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Regulatory Role of Gut Microbiota in MRSA Colonization and Horizontal Transfer of Antibiotic Resistance Genes

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Abstract:

Antimicrobial resistance (AMR) has become a major global health threat, seriously impairing the efficacy of modern medical anti-infective therapies. With the continuous emergence of multi-drug resistant (MDR) bacteria, the difficulty of clinical infection diagnosis and treatment is increasing, leading to a continuous rise in morbidity, mortality and medical burden. Against this backdrop, the human intestinal microbiota, as a vast microbial ecosystem, plays a key role in regulating host immunity, metabolism and resisting pathogen colonization. The intestinal microbiota is also an important hub for the transmission of antibiotic resistance genes (ARGs). Once the microbiota is imbalanced, that is, when the composition and function of microorganisms are disrupted, it may promote the horizontal transfer of resistance genes, accelerating the colonization and spread of MDR bacteria. This review will systematically elaborate on the role of the intestinal microbiota in the colonization of methicillin-resistant Staphylococcus aureus (MRSA) and how it affects the horizontal transfer of resistance genes, and discuss how to use therapeutic strategies such as probiotics, bacteriophage therapy (BT) and fecal microbiota transplantation (FMT) to curb the spread of resistance, gradually restore microbiota homeostasis, and potentially provide a theoretical basis for the development of targeted microbiota-based personalized treatments to address the increasingly severe problem of resistant bacterial infections.

Keywords: Gut microbiota; MRSA; MRSA colonization; HGT.

1. Introduction

AMR has become a major challenge to global public health. According to in-depth analysis of global antibiotic resistance, it is estimated that over 39,000,000 people will die from antibiotic-resistant infections by

2050 [1]. The excessive and improper use of antibiotics, the lag in the development of new antibacterial drugs, and the prevalence of ESKAPE pathogens are the fundamental factors contributing to this problem. Staphylococcus aureus, as a conditional pathogen, can cause various severe infections, among which the emergence of MRSA is particularly concerning. In some regions, nearly 90% of Staphylococcus aureus infections are caused by MRSA, which cannot be treated with standard antibiotics [2]. Recent studies have revealed that the prevalence of MRSA not only depends on its inherent resistance mechanisms and virulence factors but is also closely related to the host's intestinal microbiota. The human intestine is a vast microbial system, and these pathogens usually maintain a symbiotic relationship with the host under normal circumstances. However, when the host's immune defense is weakened, they can rapidly transform into opportunistic pathogens, causing intestinal microecological imbalance and related infections. After dysbiosis, not only does the host's ability to resist MRSA colonization weaken, but also mobile genetic elements such as plasmids and bacteriophages may promote the horizontal transfer of ARGs among different strains, accelerating the spread of MRSA resistance [3]. In addressing this challenge, the intestinal microbiota plays a crucial role in maintaining the host's immune homeostasis and resisting pathogen invasion. Therefore, intervention strategies based on the intestinal microecology have gradually attracted attention. This review will take MRSA as a starting point to explore the regulatory role of the intestinal microbiota in its colonization and the horizontal transfer of resistance genes, and review innovative treatment strategies based on microbiota intervention.

2. The Impact of Gut Microbiota on MRSA Colonization Under Various Physiological States

2.1 The Impact of a Healthy Gut Microbiota on MRSA Colonization

MRSA colonization has significant epidemiological implications in the population. Although colonized individuals may not show obvious symptoms or clinical infection manifestations, their risk of future infection is significantly increased. In 50-80% of cases, the clinical infection strain is consistent with the previously colonized strain, suggesting that colonization is an important prerequisite for subsequent infection. A balanced and diverse gut microbiota can inhibit the colonization of pathogenic bacteria.

The human gastrointestinal microbiota is a dynamic ecosystem composed of bacteria, fungi, viruses, and protozoa, which forms a mutualistic relationship with the host and promotes overall health through immune regulation, metabolic support, and resistance to pathogens. One of the mechanisms by which it combats MDR infections is colonization resistance, which prevents the colonization and overgrowth of exogenous and endogenous pathogenic bacteria through multiple pathways. The specific defense strategies include the following: 1. Nutritional competition. The intestinal microbiota limits pathogen growth by competing for essential nutrients. For example, Dubosiella newyorkensis (L8) is a mouse intestinal symbiotic bacterium that reduces MRSA colonization by competing for fucose with MRSA [4]. 2. Ecological niche exclusion refers to the exclusion of pathogens by the microbiota through occupying physical space, consuming resources, or producing inhibitory substances. For example, probiotics inhibit the adhesion of harmful bacteria by competing for binding sites on the intestinal mucosal surface; An acidic pH environment has been proven to be a major environmental stress factor that prevents the formation of MRSA [5]. Meanwhile, Bifidobacterium and Lactobacillus acidophilus can secrete substances such as lactic acid and acetic acid (short-chain fatty acids, SCFAs) to create an acidic environment and inhibit the growth of MRSA. 3. The intestinal mucosal barrier is composed of multiple immune components and can resist pathogen invasion. Among them, Mucin 2 (MUC2), the core component of the mucus layer, is crucial for maintaining barrier integrity. MUC2 is secreted by goblet cells and forms the basis of the intestinal mucus layer. It builds a physical barrier within the intestine, serving as a filter between the external environment and host tissues while regulating the accessibility of substances in the intestinal lumen, thereby protecting the mucosa from irritation and pathogen attack. This function relies on continuous secretion at baseline levels to ensure long-term coverage of the mucus layer. In addition, the intestine has another more intense defense mechanism. When stimulated or threatened by pathogens, it rapidly releases stored MUC2 granules to remove bacteria and other pathogenic factors from the mucus layer, further strengthening the barrier function [6].

2.2 The Impact of Gut Microbiota Dysbiosis on MRSA Colonization

Dysbiosis, in simple terms, refers to a reduction in beneficial bacteria in the gut and an overgrowth of pathogenic bacteria. It is usually caused by improper antibiotic use, immune disorders, metabolic abnormalities, and environmental factors.

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Studies have shown that the absence of the intestinal microbiota affects the adaptive evolution of MRSA in the gut [7]. Experiments with germ-free mice have demonstrated that MRSA enhances carbohydrate transport through continuous mutations and later differentiates into evolutionary paths mainly focused on arginine metabolism or cell wall synthesis, thereby enabling normal growth in the gut without a microbiota. Among them, arginine metabolism mutations improve the redox balance and energy production under hypoxic conditions. Notably, these adaptive changes are often accompanied by a decrease in drug resistance and virulence, suggesting an evolutionary trade-off. The normal intestinal microbiota can inhibit these adaptation processes and maintain a balance between virulence, drug resistance, and colonization adaptability of MRSA, highlighting the key role of a healthy microbiota in regulating the evolutionary direction of MRSA. Additionally, when the intestinal microbiota is imbalanced, the reduction of beneficial bacteria and the vacancy of ecological niches allow pathogenic bacteria such as MRSA to occupy more ecological niches and increase the speed of colonization. Microbiota imbalance also hinders the production of secondary bile acids and the activation of bile acid membrane receptors, and these changes may affect the tolerance and transduction efficiency of MRSA. At the same time, the reduction of probiotics directly reduces the ability of the intestinal microbiota to resist the production of enterotoxins by Staphylococcus aureus. All these changes indicate that intestinal microbiota imbalance increases the rate of colonization and increases the risk of infection.

3. Mechanisms of Horizontal Transfer of ARGs in the Gut Microbiota

The gut microbiota, with its high bacterial density, provides an ideal environment for bacterial interactions, and the frequency of horizontal gene transfer (HGT) is over 25 times higher than that in soil. Gut microbiota dysbiosis can lead to a decrease in colonization resistance and an increased risk of opportunistic infections. In intestinal bacteria, drug resistance is often acquired through HGT from other bacteria to obtain resistance genes. Resistance genes are often transferred through the following pathways.

Conjugation refers to the transfer of DNA between two bacteria through the use of pili or direct adhesion, via mobile elements such as plasmids and transposons [8]. The intestinal environment, with its high-density bacterial communities and mucus layer, provides ideal conditions for conjugation. Among these conjugative elements, conjugative plasmids have the closest relationship with the spread of ARGs. This is mainly because they are usually larger and more likely to carry multiple resistance genes. Additionally, conjugative plasmids contain other functional genes besides resistance genes, which help microorganisms enhance their adaptability to the environment, such as by encoding new metabolic pathways or increasing tolerance to disinfectants and heavy metals, allowing them to be retained and spread together under different selective pressures.

Transduction is the process of transferring chromosomal or extrachromosomal DNA between bacteria mediated by bacteriophages, including generalized transduction, specialized transduction and lateral transduction [8]. These mechanisms collectively enable the movement of bacterial genomes at any segment. Generalized transduction occurs during the lytic cycle of bacteriophages, when DNA fragments of the bacterial host are packaged into the capsid during the assembly of the capsid. Specialized transduction is the process of excising the region adjacent to the integration site of the lysogenic bacteriophage and packaging it into the capsid. Lateral transduction initiates DNA replication before the bacteriophage is excised from the host genome. During replication, not only the bacteriophage DNA is replicated, but also large segments of the host genome near the integration site are replicated together, generating multiple copies containing host sequences. Subsequently, these DNA fragments are encapsulated into new bacteriophage particles and spread to other bacterial populations.

Transformation refers to the absorption of free extracellular DNA by bacterial cells, that is, the uptake and integration of DNA from the environment [8]. This process requires that the bacteria have natural transformation ability. Currently, the exact stimulating factors that induce bacteria to enter the transformation state are not fully understood, but it is known that nutrient deficiency and the presence of competence-inducing peptides can trigger this process. Although deoxyribonuclease activity in the intestine can degrade most free DNA, researchers have successfully isolated intact plasmid DNA from the intestinal contents of rats fed plasmids, indicating that extracellular DNA in the intestinal environment still has the potential to be taken up by bacteria. However, the specific extent of horizontal spread of DNA through transformation in the intestine remains unclear.

In recent years, the role of membrane vesicles (MVs) in HGT has gradually been recognized [8]. Such gene exchange is more likely to occur in high-density microbial environments. MVs are mainly produced by Gram-negative bacteria and can transfer the substances they carry when fusing with target cells. In the context of HGT, MVs secreted by intestinal bacteria may also carry cytoplasmic contents. MVs containing DNA are usually formed by

protrusions of the outer and inner membranes, encapsulating cytoplasmic components within the vesicles. Although MVs are widely produced in the intestine and may regulate the host‘s immune response, their specific role in promoting HGT in the intestinal microbiota remains unclear.

4. Interventions for MRSA Based on Gut Microbiota

4.1 Probiotics

When consumed in adequate amounts, probiotics can regulate dysbiosis and bring health benefits to the host. Common types of probiotics include Lactobacillus, Bifidobacterium, and yeast. They exert their effects by secreting antimicrobial substances (such as lactic acid), competing for nutrients, and modulating the host's immune response. These mechanisms collectively reduce the risk of infection, making probiotics an important means for promoting health and preventing infections in clinical interventions and dietary management. Among them, the Lactobacillus genus shows significant antibacterial activity against pathogens and plays a key role in preventing MRSA. These strains not only enhance the host's immune response but also promote the production of SCFAs. For example, Lactobacillus plantarum can increase the abundance of butyrate-producing bacteria, reduce the levels of pro-inflammatory cytokines, and lower the risk of infection. Certain Lactobacillus strains can also alleviate antibiotic-associated diarrhea and Clostridium difficile infections.

Previous studies have innovatively adopted encapsulation strategies to alleviate the antibiotic stress on probiotics when used in combination with antibiotics [9]. They attempted to use biomimetic membrane technology to embed probiotics, encapsulating probiotic strains in alginate to protect them from the inhibition of tobramycin and ultimately achieve effective clearance of MRSA. The mechanism mainly lies in the following aspects. Firstly, the encapsulation structure prevents antibiotics from diffusing to the core of probiotics, effectively increasing the survival rate of probiotics under antibiotic pressure, allowing them to continuously function in the infection environment. Secondly, it allows the metabolic products of probiotics to diffuse outward. The surviving probiotics can secrete active metabolites such as lactic acid and bacteriocins. These metabolites not only directly inhibit the growth of MRSA but also disrupt its biofilm structure, thereby weakening the defense ability of the pathogen. Finally, the action of probiotic metabolites makes MRSA more sensitive to tobramycin, enabling the antibiotic to enter the cells more efficiently and inhibit protein synthesis, ultimately leading to bacterial death.

4.2 BT

As bacteriophages usually target only specific strains, their application can eliminate pathogenic bacteria while minimizing damage to the host's beneficial microbiota. After lysing bacteria, bacteriophages naturally degrade, thereby reducing potential toxicity risks. Unlike antibiotics, bacteriophages do not directly act on human cells, thus avoiding damage to healthy tissues. Moreover, the rate at which bacteria develop resistance to bacteriophages is typically slower than that to antibiotics, making BT a promising strategy for addressing MDR infections [10].

BT has developed various application forms for MDR bacteria. Personalized BT achieves customized treatment for MDR infections by isolating specific phages targeting the patient's infecting strain. BT combines multiple phages that act on the same pathogen to enhance therapeutic effects and reduce the risk of drug resistance. The phage-driven antibiotic strategy investigates how phages can enhance the efficacy of traditional antibiotics by weakening bacterial defense mechanisms or disrupting protective biofilms. In MDR infections, such as those caused by Staphylococcus aureus, this strategy has shown significant potential. When a mixture of four phages was applied to Staphylococcus aureus, its effectiveness exceeded 98%, and it significantly reduced the minimum inhibitory concentration of antibiotics such as vancomycin in MRSA biofilms [10].

4.3 FMT

FMT is to restore a functional microbiota ecosystem in patients by transferring the intestinal flora of healthy donors into their bodies. Through various mechanisms such as competitive inhibition, bacteriocin secretion, and immune modulation, FMT significantly enhances the clearance efficiency of MDR pathogens and achieves effective remission. The success of FMT in treating Clostridioides difficile infections has spurred its exploration in other MDR bacterial infections, such as vancomycin-resistant Enterococcus and MRSA. This therapy can restore the host's immune response and enhance the ability to clear drug-resistant bacteria, thereby effectively promoting infection control. In clinical practice, FMT has already been used to treat hospital-acquired MRSA enteritis [11].

FMT has shown promising efficacy in managing MDR infections and restoring the balance of the intestinal microbiota, especially for patients with recurrent infections. By optimizing the structure of the intestinal flora, FMT can not only regulate the immune system and enhance

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the ability to clear pathogens, but also bring about overall health improvements. However, this field still faces many challenges, including establishing ideal donor criteria, standardizing fecal preparation and administration protocols, assessing long-term safety, and deeply understanding its mechanism of action.

5. Conclusion

The gut microbiota plays a core and complex role in the host's defense against MDR bacteria such as MRSA. Although its functions in providing colonization resistance and as a medium for HGT have been confirmed, the inherent limitations of traditional antibiotics and the high variability of the microbiota itself pose significant challenges to the development of universal therapies. Our current understanding of the intricate and interactive relationship among the microbiota, pathogens, and host immunity is still incomplete, which to some extent hinders the precise application of microbiota-targeted strategies. However, modulating the gut microbiota has become a key frontier in the fight against MRSA. Interventions such as probiotics, BT, and FMT have shown potential in helping restore the balance of the gut microbiota, enhancing host defense mechanisms, and curbing the spread of antibiotic resistance.

Looking ahead, personalized medicine can be considered for infections caused by multi-drug resistant bacteria. This requires a deeper understanding of the mechanisms and the utilization of advanced technologies such as artificial intelligence and big data analysis to tailor specific intervention measures based on individual microbiome profiles. By refining these methods and ensuring their long-term safety, we can transform the prospects of microbiome-based therapies into practical and sustainable solutions, thereby making a significant contribution to reducing the global burden of AMR.

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