Journal of Microbiology, Immunology and Infection (2016) 49, 595—599

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

BRIEF COMMUNICATION

Investigation of mechanisms and molecular epidemiology of linezolid nonsusceptible *Enterococcus faecalis* isolated from a teaching hospital in China

Bin Li [a], Chuan-Ling Ma [a], Xiao Yu [b], Yao Sun [a], Mei-Mei Li [a], Jian-Zhong Ye [a], Ya-Pei Zhang [a], Qing Wu [a], Tie-Li Zhou [a,*]

[a] *Department of Clinical Laboratory, First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, China*
[b] *School of Laboratory Medicine and life Science, Wenzhou Medical University, Wenzhou, Zhejiang Province, China*

Received 23 September 2014; received in revised form 23 April 2015; accepted 21 May 2015
Available online 27 June 2015

KEYWORDS
Enterococcus faecalis;
Linezolid nonsusceptible;
Multilocus sequence typing;
Pulsed-field gel electrophoresis;
23S rRNA

Abstract The epidemiological and molecular characteristics of eight linezolid nonsusceptible *Enterococcus faecalis* isolated from a teaching hospital in China (January to July 2014) were investigated. The target site modifications and *cfr* gene associated with linezolid resistance were not found. Results of the epidemiological investigation indicated that linezolid resistance possibly occurred on several independent occasions and was often not related to linezolid administration.

* Corresponding author. Department of Clinical Laboratory, First Affiliated Hospital of Wenzhou Medical University, Nanbaixiang Street, Ouhai District, Wenzhou, Zhejiang Province, China.
E-mail address: wyztli@163.com (T.-L. Zhou).

http://dx.doi.org/10.1016/j.jmii.2015.05.010

Introduction

Enterococci have emerged in the past decade as an increasingly important cause of nosocomial infections. Linezolid, the first member of a new class of antibiotics (oxazolidinones), has been introduced into clinical practice for infections caused by multiresistant Gram-positive bacteria especially vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*.[1] However, linezolid resistant isolates had emerged in several countries shortly after the drug appeared on the market, and they have also emerged recently in several hospitals in China.[2] Vancomycin-resistant enterococci have caused hospital outbreaks worldwide; the occurrence of linezolid nonsusceptible enterococci, therefore, represents an immediate threat for patient care. A variety of mutations in domain V of 23S rRNA genes, especially G2576U and the multidrug resistance gene *cfr*, which codes for an adenine methyltransferase that modifies adenosine at position 2503 in the 23S rRNA, play an important role in linezolid resistance of enterococci. Mutations of the bacterial ribosomal proteins L3 and L4 have also been associated with resistance to linezolid,[3–5] and active efflux pump systems may also be involved.[6] Pulsed-field gel electrophoresis (PFGE) has been considered the "gold standard" for the study of hospital outbreaks because of its high degree of isolate differentiation. Multilocus sequence typing (MLST) offers the possibility to transfer typing data between laboratories or compare results via Internet, thus providing a powerful tool for global epidemiologic studies. The aim of this study was to investigate the epidemiological and molecular characteristics of linezolid nonsusceptible *Enterococcus faecalis* isolated from a Chinese hospital.

Methods

Strain selection

Eight *E. faecalis* clinical strains exhibiting linezolid Minimum inhibitory concentrations (MICs) $\geq$ 4 mg/L were collected from the First Affiliated Hospital of Wenzhou Medical University from January to July 2014. The clinical records of patients with linezolid nonsusceptible *E. faecalis* were examined retrospectively. *Staphylococcus capitis*-(*cfr*$^+$) used for positive control in this study was donated by Rong Zhang, charge technician from the Second Affiliated Hospital of Zhejiang University.

Antimicrobial susceptibility testing

Susceptibility testing was determined using the agar dilution method and broth microdilution method (linezolid and Daptomycin) according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2013). *E. faecalis* ATCC29212 was used as quality control.

Molecular detection of mutations and resistance gene *cfr*

The presence of mutations in domain V of 23S rRNA and ribosomal proteins L3 and L4, and *cfr* gene were investigated using polymerase chain reaction as described previously.[3,4] Genomic DNA extracted from *E. faecalis* ATCC29212 and *S. capitis*-(*cfr*$^+$) were used as negative and positive control for *cfr*, respectively. The positive polymerase chain reaction products were sequenced by Shanghai Majorbio Bio-Pharm Technology Co. (Shanghai, China). Nucleotide and deduced amino acid sequences were compared with *E. faecalis* V583 strain and the linezolid susceptible *E. faecalis* clinical isolates (MIC, 1 μg/mL) recovered from the same hospital during the study interval.

Effect of efflux pump mechanism

The MIC of linezolid was determined using the broth microdilution method in either the presence or absence of reserpine (20 mg/L) or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; 5 mg/L), according to Clinical and Laboratory Standards Institute guidelines. An inoculum of 0.5 Mcfarland Standard of each isolate was inoculated into Mueller—Hinton cation-adjusted broth containing serial dilutions of linezolid. Four-fold or more reduction of linezolid MICs in the presence or the absence of reserpine or CCCP was defined as a positive phenotype for efflux.[7]

PFGE

PFGE was performed on the CHEF—Mapper XA PFGE system (Bio-Rad, Hercules, CA, USA) with a running time of 20 hours and pulse duration of 3.5—23.5 seconds. *Salmonella serotype* Braenderup H9812 DNA marker was used for the standard molecular weight and size determinations. Pattern analysis was carried out using QualityOne software (Bio-Rad Laboratories). Percentage similarities were identified on a dendrogram derived from the unweighted pair group method with arithmetic averages. Band position tolerance and optimization were set at 1.5% and 0.5%, respectively. Isolates showing a similarity coefficient of $\geq$80% were considered to be genetically related.[8]

MLST

The linezolid nonsusceptible *E. faecalis* isolates were characterized by MLST using seven housekeeping genes (*gdh*, *gyd*, *pstS*, *gki*, *aroE*, *xpt*, and *yqiL*) according to the protocols and sequencing primers available at the *E. faecalis* database (http://efaecalis.mlst.net/).

Results

Clinical characteristics of patients with linezolid nonsusceptible *E. faecalis*

The clinical information of patients is given in Table 1. Two of the strains were isolated from urine, three from vaginal secretions, two from surgical wounds, and one from blood. No patients were treated with linezolid before linezolid nonsusceptible *E. faecalis* was isolated.