Gut Microbiome and Its Association with Antibiotic Resistance: A short review for general public awareness

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Abstract: The gut microbiome, a complex network of microorganisms within the gastrointestinal tract, has gained significant interest in recent years in a range of diseases and disorders, particularly in metabolic, immune, and neurological issues concerning human health and well-being. It has now emerged as a critical player in antibiotic resistance, which is a global concern for infectious disease treatment. This review aims to explore the association of the gut microbiome with antibiotic resistance and the spread of antibiotic-resistance genes in the gut through horizontal gene transfer and selective pressure that makes the infections difficult to treat. Some of the novel approaches to fight against antibiotic resistance are phage therapy, faecal microbiota transfer (FMT), and probiotics. This review highlights the necessity for a deeper understanding of this complex interaction between the gut microbiome and antibiotic resistance, paving the way for the development of novel therapeutic strategies that will help mitigate risk factors associated with antibiotic-resistant bacteria.

Keywords: Gut microbiome, Antibiotic Resistance, Antimicrobial Resistance Gene, Dysbiosis, Horizontal Gene Transfer

I Introduction

The gut microbiome consists of approximately about 100 trillion microorganisms (1) ranging from bacteria, fungi, viruses, protists, and archaea that reside in the gastrointestinal tract. It plays a crucial role in digestion, metabolic regulation, nutrient absorption, immune regulation, and neurological functions. The key microorganisms that are predominant in the gut ecosystem are – Firmicutes (Lactobacillus, Clostridium), Bacteroides species, Bifidobacterium species, Proteobacteria (E. coli), fungi (Saccharomyces cerevisae), archaea (Methanobrevibacter smithii), bacteriophage, and lesser-known eukaryotic viruses (Enterovirus) (2). A balanced gut microbiota protects against immune dysfunction, metabolic syndromes, and inflammatory bowel disease. However, disruptions particularly when caused by antibiotics, leading to dysbiosis, can have profound health issues (3). The association between antibiotics and the evolution of the gut microbiome is quite significant, especially with the global concern regarding antibiotic resistance. Significant alterations in microbial diversity are observed after antibiotic administration, causing a drift in the microbial composition while fostering a suitable scenario for Antimicrobial-Resistant Genes (ARG) to flourish (4). For instance, a decrease in microbial diversity was observed with piperacillin-tazobactam and meropenem while 40% increase was observed when postbiotics were co-administered during antibiotic therapy (5),(6). Overuse or misuse of antibiotics can lead to long-term effect on the composition of the gut ecosystem (7). These ARGs have the ability to spread in the gut ecosystem through horizontal gene transfer, thus increasing the risk of antibiotic resistance. A comprehensive understanding of the complex relationships among antibiotics, microbial modifications, and resistance development is the need of the hour. The current review aims to present a quick overview on the topic to generate general awareness on the threats of the development of antibiotic resistance with the involvement of the gut microbiome and its potential impact on regular lifestyle and disease management.

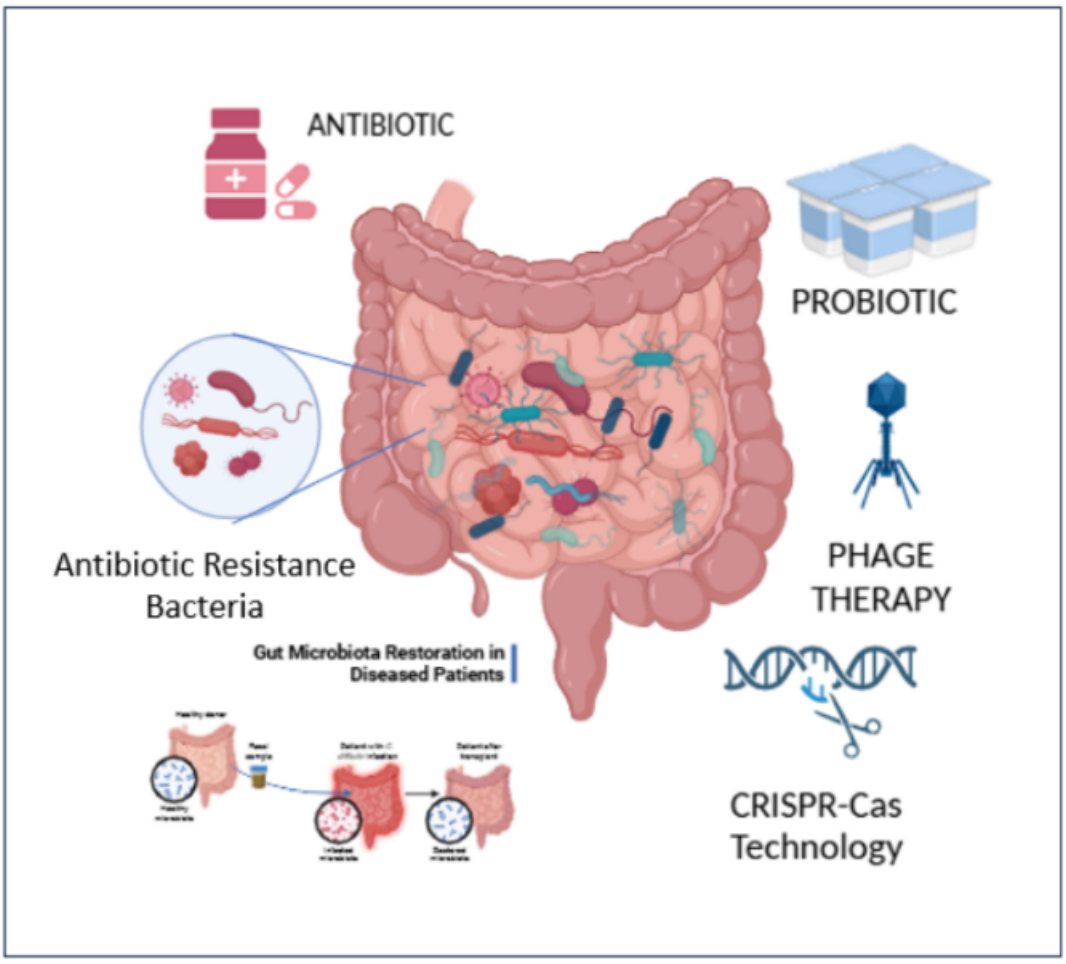

Figure 1: **Graphical abstract**

II Influence of Antibiotics on Gut Microbiome

Bacterial infections are treated by antibiotic administration, which destroys or inhibits the growth of the bacteria. Indiscriminate use of antibiotics causes dysbiosis, i.e., overpopulation of harmful bacteria and reduction in beneficial ones. In the short-term, a decrease in bacterial diversity is observed as antibiotics may kill both beneficial and harmful bacteria in the gut, such as certain species of Bacteroides, while increasing the number of other bacteria, such as certain species under the phylum Firmicutes (Lactobacillus) (3),(7). Beyond this, several long-term effects include, obesity, allergies, irritable bowel syndrome (IBS), autoimmune disorder and most importantly, antibacterial resistance in the gut for which the understandings are still relatively limited. Antimicrobial-resistant genes (ARG) arise as one of the most drastic long-term effects of dysbiosis which again gets distributed in the gut via horizontal gene transfer. This changes the microbiome composition and contribute to the increased risk of infections that are more challenging to treat. However, a few questions still remain unanswered:

1. What kind of microbiome shift occurs in the gut post-antibiotic treatment?
2. How do these changes in the gut predispose an individual to chronic and recurrent infections?
3. How do these changes influence the gut's resilience to disruptions of homeostasis?

Do different classes of antibiotics together shape the constituents of the gut and impact the acquisition

of ARGs?

Addressing these questions might help us understand more about the long-term effects of antibiotics in the gut and approach therapeutic strategies in a more targeted way.

III Mechanism of Antibiotic Resistance Development in the Gut Microbiome

The underlying mechanisms of the development of antibiotic resistance in the gut involve both the alteration of the microbiome community as well as the acquisition of resistant genes, which leads to modifications in the gut flora. Acquisition of ARGs can occur via horizontal gene transfer, mobile genetic elements, and selection pressure. The process of horizontal gene transfer involves the acquisition of ARGs from other bacterial species either via conjugation, transformation, or transduction. Conjugation occurs when DNA from one bacteria transfers through direct contact with the recipient bacteria (same genus or different species), mainly through pilus or pore by small circular DNA called plasmids (8). Transformation takes place when bacteria die, and the recipient bacteria take up the released genetic material in the environment, incorporating it into their genome as extrachromosomal genetic elements. If the free DNA contains ARGs, it is possible for the recipient bacteria to acquire those genes by this process (9). Lastly, bacteria can transfer ARGs via the transduction process where bacteriophage containing ARGs attaches with the recipient bacterium and injects the DNA into the bacterial cytoplasm, allowing it to acquire the resistant traits. ARGs can spread either by specialized transduction, where bacterial DNA is exclusively packaged near the attachment site of the phage, or by generalized transduction, where any gene from the host can be randomly packaged into the phage head (10). However, significant aspects regarding horizontal gene transfer still need much exploration to address a few hypotheses, such as the association of environmental factors, diets, and epigenetic modifications affecting the efficiency and frequency of gene transfer in various bacterial strains. Coming to the mobile genetic elements – bacteriophages, integrons, and plasmids, also help in distributing resistant strains across the gut environment. Among these, integrons are particularly noteworthy as they act as a mediator to acquire and express ARGs in commensal bacteria, specifically after antibiotic treatment, thereby maintaining the resistant genes' reservoir in the gut, which can be mobilized during selective pressure (11). The mobile genetic elements further allow the commensal bacteria to adapt themselves and survive during antibiotic exposure, thus posing health risks to patients (12). They themselves have a lot of aspects to be investigated and their role in the gut microbiome can bring an interesting perspective in shaping theranostics strategies if their regulation and expression can be targeted to reduce ARG spread in the gut.

Mobilization of ARGs facilitated by mobile genetic elements sets the stage for selective pressure. When susceptible microorganisms are eradicated by antibiotics, either by killing or inhibiting their growth, an ecological gap develops as resistant bacteria get an opportunity to multiply themselves. Resistant strains, which survive due to their ARGs, encounter less competition and proceed to spread, establishing dominance by the process of selective pressure . Studies more focused on the molecular level can help in addressing questions regarding the mechanisms of development of dominant resistant strains in an individual's gut by selective pressure, the influence of the gut composition on these dominant strains and vice-versa.

IV Examples of Antibiotic-Resistant Bacteria in the Gut Microbiome

The emergence of a few dominant resistant strains in the gut is mostly well documented with respect to their resistance profile. The following table summarizes a few antibiotic-resistant bacteria found in the gut microbiome with their associated infections.

Table 1: List of a few antibiotic-resistant bacteria predominantly found in the gut with their resistance profiles and associated infections.

Antibiotic-Resistant Bacteria	**Resistance Profile**	**Associated Infections**	**References**
E. coli	Amoxicillin, Fluoroquinolones, Aminoglycosides	Urinary Tract Infections (UTI), GI infections	(13)
Enterococcus spp. (E. faecium)	Penicillin, Lacosamide, Macrolides	Endocarditis, UTI, bloodstream infections	(14; 15)
Klebsiella pneumoniae	Cephalosporins, Carbapenems	Pneumonia, sepsis, bloodstream infection	(16; 13)
Clostridioides difficile	Fluoroquinolones, Rifampicin	Diarrhea, colitis	(17),(14)
Pseudomonas aeruginosa	Carbapenems, Ciprofloxacin	Wound infection, respiratory tract infections	(18),(19)
Staphylococcus aureus	Methicillin, Vancomycin	Skin infections, pneumonia, gastroenteritis	(20),(21)
Acinetobacter baumannii	Ceftriaxone, Gentamicin	Pneumonia, wound infection, sepsis	(22)
Salmonella spp.	Sulfamethoxazole, Sulfonamides	Gastroenteritis, sepsis	(23),(24)
Campylobacter jejuni	Ciprofloxacin	Gastroenteritis, systemic infections	(25),(26)

V Current and Emerging Strategies to Combat Antibiotic Resistance and Limitations

The growing concern of antibiotic resistance in the gut necessitates novel strategies to mitigate health risks. Several innovative and advanced technologies are emerging against antibiotic resistance. This review explores some of the current strategies like Pro- and Prebiotics, Phage therapy, FMT, and CRISPR-Cas, etc.

V.a Probiotics and Prebiotics

Probiotics, mostly found in fermented food products, help in promoting beneficial bacteria in the gut, improving gut health and competing against resistant strains. Similarly, prebiotics, predominantly found in plant-fibre rich food, can also nourish beneficial microorganisms in the gut, thus balancing with the harmful ones. These adjuvant therapies can reduce the adverse effects of antibiotics (27). However, there are under-researched areas concerning probiotic treatment –
1.What are the long-term consequences of probiotic treatment?
2.What is the effect of the interaction of probiotics with antibiotics?
3.What are the effects of probiotics on different microbial strains? The answers to these questions are needed for widespread commercialization of the market regimes.

V.b Natural Products

Natural products like phytochemicals (alkaloids, flavonoids, phenolic compounds) and essential oils are less studied therapeutic avenues that can be used as promising tools to combat antibiotic resistance. Herbal extracts containing metabolites have synergistic effects with antibiotics, enhancing the effectiveness against pathogenic E. coli and Clostridioides difficile. Some of the plant-based products are

Eugenol inhibiting toxin production in enterohemorrhagic E. coli, Geraniol disrupting bacterial cell walls and cytoplasmic integrity, and Zingerone having antibacterial functions against GI pathogens (28). Alkaloids and phenolic compounds have diverse pharmacological effects by interfering with bacterial metabolism and modulating bacterial resistance mechanism (29). Plant-derived essential oils, like tea-tree oil disrupts bacterial cell membrane and oregano oil inhibits bacterial efflux pumps through which bacteria prevent themselves from antimicrobial agents (30). These mechanisms can selectively target resistant strains in the gut microbiome while protecting beneficial ones. Minimum Inhibitory Concentration (MIC) has been reduced by terpenoids and flavonoids for tetracycline antibiotic (31). Bacteria can communicate and co-ordinate among themselves via quorum sensing technology, thus increasing pathogenicity. Essential oils from Lippiaoriganoides demonstrated promising results inhibiting quorum sensing and reducing virulence factor in bacteria (32). Although phytochemicals and essential oils have broad antimicrobial properties with their ability to target multiple bacterial pathways, standardizing and delivering such natural products along with their large-scale production is still a hassle. Research focusing on optimizing formulations and improving bioavailability of these products can further help in conducting clinical trials for therapeutic advancements.

V.c Phage Therapy

It is a growing field of therapeutics with respect to antibiotic resistance, as the use of bacteriophages can selectively destroy the resistant strains without disrupting the normal gut flora. Moreover, with reduced antimicrobials and minor side effects, phage therapy can also minimize the transfer of ARGs. The specificity and selectivity of the bacteriophages pave the path for personalized phage therapy in the case of individual bacterial infections (33). Nonetheless, more research is needed on bacteriophage delivery and selectivity of bacterial strains. It is needed to be better understood whether gut microbes can develop resistance towards phage also, as they have developed towards antibiotics.

V.d Fecal Microbiota Transplantation (FMT)

It is a field of therapeutics where stools from healthy individuals are transplanted to restore the balance in the gut environment by re-establishing microbial diversity and reducing the extensiveness of ARGs. FMT is a novel and promising approach that can be studied further to manage recurrency of multidrug-resistant infections However, FMT is quite complex considering the diversity of the gut microbiome composition, which again varies from person to person. Hence, selecting a donor and standardizing can be difficult. Additionally, long-term consequences remain unanswered, along with the risk of developing adverse effects in the gut landscape.

V.e CRISPR-Cas Technology

The precise nature of this technology allows to specifically target and cut the DNA of the bacteria containing ARG. CRISPR-Cas has the potential to target multi-drug-resistant bacterial strains, thus restoring the effectiveness of existing antibiotics (34). Future research is needed to optimize CRISPR-Cas system for improving its efficacy and safe delivery to patients along with preventing the transmission of ARGs in the gut (35).

VI Conclusion and Future Directions

The upcoming research in the domain needs to explore more on the underlying molecular mechanisms, pathways, and environmental factors that might affect the transmission of ARGs through horizontal gene transfer in the gut microbiome. Additionally, metagenomics and metabolomics studies have the potential to emphasize more into personalized antibiotic therapies to reduce the burden on beneficial bacteria. Emphasis on antibiotic stewardship programs through public education and strong policies are needed to reduce the exposure and misuse of antibiotics. The author believes a multifaceted approach

will surely help in improving the efficacy of existing antibiotics, maintaining a balanced gut flora, and reducing the risk of redundant antibiotic-resistant infections affecting the general public health.

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