

Dissemination of *cfr*-mediated linezolid resistance among *Staphylococcus* species isolated from a teaching hospital in Beijing, China

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Jiyong Jian^{1,2,3,#}, Liang Chen^{1,2,3,#},
Zejiang Xie^{1,2,3} and Man Zhang^{1,2,3}

Abstract

Objective: The aim of the present study was to report the dissemination of *cfr* and *fexA* genes mediated by linezolid resistance among *Staphylococcus* species.

Methods: Three methicillin-resistant staphylococci that were collected from a teaching hospital in Beijing were identified as linezolid-resistant. These three staphylococci were *Staphylococcus aureus*, *S. haemolyticus*, and *S. cohnii*. Mutations in domain V of 23S ribosomal RNA, ribosomal proteins, and the *cfr*, *fexA*, and *optrA* genes were analysed.

Results: The three isolates had no mutations of 23S ribosomal RNA, but showed mutations in the *cfr* and *fexA* genes. Mutations in the gene for ribosomal protein L3, which resulted in the amino acid exchanges Gly108Glu, Ser158Phe, and Asp159Tyr, were identified in *S. cohnii* X4535.

Conclusions: This is the first report of the *cfr* gene in clinical linezolid-resistant methicillin-resistant *S. aureus* isolated from Beijing. L3 mutations coupled with the *cfr* and *fexA* genes may act synergistically. Potential transmissibility of this agent, even without prior exposure to linezolid, may have serious epidemiological repercussions.

Keywords

Linezolid resistance, staphylococci, *cfr* gene, ribosomal protein, 23S rRNA, mutation

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¹Clinical Laboratory of Medicine, Beijing Shijitan Hospital, Capital Medical University, Beijing, China

²Peking University Ninth School of Clinical Medicine, Beijing, China

³Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics, Beijing, China

[#]These authors contributed equally to this work

Corresponding author:

Man Zhang, Clinical Laboratory Medicine, Beijing Shijitan Hospital, Capital Medical University, No. 10, Tie Yi Road, Yang Fang Dian, Haidian District, Beijing, 100038, China. Email: zhangman@bjsjth.cn



Introduction

Linezolid, the first clinically used oxazolidinone antibiotic, has a broad spectrum of activity against a variety of Gram-positive pathogens, especially methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (CoNS), penicillin-resistant *S. pneumoniae*, and vancomycin-resistant enterococci.¹ The first report of linezolid resistance was in a staphylococci clinical isolate in 2001.² Since this time, linezolid-resistant MRSA^{3–6} and the linezolid-resistant CoNS strains^{7–8} have been increasingly isolated from the health care setting.

Linezolid binds to ribosomal RNA (rRNA), specifically to domain V of the 23S rRNA of the 50S ribosomal subunit, and inhibits protein synthesis.⁹ Mutations in domain V of 23S rRNA, and ribosomal proteins such as L3 and L4, predominantly mediate resistance to linezolid.^{10,11} Nonmutational oxazolidinone resistance is due to the chloramphenicol–florfenicol resistance (*cfr*) gene. The *cfr* gene is a horizontally transferable resistance gene that encodes a ribosomal methyltransferase, conferring cross-resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (PhLOPS_A phenotype).¹² In staphylococci, florfenicol resistance can also be mediated either by the *fexA* gene (coding for a phenicol-specific efflux pump) or the *optrA* gene (coding for an ABC transporter), all of which mediate combined resistance to phenicols.^{13,14}

In China, linezolid was granted a license for clinical use in 2007. Since then, linezolid-resistant MRSA and CoNS have emerged in China.^{4,15} In the present report, we describe three linezolid-resistant *Staphylococcus* species (*S. aureus*, *S. haemolyticus*, and *S. cohnii*) that were isolated from a teaching hospital. These three *Staphylococcus* species were all positive for

the *cfr* and *fexA* genes, and mutations in ribosomal proteins were found in *S. cohnii*. The three isolates were collected from three inpatients who had never been treated with linezolid.

Materials and methods

Bacterial strains

Three linezolid-resistant clinical isolates were isolated from Beijing Shijitan Hospital, Capital Medical University (1000-bed tertiary care hospital). *S. aureus* 12223 was obtained from sputum culture in July 2017 and was isolated from the Emergency ward. *S. haemolyticus* 1760 was obtained from wound secretion culture in January 2016 and was isolated from the Spine Surgery ward. *S. cohnii* X4535 was isolated from an inpatient's blood culture in the Intensive Care Unit in May 2016. The study was approved by the Medical Ethics Committee of Beijing Shijitan Hospital, Capital Medical University.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test (AST) results were determined by VITEK 2 Compact (bioMérieux, Marcy L'Etoile, France). The minimum inhibitory concentrations (MICs) of linezolid were confirmed using the E-test (bioMérieux). *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used for quality control in the AST. The results of the AST were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2016). The results of the AST for tetracycline were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016).

DNA extraction

Briefly, colonies of clinical strains were transferred to sterile distilled water solution

in a microcentrifuge tube. The samples were boiled to prepare the DNA templates for polymerase chain reaction (PCR).

16S rRNA gene sequencing

Primers were used to amplify and sequence the 16S rRNA gene.¹⁶ BLAST analyses were applied to the sequencing results.

Molecular detection of resistance genes

Domain V of the 23S rRNA gene spanning 2011 to 2699 bp (*Escherichia coli* GenBank accession no. AJ278710) was amplified and sequenced using previously described primers.¹⁷ Genes encoding the ribosomal proteins L3 (*rplC*), L4 (*rplD*), and L22 (*rplV*) were amplified and sequenced using primers and under previously described conditions.¹⁸ Sequence data were analysed using DNAMAN (Lynnon Biosoft, Quebec, Canada). The presence of the *cfr*, *fxaA*,

and *optrA* genes were tested using PCR with primers as previously described.^{19–21}

Results

The multidrug-resistant phenotypes of the isolates 12223, 1760, and X4535 are shown in Table 1. All of the isolates were methicillin-resistant and exhibited resistance to clindamycin and erythromycin. The VITEK 2 Compact system showed MIC values of ≥ 8 $\mu\text{g/mL}$ for linezolid for all of the isolates. MICs of linezolid as determined by the E-test were 32, 16, and >256 $\mu\text{g/mL}$ for the isolates 12223, 1760, and X4535, respectively.

Further 16S rRNA sequencing and analysis showed that the three isolates were *S. aureus* (12223), *S. haemolyticus* (1760), and *S. cohnii* (X4535). These findings were in accordance with the VITEK 2 Compact results.

To determine the mechanism of linezolid resistance, we initially investigated the

Table 1. MICs and resistant determinants of the three clinical isolates

Parameter	Results for isolates			Breakpoint ($\mu\text{g/mL}$)
	12223	1760	X4535	
MICs ($\mu\text{g/mL}$)				
Linezolid	≥ 8	≥ 8	≥ 8	≥ 8
Linezolid*	32	16	>256	≥ 8
Ciprofloxacin	≥ 8	≥ 8	≥ 8	≥ 4
Clindamycin	≥ 8	≥ 8	≥ 8	≥ 4
Erythromycin	≥ 8	4	≥ 8	≥ 8
Gentamycin	≥ 16	≥ 16	2	≥ 16
Levofloxacin	≥ 8	≥ 8	≥ 8	≥ 4
Oxacillin	≥ 4	≥ 4	≥ 4	≥ 4
Benzylpenicillin	≥ 0.5	≥ 0.5	≥ 0.5	≥ 0.25
Rifampin	≤ 0.5	≤ 0.5	≤ 0.5	≥ 4
Tetracycline	≥ 16	2	≤ 1	≥ 16
Vancomycin	≤ 0.5	1	1	≥ 16
Quinupristin/dalfopristin	0.5	1	8	≥ 4
Tigecycline	0.5	≤ 0.12	0.25	>0.5
Moxifloxacin	4	≥ 8	≥ 8	≥ 2
Trimethoprim-sulfamethoxazole	≤ 10	≤ 10	≤ 10	$\geq 4/76$
Inducible clindamycin resistance	NEG	NEG	NEG	

*MICs were determined by the E-test. MIC: minimum inhibitory concentration; NEG: negative.

presence of mutations in genes encoding domain V of 23S rRNA (the most common mechanism found in clinical isolates) and in the ribosomal protein genes *rplC*, *rplD*, and *rplV*. We did not detect mutations in domain V of 23S rRNA in the three isolates. Moreover, no mutations in *rplC*, *rplD*, and *rplV* were detected in the 12223 and 1760 isolates in our study. However, alterations were detected in the *rplC* gene, which resulted in the amino acid substitutions Gly108Glu, Ser158Phe, and Asp159Tyr in ribosomal protein L3 of *S. cohnii* X4535. The three isolates were all PCR-positive for the *cfr* and *fexA* genes. Results for the main antibiotic resistance genetic determinants that were investigated are shown in Table 2.

Discussion

In the present study, MICs of linezolid were high for the three studied isolates. These elevated MICs of linezolid may have been attributed to the occurrence of *cfr* and *fexA* genes and mutations in ribosomal protein genes. The *cfr* and *fexA* genes were possible resistance mechanisms in 12223 and 1760, with MICs of 32 and 16 µg/mL, respectively. The combination of *cfr* and *fexA* genes and mutations in ribosomal protein genes were found in X4535, and the MIC was higher (>256 µg/mL) than that in the 12223 and 1760 isolates. Staphylococci carrying *cfr* display a multidrug resistant

phenotype, which is in agreement with the resistance profiles of these isolates.

The most frequent resistance mechanisms of linezolid are mutations in domain V of 23S rRNA and in ribosomal proteins, which are not transmissible and associated with previous use of linezolid. The *cfr* gene is usually located in an unstable genetic environment either in the chromosome or in multidrug resistant plasmids.²² Additionally, *cfr* is typically associated with transposons and is plasmid borne, which could result in ready exchange between Gram-positive strains.^{7,23} This would facilitate easy spreading of *cfr* into susceptible populations and other pathogenic bacteria. Furthermore, *cfr*-mediated resistance limits therapeutic options because it encodes resistance to an array of antibiotics. The *fexA* gene has been detected either as part of the small non-conjugative transposon Tn558 or in combination with the *cfr* gene in transposition-deficient Tn558 variants in several staphylococcal species.¹⁹ In this study, linezolid was not used before we identified the three isolates. Therefore, occurrence of the *cfr* and *fexA* genes may be the mechanisms of linezolid resistance in 12223 and 1760. Additionally, in our study, the Ser158Phe and Asp159Tyr substitutions in the L3 protein of *S. cohnii* X4535 involved two residues that were located in close proximity to the residues Gly155

Table 2. Genotypic characteristics of the isolates

Strain	23S rRNA gene mutation	Mutations in ribosomal proteins			Resistance gene		
		L3	L4	L22	<i>cfr</i>	<i>fexA</i>	<i>optrA</i>
12223	—	—	—	—	+	+	—
1760	—	—	—	—	+	+	—
X4535	—	Gly108Glu, Ser158Phe, Asp159Tyr	—	—	+	+	—

rRNA: ribosomal RNA.

and Ala157. Gly155 and Ala157 were previously found to be associated with linezolid resistance by abolishing linezolid binding to its target.²⁴⁻²⁶ The combination of the *cfr* gene and L3 substitutions can act synergistically.²⁷ The mechanisms of linezolid resistance in X4535 may be the combination of *cfr* and *fexA* genes and mutations in *rplC*.

In conclusion, this is the first report to document the *cfr* gene in clinical linezolid-resistant MRSA isolated from Beijing. The presence of the *cfr* and *fexA* genes and L3 substitutions in *S. cohnii* X4535 may act synergistically. Identification of the *cfr* and *fexA* genes in *S. aureus* (12223), *S. haemolyticus* (1760), and *S. cohnii* (X4535) suggests horizontal gene transfer in our hospital. This possibility indicates the need for strengthening implementation of infection and control measures.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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