Tackling the Antibiotic Resistance: The "Gut" Feeling

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Abstract Antibiotic resistance is a threat to human health worldwide. The ever-increasing multidrug-resistant (MDR) strains of many bacterial pathogens are paralyzing our efforts to treat many deadly infections. An important measure to deal with the menace is to better understand the process and manage the reservoirs or risk areas. Over the recent past, the importance of gut bacteria in various aspects of human health and physiology has been highlighted. Also, studies are now being carried out to better understand its role in antimicrobial resistance and grave consequences of antibiotic exposure on gut microbiota. This chapter highlights the importance of gut microbiota in better understanding of antibiotic resistance and summarizes the burden imposed by antibiotic use in the healthcare sector. Due to close contact of pathogens with dense human microbiota during the disease progression, gene transfer events might occur frequently. In this context, our microbiome warrants special attention since it can possibly act as one of the most accessible reservoir of antibiotic resistance genes. It seems pertinent to evaluate antimicrobial therapies in the context of this microbial framework, as many life-threatening infections can arise due to antibiotic-associated alterations in the gut microbiota.

1 Introduction

In 1928, when Alexander Fleming discovered penicillin, the first natural chemical compound with antibiotic properties (Fleming 1980), the world rejoiced and called it a "wonder drug." Since then, the discovery of antibiotics has probably been one of

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the most successful forms of antimicrobial treatment in the history of medicine. Little did we know that unaccounted use and misuse of many of these wonder drugs would lead to a grave situation of "antimicrobial resistance," where the deadly pathogens would smartly change in ways that make the drugs ineffective! Resistant microorganisms stop responding to the standard drugs, which were originally effective for treatment. This increases the burden of the disease, as it not only lengthens the regular time course of treatment, which in turn increases the risk of spread to others. Moreover, in case of serious pathological conditions, this long treatment exposure also increases the chances of death due to susceptibility to other opportunistic infections.

1.1 Antibiotics

Selman Waksman, the famous soil microbiologist who discovered streptomycin, proposed the term "antibiotic." Although he and his colleagues referred this term to the activity of the compound produced by microbes against other microbes, the term has since then popularly been used for any organic molecule with antibacterial properties (Waksman and Flynn 1973; Davies and Davies 2010). Microbes producing antibiotics are studied largely for exploiting their therapeutic potential; however, these compounds are known to play multifaceted role in microbial community including acting as signaling molecules (Kalia et al. 2007; Kalia and Purohit 2011; Kumar et al. 2013; Sengupta et al. 2013; Kalia 2014; Sajid et al. 2015; Koul et al. 2016). We now understand that antibiotics and antibiotic resistance genes coevolved in the organism to confer a trait, advantageous to the organism in face of selection pressure (Kalia 2013; Arora et al. 2014; Bhaduri et al. 2014; Kalia et al. 2014). So, antibiotic resistance is a naturally evolved phenomenon, without any role of human intervention. However, we also know that the recent upsurge in antibiotic resistance is due to the immense selection pressure created at various levels by anthropogenic activities. The unaccounted use of antibiotics that has resulted in the emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a grim reminder of powerful adaptation of nature (Velayati et al. 2009).

Administration of most antibiotics is associated with adverse effects that range from nausea, fever, allergic reactions, and diarrhea. While antibiotics inhibit pathogenic bacteria, they also exert detrimental effects on the commensal bacterial community that contribute to human health. One such widely studied condition is the antibiotic-associated diarrhea (AAD). Antibiotics affect the human microflora by disrupting the species composition in the gastrointestinal (GI) tract or gut, which leads to overgrowth of pathogenic bacteria, such as *Clostridium difficile*, which is responsible for conditions like diarrhea to pseudomembranous colitis in patients (Cotter et al. 2012). As compared to its presence in healthy individuals, *C. difficile* population is reported to increase in number due to antibiotic-induced disturbances. Most antibiotics have the potential of creating a bacterial imbalance in intestines, and exposure to antibiotics early in life has been implicated to lead to long-term

health effects such as development of allergic sensitization and pathogen-induced colitis (Willing et al. 2011). This is most pronounced in case of antibiotics with broad target range. The molecular targets aimed by these broad-spectrum antibiotics (e.g., cell wall components, RNA polymerase, DNA gyrase, etc.) are often highly conserved across many bacterial species, genetically as well as structurally. So, the use of an antibiotic against a pathogenic bacterium most likely also targets the bacterial community sharing the niche with the pathogen. The brazen use of broad-spectrum antibiotics has been pushed in part by the pharmaceutical industry, which focuses largely on the development of broad-spectrum antibiotics, which can be utilized to treat a variety of different infections. Moreover, most of these are available as "over-the-counter" medicines in some countries and are often administered to treat infections of unknown/little known etiology. However, it is only in recent years that the consequence of widespread use of antibiotics has caught our attention. With the help of high-throughput DNA sequencing and related technologies, we are just beginning to comprehend the effect of antibiotic use on the gut microbiota of humans. Over the years, the consumption of antibiotics has led to collateral damage faced by indigenous host-associated microbes, which results in physiological turmoil.

1.2 Microbiome

The collection of microorganisms present in our body is commonly referred as human microbiota/microbiome. It is estimated that microbes constitute 100 trillion cells in our body, almost tenfold the number of human cells (Eckburg et al. 2005; Sears 2005). These microbes colonize mostly all body surfaces like the gut, oral cavity, auditory canal, nares, and skin surfaces (Costello et al. 2009). Of all the microbial population associated with our body, 99 % of the population is bacterial and the majority resides in the gut (Gill et al. 2006; Qin et al. 2010). Of the known phylogenetic categories, bacterial phylotypes, *Bacteroidetes*, and the *Firmicutes* constitute over 90 % of the distal gut microbiota. Populations of *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucobacteria* are present in minor proportions, and change in their relative proportions is indicative of some pathological conditions (Eckburg et al. 2005; Sears 2005; Kalia 2014). It is now well appreciated that these microbes play an important role in the human physiology and health. Some of the crucial processes governed by gut microbes include nutrition, modulation of immune system, and pathogen invasion (Round and Mazmanian 2009; Qin et al. 2010). Since we have been unable to culture most of these bacteria, our understanding of this microbial pool began with the advent of high-throughput sequencing technologies. The microbiome studies have shown substantial diversity of gut microbes between healthy individuals, with lifestyle and diet playing a crucial role in establishing the diversity (Dicksved et al. 2007; Flint et al. 2007; Jernberg et al. 2010). It is now known that the GI tract of a fetus is sterile, and microbiota is acquired primarily during passage through the birth canal in an infant,

with the mother's vaginal microbiota being the most common influence. In this context, the mode of infant delivery also governs the microbe diversity and, in turn, susceptibility to various infections (Mändar and Mikelsaar 1996; Huurre et al. 2008). Apart from this, the illness and use of antibiotics are known to cause a drastic change in microbiota or dysbiosis (Dethlefsen and Relman 2011; Faith et al. 2013).

2 Burden of Antibiotic Resistance

Since the first report of antibiotic usage and resistance, the burden of resistance among bacteria has progressively increased and has accelerated within the last decade. In an alarming trend, a survey carried out to account for global consumption of antibiotics from year 2000 to 2010 revealed an increase of 36 % in antibiotic consumption in 71 countries over this period, with India, Brazil, China, Russia, and South Africa accounting for 76 % of this increase. India also emerged as the largest consumer of antibiotics (Van Boeckel et al. 2014). Antibiotic resistance is now established to be a cause of grave concern to human health and welfare. The problem is even intense in developing countries such as India where comprehensive surveillance system is lacking in healthcare sector to monitor the burden of antimicrobial resistance. Due to lack of surveillance data, often the scenario is underrepresented. Depending upon the degree of resistance, most people with antibiotic resistance remain infected for longer. This not only increases the cost of healthcare in medical centers but also increases the chance of death. For example, according to the World Health Organization (WHO), methicillin-resistant *Staphylococcus aureus*-infected patients are 64 % more likely to die as compared to patients infected with a nonresistant form of the bacteria. Similarly, people infected with multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* require much longer courses for treatment of tuberculosis (http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf). According to the US Center for Disease Control and Prevention (CDC), treatment of *C. difficile* infections requires at least $1 billion in excess medical costs per year (CDC, Antibiotic Resistance Threats in the United States, 2013).

Bacterial infections can be broadly classified into healthcare-associated infections (HAIs) or nosocomial infections and community-acquired infections (CAI). CAIs are infections developed outside of a healthcare setting or an infection present on admission, while HAIs are infections that patients contract during the course of receiving treatment for other conditions within a healthcare setting. A large proportion of infections reported in hospitals reveal that HAIs are on a rise, and, usually, strains causing HAIs tend to be naturally more resistant to antibiotics. In a review by Wattal et al., the authors have provided a beautiful summary of the burden of resistance, associated with HAI and CAI, in India (Wattal and Goel 2014). According to the CDC report, at least two million people in the United States acquire bacterial infections that are resistant to one or more of the antibiotics

designated to cure those infections, each year (CDC, Antibiotic Resistance Threats in the United States, 2013). The WHO also in its report on resistance surveillance highlighted the serious threat to public health globally due to antibiotic resistance (World Health Organization, "Antimicrobial resistance: Global report on surveillance, 2014").

Some of the key findings from these reports and others are summarized below:

- Resistance to carbapenem antibiotics in *Klebsiella pneumonia*, a common cause of HAI, has spread all over the world.
- One type of HAI—*C. difficile*—is directly associated with administration of antibiotics and may prove to be life-threatening, especially in older adults under medical care.
- Resistance to first-line drugs to treat infections caused by *S. aureus*—a common cause of CAI and HAI—is also widespread.
- MDR and XDR *M. tuberculosis* infections are an increasing threat, globally.
- *Acinetobacter* strains, a cause of pneumonia or bloodstream infections among critically ill patients, are resistant to nearly all antibiotics including carbapenems.
- *Enterococcus* strains have become resistant to vancomycin, making treatment difficult.
- *Salmonella typhi* and non-typhoidal *Salmonella* infections are also reported to be resistant to drugs such as ceftriaxone and ciprofloxacin.
- In 10 countries, millions of people die of gonorrhea, a sexually transmitted disease, because *Neisseria gonorrhoeae* has become resistant to even the last recourse of treatment—the third-generation cephalosporins. An impending danger looms wherein gonorrhea may soon become untreatable, as no alternative treatment measure is currently under development.
- The treatment of urinary tract infections, mainly due to strains of *Escherichia coli*, involves administration of one of the most widely used antibiotic—the fluoroquinolones—resistance to which is very widespread.
- Resistance in *Vibrio cholerae* has also been reported against commonly used antibiotics like furazolidone, co-trimoxazole, and fluoroquinolones (Sharma et al. 2007).
- High prevalence of resistance to first-line drugs is reported in *Shigella* spp., and the recent emergence of ceftriaxone resistance in *Shigella* has led to the use of carbapenems for the treatment of a simple community-acquired diarrheal disease (Taneja et al. 2012).
- Many other infectious agents are increasingly bearing multidrug resistance traits such as *Campylobacter* spp. and *Aeromonas* spp.

3 Source of Antibiotic Resistance in Pathogens

Antibiotic resistance genes (ARGs) have been present in bacterial populations from ancient times (D'Costa et al. 2011), and cycling of ARGs in environmental settings is well documented (Vaz-Moreira et al. 2014; Berendonk et al. 2015). Resistance is known to arise in bacteria either by spontaneous mutations in their genomes (Bagel et al. 1999) or by means of horizontal gene transfer (Frost et al. 2005; Modi et al. 2014) (Fig. 1). Antibiotic resistance genes are often encoded on mobilizable genetic elements, called integrons, in environmental bacteria and commensals and can move between diverse bacteria to disseminate resistance genes, when microbial communities communicate with each other under high selection pressure (Alekshun and Levy 2006). These conditions include communication under highly dense bacterial populations subjected to sub-therapeutic antibiotic concentrations. These interactions can be seen not only in environmental conditions that are subjected to anthropogenic pressure, such as municipal wastewater systems, pharmaceutical manufacturing units, and animal husbandry facilities, but also in hospital settings (Berendonk et al. 2015). The occurrence of New Delhi metallo-beta-lactamase-1 (NDM-1) containing bacteria in water samples in New Delhi is one such stark example of the gravity of the situation (Walsh et al. 2011). The use of sub-therapeutic doses of antibiotics or related compounds in the agricultural industry and animal husbandry to promote animal growth creates a perpetual selective

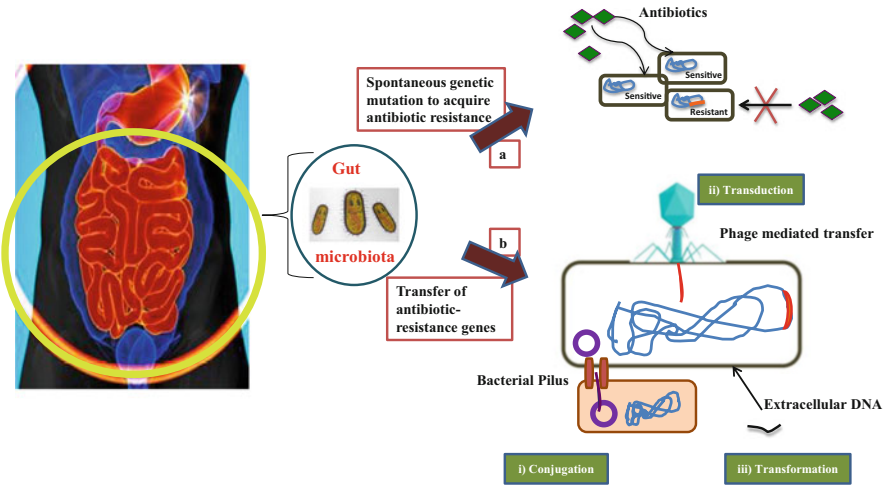

Fig. 1 Acquisition of antibiotic resistance in bacteria. Bacteria are known to acquire resistance to antibiotics by two mechanisms: (a) *spontaneous genetic mutations* which enable bacterium to resist antibiotics either by inactivating them directly or indirectly by modifying the targets/route of entry and (b) acquired resistance genes from another source by means of (i) *conjugation*, which involves direct cell–cell contact with another bacteria; (ii) *transduction*, which is virus-mediated transfer of DNA; and (iii) *transformation*, which is the ability to acquire naked DNA from the environment

pressure which in turn facilitates resistance to develop and may possibly contribute to a larger global resistance reservoir (Heuer and Smalla 2007; Berendonk et al. 2015). In fact, a study reveals that usage of antibiotics in beekeeping in the United States has led to accumulation of extensive tetracycline resistance genes in the microbiota of honeybees (Tian et al. 2012). There is evidence of horizontal transfer of genes encoding carbohydrate-active enzymes between marine bacteria and gut microbiome of Japanese individuals with seaweed-rich diet (Hehemann et al. 2010), which suggests that one such niche can also be the human gut microbiome of healthy individuals, which acts as a reservoir of ARGs, although the direct experimental evidence of in vivo transfer of antibiotic resistance genes within the human microbiome is very limiting (Doucet-Populaire et al. 1991; Ley et al. 2006; Smillie et al. 2011). There is enough alarming evidence to endorse the view that the transfer of ARGs in humans is possible to overcome persistent antibiotic selective pressure, and our urgent attention is warranted to tackle the situation.

4 Effect of Antibiotics on Gut Microbiota

Antibiotics affect the microbial composition in two different ways. Firstly, it decreases the competition for resources for microbes in turn opening up many ecological niches for opportunistic pathogens. In addition, lysis of susceptible bacteria releases carbon sources which can then be utilized by the remaining members of the microflora (Willing et al. 2011). For example, upon disruption of resident microbiota due to antibiotics, two distantly related antibiotic-associated pathogens, *S. enterica* serovar *Typhimurium* and *C. difficile*, utilize the microbiota-liberated sialic acids for their proliferation (Ng et al. 2013). With the help of culture-based approach and next-generation sequencing technologies, many studies have now investigated the effect of administration of antibiotics on persistence of resistance in gut microbiota. These studies have highlighted the potential of gut microbiota as a reservoir accessible to pathogens under extreme antibiotic selection pressure. Some of the key examples from various studies are:

- *Helicobacter pylori*, a gram-negative bacterium colonizing the gastric mucosa, is the main causative agent of peptic ulcer and gastric cancer. Its treatment requires a triple therapy combination with clarithromycin, metronidazole, and omeprazole. It was revealed in a study that apart from the short- and long-term disruption of indigenous microbiota in the throat and in the lower intestine region, there was enrichment of macrolide resistance gene, even after 4 years of treatment (Jakobsson et al. 2010). In an independent study, this treatment regimen was compared with another combination containing amoxicillin and was suggested better for treatment of *H. pylori* since it resulted in emergence of lesser resistant strains, so it can be considered better from an ecological perspective (Adamsson et al. 1999).

- An overgrowth of resistant enterobacterial species was observed on administration of amoxicillin with or without clavulanate (Sullivan et al. 2001).
- Administration of cephalosporin leads to decrease in the abundance of enterobacteria and increased the levels of enterococci, which are known to be intrinsically resistant to this antimicrobial agent (Rafii et al. 2008).
- Another report showed that even short-term exposure to clindamycin treatment led to long-term impacts on the intestinal microbiota and results in dramatic increase in levels of specific resistance genes (Jernberg et al. 2007).
- Karami et al. have demonstrated β-lactamase gene transfer between two *E. coli* strains co-residing in the human gut of a child who was administered ampicillin (Karami et al. 2007).
- Sommer et al. characterized the gut microbes of unrelated healthy individuals who for at least 1 year were not exposed to antibiotics. Surprisingly, with the help of metagenomic as well as culturing approaches, they could decipher many resistance genes that were identical to genes harbored by pathogens, while many were evolutionarily distant from known resistance reservoir. This alarming fact underscored the potential of commensal microbiota of healthy individuals acting as reservoirs of resistance genes, with an imminent contribution to antibiotic resistance (Sommer et al. 2009).

The disturbance in microbiota has also been associated to many disease phenotypes. In many instances, downstream regulation of innate defenses has been associated with antibiotic-induced disruption of microbiota, leading to colonization of other microbes (Brandl et al. 2008; Willing et al. 2011). Antibiotic-associated diarrhea (AAD), a condition characterized by administration of antibiotics and not any other obvious causes, is one of the most common complications arising in hospitals. Although the frequency of AAD can vary between different antibiotics, it is believed that AAD can affect up to 25 % of the patients receiving a particular antibiotic (Young and Schmidt 2004). It is also reported that cephalosporin use during early childhood leads to increased susceptibility to asthma (Kozyrskyj et al. 2007). Kanamycin administration during infancy led to modulation of gut microbiota and was associated with the development of atopic dermatitis-like skin lesions in a mice model (Watanabe et al. 2010). Furthermore, importance of gut microbiota is also emphasized by the fact that fecal microbial transplantation (FMT) has proved to be successful in treatment of recurrent *C. difficile* infection (Seekatz et al. 2014). However, the mechanism and long-term effects of this treatment still remain to be elucidated. In addition, effectiveness of FMT in other diseases still needs to be ascertained.

5 Road to Future

Many steps need to be taken worldwide to tackle the emergence and spread of antibiotic resistance. Firstly, we need to have better understanding about the health and physiology of healthy individuals and the tussle between host and pathogen by modulating various physiological processes (Sachdeva et al. 2010; Maji et al. 2015). Furthermore, detailed understanding about the relationship between the various human-associated and environmental reservoirs that harbor distinct resistance genes need to be attained. With advent of metagenomic approach and advancement in whole-genome sequencing methods, our knowledge about the human microbiome functional diversity and impact of antibiotic treatment on microbial community is improving (Fig. 2). This information needs to be expanded further and applied for effective monitoring of antibiotic load and better formulation of guidelines for therapies. In view of the challenges faced by the community, the WHO focus for the World Health Day in 2011 was antimicrobial resistance, and it also recently concluded the first "Antibiotic Awareness Week" in November 2015. The campaign was aimed to increase awareness in people about the rising antibiotic resistance globally and to encourage good practices among the health practitioners as well as general public to avoid further emergence and spread of drug resistance. However, poor implementation poses a significant challenge toward reaping the benefits of these programs. In order to turn the tide on

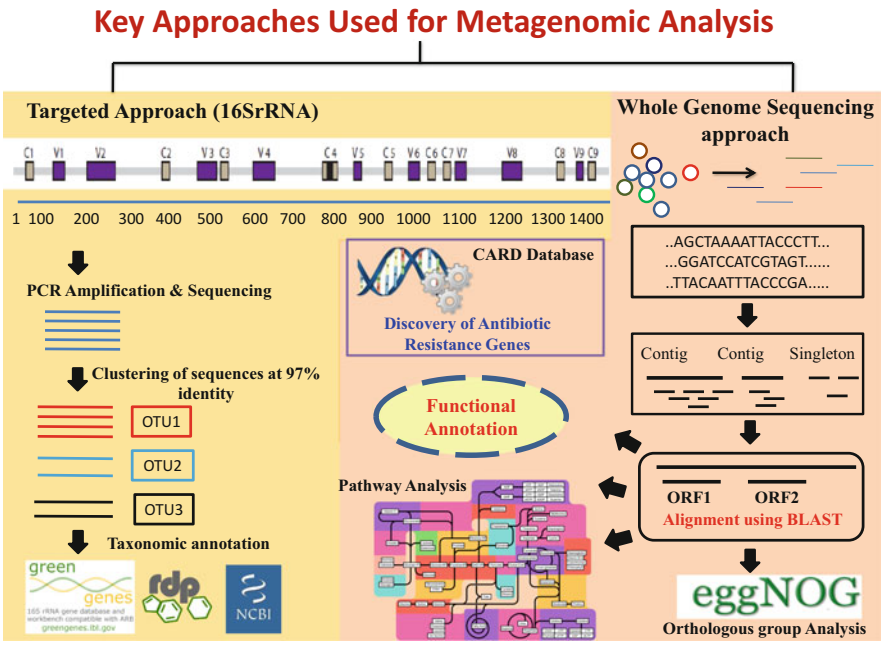

Fig. 2 Schematic representation of metagenomics study

antimicrobial resistance, we need to have a better infrastructural framework, which ensures strict implementation of good practices. Unrestricted use of antibiotics in all sectors, such as healthcare, agricultural, etc., should be curtailed. Examination of antibiotic resistance in environmental samples like sludge, soil, and manure samples should be carried out. Greater importance should be given toward strict implementation of these guidelines for antibiotic therapies. We need to formulate and follow laws against antibiotic dispensing without prescription. The general public should be educated and made aware of the flip side of using antibiotics for any medical problem. Moreover, mandatory refresher courses should be framed for medical practitioners, both in rural and urban settings, to update them about the global medical trends. Improvement in diagnostic facilities, treatment facilities, and overall infrastructural advancement can go a long way in curtailing this deadly menace.

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References

Adamsson I, Nord CE, Lundquist P, Sjöstedt S, Edlund C (1999) Comparative effects of omeprazole, amoxicillin plus metronidazole versus omeprazole, clarithromycin plus metronidazole on the oral, gastric and intestinal microflora in *Helicobacter pylori*-infected patients. J Antimicrob Chemother 44:629–640. doi:10.1093/jac/44.5.629

Alekshun MN, Levy SB (2006) Commensals upon us. Biochem Pharmacol 71:893–900. doi:10.1016/j.bcp.2005.12.040

Arora G, Sajid A, Singhal A, Joshi J, Virmani R, Gupta M, Verma N, Maji A, Misra R, Baronian G, Pandey AK, Molle V, Singh Y (2014) Identification of Ser/Thr kinase and forkhead associated domains in *Mycobacterium ulcerans*: characterization of novel association between protein kinase Q and MupFHA. PLoS Negl Trop Dis 8, e3315. doi:10.1371/journal.pntd.0003315

Bagel S, Hüllen V, Wiedemann B, Heisig P (1999) Impact of *gyrA* and *parC* mutations on quinolone resistance, doubling time, and supercoiling degree of *Escherichia coli*. Antimicrob Agents Chemother 43:868–875

Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons MN, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL (2015) Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 13:310–317. doi:10.1038/nrmicro3439

Bhaduri A, Misra R, Maji A, Bhetaria PJ, Mishra S, Arora G, Singh LK, Dhasmana N, Dubey N, Virdi JS, Singh Y (2014) *Mycobacterium tuberculosis* cyclophilin A uses novel signal sequence for secretion and mimics eukaryotic cyclophilins for interaction with host protein repertoire. PLoS One 9, e88090. doi:10.1371/journal.pone.0088090

Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, Schnabl B, DeMatteo RP, Pamer EG (2008) Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature 455:804–807. doi:10.1038/nature07250

Centers for Disease Control and Prevention (CDC) (2013) Antibiotic resistance threats in the United States, 2013. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R (2009) Bacterial community variation in human body habitats across space and time. Science 326:1694–1697. doi:10.1126/science.1177486

Cotter PD, Stanton C, Ross RP, Hill C (2012) The impact of antibiotics on the gut microbiota as revealed by high throughput DNA sequencing. Discov Med 13:193–199

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433. doi:10.1128/MMBR.00016-10

D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD (2011) Antibiotic resistance is ancient. Nature 477:457–461. doi:10.1038/nature10388

Dethlefsen L, Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A 108(Suppl 1):4554–4561. doi:10.1073/pnas.1000087107

Dicksved J, Flöistrup H, Bergström A, Rosenquist M, Pershagen G, Scheynius A, Roos S, Alm JS, Engstrand L, Braun-Fahrländer C, von Mutius E, Jansson JK (2007) Molecular fingerprinting of the fecal microbiota of children raised according to different lifestyles. Appl Environ Microbiol 73:2284–2289. doi:10.1128/AEM.02223-06

Doucet-Populaire F, Trieu-Cuot P, Dosbaa I, Andremont A, Courvalin P (1991) Inducible transfer of conjugative transposon Tn1545 from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. Antimicrob Agents Chemother 35:185–187

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308:1635–1638. doi:10.1126/science.1110591

Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI (2013) The long-term stability of the human gut microbiota. Science 341:1237439. doi:10.1126/science.1237439

Fleming A (1980) Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae* by Alexander Fleming, Reprinted from the British Journal of Experimental Pathology 10:226–236, 1929. Rev Infect Dis 2:129–139

Flint HJ, Duncan SH, Scott KP, Louis P (2007) Interactions and competition within the microbial community of the human colon: links between diet and health. Environ Microbiol 9:1101–1111. doi:10.1111/j.1462-2920.2007.01281.x

Frost LS, Leplae R, Summers AO, Toussaint A (2005) Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 3:722–732. doi:10.1038/nrmicro1235

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359. doi:10.1126/science.1124234

Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G (2010) Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature 464:908–912. doi:10.1038/nature08937

Heuer H, Smalla K (2007) Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol 9:657–666. doi:10.1111/j.1462-2920.2006.01185.x

Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E (2008) Mode of delivery – effects on gut microbiota and humoral immunity. Neonatology 93:236–240. doi:10.1159/111102

Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5, e9836. doi:10.1371/journal.pone.0009836

Jernberg C, Löfmark S, Edlund C, Jansson JK (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 1:56–66. doi:10.1038/ismej.2007.3

Jernberg C, Löfmark S, Edlund C, Jansson JK (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156:3216–3223. doi:10.1099/mic.0.040618-0

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245. doi:10.1016/j.biotechadv.2012.10.004

Kalia VC (2014) Microbes, antimicrobials and resistance: the battle goes on. Indian J Microbiol 54:1–2. doi:10.1007/s12088-013-0443-7

Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140. doi:10.3109/1040841X.2010.532479

Kalia VC, Rani A, Lal S, Cheema S, Raut CP (2007) Combing databases reveals potential antibiotic producers. Expert Opin Drug Discov 2:211–224. doi:10.1517/17460441.2.2.211

Kalia VC, Wood TK, Kumar P (2014) Evolution of resistance to quorum-sensing inhibitors. Microb Ecol 68:13–23. doi:10.1007/s00248-013-0316-y

Karami N, Martner A, Enne VI, Swerkersson S, Adlerberth I, Wold AE (2007) Transfer of an ampicillin resistance gene between two *Escherichia coli* strains in the bowel microbiota of an infant treated with antibiotics. J Antimicrob Chemother 60:1142–1145. doi:10.1093/jac/dkm327

Koul S, Prakash J, Mishra A, Kalia VC (2016) Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria. Indian J Microbiol 56:1–18. doi:10.1007/s12088-015-0558-0

Kozyrskyj AL, Ernst P, Becker AB (2007) Increased risk of childhood asthma from antibiotic use in early life. Chest 131:1753–1759. doi:10.1378/chest.06-3008

Kumar P, Patel SK, Lee JK, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. doi:10.1016/j.biotechadv.2013.08.007

Ley RE, Peterson DA, Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124:837–848. doi:10.1016/j.cell.2006.02.017

Maji A, Misra R, Kumar AM, Kumar D, Bajaj D, Singhal A, Arora G, Bhaduri A, Sajid A, Bhatia S, Singh S, Singh H, Rao V, Dash D, Baby SE, Michael JS, Chaudhary A, Gokhale RS, Singh Y (2015) Expression profiling of lymph nodes in tuberculosis patients reveal inflammatory milieu at site of infection. Sci Rep 5:15214. doi:10.1038/srep15214

Mändar R, Mikelsaar M (1996) Transmission of mother's microflora to the newborn at birth. Biol Neonate 69:30–35. doi:10.1159/000244275

Modi SR, Collins JJ, Relman DA (2014) Antibiotics and the gut microbiota. J Clin Invest 124:4212–4218. doi:10.1172/JCI72333

Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL (2013) Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 502:96–99. doi:10.1038/nature12503

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Meta HITC, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65. doi:10.1038/nature08821

Rafii F, Sutherland JB, Cerniglia CE (2008) Effects of treatment with antimicrobial agents on the human colonic microflora. Ther Clin Risk Manag 4:1343–1358. doi:10.2147/TCRM.S4328

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. doi:10.1038/nri2515

Sachdeva P, Misra R, Tyagi AK, Singh Y (2010) The sigma factors of *Mycobacterium tuberculosis*: regulation of the regulators. FEBS J 277:605–626. doi:10.1111/j.1742-4658.2009.07479.x

Sajid A, Arora G, Singhal A, Kalia VC, Singh Y (2015) Protein phosphatases of pathogenic bacteria: role in physiology and virulence. Annu Rev Microbiol 69:527–547. doi:10.1146/annurev-micro-020415-111342

Sears CL (2005) A dynamic partnership: celebrating our gut flora. Anaerobe 11:247–251. doi:10.1016/j.anaerobe.2005.05.001

Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, Young VB (2014) Recovery of the gut microbiome following fecal microbiota transplantation. mBio 5:e00893-14. doi:10.1128/mBio.00893-14

Sengupta S, Chattopadhyay MK, Grossart HP (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 4:47. doi:10.3389/fmicb.2013.00047

Sharma NC, Mandal PK, Dhillon R, Jain M (2007) Changing profile of *Vibrio cholerae* O1, O139 in Delhi & its periphery (2003–2005). Indian J Med Res 125:633–640

Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244. doi:10.1038/nature10571

Sommer MO, Dantas G, Church GM (2009) Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 325:1128–1131. doi:10.1126/science.1176950

Sullivan A, Edlund C, Nord CE (2001) Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 1:101–114. doi:10.1016/S1473-3099(01)00066-4

Taneja N, Mewara A, Kumar A, Verma G, Sharma M (2012) Cephalosporin-resistant *Shigella flexneri* over 9 years (2001–09) in India. J Antimicrob Chemother 67:1347–1353. doi:10.1093/jac/dks061

Tian B, Fadhil NH, Powell JE, Kwong WK, Moran NA (2012) Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. mBio 3:e00377-12. doi:10.1128/mBio.00377-12

Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis 14:742–750. doi:10.1016/S1473-3099(14)70780-7

Vaz-Moreira I, Nunes OC, Manaia CM (2014) Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol Rev 38:761–778. doi:10.1111/1574-6976.12062

Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, Ziazarifi AH, Hoffner SE (2009) Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 136:420–425. doi:10.1378/chest.08-2427

Waksman SA, Flynn JE (1973) History of the word 'antibiotic'. J Hist Med Allied Sci 28:284–286. doi:10.1093/jhmas/XXVIII.3.284

Walsh TR, Weeks J, Livermore DM, Toleman MA (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 11:355–362. doi:10.1016/S1473-3099(11)70059-7

Watanabe J, Fujiwara R, Sasajima N, Ito S, Sonoyama K (2010) Administration of antibiotics during infancy promoted the development of atopic dermatitis-like skin lesions in NC/Nga mice. Biosci Biotechnol Biochem 74:358–363. doi:10.1271/bbb.90709

Wattal C, Goel N (2014) Tackling antibiotic resistance in India. Expert Rev Anti Infect Ther 12:1427–1440. doi:10.1586/14787210.2014.976612

Willing BP, Russell SL, Finlay BB (2011) Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol 9:233–243. doi:10.1038/nrmicro2536

World Health Organization (2014) Antimicrobial resistance: global report on surveillance. WHO Press. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf

Young VB, Schmidt TM (2004) Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol 42:1203–1206. doi:10.1128/JCM.42.3.1203-1206.2004