Chapter 1

Metagenomics of Antimicrobial Resistance in Gut Microbiome

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76214

Abstract

A healthy human body functions in sync with a wide array of gut microbes collectively known as human gut microbiome. They complement in a number of functions which are essential in our daily life such as in food metabolism. Various illnesses including colon cancer, autism, obesity, and autoimmune diseases have been linked to an imbalanced gut microbiota. Antibiotics are indispensable drug; however, the administration of antibiotics in humans as well as in animal farms has shown to increase antimicrobial resistance genes (ARGs) in gut microbiome. This is of serious concern since the commensals in gut microbiome could capture ARGs through horizontal gene transfer which in turn could cause postsurgical infections. In addition, numerous studies have consistently shown that the gut microbiome is unique to each individual. Hence, in-depth knowledge on the gut microbiota community and the factor responsible for shaping and spreading of ARGs is essential. This would in turn enable the development of custom-tailored personalized food and drugs in the future.

Keywords: metagenomics, gut microbiome, antimicrobial resistance genes (ARGs) and gut resistome

1. Introduction

1.1. The gut microbiome and its significance

The human gut microbiome, also known as "second genome" [1], hosts over 100 trillion microorganisms [2] collectively covering over 150 folds more unique genes than the host [3, 4]. Several projects such as the Human Microbiome Project, MyNewGut, and Meta-HIT have been initiated with the aim to understand the entirety and the functional potential of gut microbiome and to find possible strategies to benefit the host though the alteration of gut

microbiome [5]. The gut microbiome has been linked to various functions, some of which are discussed subsequently.

1.1.1. Gut microbiome is a necessary digestive "organ"

The gut microbiome is also considered as a "metabolically active organ" [6]. The distal human intestine is an anaerobic bioreactor consisting of numerous microbes having the ability to degrade and harvest nutrients which are otherwise inaccessible to the host [7]. In return, the host provides the raw materials and shelter to the microbiome. In this way, the host is relieved of various genotypic attributes which the microbiome complements. Studies have shown that the microbiome coevolved with us by having a mutualistic association [8]. It would seem that the microbiome might compete with the host for food and nutrients. However, conventional animals require 30% more calorie intake than the germfree counterparts in order to maintain the same body weight, implying that the microbiome actually aid in the host metabolism [9, 10].

1.1.2. Personalized gut microbiome

The gut microbiome, similar to fingerprint, has its own unique signature for every individual which is, however, very dynamic [11, 12]. The changes in the microbiota, also called dysbiosis, have also been associated to several health issues [13]. This has led to the possibility of personalized medicine and diet tailored uniquely for every individual depending on his/her unique microbiome [14].

1.1.3. The gut-microbiome-brain connection

Alterations in gut microbiota have also been linked to autism spectrum disorder (ASD) and gut-microbiome-brain connection. Maternal immune activation (MIA) mouse exhibits similar symptoms to ASD such as neurodevelopmental disorders, dysbiosis, alterations in gastrointestinal (GI), and serum metabolites [15–18]. Such MIA mouse when treated with *Bacteroides fragilis* improves intestinal permeability, tight junction proteins, and colon cytokines IL-6 which is required by the MIA offspring for the development of behavioral deficits [9, 19]. Precursor 4-ethylphenol (4EP) found in MIA mice have also been shown to increase anxiety-like behavior in naïve mice. 4EP is produced by several species of *Clostridium* which are also abundant in MIA mice. Treatment with *B. fragilis* resorts the 4EP level in MIA further supporting the role of microbiota in behavioral development [9].

1.2. Types of ARGs in gut microbiome resistome

The gut microbiome resistome can be broadly classified into intrinsic and mobile resistance genes [20]. As the name suggests, intrinsic resistance genes are non-mobile resistance genes which are inherited and render tolerance to a particular drug without prior exposure. Although less mobile, there are possibilities of intrinsic resistance genes getting captured into mobile genetic elements (MGEs). Such events would turn it into mobile-resistant genes. Hence, studying such intrinsic resistance would provide knowledge on the mechanism and the possible treatment to tackle in case of outbreaks [20]. On the other hand, mobile resistomes are the resistance genes which are encoded in the highly mobile mobile genetic elements (MGE).

Mobile genetic elements include plasmids, transposons, integrons, integrative conjugative elements, genomic islands, and phages [20–25]. Resistance genes can get accumulated into a particular segment of DNA forming a special genomic island encoding multiple antimicrobial resistance genes (ARGs) called resistance islands (RIs). For instance, *Acinetobacter baumannii* Resistance Island of 86 kb is the largest known RI harboring 45 ARGs [26]. Resistance genes encode for proteins that render the microbe resistance to various antibiotics (**Figure 1**).

1.3. Factors that shape and spread gut microbiome ARGs

It is essential to understand the factors that shape and spread ARGs in the gut microbiome since gut microbiota regulates the human body in a diverse way, many of which are yet to

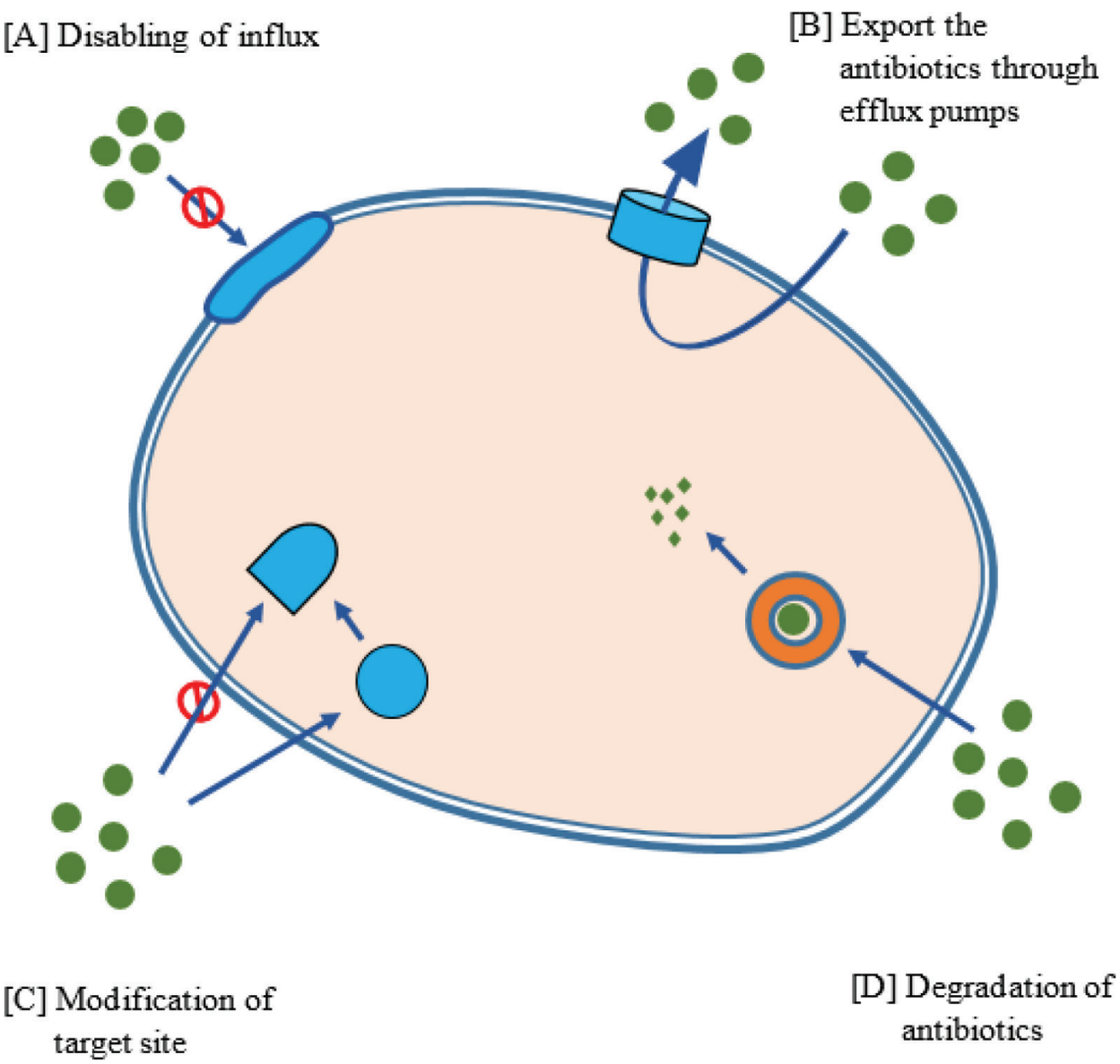

Figure 1. Antibiotic resistance mechanisms: They are broadly classified into four types: (A) the influx of the antibiotics is disabled into the cell, (B) the antibiotics that manage to get into the cell is pumped out by active efflux pumps, (C) the target site for antibiotic in the cell is modified so that the antibiotic cannot bind, and (D) the antibiotics that enters the cells are degraded by the cell machinery.

be known. It is indeed an important part of our body as discussed earlier which need special attention. However, the human gut microbiome is exposed to every food and drugs we consume. The microbiota is, therefore, reflected by the dynamic nature it faces. Cataloging ARGs in gut microbiome is essential in order to study and determine the source and the possible measure to tackle the problem.

1.3.1. Horizontal gene transfer through mobile genetic elements

Mobile genetic elements (MGEs) are transferred between microbes through horizontal gene transfer (HGT) involving conjugation, transduction, and transformation. Transformation is the capturing of naked DNA from the environment into the microbe. If the naked DNA has ARG encoded in it, the microbe taking up the naked DNA would gain resistance owning to the resistant gene encoded in the naked DNA. However, such events are found to be considerably rare in the mammalian gut [27]. Hence, comparatively, conjugation and transduction seem to have a higher impact in ARG horizontal gene transfer [28]. Conjugation involves the formation of mating bridge though which the ARGs are transferred from the donor to the recipient cell. Bacterial HGTs are more common among the same phylogenetic taxa [29]. ARG transfer was boosted between the commensal *Escherichia coli* and other pathogens during gut inflammation [30]. However, ARG transfer through conjugation was significantly reduced between *E. coli* strains in the healthy human gut since the intestinal epithelial cells produce a proteinaceous compound [28, 31] which could interfere with the conjugative process. In transduction, the ARG is encoded in the bacteriophages which get incorporated into the host once the bacteriophages invade a bacterium. It is postulated that transduction could be a major player in gut resistome [32] since the amount of phages and bacteria is equivalent in the intestinal tract [33, 34]. This is supported by the work of Goren et al. [35] showing that the phages isolated from antibiotic-treated mice when inoculated to aerobic microbiota culture showed higher ARG isolates when compared to culture which was treated with non-antibiotic-treated mice.

1.3.2. Gut resistome and antibiotic usage in farm animals

In the United States, nearly 80% of the antibiotics produced is used up in animal farm for treatment purposes [36]. As a result, the gut microbiome of farm animals is highly enriched in ARGs due to regular antibiotics treatment [37, 38]. ARGs enrichment up to 28,000 folds, including numerous unique ARGs, were detected in Chinese Swine farm [38] having efflux pumps, antibiotic deactivation, and cellular protection resistance mechanism. However, antibiotic-free organic pig guts were also found to harbor novel genes encoding resistance to the tetracyclines which were associated with putative mobile genetic elements [39]. Tetracycline resistance gene had the highest ratio of total ARGs according to a large-scale human gut microbiome analysis within the population from Denmark, Spain, and China. The study suggests the possibility of tetracycline resistance gene being transferred from animals since tetracyclines were highly used in animal farms [40, 41]. Subjects from country with comparatively tighter policies on antibiotic usages in humans and animals have considerably lesser ARG levels [42]. In addition, the antibiotic resistance genes revealed signature clustering of Chinese samples separate from other European countries thought single nucleotide polymorphisms (SNPs) analysis [41]. An independent study [43] on another population further supports this

idea of ARG signature. The country-wise signature patterns could be linked to different policies adapted in different countries [28].

1.3.3. Travelers and migratory birds spread ARGs

ARGs can also spread through traveling. In a study involving Swedish students exchange programs to India or Central Africa, the level of sulfonamide, trimethoprim, and beta-lactams were increased after the completion of the exchange programs [44]. The spread of ARGs can also be affected widely by migratory birds, which fly long distances [45].

1.3.4. Antibiotic therapy enriches ARGs

Gut microbiome is a reservoir of ARGs which can indirectly pass the ARGs into the environment. The application of antibiotics has been largely linked to increase in ARGs. Resistance to aminoglycosides was found to increase after admitting to intensive care unit (ICU) [46]. ARGs were also found to increase on patients after treatment with antibiotics [47]. Studies on large-scale human gut samples from 10 different countries have shown that the ARGs in gut microbiome are highly influenced by the antibiotic usage and food products [48] while other factors such as age, sex, body mass index (BMI), and health status did not show significant contribution to ARGs level. The administration of cephalosporin, cefprozil, increased *Lachnoclostridium bolteae* in 16 out of 18 participants, as revealed from a study by Raymond et al. [49]. It also increased opportunistic pathogen *Enterobacter cloacae* in those participants whose initial microbiome diversity was comparatively lower. The treatment also enriched ARGs which were undetectable before the treatment. The alternation in the microbiome was specific to each subject, however, in a specific and reproducible manner. The authors, Raymond et al. [49], hypothesized that the initial analysis of microbiome before the treatment of antibiotics could bypass adverse effects during and after the antibiotic treatments. Nonetheless, the reduction of ARGs was seen in some studies when combinatorial antibiotic treatment was administrated [28, 46, 47]. This could happen when the resistant microbe is susceptible to another antibiotic when given in combination [28]. The application of antibiotic treatment, in addition to alteration of gut microbiome, can also cause long-term persistence of the ARGs in the gut microbiota [50]. Hence, alternative approach to antibiotic therapy is of urgent need to avoid undesirable effects to the microbiota. Alternative therapies such as probiotic intervention, vaccination, and bacteriotherapy [51–54] have been developed. However, such alternative strategies are still at infancy stage; hence, focus on such strategies have to be encouraged.

1.4. Gut microbiome ARGs

Human gut microbiota is a home to numerous commensals, microbes that derive benefit from the host without causing harm. However, such commensals can acquire ARGs from microbes that are merely passing through the gut which can cause serious postsurgical infections [20]. In addition, disruption in the composition of gut microbiome in animal models has shown to cause non-communicable diseases (NCDs) such as colon cancer, autism, obesity, and autoimmune diseases [55, 56]. Salyers et al. [57] proposed the concept of ARGs in human gut microbiome. Since then, the technological advancement in high-throughput robotic screening

and next-generation-sequencing (NGS) technologies in the last decade has pushed the gut microbiota research into full swing [20].

1.4.1. The infants' gut resistome

The infant microbiota is highly dynamic and susceptible to antibiotics [58]. The disruption of microbiota at such stage could have significant ill effects throughout life by interfering with the metabolic and immune system [59]. The infant microbiota development is linked to various factors such as the host genetic makeup, nutrition, and environment [60–62]. The microbiota of a new born baby, even without antibiotic treatment, harbors a diverse resistance gene in their resistome [63, 64]. However, antibiotic treatments increase the abundance of pathogenic *Enterobacteriaceae* and lower healthy microbiota such as *Bifidobacteriaceae*, *Bacilli*, and *Lactobacillales* spp. [59, 65–67]. It is believed that the *Lactobacillus* and *Bifidobacterium* spp. are originated from maternal microbiome which is an essential component for the development of infant gut microbiome [62, 68]. The treatment of *L. acidophilus* and *Bifidobacterium* as probiotics in low birthweight infants increases the daily weight gain and recedes morbidity [69, 70], possibly by promoting the healthy gut microbiome and intestinal epithelial layer [58, 71]. The two modes of delivery, vaginally and C-section, can also distinctly affect an infant's microbiota in the first year after delivery. Vaginally delivered infants harbor comparatively higher resemblance to mother's microbiota [72]. Microbes such as *Bacteroides* and *Bifidobacterium* are less frequent in C-section-delivered infants; however, an increased frequency of bacteria is associated to oral and skin [73]. Studies have also found that the microbiota of a 2-month infant and their mother shares distinction in resistome which includes broad-spectrum beta-lactam antibiotics to be found only in the infants [74]. In fact, comparison between infant and their mother to an unrelated infant showed no significant difference [74]. It is proposed that the host genetic makeup and the environmental factors could play a role in the shaping resistome [74]. Infant microbiota shapes into an adult-like by increasing the alpha diversity while reducing the beta diversity which continues until the age of 3 [60]. Maturation of the infant microbiota is also driven by the feeding habit. The addition of solid food does not induce the maturation of microbiota significantly. However, cessation of breastfeeding enriches the gut microbiota to adult-like [72]. Infants with breastfeeding are enriched by *Bifidobacterium and Lactobacillus* even at the age of 1 while infants who no longer breastfeed are enriched with *Roseburia*, *Clostridium*, and *Anaerostipes*, which are prevalent in adults. Functionally, polysaccharide-degrading genes are enriched only after the cessation of breastfeeding [72]. The microbiota also acquires significant essential amino acids, irons, and vitamins genes after 4 months, which are essential for normal brain development [9, 75]. Functional metagenomics from healthy infants and children isolated three novel ARGs and also demonstrated that the ARG in gut resistome is significantly higher than previously estimated [64, 76].

1.4.2. The adult gut resistome

Large-scale metagenomic study of 252 fecal metagenomes samples identified 50 antibiotic classes [42]. Tetracycline resistance gene, tetQ, is the most abundant resistance gene in fecal samples of Chinese, Danish, and Spanish individuals. In fact, tetracycline resistance genes

were the most abundant genes in multiple studies [41, 42]. Although sufficient evidence for the diversity and abundance of ARGs have already been shown to light, the numbers could still be underestimated since during the annotation of metagenomic data, only those ARGs which have been identified and added into the database would yield a positive hit. This would exclude all the ARGs which have not yet been identified. For instance, 290 ARGs having an average similarity of only 69.5% against the GenBank were isolated using functional metagenomics of fecal samples from two healthy individuals [77].

2. Conclusion

Gut microbiome is an essential "organ" without which the host would be deprived of various benefits derived from the numerous gut microbes. The benefits range from food metabolism to the mental health of the host. Hence, it requires attention as much as any other organ in our body. Various studies have, however, noticed the dynamic nature in the compositing and diversity of the gut microbiome making it one of the most dynamic "organs" in us. In addition, the wide application of antibiotic treatment for human as well as animals has enriched the gut ARGs. Hence, strict polices has to be implemented in order to maintain a moderate antibiotics usage. In addition, the surge in ARGs is a clear indication that the research on antibiotic alternative is a necessity for the coming future.

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