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REVIEW ARTICLE

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Exploring the role of gut microbiota in antibiotic resistance and prevention

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ABSTRACT

Background/Introduction: Antimicrobial resistance (AMR) and the evolution of multiple drug-resistant (MDR) bacteria is of grave public health concern. To combat the pandemic of AMR, it is necessary to focus on novel alternatives for drug development. Within the host, the interaction of the pathogen with the microbiome plays a pivotal role in determining the outcome of pathogenesis. Therefore, microbiome-pathogen interaction is one of the potential targets to be explored for novel antimicrobials.

Main Body: This review focuses on how the gut microbiome has evolved as a significant component of the resistome as a source of antibiotic resistance genes (ARGs). Antibiotics alter the composition of the native microbiota of the host by favouring resistant bacteria that can manifest as opportunistic infections. Furthermore, gut dysbiosis has also been linked to low-dosage antibiotic ingestion or subtherapeutic antibiotic treatment (STAT) from food and the environment.

Discussion: Colonization by MDR bacteria is potentially acquired and maintained in the gut microbiota. Therefore, it is pivotal to understand microbial diversity and its role in adapting pathogens to AMR. Implementing several strategies to prevent or treat dysbiosis is necessary, including faecal microbiota transplantation, probiotics and prebiotics, phage therapy, drug delivery models, and antimicrobial stewardship regulation.

KEY MESSAGES

- The gut microbiota is crucial for developing local and systemic immunity and prevents a wide array of bacterial infections.
- Numerous factors, such as antibiotic use, physical and mental stress, radiation, gastrointestinal infections, dietary changes, pollution, hospital exposure, etc., can damage the beneficial gut microbiota.
- Influx of substantial inoculums of highly pathogenic bacteria can overwhelm the body defence systems, disrupt the balance of the gut microbiome, and cause various clinical symptoms.
- Knowledge about the interactions between the microbiota and the host, microbial quorum sensing, and the elements that lead to colonization resistance, it will be possible to eradicate pathogenic bacteria based on their preference and preserve gut microbiota homeostasis with minimal disturbance.

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1. Introduction

1.1. Era of antimicrobial resistance (AMR)

The introduction of antibiotics after World War I resulted in a dramatic decrease in the number of deaths due to bacterial infections. Today, antibiotics have lost their status as the 'miracle cure', and 'treatment failure' is a new and often-seen situation. When bacteria, fungi, viruses, parasites, and other microbes evolve to the point that they eventually develop resistance to the antimicrobials that are used to treat the associated diseases, it is known as antimicrobial resistance (AMR). The increase in antibiotic resistance is to blame for this medical emergency. The misuse of antimicrobials in clinical practice and animal husbandry has been prevalent in both the developed and developing nations which has led to the prevalence of bacterial pathogens resistant to multiple antimicrobials. The widespread of AMR bacterial strains has now been converted to a pandemic; therefore, it's a matter

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of public health concern globally [1]. According to a survey in India, 45% of participants use antibiotics to cure colds, and 49% of respondents said they could heal viral illnesses. India is consequently noted as having one of the highest incidences of infectious diseases, especially those brought on by viruses that are multi-resistant. These studies show a connection between public awareness and AMR [2]. Sub-Saharan Africa has the highest rate of all-age deaths in the Global Burden of Diseases (GBD) area that is directly linked to or related to AMR, in contrast to Australia, which had the lowest rate of AMR-associated mortality in 2019 [3].

1.2. Understanding Microflora in AMR

Any niche that can accommodate a wide range of bacterial flora can, in turn, act as a potential storehouse for AMR genes. One such niche is the host-associated environment, which is comparable to the natural world and includes the human intestine. Investigating the gut microbiota, AMR profile, and variables influencing mobility, diversity, and the consequences of AMR gene expression is crucial. The gut 'core microbiota' of many healthy adults is primarily made up of bacteria from the Firmicutes and Bacteroidetes genera, followed by Actinobacteria, Cyanobacteria, Fusobacteria, Proteobacteria, and Verrucomicrobia [4]. The endogenous gut microbiota is dynamic and varied consisting of smaller quantities of enteropathogens from the *Enterobacteriaceae* and *Enterococcaceae*. This is intriguing because our gut can act as a reservoir for these bacteria, which have become source of potent nosocomial infections [5].

2. Microflora and pathogen adaptation

2.1. Composition of microflora

The term 'human gut microbiota' refers to microorganisms like bacteria, fungi, viruses, and protozoans. They live inside the gut and perform a variety of helpful tasks for their hosts, such as fermenting food, producing vitamins and amino acids, preventing the colonization of enteropathogenic bacteria, maturing and regulating the immune system, controlling the release of hormones from the gastrointestinal (GI) tract, and controlling behaviour in the brain [6]. Two major phyla—Bacteroidetes and Firmicutes—and two minor phyla—Actinobacteria and Proteobacteria—make up the normal human gut microbiota. The gut microbiota shows both temporal and spatial changes in distribution according to pH and aerobic conditions at the genus level and beyond, even though this general profile does not change. There will be a noticeable shift in the variety and quantity of bacteria as one moves from the distal oesophagus distally to the rectum; in the former, there are 10^{1} bacteria per gram of contents, while in the latter, the colon and distal gut have 10^{12} bacteria per gram of contents [7].

2.2. Interactions between pathogens and microflora

The group of microorganisms that inhabit the human GI system is referred to as the 'human gut microbiota'. The gut microbiota normally coexists in a symbiotic relationship with the human host, offering mutual protection against harmful organisms and nutrition [8]. However, gut microbiomes can also harbour opportunistic infections. In individuals with good health, they are primarily carried asymptomatically; nonetheless, they can cause infections, especially in cases where the host's immune system is impaired. These opportunistic, gut-dwelling pathogens include *Enterococcus faecium*, *Escherichia coli*, and *Clostridium difficile* [9]. Furthermore, although Bacteroides species are often considered commensal members of the gut microbiota, *Bacteroides fragilis* is one of the most common anaerobic sources of infection [10]. Humans get colonized with MDR pathogens by encountering hospital environments, contaminated food and water, animals, etc. These pathogens can be below the detection level; however, antimicrobials, host factors, and metabolites can exert pressure on the gut microbiota and lead to MDR bacteria dominance [11]. As per recent research, commensals in the gut produce short-chain fatty acids (SCFA), which may be crucial in preventing colonization by the MDR pathogen. Although a balanced gut microbiota produces enough SCFA to maintain the acidic gut's pH, antibiotic treatment causes dysbiosis of the gut microbiota, which lowers SCFA production and raises pH. Gram-negative pathogens have a higher advantage of colonizing and taking over the gut in these circumstances [12].

2.3. Role of microflora in pathogen evolution and adaptation to AMR

Antibiotic resistance can be induced by various processes, the three most important of which are inhibiting the antibiotic from binding to its target, degrading the antibiotic, and preventing the antibiotic from entering the target. Bacteria can develop resistance through mutations, and during cell division, this

resistance can then spread vertically to daughter cells. Alternatively, bacteria can acquire mobile genetic elements (MGEs) containing antibiotic-resistance genes through horizontal gene transfer (HGT). Transformation, transduction, conjugation, and membrane-vesicle-mediated DNA transfer are the mechanisms that underlie HGT. All these HGT pathways work together to promote the global spread of genes that confer antibiotic resistance in microbial environments. It is anticipated that the spread of genes resistant to antibiotics will be especially strong in environments with wide microbial variety and abundance, such as the bacterial communities present in the human gut [13,14]. Research highlighted that a significant proportion of Taiwanese adults had third-generation cephalosporin-resistant (3GC-R) *Enterobacteriaceae*. The gut microbiota of carriers harbouring 3GC-R *Enterobacteriaceae* was shown to be more diverse than that of non-carriers [15].

3. Mechanisms of microflora-mediated AMR

3.1. Horizontal gene transfer in microflora

The lateral transfer of genes between unicellular or multicellular organisms is known as HGT. It permits the transmission of genetic sequences across distant species, compared to vertical gene transfer, which occurs between the generations. There are several mechanisms by which ARGs can be transported horizontally, the most significant ones being conjugation, transduction, and transformation. The function of membrane vesicles (MVs) in HGT has also come to light more recently. MVs deliver their cargo by fusing with the cells that make up their target. Beta-lactamases are present in Bacteroides, and these vesicles shield target cells against beta-lactam drugs [16]. It is prudent to understand that the genomes of methanogenic archaea in the gut have inherited the capacity to survive and multiply through interdomain HGT from the microbial counterpart that dominates this niche, given that the gut is primarily colonized by a plethora of microbial species. Human gut conditions are highly favourable to HGT because of the constant flow of food, optimum temperature, biofilm formation, high bacterial density, and the wide variety of enteric bacteria [17]. The probiotic and indigenous strains can exchange genes by HGT. The importance of gene acquisition or loss within or across different probiotic strains is highlighted in previous studies mediated *via* interspecies exchange of genes. There have been reports of HGT in probiotic strains of *Lactobacillus rhamnosus*, etc. [18]. For instance, it has been documented that probiotic *L. reuteri* can transfer a tetracycline resistance gene to bacteria in the human gut [19].

Numerous bacteriophages inhabit the human gut, and both the gut and other surroundings harbour a population of phages that carry ARGs. After receiving antibiotics, the number of these ARG-carrying phages in the human gut rises. Transduction is a key factor in the genetic diversity of gut-colonizing *E. coli* strains and can influence the development of drug resistance in gut bacteria, according to studies conducted in mouse models. The invader's genetic evolution was influenced by HGT, which provided lysogenic invaders with both phage-killing and adaptive metabolic advantages. The prophages probably are responsible for the modification of the host's outer membrane proteins, allowing enhanced nutrient intake [20]. DNA migrates *via* a pilus between bacteria that are near one another during conjugation, a complicated, multistage, and contact-dependent process. An environment favourable to conjugation is provided by the gut's thick layer of mucus and high bacterial cell density. It has been noted that commensals and opportunistic microorganisms that colonize the human gut can transfer antibiotic resistance plasmids and conjugative elements (ICEs). Conjugative plasmids can mobilize non-self-transmissible DNA, significantly raising the possibility of HGT of resistance genes [21]. In an investigation, human feces were cultured on a selective medium, which showcased the presence of vancomycin resistance (*van*B) transposon as novel hosts of ARGs in the gut commensals *Eggerthella lenta* and *Clostridium innocuum* [22]. A clinical study revealed that three *Enterobacterales* (*Enterobacter cloacae, E. coli*, and *K. pneumoniae*) co-infected a patient, and all carried a blaOXA-48-harboring IncL/M-type plasmid, suggesting that the plasmid was acquired by two of the strains inside the patient's gut microbiome [23]. The efflux pump genes in bacteria are also found on the chromosome or plasmids and transposable elements. Efflux pumps associated with the MDR phenotypes in bacteria, such as multi-antimicrobial extrusion (MATE) pumps and macrolide-specific efflux (Mac) pumps, are widespread in the genome of most of the gut commensals. It confirmed that these efflux pumps played an important role in the resistance phenotypes of commensal microbiota [24].

3.2. Biofilm formation and resistance

Bacterial biofilms are structured polymicrobial communities encapsulated in an autogenic extracellular matrix and are significant in AMR as they confer protection to bacteria from the host environment and antimicrobials [25]. The extracellular matrix composed of complex polymeric substances plays an important role as the

physical barrier preventing the penetration of antibiotics, rendering the drug less effective [26]. Moreover, the biofilms serve as the epicentre for HGT. Madsen et al. confirm this by exploring the spread of extended-spectrum β-lactamase and carbapenemases among *E. coli* and *K. pneumoniae* in the biofilm communities [27]. It has been demonstrated that biofilms, common in industrial, clinical, and natural settings, are essential for the development of many chronic infections.

Commensal and pathogens can form biofilms in the gut. Beneficial gut bacteria such as *Bifidobacterium* and *Lactobacillus* form biofilms that support gut health by obstructing the colonization of pathogens [28]. However, disruption in the gut microbiota due to the action of antibiotics, diet, or infection can alter the bacterial load of the gut, favouring pathogens to thrive [25]. *C. difficile*, *Helicobacter pylori* and *E. coli* are pathogens capable of forming biofilms that contribute to persistent infections and antibiotic resistance by shielding bacterial cells from antimicrobial agents and HGT of resistance genes. *C. difficile* forms biofilms in the colon, leading to recurrent infections that are difficult to treat [29], while *H. pylori* line the stomach lining, contributing to chronic gastritis and resistance to antibiotics [30]. A subset of gut microbiome *E. coli* strains was found to have a variety of unusual genetic components, such as the well-known biofilm gene *pga*ABCD, which encodes functions related to the formation of biofilms. As a result, during antimicrobial therapy, these bacteria develop resistance to antibiotics and have a markedly increased capacity to endure their deleterious effects [31]. Within the GI tract, a complex and integrated pattern of environmental factors, host signals, and commensal bacterial signals determines the production of *E. coli* biofilms within the multifaceted multicellular communities. A study suggests that bile salts may enhance *B. fragilis* intestine colonization through a variety of mechanisms, including biofilms [32]. Additionally, biofilms promote the formation of metabolically inactive bacteria known as persister cells. These cells survive antibiotic treatment, and when the conditions are favourable, the biofilm repopulates, contributing to recurrent and chronic infections [33]. Moreover, biofilm dispersal enables resistant bacteria to colonize new niches, contributing to the spread of AMR within different settings [34].

3.3. Quorum sensing

Quorum sensing (QS) is a way through which bacteria communicate by regulating gene expressions in response to their cell population density. It plays a crucial role within the gut microbiome in harmonizing microbial interactions, virulence, biofilm formation, motility, and AMR. Also, these bacteria use QS to enhance their survival under stress conditions [35]. The significant phenotypic changes that a bacterium goes through when transitioning between the lag phase, exponential phase, and stationary phase of growth are extremely crucial for bacterial physiology [36].

One of the major ways QS contributes to AMR development is through the formation of biofilms, which build a protective environment shielding the microbial community from the effects of antimicrobials and host immune responses and serve as reservoirs of resistance genes [37] Through QS-facilitated signalling, bacteria can upregulate the expression of efflux pumps, resulting in increased antibiotic tolerance [38]. Interspecies communication through QS enhances the exchange of resistance determinants among commensal and pathogenic microorganisms [39]. The presence of autoinducers may induce genetic changes that promote persistence against antibiotics [40] (Figure 1). These diffusible molecules are produced by bacteria to indicate population density, and an increase in their concentration in response to density triggers intracellular signalling pathways that alter gene expression [36]. The majority of research on the significance of the bacterial QS system for the health of the host has been conducted on both healthy and disease-modelling animals. These investigations unequivocally demonstrate that QS-regulated compounds from (opportunistic) pathogens and AHL themselves play a significant, if not crucial, role in the development of disease. 3-o-C12-HSL therapy led to a significant reduction in body weight, GI inflammation, and enhanced GI permeability in pathogen-free (SPF) mice. Replicating this result were germfree mice that received transplants of faecal microbiota from animals treated with 3-o-C12-HSL, indicating that the effects of 3-o-C12-HSL are mediated through microbiome modification [41]. According to a recent study, there is a link between indole and *C. difficile* infection (CDI). Individuals with CDI have higher indole concentrations than patients with diarrhea who do not have CDI. By overexpressing the tryptophanase gene *tna*A in enterotoxigenic *E. coli* and other indole-producing anaerobes, *C. difficile* stimulates the production of indole. It has been demonstrated that some beneficial bacteria are negatively impacted by this elevated indole level, which also promotes *C. difficile* colonization [42].

3.4. Metabolic interactions and resistance development

The gut commensal microbiota can operate as a barrier in the GI tract to keep out potentially harmful microorganisms. Interactions between immune cells and gut microorganisms support intestinal

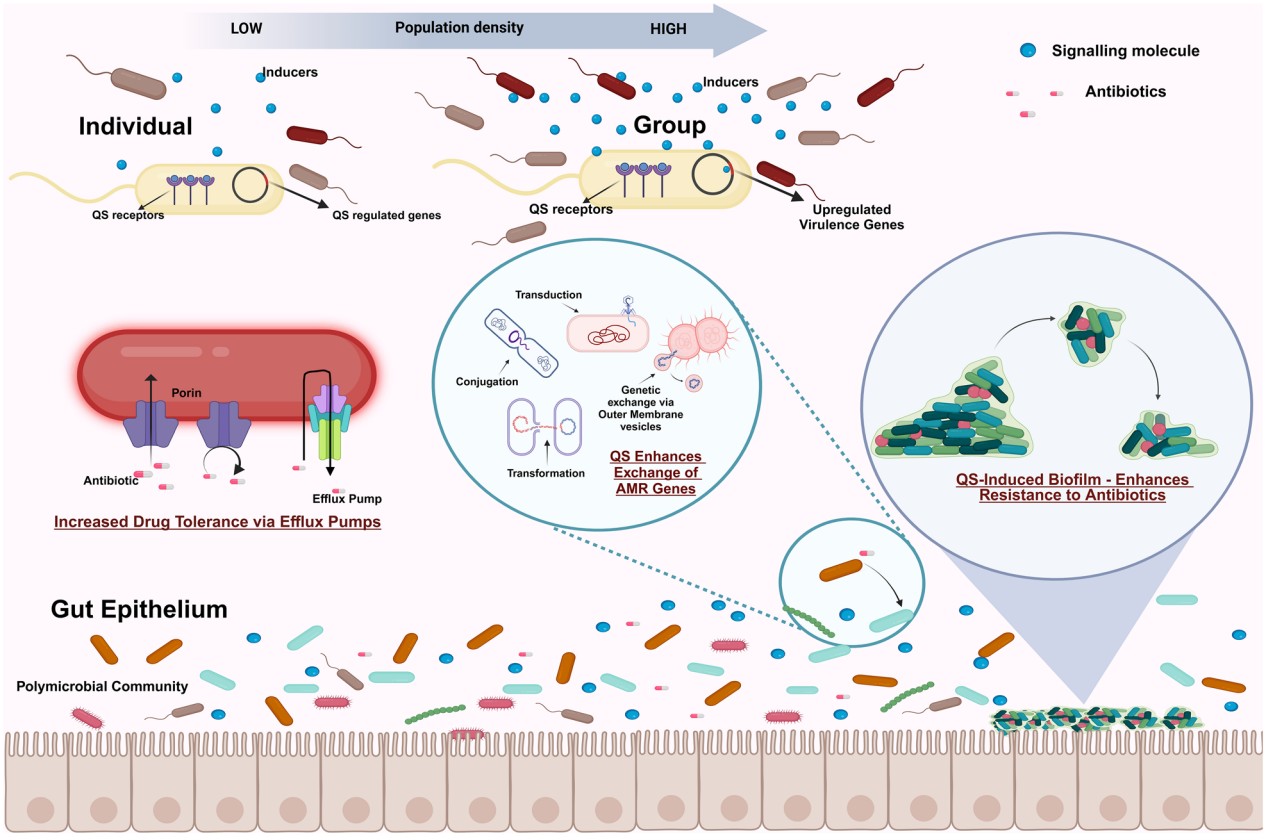

Figure 1. Role of quorum sensing mechanisms in the development of AMR of pathogens in gut microbiota. Bacterial communication *via* quorum sensing (QS) influences antimicrobial resistance (AMR) in gut pathogens. QS signalling molecules regulate gene expression in bacterial populations, leading to biofilm formation, efflux pump expression, etc., promoting horizontal gene transfer (HGT) of resistance genes and factors like antibiotics shape QS-mediated AMR development.

homeostasis in humans. Numerous metabolic diseases in humans, including obesity, hepatic steatohepatitis, and insulin resistance in type 2 diabetes, have been linked to dysbiosis of the gut microbiota [43]. A microbiome ailment such as irritable bowel syndrome (IBS) has been proposed, and based on prior work, it would typically comprise lower total microbial diversity as well as an abundance of methanogenic or *Clostridium* spp., which are more usually related to the increasing severity of IBS symptoms. *Clostridium* spp. have been demonstrated to have a deleterious effect on normal GI activity because they participate in serotonin (5-hydroxytryptamine [5-HT]) synthesis; however, further research is needed to understand the molecular mechanisms [44].

3.5. Microflora and humans

Bacteria that exist as free-living or commensals might become obligatory pathogens after acquiring certain virulence factors, frequently transferred to mobile genetic elements. These genes might, for instance, be responsible for coding of bacterial toxins, extracellular enzymes, or cell surface components such as membrane proteins and polysaccharides, which are directly involved in pathogenesis. Several studies have documented that ARGs can circulate in hospitals, animal farms, sewage treatment plants, and other environments. Wastewater discharge from hospitals is found to be rich in ARGs [45].

ARGs pose an urgent threat that must be addressed from a One Health perspective. The first factor in analyzing the emergence of ARGs is in the life cycle of antimicrobials. In this process, the active intermediate products or drugs can be discharged into the environment. There have been reports of antibiotics accidentally leaking into the environment during manufacture, transportation and sale. However, the major cause of antibiotic resistance is the indiscriminate usage of antibiotics in clinical settings. Also, antibiotics, when consumed, are not completely metabolized [46]. They are only partially digested and absorbed in the body; the rest are excreted through urine and feces within 8–24 h after consumption [47]. These unmetabolized residues or antibiotics from pharmaceuticals are not generally biodegradable, finding entry into the natural

environment (soil, surface runoff, and groundwater). These antibiotics accumulated in the environment find their way back to humans and animals through the food chain, further expanding the distribution of ARGs. The ARGs are said to be adsorbed onto urban dust and spread as aerosols. Besides, ARGs are also found in the ocean and eventually in sea animals, and the human intestinal flora can acquire resistance through the ingestion of these foods [48].

To study the healthy human microbiome, the focus has always been on bacteria, with negligible attention given to other microflora like archaea, viruses, fungi, and eukaryotes. While the microbiome covers all the microorganisms present in a particular environment, most of the available data describes the bacterial composition, especially in the gut [49]. Previous investigations aimed to identify a 'core' set of bacterial groups universally present in healthy beings. Hence, defining the core bacterial microbiota is crucial for discriminating between the stable, constantly associated microbiome and the transient, environmentally influenced microbiome [50,51].

The human microbiome is all-encompassing and thus includes archaea, viruses, and eukaryotes. Archaeal genera are thinly populated and have been identified as a part of a healthy human microbiome, predominantly in the gut [49]. Although eukaryotic microorganisms like fungi and protists found in or on the human body are typically pathogens, it is important not to neglect that many such eukaryotes, in particular *Candida*, *Malassezia*, and *Saccharomyces*, are identified even in healthy populations [52].

Although a few studies have been reported regarding direct mutualistic relationships between humans and fungi, the best-studied being of the probiotic *Saccharomyces boulardii*, isolated to resist cholera. Protozoa are even more common inhabitants of healthy microbiomes, with even greater interspecies differences than bacteria. Further, the presence of the common protozoa *Blastocystis* has been linked with a reduced risk of GI disease. Looking back at recent evolutionary history, helminths, a multicellular eukaryote, have been a component of the gut microbiome for a significant portion and have been eliminated from gut microbiomes in Western cultures. Given their potential to exhibit immunomodulatory capabilities and interactions with other inhabitants of the normal gut microbiome, their elimination may have removed a crucial repository of information regarding our immune systems [49]. On the contrary, a lack of diversity is apparent in the gut microbiome in diseases ranging from obesity to inflammatory bowel disease and type 1 and 2 diabetes and in skin microbiome in atopic dermatitis and psoriasis. The administration of antibiotics also causes a drastic reduction in the diversity of the microbiome with highly variable recovery dynamics, potentially weakening the community's ability to exclude pathogens. This may clear the way for infection by pathobionts—opportunistic microbial community members that, under perturbation, become harmful, such as *Candida albicans* [53].

Understanding the factors that determine adaptive potential will focus efforts seeking to link in-person mutations to health and disease [49].

4. Impact of microflora diversity on AMR

4.1. Microbiome diversity influences pathogen adaptation

A healthy microbiome, the vast community of microbes residing within us, plays a crucial role in defending against invading pathogens. The beneficial microbes are capable of producing antimicrobials that directly kill or inhibit pathogen growth. They can also modulate the immune system, making the host more resistant to infection. However, when the microbiome is at low diversity, pathogens face less competition and immune pressure, allowing them to flourish and potentially evolve resistance to antibiotics or host defences. As an illustration, a disruption of the gut microbiome can lead to the overgrowth of *Clostridium difficile*, a pathogen that causes diarrhea [12].

The gut serves as a pool of β-lactam and plasmid-mediated quinolone resistance (PMQR) genes, often located on mobile genetic elements. β-lactams, the choice of prescribed antibiotics, are significant due to their global use and essential role in treating various bacterial infections. Mobile elements like plasmids enable the easy transfer of resistance genes between commensal and pathogenic bacteria. *Faecalibacterium prausnitzii* and *Prevotella copri,* isolated from faecal samples of healthy adults, were found to be resistant to cephalosporins, ceftriaxone, and cefotaxime. Metagenomic examination of the sequenced isolates from a study revealed that many of their ARGs were found near mobilization elements like integrase or on plasmids, indicating gene transfer [54].

Recent research has explored that harmless or beneficial bacteria may also contribute to disease *via* mechanisms such as mutations, HGT, and immune evasion. Pathoadaptive mutations are genetic changes that increase the virulence of bacteria as seen in *E. coli* strains causing bone and joint infections where mutations enable them to evade the immune system and establish persistent infections; essentially, these

mutations help the bacteria adapt to the host's defences, making them more pathogenic. Studies have shown that a single transposon insertion can convert a benign bacterium into a pathogen, emphasizing the role of genetic mobility in microbial communities. Additionally, some commensals adapt to evade immune defences, facilitating chronic infections, as demonstrated in *Staphylococcus aureus*, where host-adaptive evolution led to changes in toxin production and immune evasion strategies. These findings highlight the complex and dynamic nature of host-microbe interactions, emphasizing the need to maintain a balanced gut microbiota and develop targeted therapies to prevent the emergence of pathogenic traits in commensal bacteria [55,56].

4.2. Gut microbiota and AMR development

In its absolute lifespan, the human GI tract experiences around 60 metric tons of food passing through it, along with a plethora of microorganisms from the environment that pose a great threat to gut integrity. It has been suggested that the ratio of human to bacterial cells is closer to 1:1 but over 100 times the amount of genomic content (microbiome) as compared to the human genome. This microbiota is helpful to the host given that it performs physiological activities such as enhancing gut integrity, modifying the intestinal epithelium, accumulating energy, protecting against infections, and regulating host immunity. Along with the benefits, the role of microbiota in intestinal and extra-intestinal diseases has become steadily apparent [57].

The myriads of microorganisms colonizing the GI tract live in close contact with each other in a complex and dynamic relationship and participate in direct or indirect interactions. These interactions between the gut bacteria provide insight into diverse competitive and cooperative interactions among the dominant gut flora, helping to establish the dynamics of the gut ecosystem. Encounters with various environmental factors can influence microbial interactions, resulting in positive, negative, or neutral outcomes. Competitive interaction is when a strain shows a phenotype that abates the survival of other organisms. Bacteria within the same ecosystem can also interact positively, which benefits each other, either through commensalism, mutualism, or cooperation [58]. Co-operation generally happens between organisms belonging to the same taxa. Co-culturing *E. coli* antibiotic-resistant strains can cross-protect, where they defend each other from the drug being administered, which, in contrast, would have wiped out the bacteria when allowed to grow alone [59].

As mentioned earlier, phages coexist with the gut microbiome, the same ecological environment where the ARGs also exist, hinting at the role of phages in the spread of ARGs [60]. Human guts contain many ARG-carrying phages, and their abundance in the gut increases upon antibiotic treatment [61].

Plasmids and integrating and conjugation elements (ICEs) are transferred to the target through a pilus or pore by being in proximity. The transmission of ARGs is observed in commensal and opportunistic pathogens while colonizing the human gut *via* this mechanism. This can be among the different species of the same genus or in different genera. Studies have confirmed the transfer of plasmid-encoded carbapenem-resistance, OXA-48, from *Enterobacter cloacae* to other membranes of the *Enterobacteriaceae* family in the GI tract, possibly through conjugation. ICE-mediated drug resistance dissemination mechanisms are also found in gram-positive bacteria, such as *Streptococcus* spp. [60]. MVs isolated from the genera *Acinetobacter* and *E. coli* can transfer antibiotic resistance plasmids. In addition to their roles in pathogenesis, cell-to-cell communication, and stress responses, OMVs play important roles in immunomodulation and the establishment and balance of the gut microbiota [61].

4.3. Factors influencing microflora-driven AMR

4.3.1. Diet and nutrition

The lifestyle of an individual drives the composition of human resistance. One of the most important lifestyle habits is a person's diet. Diet is considered one of the main drivers in shaping gut microbiota across the lifespan [57]. For example, populations from rural or unindustrialized societies, even though they have no access to modern medicines, show evidence of abundant and diverse reservoirs of ARGs within faecal metagenomes. In industrialized populations, the composition and diversity of ARGs are significantly different from those in nonindustrialized populations. One reason behind the difference in microbiomes could be dietary changes. A study aimed to explore the dietary and lifestyle relationship with the AMR in the United States. The diet of 290 healthy adult participants was examined. It was found in this study that aminoglycoside-*O*-phosphotransferases (*aph3-dprime*) were negatively correlated with total calories and soluble fibre intake. Participants with low ARG consumed significantly more fibre in their diets than medium- and high-ARG individuals, collaterally showing

increased abundance of the family *Clostridiaceae* (obligate anaerobes) in their gut microbiota [62].

Post-antibiotic treatment shifts the gut microbiota, having a profound impact on the selection of the microbiota that alters the nutritional landscape of the gut and leads to the expansion of pathogenic populations. For example, the increase in sialic acid levels post-antibiotic treatment favours the expansion of enterohaemorrhagic *E. coli, S. Typhimurium, and C. difficile* within the gut [57].

The food production chain has a role in the selection and spread of ARG in the environment. This is achieved by the food commensal or contaminant microorganisms generally recognized as the source of ARG. Tetracyclines, macrolides, β-lactams, and sulfonamides, frequently used in livestock, contribute to widespread ARG. Considering the adverse effects, the growth promoters were banned, yet high antimicrobial use continues, predicting an increase in consumption by 2030. The detection of ARGs, *tet(Q) and tet(O)*, associated with mobile genetic elements, in farm animal feces were found in the human gut resistome. *Erm* genes, frequent in the human gut, spread *via* HGT. Despite all the data available, there is still a need to map and understand ARG transfer routes within the gut microbiota [63].

4.3.2. Travel

Age-old permafrost of microbiomes revealed enzymes within the TEM family, which is responsible for resistance to β-lactams. These can be easily spread, with evidence of transmission of these resistance elements from one location to another, with humans serving as vectors by traveling. In a study conducted by Bengtsson-Palme J et al. 12 of 18 Swedish students tested positive for ESBLs after visiting the Indian peninsula, in contrast to negative tested bacterial isolates in the gut microbiome before travel. Transmission of ESBLs *via* plasmids has been identified, with community-associated ESBL infections in the United States responsible for more than one-third of total ESBL infections. Apart from single-nucleotide polymorphisms, plasmid-mediated quinolone resistance (PMQR) is a new highlight. Travelers from the Netherlands were found to have a significant acquisition of PMQRs after returning from Southeast Asia and India, indicating that travel to an area of high endemic resistance can act as a vector for transmitting PMQRs [54]. *Campylobacter* infections, common among international travellers, are a leading cause of diarrheal diseases globally, with an increasing prevalence of drug resistance across high- and low-income countries. This prevalence has a higher rate of resistance in travel-associated cases in contrast to domestic infections reported in the USA, where 62.4% of travel-associated *Campylobacter* infections were ciprofloxacin-resistant compared to 14.4% of domestic cases [64]. The mass movement of military personnel globally can also spread resistance genes. Over 90% of enteric fever cases in the UK are acquired primarily from Pakistan, India, and Bangladesh [65].

4.3.3. Hospital exposure

Recent decades have seen a global rise in infections caused by antibiotic-resistant clones of *E. coli*, *K. pneumoniae*, and *E. faecium* [61]. Around 5–15% of all patients are subjected to healthcare-associated infections (HAIs), which are generally sustained by multidrug-resistant (MDR) or even pan-drug-resistant microorganisms, making the therapeutic approach very difficult. AMR in hospitals due to this reason is dangerous. The hospital environment represents a reservoir of HAI-associated pathogens and plays a role in HAI transmission [66].

A recent study reports a high occurrence of bacteria carrying $bla_{CTX\text{-}M\text{-}15}$, $bla_{NDM,}$ and $bla_{OXA\text{-}48}$-like genes colonizing hospital surfaces and patient care equipment across 6 low- and middle-income countries. They reported the presence of a high-risk ST15 *K. pneumoniae* clone carrying bla_{NDM} and $bla_{OXA\text{-}48}$-like genes from hospital surfaces and blood cultures of septic neonates in Pakistan over 2 years, indicating transmission between the ward environment and neonates. Multiple distinct strains of gram-negative bacteria were detected on hospital surfaces in Bangladesh and Rwanda, suggesting widespread bacterial colonization and transmission of these pathogens. *K. pneumoniae, E. hormaechei, A. baumannii, S. marcescens,* and *L. adecarboxylata* were the most prevalent species carrying the AMR genes across countries. The whole genome sequence exposed diverse strain types of *E. hormaechei* and *K. pneumoniae* that co-occurred. The study highlighted the widespread contamination of hospital surfaces by bacteria carrying multiple antibiotic-resistant genes, suggesting its dissemination [67]. These pathogens are easily spread by patients, visitors, and hospital staff. They can survive for long periods on surfaces, from where they can be easily transmitted to patients by direct or indirect contact.

In HAIs, the causative agent of AMR is mainly the extensive use of broad-spectrum antibiotics. Some of the factors that contribute to the development of HAIs include hospital design factors (e.g. ventilation and an adequate number of handwash basins), longer

hospital stays, gender, surgery since admission, intubation, mechanical ventilation, age of the patient, type of hospital, and urinary catheter and hygienic practices [68].

4.3.4. Pollution

Pollution also has a role in influencing gut microbiota. Though the process can be slow, exposure to various pollutants can alter the composition and diversity of the gut microbiota, leading to changes in the metabolic processes and immune responses within the body. Exposure to particulate matter (PM) has been linked to alterations in the gut microbiota. An investigation shows that exposure to PM 2.5 was associated with a lower abundance of beneficial bacteria, such as *Bacteroidetes,* and a higher abundance of potentially pathogenic bacteria, like *Firmicutes* [69]. Ozone exposure has reduced gut bacterial diversity and increased the presence of certain species linked to obesity and disease. Higher ozone exposure was associated with a lower Shannon diversity index, higher *Bacteroides caecimuris*, and multiple gene pathways involved in L-ornithine de novo biosynthesis and pantothenate and coenzyme A biosynthesis [70]. Exposure to a higher concentration of nitrogen oxide has been associated with fewer taxa, including higher *Firmicutes*, and altered gene pathways involved in fatty acid synthesis and degradation. Exposure to sulphur dioxide and traffic-related air pollution is associated with a higher abundance of *Coriobacteriaceae* and a lower abundance of *Bacteroidaceae* [69]. Exposure to air pollutants has been linked to an increased risk of chronic diseases such as obesity, diabetes, and GI disorders [71].

4.3.5. Agriculture and animal husbandry

Agriculture and animal husbandry play significant roles in influencing AMR in the microflora. The widespread use of antibiotics in animals and counterfeit and substandard drugs are associated with the rise in resistance [65]. Antimicrobials are predominantly used as prophylaxis in animals to ensure good health. Antibiotics are also used as therapeutics in preventing the spread of diseases in animals and poultry. The therapeutics are currently used on a large scale as growth promoters to enhance feed efficiency and growth rates in livestock, which can lead to the selection of resistant bacteria. The use of antibiotics exerts selective pressure on microbial communities, promoting the survival and growth of resistant microorganisms, while susceptible ones are inhibited or killed [72]. Continuous use of antimicrobials in food-producing animals for growth promotion, feed proficiency enhancement, and prophylaxis is a significant contributing factor to increasing AMR. The administration of antimicrobials in food-producing animals can result in the presence of residues of these antimicrobials in food products, focusing on the products consumed by humans, such as milk, meat, eggs, and their byproducts. This can contribute to a range of health issues in humans, mainly allergic reactions, mutagenicity, and carcinogenicity. Moreover, it can lead to alterations in the natural balance of microflora in the body [67].

The use of fertilizers, including cattle manure, chicken manure, swine manure, and sewage sludge, has been connected to the presence of antibiotics and corresponding antibiotic-resistance genes in the soil, as antibiotics are widely used in all farm animals [73]. The presence of antimicrobial residues in food products poses significant public health concerns, highlighting the need for prudent use of antimicrobials in food-producing animals and effective monitoring of antimicrobial residues in food products to mitigate potential health risks [74]. Strategies targeting farmers, veterinarians, and other stakeholders, as well as supporting science-based practices that can be translated at the local level, are crucial for mitigating AMR [75].

4.3.6. Antimicrobial use

Antibiotic usage rates and ARG abundance and diversity have been associated across different populations and continents, underscoring the global impact of ubiquitous antibiotic use on the gut microbiome and resistance. Use, overuse, and misuse of antibiotics are critical factors in the microflora-driven evolution of AMR [76]. Even though species diversity is reduced after the antibiotic treatment, the total microbial load may increase. Antibiotic exposure also alters the gut metabolome and shows a shift in the metabolites, affecting host physiology by altering host health and immune responses and increasing infection susceptibility [77,78]. The undesirable use of antibiotics, fuelled by their availability over the counter, is the primary driver of antibiotic resistance [79]. Bacteria have developed mechanisms like decreased antibiotic uptake through cell wall modification, producing enzymes to modify or degrade the antibiotic, and actively removing antibiotics using efflux pumps to eliminate the effect of antibiotics. These microscopic organisms can also mutate the targets of antibiotics. For example, modifications in penicillin-binding proteins reduce the efficacy of β-lactams, mutations in 23S rRNA confer resistance to macrolides, lincosamides, and streptogramin B, and mutations in DNA topoisomerase II and

IV lead to resistance to quinolones and fluoroquinolones [80]. Bacteria can eliminate antimicrobial agents by pumping them out *via* efflux proteins embedded in the bacterial cell membrane. Although these proteins can be antibiotic-specific, most are multidrug transporters.

4.3.7. Age-related alterations and genetics

Factors such as birth delivery options and feeding methods, age, perinatal period, maternal factors, and an individual's sociocultural environment contribute to the microbiota's makeup. The perinatal period and maternal factors, in babies born at term by vaginal delivery and breastfeeding, their gut microbiota is primarily composed of *Enterobacter*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Actinobacteria*, *Firmicutes*, *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, and *Clostridium* [4]. The culture-based paper by Mitsuoka in 1990 described that, in comparison to young adults, the faecal microbiota of the elderly constituted a lower abundance of *Bifidobacteria,* whereas *Clostridia, Lactobacilli, Streptococci,* and *Enterobacteriaceae* were increased [81]. These age-related shifts in gut microbiome composition are influenced by a variety of factors. Lifestyle factors like diet, physical activity, medication use, and social interactions also tend to change with age and influence the gut microbiome [4,82].

The age-related imbalance in the gut microbiome has been associated with increased infection susceptibility, metabolic disorders, and neurodegenerative diseases in the elderly population. The loss of microbial diversity and beneficial microbes may contribute to systemic inflammation, immunosenescence, and other signs of the aging process [83]. Hence, age is a key factor that shapes the composition and function of the gut microbiome over the lifespan.

4.3.8. Gender

Sex hormones, distinctly estrogen and testosterone, play a crucial role in shaping the gut microbiota. Studies have often shown that there are gender-specific differences in the microbiota influenced by sex hormones. The gut microbiota undergoes significant changes during puberty in both sexes [84,85]. Research has shown that the gut microbiota is dominated depending on the sex of a person, with certain bacterial genera being more abundant in men than women. For instance, men have higher levels of *Acinetobacter, Dorea, Ruminococcus, and Megamonas*, while women have higher levels of *Slackia and Butyricimonas* [85]. The interaction between the gut microbiota and sex hormones has been involved in various sex hormone-related diseases, such as PCOS, endometriosis, and breast cancer. The differences in gut microbiota composition may also contribute to sex-specific health risks, such as the higher incidence of certain diseases in men than in women and vice versa [86].

4.3.9. Pregnancy

Most of the immune system and metabolic changes occur during pregnancy [87]. Healthy pregnancy is often associated with an increase in the bacterial load and profound alterations in the composition of gut microbiota [89]. During the first to third trimesters, an increase in Proteobacteria and Actinobacteria and reduced richness have been reported [87]. In a human cohort study, 41 mothers who delivered preterm showed a change in the gut microbiome with an increase in commensal oral bacteria in their gut microbiome as compared to the gestational-age-matched controls who delivered at term. Also, changes in the gut microbiota have been associated with pregnancy loss [88].

The overview of different factors affecting the gut microbiome is listed in Table 1.

5. Clinical implications and challenges

5.1. Role of microflora in healthcare-associated infections (HAIs)

HAI represents the most frequent adverse event during care delivery, with the majority caused due to the spread of microorganisms in the health care settings [90]. Microorganisms associated with HAIs include *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Coagulase-negative staphylococci* (CoNS)*, Methicillin-resistant Staphylococcus aureus* (MRSA)*, Candida* spp., and *Vancomycin-resistant Enterococcus* (VRE) [91,92]. These bacteria are said to be able to develop antibiotic resistance, making them difficult to treat and contributing to the high morbidity and mortality associated with HAIs. The gut microbiota plays a key role in the development and progression of infections, particularly considering antibiotic resistance and the dissemination of pathogens. Factors influencing the gut microbiota and increase in the risk of HAIs include antibiotic use, the hospital environment, age of the patient, sex, and underlying medical conditions [93]. To prevent HAIs, healthcare providers must focus on the gut microbiota by practicing proper hand hygiene, implementing infection control measures, and implementing antibiotic stewardship programs.

Table 1. Overview of gut microbiome, infections and antibiotic resistance.

Factor	Role in the Gut Microbiome	Impact on Infections	Influence on Antibiotic Resistance (AMR)	References
Microbial Diversity & Composition	The gut microbiome consists of commensal, symbiotic, and pathogenic microbes; Bacteroidetes and Firmicutes are dominant phyla. Proteobacteria increase during dysbiosis.	Dysbiosis increases susceptibility to infections like *Clostridium difficile*, *Enterococcus*, and MDR *E. coli*.	Loss of beneficial species enhances HGT among pathogens, increasing ARGs.	[57, 4]
Microbial Interactions & Competitive Exclusion	Beneficial microbes (e.g. *Lactobacillus*, *Bifidobacterium*) produce antimicrobial peptides, compete for nutrients, and enhance immune responses.	Disruption of competitive exclusion allows opportunistic pathogens (e.g. *K. pneumoniae*, *Pseudomonas aeruginosa*) to colonize and cause infections.	Commensals can harbour resistance genes (e.g. β-lactamases), which can be transferred to pathogens via conjugation and transformation.	[82, 76]
Dietary Influences	High-fibre diets promote SCFA production, supporting beneficial microbes; Western diets (high fat, sugar) induce gut inflammation.	Low-fibre diets reduce colonization resistance, increasing the risk of enteric infections (e.g. *Salmonella*, *Enterobacteriaceae*).	Fiber reduces ARGs by enhancing microbiome resilience; high-fat diets promote the selection of resistant bacteria.	[62, 63]
Antibiotic Exposure & Selective Pressure	Broad-spectrum antibiotics reduce microbial diversity, leading to dysbiosis and overgrowth of opportunistic pathogens.	Antibiotic-induced dysbiosis lowers resistance to infections, leading to *C. difficile* overgrowth and vancomycin-resistant *Enterococcus* (VRE) infections.	Selective pressure increases ARG retention (e.g. bla_{NDM}, bla_{KPC}, *mecA*), facilitating resistance spread via MGEs.	[79, 78]
Hospital Environment & Nosocomial Infections	Frequent antibiotic use and close patient interactions alter microbiome composition, favouring MDR organisms.	Increased hospital-acquired infections (HAIs) from MDR pathogens like A. *baumannii*, *K. pneumoniae*, and carbapenem-resistant *Enterobacterales* (CRE).	Hospital surfaces and medical devices act as ARG reservoirs, facilitating spread through biofilms and plasmid-mediated transfer.	[61, 67]
Travel & Globalization	Exposure to new microbial populations influences gut microbiota composition.	International travel increases colonization with ESBL-producing *Enterobacteriaceae* and fluoroquinolone-resistant *Campylobacter*.	Travel accelerates the global dissemination of ARGs, particularly bla_{CTX-M}, *qnr*, and *mcr* genes.	[54, 64]
Environmental Pollution & ARG Dissemination	Pollutants (e.g. heavy metals, pesticides, industrial waste) impact microbiome stability.	Pollutant-induced dysbiosis enhances susceptibility to opportunistic infections.	Heavy metals (e.g. mercury, arsenic) co-select for ARGs by promoting integrons and transposons carrying AMR determinants.	[69, 70]
Agriculture & Food Chain Transmission	Antibiotics used in livestock alter gut microbiota and introduce resistant strains into the human gut.	Zoonotic pathogens (e.g. *Salmonella*, *Campylobacter*) gain resistance and infect humans via contaminated food and water.	ARGs (e.g. *tet*, *sul*, and *bla* genes) spread from livestock microbiomes to human gut bacteria through foodborne transmission.	[73, 74]
Age-Related Microbiome Shifts	Neonates have a developing microbiome dominated by maternal and environmental influences; aging reduces microbial diversity.	Infants are susceptible to neonatal sepsis due to underdeveloped microbiomes; elderly individuals show increased risk of infections (e.g. UTI, pneumonia).	Aging-associated dysbiosis favors colonization by MDR organisms (e.g. ESBL-producing *E. coli*, VRE).	[83, 4]
Gender & Hormonal Influence	Sex hormones (e.g. estrogen, testosterone) shape microbiome composition differently in males and females.	Gender-related microbiome variations affect susceptibility to UTIs (higher in females) and *Helicobacter pylori* infections (higher in males).	Hormonal differences influence ARG expression; estrogen may modulate bacterial resistance phenotypes.	[86, 85]
Pregnancy & Maternal Microbiome Transmission	Microbial shifts during pregnancy affect maternal-fetal microbial transfer, impacting newborn immunity.	Dysbiosis is linked to preterm births, gestational diabetes, and increased neonatal infections.	Vertical transmission of maternal microbiota may include ARG-carrying bacteria, influencing neonatal resistome.	[87, 89]

5.2. Therapeutic strategies targeting microbiome to combat AMR

5.2.1. Faecal microbiota transplantation (FMT)

It involves the administration of fresh, frozen, or encapsulated faecal matter from a suitable donor to restore the natural balance of the gut microbiota in a patient. This therapy has proven to displace vancomycin-resistant *Enterococcus* when they are predominant and efficient in treating infections with *C. difficile* [94]. FMT has also been used to decolonize AMR bacteria from the gut. Studies have demonstrated the significance of FMT in reducing AMR colonization in the gut, with a difference in AMR remission observed between FMT and non-treated patients [95]. When used in combination with antibiotics, the efficacy of the FMT is enhanced, and the risk of resistance development is reduced. FMT and antibiotic combination therapy have been effective in treating infections caused by *P. aeruginosa and K. pneumoniae* [94]. Therefore, FMT has been demonstrated to reduce antibiotic resistance by re-establishing the natural flora in the gut and minimizing the selection pressure for resistant bacteria.

5.2.2. Bacteriophage therapy

In 1917, Félix d'Hérelle showed the bactericidal effect of the bacteriophages isolated from the feces of patients recovering from dysentery, which encouraged the development of phage therapy in parts of the world, majorly Europe, the US, and the Soviet Union. With the discovery of antibiotics, the development of phage therapy was reduced [96]. As the world recognizes the increase in antibiotic resistance due to the failure of antibiotic activity and the reduced development of new antibiotics, phage therapy stands a chance in the treatment of AMR bacterial infections. The family of small lytic phages *Microviridae* and *Leviviridae* lyses the bacterial host by encoding a protein endolysin [94]. These phage-encoded proteins serve as a potential treatment for antimicrobial-resistant pathogens. The added benefit of using phage for treatment against bacterial infection is its specificity to the host. These phages are then administered to the patient, either by oral route, topically, or intravenously. The phages then replicate in the target bacteria, leading to their lysis and eliminating the pathogen from the body. This approach has shown promising results in the treatment of various infectious diseases [97].

5.2.3. Bacteriocins

Bacteriocins are antibacterial peptides synthesized on the ribosomes of bacteria. These represent a potential alternative to antibiotics because of their ability to inhibit the growth of similar or closely related bacterial strains. These have been used in the food industry as preservative agents and for food safety [98]. Bacteriocins have shown promising results as antimicrobials in mouse models. Pyocin has been successfully used to treat *P. aeruginosa* lung infections efficiently without any adverse effects [99]. Bacteriocins have been successfully used to treat and prevent bovine mastitis with comparable efficacy to antibiotics [100]. However, the quantitative contribution of phages to the horizontal transfer of ARGs is still to be understood.

5.2.4. Probiotics and drug repurposing

Probiotics and drug repurposing are two therapeutic strategies that are used to target the microbiome and combat AMR. Probiotics is the use of live microorganisms to restore the balance of the gut microbiome. Probiotics have been shown to intensify cognition in patients with Alzheimer's disease and improve cognitive function in them. Probiotics are also prescribed to treat various diseases by modulating the gut microbiome. Drug repurposing is the use of existing drugs for new therapeutic purposes. This approach can be used to combat AMR by identifying non-antibiotic compounds that can inhibit bacterial resistance determinants or enhance antibiotic activity. For example, it was reported that >20% of Food and Drug Administration (FDA)-approved drugs were able to influence the growth of gut bacteria, and some microbes could metabolize or accumulate drugs to affect the drug efficacy and toxicity [101].

While these therapeutic strategies are thought to be promising to combat AMR, there are several challenges to consider, like ensuring the quality and establishing regulatory frameworks of probiotics and drug repurposing products to ensure their safety and efficacy, conducting clinical trials is essential to ensuring their effectiveness in combating AMR, and combining probiotics and drug repurposing with other therapeutic strategies, such as antibiotics, may also be necessary to effectively combat AMR.

5.2.5. Drug delivery systems

Emerging research also highlights the potential of microbiota-directed therapies, including prebiotics, synbiotics, and postbiotics, which work synergistically with drug-delivery platforms to optimize gut health and combat antibiotic-resistant infections [102]. Innovations such as stimuli-responsive nanoparticles, which release their payload in response to changes in pH, temperature, or enzymatic activity, are paving the way for next-generation therapies targeting dysbiosis-associated diseases [103] (Figure 2).

The simultaneous use of active gut supplements, drug delivery systems, and strategic polypharmacy represent promising and therapeutically feasible methods for managing microbiome interactions [104]. Many studies have found that encapsulating probiotics increases their ability to thrive in the GI tract. For example, encapsulating *Lactiplantibacillus plantarum* in calcium alginate capsules enhanced the survival rate of the bacteria from 18.5% to 84.5% after transiting through an *in vitro* GIT model [105]. The most advanced nanoparticles for drug delivery impacting gastrointestinal infections are based on polylactic-co-glycolic acid (PLGA), polylactic acid (PLA), chitosan, gelatin, polycaprolactone, and poly-alkyl-cyanoacrylates [106]. Additionally, lipid-based nanoparticles, such as solid lipid nanoparticles and nanostructured lipid carriers, have shown promise in protecting bioactive compounds from degradation while enhancing their intestinal absorption [107,108]. As research continues to evolve, integrating these cutting-edge approaches will be critical in developing precision medicine strategies for gastrointestinal dysbiosis.

6. Future directions and research opportunities

6.1. Technologies for studying microflora-pathogen interactions

To study microflora-pathogen interactions, various technologies have been employed by researchers globally that provide a more thorough understanding of these complex interactions. Dual RNA sequencing is used to sequence both host and pathogen RNA to analyze their interactions and identify key biological pathways involved in disease transmission [109]. CRISPR-Cas9 screening is a genome-wide analysis tool that allows researchers to detect genes important for parasite survival, pathogenicity, and transmission, as well as genes involved in host susceptibility to infections [110]. Metaproteomics uses the proteome of microbial communities, providing insights into the functional potential of microbial communities. Metabolomics is an approach involving the analysis of the metabolic pathways and products of microbial communities, providing insights into the functional potential of microbial communities [111]. Single-cell omics is used for analysis of the gene expression and other cellular features at the single-cell level, providing insights into the heterogeneity of microbial communities [110]. The use of three-dimensional models, like the rotating wall vessel (RWV) bioreactor, extracellular matrix (ECM)-embedded organoid models, and organ-on-a-chip (OAC) models [109], which mimic the host microenvironment, allows for the study of host-pathogen interactions in a more physiologically relevant manner [112]. Next-generation sequencing (NGS) is also used to study host-pathogen interactions for the analysis of gene expression and the identification of key biological pathways involved in disease transmission [113]. Computational modelling uses computer simulations to model the interactions between microorganisms and their environments, allowing for the prediction of AMR dynamics and the evaluation of potential interventions.

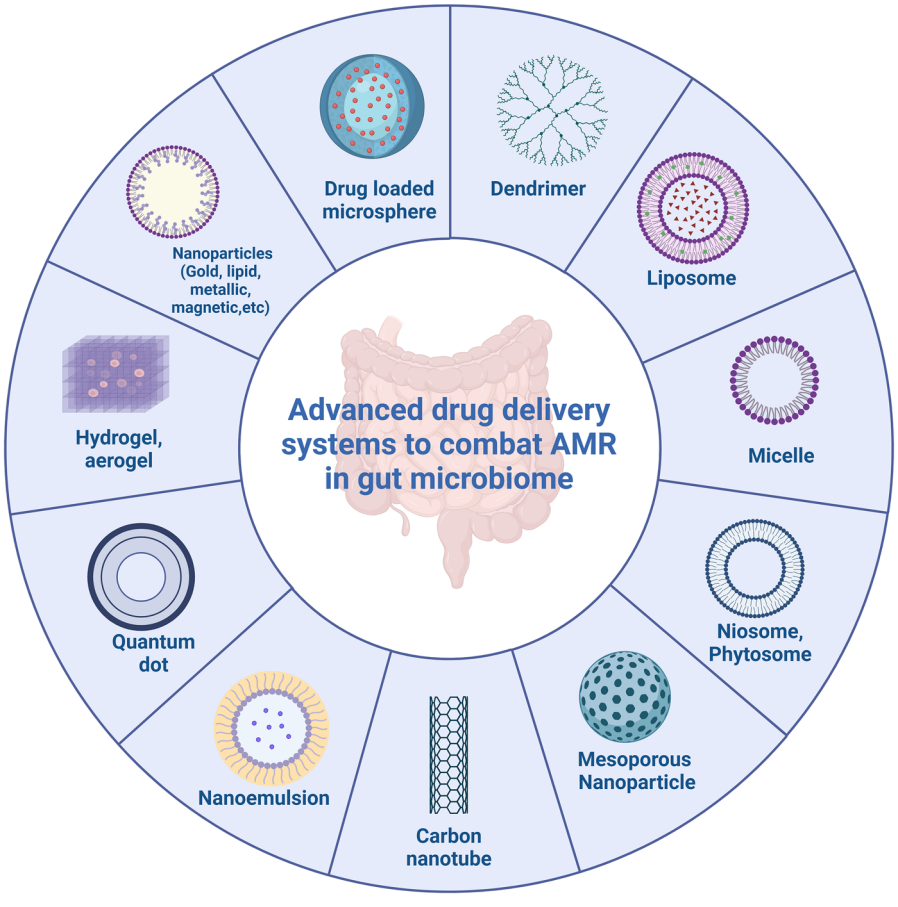

Figure 2. Recent advancements in drug delivery systems to overcome dysbiosis and AMR development by pathogens in gut microbiome. The image illustrates strategies for targeted therapy in gut health like nanoparticles, dendrimers, liposomes, micelle, niosome, phytosomes, mesoporous nanoparticles, carbon nanotube, nanoemulsion, quantum dot, hydrogel, aerogel, drug-loaded microsphere.

6.2. Areas for further investigation

A significant challenge in understanding the directionality of antimicrobial resistance gene (ARG) transmission between different metagenomes is crucial for effective AMR control. Detailed research is needed to fully understand the complex interactions between microorganisms and pathogens, particularly in the context of AMR. The impact of environmental factors, such as antibiotic pollution and anthropogenic activities, on AMR dynamics is not yet fully established and requires further investigation. Effective interventions for AMR control in low- and middle-income countries, where access to healthcare and sanitation infrastructure is limited, require further research and development.

7. Conclusion

Hippocrates' remark, 'all diseases begin in the gut', is widely acknowledged today. We learn more about the gut microbiota-gut-brain axis and its effect on human health and diseases when the association is altered. Certainly, antibiotic treatment is necessary, and the influence on the total GI flora is a matter of secondary importance but cannot be neglected. Onward transmission is influenced by infection control standards, sanitation, clean water access, quality of antimicrobials and diagnostics, travel, and migration. Minimizing and mitigating the resistance should therefore be considered comprehensively by investigating resistance mechanisms, antimicrobial drugs, host range, and context; parallel to new broad-ranging drug discovery, multidisciplinary research is needed across these levels, interlinked across the healthcare, agriculture, and environment sectors.

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Authors contributions

CRediT: **Devyani S. Dongre**: Data curation, Writing – original draft, Writing – review & editing; **Ujjayni B. Saha**: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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Data availability statement

No dataset was generated in this manuscript.

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