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Antibiotics and Resistance Genes: Influencing the Microbial Ecosystem in the Gut (Mini-Review)

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Abstract

The normal flora of human gastrointestinal tract (GIT) bacterial have many roles in human health, most of which are beneficial or neutral for the host. The intestinal bacteria play a significant role of being traffickers in antibiotics which supports the hypothesis that intestinal bacterial not only exchange resistance genes among themselves but might also interact with bacteria that are passing through the colon, causing these bacteria to acquire and transmit antibiotic resistance genes. The human gastrointestinal tract is a massive reservoir of bacteria with a potential for both receiving and transferring antibiotic resistance genes. The increased use of fermented food products and probiotics, as food supplements and health promoting products containing massive amounts of bacteria acting as either donors and/or recipient of antibiotic resistance genes in the human gastrointestinal tract, also contributes to the emergence of antibiotic resistant strains.

Keywords: Gastrointestinal tract, resistance genes, normal microbial flora, antibiotics.

1. Introduction

Antibiotic therapy can affect not only the target pathogen but also commensal inhabitants of the human host. The extent of the impact on non-target microbial populations depends on the particular antibiotic used, its mode of action and the degree of resistance in the community. Sometimes an imbalance in the commensal gut microbiota due to antibiotic administration can result in intestinal problems, such as antibiotic-associated diarrhea (AAD). An additional concern is the increase in antibiotic resistance and the potential spread of resistance genes to pathogenic bacteria.

Recently, it has been shown that even short-term antibiotic administration can lead to stabilization of resistant bacterial populations in the human intestine that persist for years [1-3]. Although the consequences of long-term persistence of antibiotic resistance in the human gut are currently unknown, there are high risks that this could lead to increased prevalence of antibiotic resistance, reduce the possibility of successful future antibiotic treatments and subsequently lead to higher treatment costs. The short-term consequences of antibiotic administration have previously been with culture-based analysis. This mini-review will focus on the long-term consequences of antibiotics on the composition, ecology and resistance of the human gut microbiota and will highlight some recent studies based on molecular methods.

2. Normal Microbial Flora of the Gut

Increasing evidence from recent ecological analysis indicates that certain bacteria might play more important roles than others in horizontal gene transfer in microbial ecosystems. For instance, antibiotic resistance *Enterococcus*, *Staphylococcus*, *Carnobacterium*, and *Lactococcus* species are prevalent in various food samples, and enterococci are also among the major culturable bacteria in antibiotic resistance populations from human stool samples.

Practically, all surfaces of the human body exposed to the environment are normally inhabited by microorganisms. The intestine constitutes an especially rich and diverse microbial habitat. Approximately 800-1000 different bacterial species and >7000 different strains inhabit the gastrointestinal tract.

These bacteria act together in many physiological processes and also interact with human cells, including those of the immune system. The diversity of the gut microbiota is relatively simple in infants but becomes more complex with increasing age, reaching a high degree of complexity in adults.

Lifestyle factors [4] and diet [5-7] can also affect the diversity and composition of the gut microbiota. Interestingly, the relative proportions of the two most dominant bacterial phyla i.e., bacteroidetes and firmicutes, were found to be correlated with obesity in mice and humans, respectively [6, 7], but a study by Duncan did not see this correlation in obese and lean humans. Molecular analyses have also revealed that the composition of the human intestinal microbiota is host-specific [2, 4] and relatively stable over time [2, 8].

Recent metagenome sequencing data of 124 individuals suggest the existence of a common core human gut microbiome, but this core may exist more at the level of shared functional genes rather than shared Taxa [9, 10].

Table 1: Commonly found bacteria in the human colon

Bacteria	Incidence (%)
Bacteroides fragilis	100
Bacteroides melaninogenicus	100
Bacteroides oralis	100
Enterococcus faecalis	100
Escherichia coli	100
Klebsiella sp	40-80
Enterobacter sp	40-80
Bifidobacterium bifidum	30-70
Staphylococcus aureus	30-50
Lactobacillus sp	20-60
Clostridium perfringes	25-35
Proteus mirabilis	5-55
Clostridium tetani	1-35
Clostridium septicum	5-25
Pseudomonas aeruginosa	3-11
Salmonella enteritidis	3-7

Source: [2].

3. Effect of Antibiotics on the Gastrointestinal Flora

The physiological effect of the bacterial flora of the gastrointestinal tract is described as “fermentation of non-digestible dietary residues and endogenous mucus: salvage of energy as short

chain fatty acids, production of vitamin K, absorptions of ions; control of epithelial cell proliferation and differentiation; development and homoeostasis of the immune system and protection against pathogen "the barrier effect".

A symbiotic relation between the bacteria and the host provides the host with optimal protection. The host provides nutrients to bacteria and bacteria repay by providing a colonization barrier.

When using antibiotics as infection treatment, a disturbance in the flora of the gastrointestinal tract is created. The barrier is broken and potentially pathogenic bacteria are allowed to colonize the intestine. Disturbances of the normal gastrointestinal flora are also seen after radical changes of the host diet of after radiation treatments.

The effect of different antibiotics on the indigenous flora was investigated and it was found that clindamycin, erythromycin, cefoperazone, cerftriaxone, and moxalactam have a pronounced influence on the flora.

Some microorganisms have become resistance to drugs, requiring a continuing search for different (and often more expensive) agents. This increase in resistance to drugs has resulted from their widespread and sometimes indiscriminate use. Bacteria undergo spontaneous mutation and exposure to it while the resistance ones survive and multiply; by such means, populations become resistant to a particular drug and sometimes to related drugs.

Furthermore, it is known that genes responsible for antibiotic resistance are present in microorganisms, providing them with self-protection to the antibiotic compounds they produce as defense mechanism against other microorganisms. If large numbers of bacteria are resistance to antibiotics, it will be more difficult and more expensive to treat human bacterial infections. When antibiotics fail to work, consequences include visits to the doctor, hospitalization or extended hospital stays, a need for more expensive antibiotics to replace the older ineffective ones, lost workdays and, sometimes, death.

Furthermore, antibiotics create a selective pressure on the intestinal flora, risking overgrowth of resistance strains. This, in turn, increases the threat of antibiotic resistant gene transfer among the indigenous flora and, at worst, transfer to other pathogenic bacteria. The risk of developing antibiotic resistant strains which can be transmitted by patient-to-patient contact and the spreading of resistance genes can be diminished by choosing antibiotics with a minimum effect on the gastrointestinal flora.

4. Emergence of Antibiotic Resistance

The introduction of antibiotics after World War I resulted in a dramatic decrease of numbers of deaths due to bacterial infections. Today, antibiotics have lost their status as the "miracle drug" [11, 12] and "treatment failure" is a new and often seen situation.

The increase of antibiotic resistance is to be blamed for this medical emergency. The sustainability of antibiotic resistance is partly due to selection of already resistant bacteria that become the new dominant population in the environment. Furthermore, antibiotic usage urges bacteria sensitive to antibiotics to become resistant in order to survive. Survival mechanism includes the acquisition of antibiotic resistance genes from other bacterial/phages (horizontal gene transfer or transduction), mutations in specific genes, and alteration of the bacterial surface.

Thus, continuous usage and accumulation of antibiotics resistant bacteria not only in Europe but also worldwide. The relationship between antibiotics used as antimicrobial growth promoters (AGPs) in production animals and the development of resistant bacteria in food products has been related to human food born infections with resistant strains. This was not easy to acknowledge. A few countries within the European Union (EU) have acted on the new research regarding the suspicious use of AGP. These countries were Sweden in 1986, Norway in 1995, and Denmark in 1998-1999 [13]. Despite a significant decrease in bacterial antibiotic resistance levels in the countries banning growth promoter products, four different AGPs were in use till January 2006, at which point the EU commission initiated the ban of all antimicrobial growth promoters.

Numerous factors influence the development of antibiotic resistance misuse being the obvious factor. The use of antibiotics is influenced by level of knowledge, expectations, choice of prescription, patient behavior economics and

health system. Patient related factors often include inappropriate antibiotics use, life self-medication or inadequate doses despite the prescription text. The prevalence of self-medication in Europe was investigated in 2006. It was concluded that the levels of self-medication were higher in Eastern and Southern Europe than in Northern and Western Europe.

Similarly, Northern countries as well as the Netherlands had the lowest frequency of antibiotic consumption and the lowest level of resistant bacteria. The prescription system for drugs is also important. In developing countries, antibiotics can be purchased in single doses, which increase the risk of the antibiotic treatment being terminated before clinical success. In some countries, antibiotics can be purchased over the counter and prescription is not even necessary, which will also contribute to the rate of incomplete treatments and self-medication. All of the above-mentioned factors can contribute to a rise in the resistance level.

However, further development of antibiotic resistance might be avoided by acquiring knowledge on the mechanisms of bacterial antibiotic resistance. Furthermore, regulatory agencies can set up guidelines and measures, in order to use antibiotics adequately. For example, some countries found that advertising against inappropriate use of antibiotics in national campaigns can reduce the total amount used due to awareness and proper information of the public [14]. During the last decades, there has been an increased focus on persistent bacterial biofilm formation on medical devices, implants, and environmental biofilms [15].

Interesting, it has been shown that biofilms were hot spot for horizontal gene transfer. Thus, promoting development of antibiotic resistance in bacteria. Changes in living standards have resulted in a large, ageing human population, and in increased usage of antibiotics. Intensive and long-term hospitalizations due to new advances in medicine often result in new infections (hospital-acquired infections) that are expensive to control and difficult to eradicate. These occur worldwide due to failures in simple infection control, such as inadequate hand hygiene and changing of gloves.

Increased usage of broad-spectrum antibiotics in order to avoid treatment failure created a vicious circle in the hospitals, as the use of broad-spectrum antibiotics influenced the level of multi-resistant bacteria and their presence [16].

5. The Gastrointestinal Tract as a Reservoir of Antibiotic Resistance Genes

The human intestine is a complex ecosystem with a large species diversity, of at least 400 different bacterial species. The density varies through the different parts of the gastrointestinal tract from 104 bacteria/ml in the stomach to 1012 bacteria/g feces in the distal part of the colon.

By classical culture techniques, approximately 5-15% of the species present in the GI tract are detected [17]. New estimates by metagenomic approaches of the bacterial flora have suggested that the presence of bacterial species might be as high as 1150 bacteria/g. Despite attempts to stop the antibiotic resistance development, the level of resistant bacteria is on the rise and the hypothesis that our gastrointestinal tract acts as reservoir of antibiotic resistance genes is widely accepted.

> "Could the microflora of the human colon, normally considered innocuous or beneficial, be playing a more sinister role in human health as a reservoir for antibiotic resistance genes?" Was the hypothesis set by Salyers and co-workers. [18]

Furthermore, it is known that genes responsible for antibiotic resistance are present in microorganisms, providing them with self-protection to the antibiotic compounds they produce as defense mechanism against other microorganisms. Similarities among the genes and resistance mechanisms found in the antibiotic producers and in the human pathogenic bacteria suggest that the producer bacteria are the pool of origin of antibiotic resistance genes.

During antibiotic treatment, all bacteria in the human/animal body are exposed to selective pressure of the antibiotic. Consequently, the GI is highly exposed, especially during oral therapy. This results in the selection of naturally resistant strains carrying an important genetic pool that might be capable of transferring antibiotic resistance genes to other strains present in the human intestine. Moreover, resistant food contaminants that originates from animals and are consumed by humans, can also act as a gene pool (donors) of antibiotic resistance genes [19].

In general, becoming resistance towards antibiotics has been associated with a biological fitness cost. The cost weakens the bacteria's ability to multiply and survive within a host [20]. The connection between resistance and decreased fitness has stimulated the idea that a reduction in the use of antibiotics would lead to a reduction in the frequently of resistant bacteria through natural selection.

Furthermore, resistance is of importance. Both in vitro and in vivo studies have illustrated that

> "compensatory evolution can stabilize resistant bacterial populations in the absence of antibiotics by making them as fit as susceptible clones". [21]

In addition, resistant bacteria can alleviate the cost of resistance by acquiring additional fitness compensatory mutations [21]. The importance of environmental conditions affecting the fitness costs was also shown [22, 23]. Mutations that have occurred in clinical isolates are seen to compensate for fitness cost in order to stabilize the resistant pathogens in the population.

Still, this reversibility in clinical settings is expected to be slow or nonexistent. Resistance genes from both Gram-positive and Gram-negative pathogenic bacteria have revealed almost identical sequences, suggesting that transfer of antibiotic resistance genes across genera has occurred. Furthermore, it is suggested that transfer events have occurred recently and are evolutionary identity.

It is also suggested that a genes flux occurs in nature from Gram-positive cocci, (*Enterococci*/*Streptococci*) to Gram-negative bacteria, with genes coding for streptogramins being described as examples.

6. Sources of Antibiotic Resistance Genes in the Gastrointestinal Tract

The source of resistant bacteria and resistance genes depends on different factors but the major pressure is antibiotic usage. Additional factors include the ability of resistant strains to colonize the Gut, their relative fitness; mutation rates and efficiencies genes. Under the selective pressure of an antibiotic, a bacterium that acquires a resistance gene is often compared with its susceptible counterpart. However, this less competitive clone might compensate for this less of fitness.

A few studies have investigated the impact of antibiotics on long-term persistence of antibiotic resistance. The prevalence of erythronyin-resistant enterococci was investigated in subjects treated with clarithromycin [24].

It has been shown that three of five subjects carried highly resistant enterococci clones 1-year post-administration and that their clones carried the *ermB* gene, conferring resistance to macrolides such as clarithromycin. In one patient, a specific resistant clone was detected 3 years after treatment in the absence of antibiotic pressure. Another study revealed that macrolide resistant *Staphylococcus epidermidis* was detected up to 4 years after patients had taken clarithromycin [25].

There is a high level of transfer of resistance genes of intestinal, as shown by several studies, but the picture is far from complete [18, 26-28]. The intestine is apparently an ideal location for efficient transmission of resistance genes. The moist, warm environment with nutrients in abundance is comprised of high numbers of bacterial cells that are potential targets for resistance determinants. After initial selection of resistance genes in the commensal microbiota, they may then potentially be transferred to pathogens. This is exemplified by a study that demonstrated transfer of a plasmid carrying a β-lactamase gene from a resistant *Escherichia coli* strain to an initially susceptible strain in a child treated with amplicillin [29]. The authors however suggested that the antibiotics may not only select for resistant bacteria but also consequently increase the number of transfer evens from the increased pool of resistant cells.

Some bacteria are only transient inhabitants of the intestine and are resistant to colonization, such as many that originates from ingested foods. However, they can still play a key role in the introduction of resistance determinants that have the potential to be transferred to the commensal microbiota in the intestine during passage [18, 30]. Another factor that might be contributing to the emerging resistance problem is the use of antibiotics or analogous compounds in agriculture. The use of these compounds in agricultural settings may lead to a more constant selective pressure for resistance to develop and could

potentially contribute to a larger global resistance reservoir with potential introduction.

7. Major Routes of Antibiotic Resistance Dissemination to Humans

The major pathways of antibiotic resistance dissemination to humans involves:

I. **Clinical Environment**: it is well established that the clinical environment has a high concentration of antibiotic resistant pathogens, giving rise to an increased chance of infection in hospital and clinic patients. It is anticipated that in such environments, the antibiotic resistant commensal bacterial concentration would be even higher than that of the antibiotic resistant pathogens, and certainly above the levels found in the community environment. Therefore, hospital and clinical contacts likely serve as an important avenue for antibiotic resistant dissemination.

II. **Food Chain**: it is now believed that the food chain may well play a very important role in disseminating antibiotic resistance genes and antibiotic resistant bacteria to the general public. The abundance of antibiotic resistant bacteria in the food supply can serve as a constant supply of antibiotic resistance genes to the human digestive micro flora. The colonization by antibiotic resistant bacteria and associated horizontal gene transfer events inevitably contribute to the increasing resistance to antibiotics as seen in humans.

III. **Animal Contacts**: as mentioned above, antibiotic resistant bacteria adapted to humans or animals (particularly mammals) may have a better chance of colonizing the hosts again than do endogenous environmental isolates. Companion animals are in close contact with humans, providing the opportunities for transmission of antibiotic resistant bacteria and antibiotic resistance genes.

8. Potential Breakthrough in Antibiotic Resistances

It is obvious now that multiple pathways and complicated mechanisms are involved in the development and maintenance of antibiotic resistance in microbial ecosystems; therefore, simple limiting the use of antibiotics is not sufficient to control the spread of antibiotic resistance. Meanwhile, antibiotics are still essential therapeutic agents, it is impossible to completely ban their use. Therefore, a systematic evaluation of the contributions of key factors in antibiotic resistance development, dissemination, and maintenance is necessary in order to develop control strategies to reduce problems associated with antibiotic resistance.

The following approaches may lead to potential breakthroughs in combating antibiotic resistance:

I. **Proper Monitoring System**: the development of antibiotic resistance takes time, and a proper monitoring system is essential, not only for sounding the alarm but also the application of different control strategies targeting various stages in antibiotic resistance development. As mentioned above, the size of the AR gene pool in commensal bacteria is much large than that in pathogens and is correlated with levels of subsequent HGT events. Therefore, the magnitude of ART commensal bacteria may serve as an indicator of AR status in any given microbial ecosystem. Only pathogens and Enterococcus are currently under qualitative monitoring by the National Antimicrobial resistance monitory system (NARMs). Expanded coverage, particularly the quantitative assessment of the AR status in the environmental, food, animal, and human microbial ecosystems, and enabling prediction of the forth wining risks associated with AR in targeted pathogens.

II. **Interrupting the spread of Antibiotic Resistance through main Dissemination pathways:** as discussed above, human and animal manure and other wastes, particularly those from patients being treated with antibiotics, likely contain more “contagious” ART bacteria than other environmental sources. Therefore, proper treatment of this water is critical to

interrupt the dissemination of AR bacteria. In fact, data from a recent study suggested that methods of manure storage and treatment likely have a substantial impact on the persistence and decline of the AR originating from food animals. In particular, proper treatment of hospital wastes is critical.

III. **Destabilizing of Resistance Traits:** many antibiotic resistance traits are quite stable in both the environment and the host, even in the absence of antibiotic selective pressure, mostly due to the presence of various plasmid stabilization mechanisms. Thus, development of effective means to destabilize or resensitize resistance traits could be an important control measure. For example, certain small molecular may be used to disrupt plasmid replication and resensitize bacteria to antibiotics.

IV. **Novel Antibiotics Delivery Strategies:** currently, systemic administration of antibiotics is most commonly used for the treatment of bacterial infections, including localized and/or superficial infections. Development of novel antibiotic delivery systems which allow selective tissue of pathogen targeting, similar to approaches used in modern cancer therapy, could reduce the potential for global development and dissemination of antibiotic resistance. The goal for such novel delivery systems would be to achieve a local dosage that is much higher than the minimum inhibitory concentrations for the most resistant strains, eliminating even the most resistant pathogens.

9. Model system for studying the development of antibiotic resistance in the gastrointestinal gut

In order to assess the effect of antibiotics on the gastrointestinal flora, a number of models have been developed, both in in-vitro methods, where a number of conditions can be controlled as well in-vivo animal models, resembling the human/animal host.

In vitro conjugation is conducted in liquid media, on agar plates or on filters placed on agar plates. There are often the first experiments by which frequency of transfer can be observed, since all the parameters can be controlled, that is, growth media, temperature, conjugation time, selective oppressive, and so forth. In vitro systems trying to mimic the GI tract have been successfully used, such as batch fermentors and continuous flow fermentors, to study the effects of pH on the degradation of nutrients, CO_2 production among others.

Another model is the antibiotic-treated mouse model, where the very effective colonizing barrier of conventional mice is disturbed by adding streptomycin to the drinking water. Some of the species colonizing the gut are eliminated allowing the new bacteria to colonize. When administered orally, antibiotics such as ampicillin, clindamycin, kanamycin, metronidazole, and streptomycin have been shown to be useful in this model.

A third model is the human microbiota associated rodent (HMA) model where germ free animals are inoculated with human *faecal bacteria*, thus mimicking the human flora composition in the rodent gut.

All GI tract animal models have the advantages of small differences in the bacterial flora composition within the aim due to husbandry standards compared to the variance within humans and their individual flora.

In vitro and in vivo transfer experiments are not comparable and do not always provide the same results and “frequencies”, similarly different types of in vivo transfer experiments are not always comparable. The colonizing barrier and the complexity of the flora in the antibiotic-treated mice compared to disassociated rats lacking the colonizing barrier (as they were germ free before bacterial inoculation) can be considered to be a too simple model, as it does not depict the complexity of the factors involved in transfer of antibiotic resistance genes in the GI tract.

10. Conclusions

A greatly respected scientist within the antibiotic resistance area has recently stated that “Evolution of bacteria towards antibiotic resistance is unavoidable as it represents a particular aspect of the general evolution of bacteria. Thus, at the very best, the only hope we can have in the field of antibiotic resistance is to delay dissemination of resistant or resistance genes. In addition, it is

suggested that identifying more resistance mechanisms in antibiotics producing strains might be the solution to predict the mechanisms that will be observed in the human pathogenic strains in the future. However, horizontal gene transfer does not appear to have homogenized the bacteria. Genetic diversity and a well-defined phylogenetic tree for bacteria are still the rule rather than the exception.

There is no doubt that antibiotic treatment is a necessity, and the influence on the total gastrointestinal flora is a matter of secondary importance conversely, antibiotic treatment creates a great advantage for resistant bacteria which is selected to colonize the intestine and treatment might result in antibiotic resistance transfer among gram-negative bacteria, or to the indigenous flora or even a pathogen. The ecological effects of antibiotic treatment on the commensal microflora should be the focus of more studies in the future.

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