more elaborate model of personalized medicine, where the determination of treatment regimen and therapeutic intervention are largely dependent on the nature of an individual's microbiome.

One example that has been studied in detail is the treatment regimen of diabetic foot infection (DFI). Although this is not a typical microbiome-based regimen, it is worth mentioning because the way of intervention largely depends on the types of microbes involved (Table 3). DFI is a major vascular, neuronal, and microbial complication of diabetes mellitus. Establishing a relationship between the microbiology of DFI, length of the disease course, risk factors that led to the development of the disease, and the history of antibiotic therapy will be of great help in the assessment and management of DFI. The microbiology of DFI varies according to these determinants. Needless to mention that microorganisms involved in DFI are mainly members of

the resident microbiota (Table 3). Management of DFI may range from an empiric antibiotic regimen or anti-ischemic therapy to surgical intervention according to the case status [102] and factors such as:

- hospital-related factors (prevalence and characterization of MRSA)
- patient-related factors (e.g., penicillin allergy, risk factors, and severity of the infection)
- microorganism-related factors (e.g., antibiotic resistance, and virulence factors like the coagulase activity of *S. aureus*).

Even with diseases involving the same bacteria, the treatment regimen may vary. Treatment of acne is different from treatment of *Propionibacterium acnes*-associated endocarditis in immunocompromised patients. In spite of the sensitivity *P. acnes* to beta-lactam antibiotics, as endocarditis caused by *P. acnes* is managed with penicillin or vancomycin, administration of beta-lactam antibiotics does not provide improvement in acne patients [65]. The predominance of *S. epidermidis* in sebaceous follicles of acne patients may explain this phenomenon [68]. In acne vulgaris, the contribution of *P. acnes* is mainly secondary to inflammation. Thus, in cases of mild acne, benzoyl peroxide and topical antibiotics are indicated mainly to reduce the inflammation associated with it [103] owing to their anti-inflammatory effects. In cases of moderate acne, systemic antibiotics (doxycycline or macrolides) are indicated, which may increase the incidence of resistance, adverse drug reactions, and depletion of *P. acnes* that may cause the sebaceous glands to be susceptible to infection by pathogens. For permanent remission of acne, retinol (vitamin A) is indicated [65].

Another example of a microbiome-dependent personalized treatment regimen is the treatment of bacterial vaginosis (See Table 2 for more information). Understanding the microbiology of bacterial vaginosis led to the development of diagnostic tools based on the identification of the types of amines produced by anaerobes (e.g., gas chromatography and mass spectrometry [104]), as well as the improvement of the treatment strategy through the use of vaginal-acidifying/buffering agents and probiotics as an adjuvant to antibiotic therapy [105].

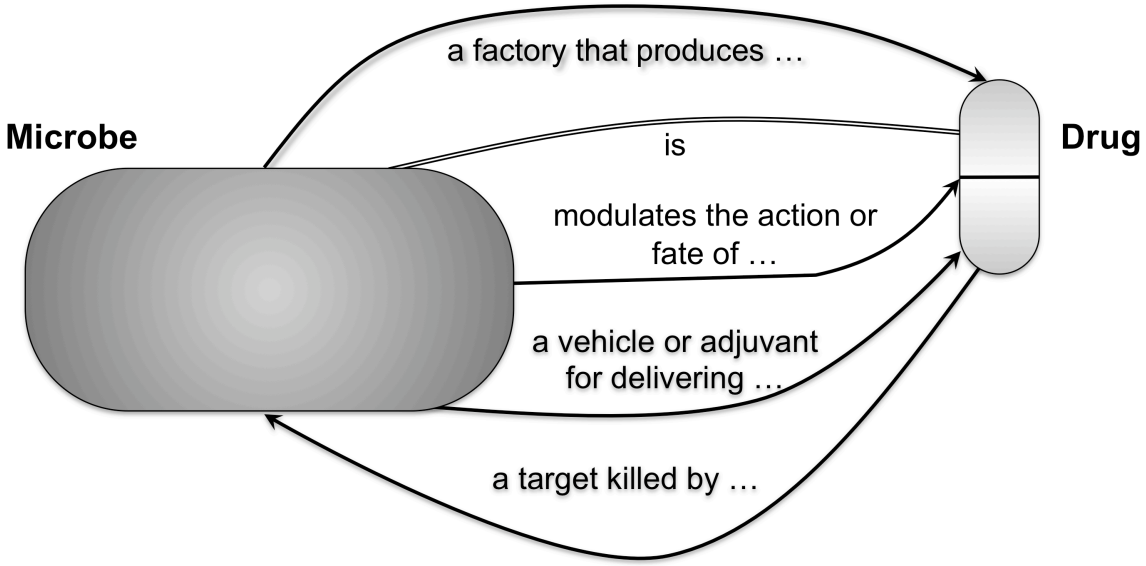
In addition to therapeutic intervention based on the determination of microbial communities and their metabolites, several modern therapeutic and preventive approaches aim to restore a healthy microbiome. This applies to the use of probiotics to control intestinal disorders (e.g., Crohn's disease and ulcerative colitis [88-90]) as well as the maintenance of good oral hygiene via restoring balance to the microbiome. Filoche and colleagues suggest that the goal of therapy in treating dental diseases (e.g., dental caries) is restoring the oral healthy state, which cannot be properly achieved without a comprehensive understanding of the etiology and progression of the disease. Restoration can take place through removal of dental plaque, use of antimicrobials and fluoride, dietary changes, and salivary stimulation [73]. Filoche *et al.* carefully note that there is no 'one-size fits all' recipe for a healthy microbiome and that "the challenge lies in determining what is normal for that particular individual" [73].

Table 3. Summary of Suggested Interventions Depending on the Microorganisms Associated with Diabetic Foot Infection (DFI) Case Status

DFI case status	Microbes involved	Suggested intervention
Absence of pus or signs of inflammation	Non-infective	No antibiotic therapy required
Acute, previously untreated, superficial infection	Mainly aerobes/ microaerophiles: *S. aureus* and beta-hemolytic streptococci	Empiric antibiotic agent active against isolated cocci e.g., dicloxacillin, cephalexin (in case of penicillin allergy), and amoxicillin/ clavulanate (for polymicrobial infection)
Recent antibiotic therapy, chronic wound or deep limb-threatening infection	Mixture of aerobes and anaerobes: *S. aureus*, beta-hemolytic streptococci, *E. coli*, *Proteus* spp., *Klebsiella* spp., *Bacteroides*, *Clostridium* spp., *Peptococcus* spp., and *Peptostreptococcus* spp.	Antibiotic agents with a wider spectrum, e.g., ceftriaxone + clindamycin or metronidazole, and anti-anaerobic therapy if gas gangrene was confirmed
Recent antibiotic therapy, or previous hospitalization	Methicillin-resistant *S. aureus* (MRSA)	Vancomycin + ciprofloxacin + metronidazole, and linezolid or daptomycin in case of penicillin allergy

This table has been compiled mostly from data reported by Bader *et al*. [102].

4.3. Pharmacomicrobiomics



From a pharmacological stand point, a microbe can be regarded as a factory to produce drugs (e.g., antibiotics, digestive enzymes); a vehicle or adjuvant for drug delivery or vaccine, respectively; a target of a drug's action (e.g., antibiotics and other antimicrobials); a positive or negative modulator of a drug's fate and action; and finally the microbe itself can sometimes be considered as a drug (e.g., probiotics).

The relationship between drugs and microbes is as old as pharmacology and can take several forms, ranging from the microbe being a drug target to being a drug itself (Fig. 1). Such complex forms of the drug-microbe relationship deserve, in our opinion, a specific branch of personalized medicine, pharmacomicrobiomics. Like pharmacogenomics, pharmacomicrobiomics will focus on variations in responses to drug disposition, action, and toxicity. However, the variable in pharmacomicrobiomics is the combined genetic makeup of the human-associated microbes (microbiome or human-associated metagenome) and their metabolic potential (meta-metabolome [106]). Below are examples that highlight the importance of the HMP in pharmacology.

4.3.1. Alteration of microbiota and the subsequently altered drug disposition processes

Alteration of the composition of gut microbiota represents an evident source of interindividual variation in drug metabolism [37]. This has been demonstrated either through microbial interaction with metabolizing enzymes or direct interaction with the drug molecules. In fact, the simplest example of microbial degradation of drugs is the antibiotic-deactivating potential of several bacterial enzymes (e.g., beta-lactamase and chloramphenicol acetyltransferase) that is a typical mechanism of drug resistance. Translating these findings into

clinical applications (e.g., determining the appropriate medication in accordance with each patient's gut microbiota) essentially implies the identification of the human gut microbes as well as their metabolic phenotype [37]. This in turn recalls the need to characterize the gut microbial communities among different populations.

There are several examples of how the extraordinary ability of bacteria to metabolize xenobitoics can affect drug pharmacokinetics. A difference has been reported between how North Americans and southern Indians metabolize digoxin. The alteration in the composition of gut microbiota, specifically the anaerobic member *Eubacterium lentum,* is claimed responsible for the difference in the concentration of the reduced digoxin inactive metabolic product. A study showed that the reduced digoxin metabolites constituted 36% and 13.7% in North Americans and southern Indians, respectively [107].

A more recent and more striking example of the microbial involvement in drug disposition is related to acetaminophen toxicity. The aromatic compound *p*-cresol, produced by some gut bacteria, reportedly competes with acetaminophen for the *O*-sulfonation–mediated catabolism, and thus will potentially predispose to acetaminophen toxicity [45]. Moreover, lower production of *N*-acetylcysteinyl conjugates of acetaminophen was shown to be associated with higher *p*-cresol sulfate excretion [45]. Clayton and colleagues, the authors of the acetaminophen study, concluded their article by recommending that "assessing the effects of microbiome activity should be an integral part of pharmaceutical development and of personalized health care" [45].

One example of gut microbiota-related modulation of drug metabolism is the action of several resident gut microbes on soy-derived phytoestrogens. These microbes can produce active metabolites and thereby enhance the efficacy of soy-derived phytoestrogens [108]. This has been reported upon observing the absence of the pharmacologically active products in germ-free rats [108]. Furthermore, it has been suggested that gut microbiota derived-phytoestrogens have an effect on cytochrome P enzymes, responsible for estrogen hydroxylation, which results in lower excretion of estrogen metabolites and thus lower toxicity [109].

Although the skin is highly exposed to external chemicals, the interactions of the skin microbiota with non-antimicrobial drugs are largely undetermined. One area that needs further exploration, for example, is whether the skin microbes contribute to the enzymatic degradation of labetalol or levodopa transdermal patches, or affect the duration of transdermal contraceptive devices. An article by Cevc and Vierl [110] discusses the effect of skin microbiota on nanotechnology-based targeted drug delivery through the transdermal route. Yet, the authors of that article were more concerned with the bacterial density and with keeping the skin as intact as possible during pore creation than with the metabolic properties of the skin microbiota [110].

Most skin microbes possess azoreductase activity, and are thus capable of reducing azo dyes Methyl Red and Orange II into N,N-dimethyl-p-phenylene diamine and 2-aminobenzoic acid [111]. Azo dyes are widely used as food and pharmaceutical colorants e.g., amaranth and tartrazine [112], and malachite green and crystal violet were previously used to treat *S. aureus* skin infections [113].

In addition to the effects of human-associated microbes on modulating drug action, these microbes can be exploited as drug vehicles or adjuvants (Fig. 1). For example, genetically

modified nasal microbes can be explored as novel targeted drug-delivery systems [114]. In addition, *S. gordonii* has been suggested as a vector for anti-group A streptococci vaccines [115]. Introducing modified microbes, though, adds to the complexity of the interaction between the human host and its resident microbes, and may affect the balance of the resident microbiota. The drug delivery vectors may have some growth advantage or disadvantage compared to bacteria belonging to the same species. Again, these interactions will be better understood when the HMP provides a comprehensive catalogue of the resident microbiota.

4.3.2. Effects of altered microbiota on drug pharmacodynamics

Whenever microbes or microbial communities are drug targets (e.g., in antimicrobial chemotherapy, antibiosis, or probiosis), it is obvious that any genetic variations in these targets will affect the drug efficacy, and consequently dosage and toxicity. However, most antibiotics and antibiotic regimens are developed and optimized against exogenous, pathogenic microorganisms. Yet, in diseases caused by human-associated microbes directly (e.g., wound infection) or indirectly (e.g., ulcerative colitis) the drug target is a population of resident microbes that are well adapted to the human immune system, and the therapy is thus more complex, and almost always personalized.

Alteration in the gut microbial population structure can be possibly associated with interindividual response variation through hindering or potentiating the drug efficacy. For example, warfarin toxicity has been reported upon concomitant use of amoxicillin/clavulanate that has been related to decrease in vitamin K-producing microbes and subsequently lower vitamin K levels and bleeding. Additionally, excessive long-term use of antibiotics may result in an increase of the proportion of antibiotic-resistant gut microbes [116]. Such phenomenon is commonly observed upon continuous administration of ampicillin, which results in the expansion of antibiotic-resistant *E. coli* in fecal samples.

The use of topical antibiotics has been shown to exert a more significant selection pressure that leads to the emergence of resistant skin microbes than that exerted by antiseptics and wash products [117].

Bacteria that are highly resistant to systemic antibiotics were shown to be also more resistant to topical antibacterial agents [118]. This poses a continuous risk of course, given that novel strains with more antibiotic resistant potential keep emerging. For example, methicillin-resistant *S. aureus* (MRSA), known to host laterally transferred genes [119] was shown to have acquired genes from *S. epidermidis* that permit its survival on human skin [31].

5. OUTLOOK

5. 1. Beyond the human microbiome

The HMP is a major transitional step towards understanding the phenotypes associated with the human species. Its completion will help the scientific community decode a much wider gene pool than that revealed by the HGP, and thus will unveil several biochemical abilities of human systems that are not genetically inherited, yet their acquisition via microbes is almost

guaranteed. However, once the human-associated microbial gene pool/ metagenome is catalogued, several other omics will be waiting in line to be decoded as well.

A. Extended human variome:

The human microbial variome, i.e., the sum of variations in human microbiomes, will need to be estimated and catalogued. The most recent studies from skin [31] and gut microbiomes [30] seem to have different estimates of the extent of interindividual variability. Regardless of that extent, this variability will need to be catalogued in a collaborative effort akin to the HapMap and human variome projects [120-122].

B. Human virome:

So far, most of the HMP resources are focused on cellular microbes, mostly bacteria and archaea, with fewer resources directed to human-associated viruses. However, work on the microbiome will inevitably overlap with and call for a human virome project, perhaps divided into two major branches: sequencing human-associated eukaryotic viruses and sequencing human microbiota-associated bacteriophages and archaephages. Phages, in particular, are major players in diversifying the microbial communities, and are consequently involved in interindividual variations and almost certainly affect personalized medicine and pharmacomicrobiomics. Viruses go as far as introducing diversity by integrating in the human genome itself, and some of these integrated viruses are inherited causing congenital diseases, e.g., human herpesvirus 6 [123].

C. Human-associated mobilome:

The human-associated mobilome can be defined as the sum of mobile genetic elements associated with the human microbiome, e.g., prophages, integrons, transposons, and insertion elements [57]. Analyzing the human-associated mobilome involves not only cataloguing these mobile elements, but also studying their dynamic interactions, the rate of their exchange within and between microbial species, and the extent of the diversification they induce in the human microbiome.

D. Human microbial resistome:

The human microbial resistome [124, 125], or the sum of antibiotic resistance genes encoded by the human microbiome/mobilome as well as antibiotic resistance proteins in the human microbial metaproteomes, is another extrapolation of the HMP [59]. It is likely that many of these antibiotic resistance genes are never expressed; however, the potential of their expression cannot be ruled out and need to be studied more thoroughly at later stages. The HMP will eventually offer a catalogue of resistance genes; however, metatranscriptomics and metaproteomics are necessary to estimate the actual impact of the presence of these genes.

5.2. A glimpse into the future

We would like to conclude our review by an attempt to predict the implications of the HMP in the 21st century medicine. Below are three short cases or anecdotes, which might seem largely imaginary at the time being, but may become reality sooner rather than later.

A. Personalized probiotic cocktails:

Patient X visits a physician with personal genomics expertise. The patient's cellular device (phone) already has his genome and all his medical history uploaded, but he needs to undergo his semi-annual microbiome analysis. A certified microbiome analyst (CMA) runs micorbiome microarrays (to quickly quantify the species balance in the patient's gut) and notices an unusual deficiency in lactobacilli and an increase of bacteroides in the gut. She recommends additional screening for possible causes of such symptoms; meanwhile, she prescribes a probiotic cocktail to restore the microbial balance in the patient's gut. Similar microarrays can be designed for a rapid, semi-quantitative estimation of the gene expression of selected biomarkers. The costs of such techniques are still an obstacle to their implementation in clinical practice today; however, these costs are likely to drop sharply in the future as we are already getting closer to $1000 human genomes [126, 127].

B. Personalized phage cocktails:

Phage therapy is not fully implemented or approved in the Western world; but phage cocktails are widely used in some Eastern European countries (e.g., Georgia and Russia) [128]. Phage cocktails are usually custom-made to help restoring the balance of the microbial components of certain human tissue; their external use is thought to be much safer than internal use. It is not unlikely that, in the near future, an individual would visit the clinic to re-establish a "healthy" microbiota by the use of a phage cocktail. For example, a phage cocktail may be topically applied to selectively remove MRSA from a person's skin or nostrils. Moreover, a cocktail of phage-derived products, such as lysins, can be used to swab and eradicate a particular pathogen (e.g., *Streptococcus pyogenes* or *Streptococcus pneumoniae*) from a child's tonsils and upper respiratory mucosal epithelium, providing resistance-free prophylaxis against rheumatic fever, pneumonia, and other *Streptococcus*-associated diseases [128].

C. Resistome-o-graphy:

In the future, sensitivity testing or antibiograms may be replaced by more global "resistome-o-graphy." According to this suggested technique, a full prophylactic screening will be routinely performed on an individual's microbiota, not just on bacteria isolated from the site of infection. Additionally, metagenomic, metatranscriptomic, and metaproteomic analyses may be applied to samples from sites of infections in severe cases. Such screening will not only determine the antibiotic resistance genes, but also which of them are expressed, and will thus allow the prediction of the extent of antibiotic resistance at the site of infection. A variation on this technique may be the quick screening of all persons at risk (e.g., soldiers, elders, and people at natural disaster areas) for the spread of particular resistance genes.

6. CONCLUSION

The HMP, being still in its early stages, still faces some practical challenges but holds many promises. Once this project is standardized and its full results are published, tremendous opportunities will be offered and novel fields created with the promise of a better human health, improved prevention and treatment, and enhanced drug therapy with minimal side effects, the ultimate goal of biomedical and health-care research!

LIST OF ABBREVIATIONS

DFI = Diabetic foot infection

ELSI = Ethical, legal, and social implications

IHMC = International Human Microbiome Consortium

HGP = Human Genome Project

HMP = Human Microbiome Project

MetaHIT = Metagenomics of the Human Intestinal Tract

MRSA = Methicillin-resistant *Staphylococcus aureus*

NIH = National Institutes of Health

TLRs = Toll-like receptors

CONFLICT OF INTEREST

The authors have no conflicts of interests to declare

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