Antibiotic treatment and stewardship in the era of microbiota-oriented diagnostics

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Some definitions

Antibiotics are key to the battle against infectious diseases by their capacity to completely block or attenuate microbial growth. Their use is widely accepted and has saved many lives. Still, antibiotic treatment has obvious downsides. For instance, (severe) allergic reactions have been documented and most if not all antibiotics have toxic side effects [1]. Moreover, antibiotics do not specifically target single bacterial species, instead they may extinguish entire (mixed) populations of bacteria. Many of such populations are considered physiologically important. These so-called microbiota, inhabiting all niches in and on our body, are known to have clear and mostly beneficial health effects. Our microbiota, defined as the total ecological community of commensal, symbiotic, and pathogenic microorganisms that inhabit us, are most commonly composed of a mixture of Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Recent nucleic acid sequencing-based efforts have shown great progress in cataloguing this diversity of microbial species: this made us realize that until then, we had only been scratching the surface in identifying human-associated microbes, and had only been assured that many more species are still left to be discovered [2].

In addition to the downsides listed above, the use of antimicrobial drugs results in selection for less susceptible and even resistant microorganisms. The efforts employed to prevent this side effect are collectively called ‘antimicrobial

stewardship’ and cover the design and implementation of prudent antibiotic usage guidelines to prevent bacterial drug resistance, multi-drug resistance, and even extensive- or pan-drug resistance. Antibiotic treatment, selection for drug resistance, and negative effects on microbiota are, however, interdependent and therefore deserve further integrated discussion (see Fig. 1 for a visual summary).

Antibiotic resistance and antibiotic stewardship

The number of different mechanisms resulting in antibiotic resistance has been quantified as being over 6000 with more to come. This includes among others adaptive gene expression, regulation, effect of mutations, and acquisition of new genes. Resistance is therefore unavoidable and even combinations of antibiotics can ultimately be withstood because of the accumulation of multiple bacterial adaptations. Therefore, prudent use of existing and new drugs is mandatory to reduce emergence and spread of (new) resistances. The Society of Healthcare of the Americas (SHEA) defined antibiotic stewardship as a set of coordinated strategies aimed to improve antibiotic medication while enhancing patient’ outcome, reducing resistance development, and decreasing unnecessary costs. In short, antibiotic stewardship aims at patient care assuring that the right antibiotic is administered to the right person at the right dose and for the right duration. Only in this way therapeutic side effects including the selection for resistant microbes can be minimized. Obviously, diagnostics in general and antibiotic susceptibility testing more specifically must play important roles.

Currently, research into antibiotic resistance and antibiotic stewardship rarely takes aspects of microbiota characterization and (antibiotic-induced) microbiota alterations into account, although this would be a highly rational way forward [3]. First of all, the microbiome hosts the natural pool of antimicrobial resistance genes (i.e., the resistome) and their microbial owners. Second, microbiota may harbor and promote

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Fig. 1 The complex interplay between antibiotics, microbial pathogens, and the microbiome

intrinsic mechanisms to exchange resistance genes towards (potentially) pathogenic bacteria when under ecological pressure. Third, microbiota provide indirect resistance against acquisition of, overgrowth by and invasion by, drug-resistant bacteria. This suggests that development and adaptation of antibiotic treatment towards being less detrimental to the microbiota is desirable: actually, the first attempts to do so have already proven successful. Nobel et al. [4] showed that pulsed antibiotic treatment may result in lesser microbiota disruption and enhanced recovery and restructuring of the original microbial community. Such links between therapy management and microbiota preservation may become very important in a medical environment where new antibiotics are rare and require time to become available where needed.

Antibiotics and microbiota

Antibiotic treatment affects the composition of microbiota and may do so in a significant and antibiotic class-dependent manner [5]. This results in major changes in bacterial populations in and on our body, reducing diversity and skewing the usually diverse composition towards homogeneity, thereby, affecting the essential functions of our microbiota and leading to microbiological imbalance. Microbiota have been associated with modulation of bone density, promotion of fat storage, angiogenesis, biosynthesis of vitamins and essential amino acids, metabolism of therapeutics, degradation of food ingredient, protection of

epithelial injury, and more. Very importantly, our microbiota also govern the development and training of our immune system and provide important intrinsic resistance to pathogens. In a sense, our microbiota even display antibiotic activities themselves. Therefore, one could claim a healthy diverse microbiome defines an individual's health status [6]. Better understanding of the changes in this diversity and shifts in microbiota composition by antibiotic usage will protect or even improve our health status [7]. But also vice versa, microbiota-based diagnostics rather than providing information on single pathogen presence may direct the use of antibiotics: as such, Haldar et al. [8] have already shown that balance between gamma-proteobacteria and firmicutes in sputum of patients with chronic obstructive pulmonary disease (COPD) can be used, independent of pathogen presence, to predict the need for antibiotic treatment during exacerbations in these patients.

Consequences of antibiotic-induced microbiota changes

Antibiotics may not only lead to selection of and infection with multi-drug resistant microorganisms, but they may also induce antibiotic-associated disease such as diarrhea, a direct effect of the elimination of beneficial commensal bacteria in the gut. However, other side effects of antimicrobial treatment including vitamin deficiency, obesity and even asthma, and atopic disease are less well-known, though may have significant clinical impact [9]. It is common knowledge that antibiotics target different bacterial species and, consequently, have variable side effects on different classes of microbiota. Hence, different antibiotic families associate with distinctive risks for microbiota-mediated sequelae [10] and with affecting distinct physiological processes [9]. Additionally, host-characteristics such as age will affect antimicrobial side effects. For instance, younger age partially protects against antibiotic-induced *Clostridium difficile* infection and severity [11]. In contrast, age at first antibiotic treatment has been inversely associated with risk of childhood obesity [12]. Being aware of this large degree of variability makes us realize that microbiota changes must be taken into account when deciding on what antibiotic to be used for a specific patient with a given disease under given medical conditions.

An example to make this more explicit: in cases of neonatal sepsis, it is surprising to note that a variety of treatment regimens, all quite effective in their own way, are proposed in international literature and differentially promoted for use in different medical institutions [13]. Combination therapies including penicillin/gentamycin, amoxicillin/clavulanic acid/gentamycin, or amoxicillin/cefotaxime are just three examples. Depending on diagnostic work-up and/or the state of the patient after initial treatment, there are various options for

follow up treatment including the use of cephalosporins or carbapenems. Given this diversity of empiric treatment protocols, it is worthwhile to include microbiota effects in the therapeutic decision on what antibiotic treatment to use. Obviously, information on local prevalence of certain resistance traits in likely pathogens or lingering (second hit) pathogens need to be included. One should consider excluding antibiotics for which there is high-level endemicity of resistant organisms including those with inducible resistance [14]. However, second, we need to consider which treatment will harm microbiota composition the least. Which are the antibiotics that protect bacterial diversity, save the functionally important gram-positive and gram-negative anaerobes, and limit the outgrowth of the group of pro-inflammatory proteobacteria? Ongoing studies in The Netherlands have set out to provide answers to this question but more studies are urgently required (DB, personal communication).

In contrast, ecological effects could also complicate infection treatment in case of (unknown) polymicrobial infections. For example, a tobramycin-susceptible *Pseudomonas aeruginosa* strain may unexpectedly show resistant behavior during treatment with tobramycin as a consequence of interaction with a co-colonizing *Staphylococcus aureus* strain [15]. Alternatively, resistance mechanisms such as antibiotic inactivation present in one bacterial (commensal) species can indirectly protect pathogenic bacteria living in the same niche from elimination by that therapeutic drug: a mechanism that has been suggested for protection of *P. aeruginosa* against cephalosporins mediated by the common intrinsic ESBL activity of *Prevotella* spp. strains [16]. Similarly, certain bacterial commensals seem to provide a more fertile environment for certain pathogenic species, thereby, potentially increasing their virulence and promoting their outgrowth [17]. Under such circumstances, therefore, ecological (side) effects of antimicrobial drugs could also benefit the patient, but only when co-consideration of commensal co-colonizers could actually become a deliberate part of diagnostics and thereby a target of therapy.

Are we ready for improved diagnostics?

Assessment of microbiota diversity and more precise composition of microbiota currently requires relatively sophisticated technology [18], although culture-based testing can be used as “poor man’s alternative” [19]. Furthermore, there is a continuous need for procedural standardization to allow for comparison of studies from diverse origins. As an example, standardization of sampling, preservation of samples, and storage of samples are urgently needed [20].

Momentarily, next generation sequencing of amplified ribosomal RNA genes or the direct sequencing of all microbial DNA in a clinical or screening specimen is time consuming and expensive [21]. However, when the technology push as

witnessed over the past decade is extrapolated to the future, we expect within 5 years from now to be able to characterize microbiota in the laboratory for less than 10 € in less than half a day using less complicated technology (author’s personal extrapolation). The other omics technologies will start to chip in as well. Defining local microbial community diversity using mass spectrometry has already been shown feasible [22, 23]. The moment we will have low-budget options to study the consequences of microbiome changes and the relevance of certain key bacterial species in health and disease, the application of these tools as clinical diagnostics to detect changes in abundance of and balance between those organisms could already guide proper (real-time) choice and duration of certain antibiotic treatments while avoiding others. With the advent of microbiota-based therapeutic procedures, monitoring not only the human microbiota itself but also the quality and (personalized) matching of for instance fecal transplants will become more pertinent as well, especially when precise modulation of the microbiota is indicated [24, 25]. Last but not least, there is a multitude of items to be solved around legal and regulatory aspects of in vitro diagnostics (IVD). Currently, even the definition of a fecal transplant as either a drug or a biological product or a tissue is already complicated [26]. Education of regulators and detailed discussion between scientists, clinicians, IVD industries, and the regulatory bodies are therefore urgently needed.

What may the future look like?

Although we may currently lack low-cost, rapid, sensitive, and specific diagnostic laboratory tools for monitoring effects of antimicrobial treatment on microbiota dynamics and diversity, without any doubt, such tools will become available over the years to come. Monitoring bacterial diversity, quantifying (opportunistic) pathogens and their genetic capacity, and using such information for targeted antibiotic intervention will become common, and this will assure the prolonged clinical use of not only antibiotics, but essentially all other methods used to combat infections and to prevent the development of resistant pathogens. Whereas, the current technology focuses on the detection and characterization of a quite distinct panel of pathogens, future methods will further integrate close analysis of aberrations in our various microbiota as well as microbiota-broad responses to therapy. This may ultimately facilitate the necessary personalized antibiotic treatment type and duration, and thereby the optimized antibiotic stewardship we are aiming for.

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