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Unraveling the emergence and mechanisms of carbapenema resistance in *Klebsiella pneumoniae*

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Abstract. The emergence and rapid spread of carbapene-resistant klebsiella pneumoniae has become a severe clinical concern. The acquisition of carbapenemase enzymes is a common mechanism contributing to resistance. Alterations in outer membrane permeability and upregulation of efflux pumps, have also been implicated. In addition, approaches to combat CRKP infections may involve the use of combination therapy, development of new antibiotics, scientific prevention and phase therapy which targets multiple bacterial mechanisms simultaneously. This review aims to look into the drug resistant mechanism and some possible therapies of anti-CRKP infection, provided valuable information on the mechanism of Carbapenem Resistance, enhancing the progress of novel strategies targeting CRKP.

Keywords: Carbapenemases, Carbapenem-resistant Klebsiella pneumoniae, Drug resistance mechanism, therapy.

1. Introduction

Carbapenems, as potent broad-spectrum β-lactam antibiotics, stand as crucial agents of last resort in combating severe gram-negative infections. Regrettably, their immersive applications has fueled the rise of carbapenem-resistant gram-negative bacteria, negating their efficacy and magnifying the challenge of treatment.

Among these bacteria, *Klebsiella pneumoniae*, a gram-negative member of the enterobacteriaceae family, occupies a central position. The swift dissemination of drug-resistant gram-negative bacteria hinges on mobile genetic elements bearing β-lactamase genes, with *Klebsiella pneumoniae* assuming a prominent role as the foremost global contributor to transmissible *carbapenemase-producing enterobacteriaceae* (CPE) [1]. Notably, such strains commonly exhibit multidrug resistance, thereby propelling the emergence of extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacterial variants [2].

Carbapenem resistance heralds treatment shortcomings, engendering prolonged hospitalization, escalated healthcare expenses, and amplified mortality rates [3]. In fact, carbapenem-resistant *klebsiella*

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pneumoniae (CRKP) has secured a critical position on the World Health Organization's list of priority drug-resistant bacteria [4]. Grasping the intricacies behind the genesis and mechanics of carbapenem resistance in *Klebsiella pneumoniae* stands as a cardinal imperative for optimal patient care and effective infection control.

This comprehensive review is dedicated to unveiling the multifaceted nature of *Klebsiella pneumoniae*'s resistance to carbapenem antibiotics. Through a thorough examination, we will dissect the assorted elements contributing to the proliferation of carbapenem resistance, delve into the underlying mechanisms at play, and explore potential avenues for containment and management. Armed with this profound comprehension, we are poised to formulate efficient strategies to counteract the influence of carbapenem resistance, thereby safeguarding the continued efficacy of our arsenal of antimicrobial interventions.

2. Mechanism of Carbapenems Resistance

2.1. Classification of Carbapenemases

Carbapenemase production is the main mechanism of *klebsiella pneumoniae* resistance to carbapenems. Since 1993, the first *carbapenemase-producing enterobacteriaceae* (NmcA) strain was discovered [5]. After that, a variety of carbapenemases were identified in *enterobacteriaceae* bacteria. According to the differences in molecular structure, Ambler classification can divide them into four categories: A, B, C and D β-lactamases. [6,7]. Among them, the A, C, and D classes of serine β-lactamases have similar structures, while the B class of β-lactamases is a metallo-β-lactamase (MBL). The three main mechanisms of Kpn resistance to carbapenems described in this review are A, B, and D β-lactamases.

2.1.1. Class A Carbapenemases. Class A are serine proteases. Class A carbapenems are capable of hydrolyzing a variety of β-lactam drugs, including carbapenems, cephalosporins, penicillins, and aztreonam, and are inhibited to some extent by clavulanate and tazobactam [8]. Its hydrolysis mechanism involves the active site serine at position 70 [9]. *Klebsiella pneumoniae carbapenemase* (KPC) is the most common type of class A *carbapenemase*, the main producer of KPC is *Klebsiella pneumoniae* isolated in the hospital, and a small amount comes from other *Enterobacter* species [10]. The gene coding sequence of KPC is located on the transferable genetic elements such as plasmids and integrons, such as transposon (Tn4401b) and various plasmids (Inc FII, Inc L/M and Inc N),and can spread horizontally among different strains (genera) [11].

2.1.2. Class B Carbapenemases. Class B carbapenemase is Metallo Beta Lactamases (MBL), which uses Zn^{2+} as the active center and needs to be combined with Zn^{2+} to exert catalytic activity. Different Zn^{2+}-dependent enzymes have different hydrolysis mechanisms. In general, MBL uses Zn^{2+} to coordinate with water molecules to form OH groups, which nucleophilically attack the carbonyl carbon of β-lactam, causing the amide bond to be hydrolyzed [12]. MBL could hydrolyze common carbapenem antibacterial drugs, and its activity would not be inhibited by common enzyme inhibitors such as: clavulanic acid, tazobactam etc, however, e*thylene diamine tetraacetic acid* (EDTA) could inhibit MBL activity [13, 14]. MBL has been identified as a source of Kpn hospital outbreaks in different countries [10]. MBL is usually expressed by mobile genetic elements such as integrons, plasmids, and transposons [14]. When mobile genetic elements are transferred between bacteria, amino acid substitutions in the *carbapenemase* occur, which lead to changes in the affinity of the carbapenemase for carbapenems. The cross-individual genetic and carbapenemase-producing diversity of MBL deserves attention.

2.1.3. Class D Carbapenemases. Class D enzymes, also known as OXA enzymes (oxacillinase), hydrolyze β-lactams through carbamyl lysine [15]. OXA-48 is the most widely distributed OXA enzyme type, mainly detected in *Enterobacteriaceae* such as *Klebsiella pneumoniae* and *Escherichia coli* [14]. OXA-48 enzyme hydrolyzes carbapenems at low levels and can hydrolyze broad-spectrum cephalosporins such as ceftazidime and aztreonam. The activity of OXA-48 enzyme is not inhibited by

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EDTA or clavulanic acid, but when ESBL is associated with permeability defects, resistance to carbapenem drugs is usually higher [10, 16]. Since the first OXA-48 positive strains was detected in a *Klebsiella pneumoniae* strain isolated in Turkey in 2003, it was gradually found in many regions and countries, and the spread of its drug resistance was related to a 62.5 kb plasmid [10]. Since the carbapenem minimal inhibit concentration (MIC) of the OXA-48-producing strain is close to the critical value of drug resistance, it is difficult to detect, which is not conducive to the implementation of infection control, so that it can spread rapidly [17].

2.2. Deletion of Outer Membrane Porins

Deletion of outer membrane porins represents a significant mechanism contributing to the development of drug resistance in *carbapenem-resistant Klebsiella pneumoniae* (CRKP). Particularly, the porins OmpK35 and OmpK36 have been identified as crucial channels involved in the diffusion of antibiotics, including β-lactamase bacteria and fluoroquinolones, which would diffuse into the bacterial cell. These non-specific porins allow the passive entry of hydrophilic small molecules, contributing the potent antibacterial effects of these drugs. However, mutations or deletions in the coding sequences or promoter regions can disrupt the expression and function of these porins. This results in the loss or modification of porin structure in turn, impairing the ability of antimicrobial agents to penetrate the bacterial cell, and thus, developing resistance against the drugs.

Studies have shown that the absence of OmpK35 and OmpK36 in strains is associated with a significantly elevated level of drug resistance [18]. Furthermore, investigations conducted on carbapenem-resistant strains have demonstrated that the coinciding presence of extended-spectrum β-lactamases (ESBLs) or AmpC enzymes with the absence or alteration of porins OmpK35 or OmpK36 leads to the emergence of widespread resistance in *Klebsiella pneumoniae* [19]. This emphasizes the significance of outer membrane porins in mediating the effectiveness of antibacterial therapies.

Understanding the implications of outer membrane porin deletion in drug resistance is critical to the development of effective therapies, particularly when targeting carbapenem-resistant strains of *Klebsiella pneumoniae*. Further research into the molecular mechanisms underlying porin deletion and their impact on drug efficacy are warranted to combat the rising threat of multidrug resistance in CRKP.

2.3. High Expression of Efflux Pumps

The overexpression of active efflux system also has a non-negligible effect on the generation of Kpn drug resistance. The drug efflux pump is an active transport protein. After the substrate induces and activates the efflux pump gene, it can actively excrete antibacterial drugs from bacteria, reduce the drug concentration in bacteria, and make the bacteria resistant [20]. Its coding genes can be located on chromosomes or within mobile elements. There are five main families of efflux pumps that have been identified: Resistance-nodulation-cell division (RND) family, Major facilitator superfamily (MFS) family, multidrug and toxic compound extrusion (MATE) family, small multidrug resistance (SMR) family, ATP-binding cassette superfamily (ABC) family. The most important multidrug efflux system in *Klebsiella pneumoniae* is the AcrAB-TolC system of the RND family [21], which achieves efflux by consuming proton power. The AcrAB-TolC system consists of the accessory protein TolC located in the outer membrane, the AcrA protein located in the periplasm, and the secondary active transporter AcrB located in the inner membrane [22]. Among them, the trimer form of AcrB interacts specifically with AcrA, and the periplasmic head of the AcrB trimer complex docks with TolC to transport drugs with a functional rotation mechanism [22, 23]. In carbapenemase-containing *Klebsiella pneumoniae*, AcrAB mutant expression has a synergistic effect with β-lactamase activity, leading to high levels of carbapenem resistance [24].

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3. Anti-crkp Infection Therapies

3.1. Combination Therapy

Generally speaking, the multidrug-resistant nature of Klebsiella pneumoniae poses a great challenge to treatment progress. Due to its strong antibacterial activity, wide antibacterial spectrum, and the inability to distinguish between two different types of Klebsiella pneumoniae at present, direct clinical treatment poses a higher risk to patients. This restriction makes choosing the appropriate medication even more important. For example, ceftazidime avibactam and tigecycline are both active drugs against Kp strains. This constraint underscores the heightened significance of selecting the most suitable medication, exemplified by the efficacy of drugs such as ceftazidime-avibactam and tigecycline against Kp strains [25].

3.2. Development of New Antibiotics

In addition, the development of new antibiotics is a way to combat their resistance, which is tested in clinical treatment. The basic principle is to generate new types of compounds through artificial synthesis.

Carbapenemase has the effect of treating Klebsiella pneumoniae infection, but later developed resistance, and its molecular structure is divided into different types. The efficacy of carbapenem antibiotics in treating Klebsiella pneumoniae that do not produce carbapenem enzymes is still worthy of recognition. Among them, OXA-48 has relatively weak hydrolysis activity when dealing with broad-spectrum cephalosporins [26].

Avibatan can interact with β-lactam The formation of enzyme inhibitor complexes by lactamases can effectively enhance the activity of ceftazidime against carbapenemase enteric strains, as previously mentioned. He excels in the activity of CRKP, but there are differences in its therapeutic effects on patients with Klebsiella, pneumoniae infection in different states, so it is necessary to adjust the dosage when using it [27].

3.3. Scientific Prevention

We should not underestimate the role of prevention. Due to the unique multi drug resistance of this bacterium, prevention and isolation have brought many conveniences to treatment. One step is to pay attention to environmental hygiene and avoid contact with animals that may be at risk. Vaccines are one of the methods that can serve as a pathway for veterinary treatment. The vaccine against Klebsiella pneumoniae is also used in units such as CPS and LPS. Furthermore, it has been found that Chinese herbal medicines such as Chinese gallnut and black plum have chemicals like tannic acid which have significant effects in animal model treatment. This method has the special advantage of being residue free [28].

3.4. Phage Therapy

Phage therapy is a common antibiotic use that can decompose and mildly treat bacteriophages. Here, it can effectively stop the survival of Klebsiella pneumoniae, effectively eradicate its biofilm, and in combination with other drugs, more effectively prevent the production of drug-resistant mutant strains. For example, research has found that the bacteriophage NTUH-K2044 has a killing effect on the hvKP strain. But as mentioned earlier, this strain cannot be accurately identified [29].

4. Conclusion

In summary, the prevalence and spread of CRKP is an important global public health problem, and anti-CRKP infection treatment has become a global urgent priority. It is imperative to carry out research on the mechanism of CRKP's resistance to carbapenems and find effective treatment strategies. CRKP resistance mechanisms are diverse and adaptable, making early identification and control of CRKP infection difficult. At present, according to the individual differences of patients, the treatment of CRKP infection mainly adopts conventional mono-therapy or combination therapy, and more targeted new drugs and alternative therapies are in the development stage. The continuous generation of new drug

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resistance mechanisms makes anti-CRKP infection a long way to go, and preventive measures are more important than treatment methods. Therefore, the monitoring of KPC-producing bacteria should be strengthened to prevent outbreaks.

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