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Research progress on the oxazolidinone drug linezolid resistance

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Abstract. – OBJECTIVE: The oxazolidinone drug linezolid is mainly used for severe infections caused by multidrug-resistant Gram-positive bacteria. However, emerging linezolid resistance is aggravating difficulties in the treatment of certain infectious diseases. The objective of this review was to provide a reference for researchers and clinicians to be able to better face together the serious challenge of antimicrobial resistance.

MATERIALS AND METHODS: A systematic literature search was performed using PubMed, Web of Science, Google Scholar, and the China National Knowledge Infrastructure (CNKI) database. The articles were scrutinized to extract information on oxazolidinone drug linezolid resistance, and the prevalence of the resistance gene *optrA*. We reviewed the latest advances in epidemic properties, resistance mechanism, and transfer mechanism of linezolid resistance genes in different isolates isolated from various samples worldwide.

RESULTS: Initially, it was thought that linezolid resistance was related to the change in drug target mediated by mutations in the 23S rRNA gene, *rplC*, *rplD*, and *cfr. optrA* was discovered in 2015, and is a gene encoding oxazolidinone resistance, which exists in both plasmids and chromosomes, but mostly plasmids. The emergence of the novel plasmid-borne ABC transporter gene *optrA* expanded the understanding of the mechanism of linezolid resistance.

CONCLUSIONS: At present, the prevalence of linezolid resistance has become increasingly serious. The resistance gene *optrA* has been reported in Enterococcus, Staphylococcus squirrel and Streptococcus, which indicates that this gene has a strong ability to spread across bacteria, so the prevalence and spread of *optrA* gene should be monitored carefully.

Key Words:
Oxazolidinone, Linezolid, Resistance gene, *OptrA*.

Introduction

Since the application of antibiotics in the clinic, they have played an important role in the prevention and treatment of diseases. However, the problem of bacterial resistance has gradually emerged and is becoming increasingly serious, complicating clinical treatment[1]. Due to inexpedient use of antibiotics, such as large doses and abuse, the development of bacterial resistance is accelerated, and several unique resistance mechanisms cause the rapid spread of multi-drug resistant (MDR) strains, further aggravating the difficulty of disease treatment and posing a potential threat to public health[2-4].

Oxazolidinone, a new type of antimicrobial agent, achieves antibacterial effect mainly by inhibiting the synthesis of bacterial proteins. Oxazolidinone is often used for severe infections caused by Gram-positive bacteria, especially for infections caused by MDR bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and *Streptococcus pneumoniae*[5,6]. It has been proposed that the main reason for drug resistance is overuse of the drug[7]. In 2015, Chinese researchers discovered the drug resistance gene *optrA*[8]. The gene not only mediates oxazolidinone (linezolid) resistance, but also mediates phenylpropanol (such as flurbenicol) resistance, which can be transmitted horizontally. At present, many scholars in China and abroad have paid close attention to the prevalence and drug resistance of *optrA* gene.

In this study, we reviewed data on the *optrA* gene for linezolid resistance, with a view to summarize theoretical scientific basis for rational clinical drug selection and for the prevention of the spread of drug-resistant strains.

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Overview of Oxazolidinone Drugs and Their Resistance

A Brief Introduction to Oxazolidinone and Linezolid

Oxazolidinones comprise a new class of synthetic antimicrobial drugs, following in the wake of sulfanilamide and fluoroquinolones. Linezolid was the first pharmacologically active compound developed and the first synthetic oxazolidinone approved for clinical use[9]. Linezolid was approved by the US Food and Drug Administration (FDA), entered the market in the United States in 2000, and has mainly been used for severe infections due to MRSA, VRE, and *Streptococcus pneumoniae*[5]. In 2007, it was used in clinical practice. The oxazolidinones are fully synthetic antibacterial agents, achieving antibacterial effect by inhibiting the synthesis of bacterial protein, usually with a good effect on infections caused by Gram-positive bacteria and MDR bacteria[6].

Overview of the Antibacterial Activity and Mechanism of the Oxazolidinone Drug Linezolid

Linezolid can be combined with the peptidyl transferase center (PTC) of the 50S ribosomal subunit of bacteria, which is an inhibitor of bacterial protein synthesis and thus plays a critical antibacterial role[10]. Compared with chloramphenicol and lincomycin, linezolid mainly targets the initial phase of protein synthesis, which, however, does not affect the prolongation or termination of the peptide chain during protein synthesis. Even if they overlap or are close to the action sites of other antimicrobials, they can induce antimicrobial activity by inhibiting the translocation of the peptide chain[11].

Linezolid has exhibited good antibacterial effect on MDR Gram-positive bacteria (such as MRSA). It has been demonstrated that linezolid has a strong bactericidal effect on most species and strains of *Staphylococcus* and *Streptococcus*, but only has a bacteriostatic effect on *Enterococcus*. In addition, according to the results of the time-sterilization curve, the efficacy of linezolid was found to correlate with time; that is, continuous use of the drug could be conducive to the improvement of efficacy[12]. Linezolid has been reported to inhibit some staphylococci and enterococci by 100%[13]. In addition, according to *in vitro* studies, linezolid also exhibits certain antibacterial effects against *Bacillus*, gonococci, and some anaerobic bacteria[14]. However, due to the effect of the efflux pump system of Gram-negative bacteria, linezolid has little or no antibacterial effect on Gram-negative bacteria[15].

Emergence and Prevalence of Linezolid Resistant Strains

Because of the unique antibacterial effect of linezolid, resistance towards this drug should not readily develop in clinical practice. However, with the extensive application of the drug, bacterial resistance to linezolid has been observed in clinical and *in-vitro* studies, which has given rise to great concern. In 2000, only one year after the drug entered the market, linezolid-resistant strains emerged, and so, the first clinical strain of linezolid-resistant MRSA was reported in the United States in 2001. By sequencing analysis, it was verified that the strain had a G2576T point mutation in the 23S rRNA domain V region, resulting in drug resistance to linezolid[16]. In addition, *Staphylococcus aureus* resistant to linezolid was detected in the first year upon its release in China[17]. After that, linezolid-resistant *Staphylococcus* was reported in the United States[18], Mexico[19], Japan [20], Spain[21] and Italy[22].

Overview of Bacterial Drug Resistance Induced by the Oxazolidinone Drug Linezolid and its Mechanism

Induced Resistance

Upon serial passage of methicillin-susceptible *S. aureus* and MRSA in linezolid, Locke et al[23] demonstrated that the minimum inhibitory concentration (MIC) of MRSA and MSSA increased by 32 and 64 times in 30 generations, respectively. Xi[24] induced *Enterococcus* with 1/2 MIC linezolid and found that *Enterococcus faecalis* generally developed drug resistance more slowly than *Enterococcus faecalis*. According to one report, after 35 generations, the MIC of oxazolidinone-induced *Staphylococcus aureus* increased 4-32 times. However, after only 16 generations of *Enterococcus faecalis* induced by oxazolidinone, the increase in MIC was 8-32 times. Thus, the fact that members of the same class of antibiotics may show different results in terms of inducing drug resistance in bacteria of the same genus suggests that such bacteria may have diverse and complex mechanisms of drug resistance to oxazolidinone antibiotics.

Wang et al[25] compared MIC values for MRSA induced by linezolid and vancomycin. After

inducing MRSA with 1/2 MIC concentration of linezolid and vancomycin for 20 generations, respectively, they found that the MIC of linezolid was 2 mg/L, which was twice the original value, while the MIC value of vancomycin increased to 4 mg/L, which was four times the original value. It appears that the rate of MRSA resistance induced by linezolid is similar to that of vancomycin, with vancomycin being slightly faster in terms of reducing resistance in MRSA.

Resistance Mechanisms

At present, it is believed that the cause of linezolid resistance is associated with clinical overuse of the drug[7]. The suggested drug resistance mechanisms are accounted for in the following.

Mutations in the 23S Ribosomal RNA Gene

Since the V region of the 23S rRNA gene is where the action site of linezolid is located, point mutations in the region are mainly resulting in bacterial resistance to rina thiazole amine; changes in the structure of point mutations can result in the production of drug resistance, and any locus mutation in this area will to a certain extent affect the rina thiazole amine resistance[26]. Among these, the 2576 position in the 23S rRNA gene was the first mutation found, and the most common occurrence of this site can occur in single copy gene or multi-copy genes[27]. In addition, some point mutations in the 23S rRNA genes, such as T2500A, G2603T, G2215A, C2534T, G2766T, T2504C, and G2247T have been reported to be associated with resistance in clinical strains. However, so far, only G2576T and T2500A have been found in clinical isolates resistant to linezolid[16,23], while other point mutations have been found in induced strains.

Lobritz et al[28] showed that under the selection pressure of antibiotics, the genes of Protobacterium were replaced by genes from mutant strains. At the same time, *in-vitro* induction tests also showed that the number of G2576T mutations increased, and the drug resistance of the bacteria increased with the number of times passed after introduction of the first G2576U mutation. When an MIC of 2 g/mL linezolid was used to treat patients with *S. aureus* infection[29], the G2576T mutation was found. After 20 days of linezolid administration, the MIC increased to 8 g/mL, and there were 2 G2576T mutations in the *S. aureus*. After 71 days, the MIC reached 32 g/mL, and 5 G2576T mutations were found. This indicated that the increasing number of G2576T mutations positively correlated with the duration of linezolid use. Another study[30] found that when linezolid was administered for more than 20 months, a similar pattern was found in clinical strains of *S. aureus*, in which the T2500A mutation was observed in multiple copies of the gene.

Mutations in Ribosomal Proteins L3 and L4

The target site of linezolid is the 50S large subunit of the ribosomal proteins, many of which are closely related to the binding sites of linezolid drugs, especially the ribosomal proteins L3 and L4 which are encoded by the genes *rplC* and *rplD* respectively[23]. Locke et al[31] studied the DNA sequence of the *rplC* gene and found the mutation sites of ribosomal protein L3, named ΔSerl45 and Alal57Arg, which were closely related to the action sites of linezolid. In addition, in a study of *S. pneumoniae*, a 6-bp deletion was found in the highly conserved *rplD* gene encoding ribosomal protein L4. When studying the *rplD* gene of *Clostridium perfringens*, a single C-T mutation was found at nucleotide position 404, resulting in the substitution of glycine with aspartic acid. In conclusion, mutations in ribosomal proteins L3 and L4 are associated with linezolid resistance.

Non-mutated Mechanism cfr Gene Mediates Drug Resistance

The *cfr* gene, initially isolated from *Staphylococcus sciuri*, mainly confers chloramphenicol and florfenicol resistance[32]. In 2005, the *cfr* gene was first detected in clinically isolated MRSA[33]. In addition, this gene has also been found in the genus *Staphylococcus* of human origin[18]. The ubiquity of the *cfr* gene plays an important role in the spread of drug resistance. In terms of drug resistance mechanism, the *cfr* gene confers resistance through a non-mutated mechanism, which is different from linezolid resistance, which is linked to gene mutations. Specifically, the *cfr* gene belongs to the methylated transferases, which can act on the binding site of linezolid and methylate at position 2503 of the 23S rRNA gene, thus making bacteria resistant to chloramphenicol, florfenicol and linezolid[34]. Locke et al[35] found that the *cfr* gene was identified in clinical linezolid-resistant *S. aureus* isolates, indicating that the presence of the *cfr* gene is another important mechanism of bacterial resistance to linezolid.

The *cfr* gene can be carried by plasmids with a mobile function, resulting in horizontal spread

within the genus of *Staphylococcus*, causing outbreaks of infection with resistant bacteria. It was reported that 15 patients with linezolid-resistant MRSA were found in the same hospital within 3 months, and all linezolid-resistant strains carried the *cfr* gene[36]. At the same time, the *cfr* gene widely exists in various strains, which is a great threat to humans. At present, it is difficult to prevent and control this resistance mechanism.

Other Resistance Mechanisms

Ribosomal protein mutations in the L22 gene is also associated with the rina thiazole amine resistance mechanisms. Due to the action of L22 near rina thiazole amine sites, L22 protein amino acid mutations, deletions, or replacements may also affect peptide acyl transferase space structure; hence, it may also be associated with rina thiazole amine resistance. This is *S. aureus* to rina thiazole amine other mechanisms of drug resistance[22,23].

Involvement of the optrA Gene in Linezolid Resistance

Discovery of the Drug Resistance-Conferring Gene optrA

In 2015, unexpectedly high rina thiazole amine MIC values in clinical *Enterococcus* isolates has been found. In order to explore this finding further, the strain plasmid was sequenced. The authors found that the plasmid size was 36,331 bp long, carrying the *optrA* gene with a size of 1,968 bp, encoding 655 amino acids, and due to the existence of high amino acid sequence homology with ABC transporters, the gene was named *optrA*[8]. The plasmid sequence was submitted to GenBank with the accession number KP399637. The *optrA* gene can be transmitted horizontally. Linezolid is the first choice for the treatment of severe infection with some *Gram*-positive bacteria, especially MRSA infection, but the emergence of this gene poses a safety threat to human health and the development of livestock and poultry breeding industry. Therefore, many scientists in China and abroad pay close attention to the emergence and prevalence of the *optrA* gene.

The Drug Resistance Gene optrA is Prevalent in Enterococcus Isolates

Since the first report of the *optrA* gene in *Enterococcus* in 2015, Chinese scholars have repeatedly reported on observations of this gene in *Enterococcus* isolates. Cai et al[37] studied 1,159 strains of *Enterococcus* isolated from Zhejiang, Guangdong, and Henan in China, and found that nearly 3% of the strains carried the *optrA* gene. Zhao[38] conducted *optrA* gene testing in 513 samples from a pig farm in Guangdong, and 17 strains carrying the *optrA* gene were detected. At the same time, they found 11 strains of *Enterococcus* carrying the *optrA* gene, and most of these were located in plasmids. Cui et al[39] studied 2,201 strains of *Enterococcus* collected from the Chinese bacterial resistance monitoring network over a period of 10 years and found that the detection rate of the *optrA* gene was 2.0%. They also found that the positivity rate of the *optrA* gene increased from 0.4% in 2004 to 3.9% in 2014, and so the positivity rate of this gene appears to be increasing year by year.

Since the discovery of the *optrA* gene in *Enterococcus chinensis*, the samples of clinical and animal origin have been reported to carry the gene in several countries. Gawryszewska et al[40] detected five *optrA*-positive strains among 50 clinically derived linezolid resistant *Enterococcus* strains. Later, Brenciani et al[41] detected *optrA* gene in two strains among 81 clinical blood-derived *Enterococcus* isolates; however, these two strains also contained another linezolid resistance gene, *cfr*. Vorobieva et al[42] detected the *optrA* gene in a strain of *E. faecalis* in a gastric sample from Denmark, which also contained genes mediating drug resistance to aminoglycosides, macrolides, tetracyclines, and other drugs. In addition to the above countries, strains carrying the *optrA* gene have also been found in clinical *Enterococcus* strains in Ireland, Malaysia, and the United States. In the last few years since 2015, the gene has shown an emerging trend of worldwide.

optrA Is Common in Staphylococcus

The *optrA* gene has been less studied in Gram-positive bacteria other than *Enterococcus*; until now, only *Staphylococcus* of origin has been reported to carry the *optrA* gene. Fan et al[43] studied porcine methicillin-resistant *S. aureus* and coagulase-negative staphylococci isolated in 2014 and found that the positivity rate of the *optrA* gene in coagulase-negative staphylococci was 6.9%; however, no *optrA* gene was found in *S. aureus*. Li et al[44] studied 50 strains of porcine *Staphylococcus* isolated in 2013, and

only one strain of *S. sciuri* with the *optrA* gene was detected.

ATP Binding Sites of New Drug-Resistant Protein OptrA

In 2015, a new drug-resistant protein *optrA* was found in the plasmid of Enterococcus pE349 in China. *OptrA* belongs to the ABC protein family, which confers resistance to oxazolidinone and chloramphenicol drugs. At present, as a new drug-resistant protein, the research into *optrA* is in the initial stage in China and abroad, and the function and mechanism of this protein have only been scarcely reported. Since all ABC transporters belong to ATP hydrolases, however, there is no conclusive evidence on the relationship between the hydrolysis of ATP and ABC mediated resistance[45]. Zhong et al[46] showed that two glutamic acid (E) loci in the *optrA* domain exhibited ATP hydrolysis activity and demonstrated the drug resistance associated with *optrA* for the first time. After in-depth analysis of the hydrolysis of ATP locus mutation which can make the function of *optrA* mediated antibiotic resistance to further reduce or lose, the *optrA* mechanism and ATP binding sites may provide the theoretical basis for the future research.

The Mechanism of Transmission and Diffusion of Drug Resistance Gene OptrA

In recent years, the localization of *optrA* gene in *Enterococcus* has been observed on both plasmids and chromosomes. The first identified *optrA* gene in *Enterococcus* E349 is located in the 36-kb plasmid pE349, the plasmid carrying open reading frame code box 39, including 21 putative proteins; products of the remaining 18 codes include the linezolid resistance gene *optrA*, the florfenicol resistance gene *fexA*, and plasmid replication and joint transfer related proteins [7]. Since the time when *optrA* was first reported in China, *optrA*-positive *Enterococcus* strains have been detected in many countries around the world, and the plasmids carrying *optrA* genes detected in clinical and animal-derived strains in some countries have high similarity to pE349, suggesting that there may be human-to-animal transmission[40,47]. It was also reported that 17 strains of *E. faecalis* carried *optrA* (nine strains located in plasmids and eight strains located on chromosomes) were investigated for their genetic environment, and it was found that all nine plasmids contained the inserted sequence IS1216E, belonging to the IS6 family, which was located upstream and/or downstream of *optrA*. Therefore, the *optrA* gene carried by enterococcal plasmids may be transmitted between different enterococcal species through IS1216-mediated recombination[48]. For the eight strains located on the chromosome carrying the *optrA* gene, flanking sequence analysis found that there are four strains of bacteria carrying the *optrA* gene upstream the transcription regulatory gene *araC*; another four strains of bacteria had the *optrA* gene upstream for florfenicol resistance gene *fexA*. However, it remains unclear how the *optrA* gene was integrated into the chromosome[48].

Similar to *Enterococcus*, the *optrA* gene localization in *S. sciuri* may be present on both plasmids and chromosomes. By studying plasmids from four *S. sciuri* strains with *optrA*, it was found that the plasmid size was about 35 kb and all of the four strains were found to contain a 17,612-bp contig containing the resistant gene cluster *optrA-cfr-ble-aadD-aacA-aphD-fexA* and a segment sequence with another plasmid carrying *optrA* pWo28-3 corresponding almost unanimous in their area. It was suggested that the *optrA* plasmid identified in the four *S. sciuri* strains may have come from the plasmid pWo28-3, and it is possible that *S. sciuri* has spread in isolated areas[43]. Twenty-nine strains *optrA* strains were subject to genetic analysis, and the chromosome *optrA* flanking sequence can be divided into six types (I-VI), with type I (n = 12) and IV being most common (n = 10). The type I contig is shortest, contains only the *optrA* gene, and is located upstream of the transcription regulation gene *araC*. The other five types contain the *araC-optrA* area. Type IV is located upstream of the *optrA-araC*, carrying the *fexA* transposon Tn558, downstream is *mdlB1* and *mdlB2*, coding ABC transporters. Transposon Tn558 is in the upstream area of *araC-optrA* in of types II-VI, but it remains unknown whether it is associated with *optrA* in *S. scirui*[43].

Drug-Resistant Genes Transfer or Pass From One Bacterium to Another

At present, it is known from many studies that some drug-resistant genes can be transferred or transferred between bacteria. However, this phenomenon is mainly caused by drug-resistant genes existing in plasmids, and plasmid conjugation is an important pathway of gene transfer, most pathogens acquire drug resistance mainly through conjugation[49]. For example, the MDR gene *lsa (E)* was first identified in human MRSA

ST398 and *S. aureus* ST9, followed by pig and human *Enterococcus*[50]. At first, amide-alcohol resistance gene *fexA* and MDR gene *cfr* were found in *Staphylococcus*, followed by *Enterococcus* and *Streptococcus*[51]. In addition, *vanA*, a vancomycin resistant gene, was first identified in *Enterococcus*, and subsequently also found in MRSA[52]. At present, although no *optrA* gene has been reported in *S. aureus*, awareness regarding the possible introduction of *optrA* in *S. aureus* is recommended.

Conclusions and Future Prospects

OptrA, a novel gene for resistance to the oxazolidinone linezolid, not only mediates linezolid resistance but also florfenicol resistance, which is mostly present in plasmids and can be transmitted horizontally, further exacerbating the rate of transmission[53]. Linezolid is the first-line drug in the clinical treatment of some serious infections due to major drug-resistant bacteria such as MRSA and VRE; therefore, the emergence and rapid spread of this gene has attracted the attention of medical doctors and scientists in China and abroad[54-56].

Although there are some differences in the genetic background of *optrA* gene, there is a certain possibility of human and animal transmission and thereby worldwide dissemination of this gene. Therefore, vigilance is needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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