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The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China

Haiying Niu[a], *Hui Yu*[b], *Tangping Hu*[a], *Gailin Tian*[a], *Lixia Zhang*[a], *Xiang Guo*[a], *Hai Hu*[c,*], *Zhanli Wang*[a,*]

[a] *The First Affiliated Hospital, Baotou Medical College, Baotou, China*
[b] *The Second Affiliated Hospital, Baotou Medical College, Baotou, China*
[c] *Department of Pathophysiology, Baotou Medical College, Baotou, China*

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ABSTRACT

This study highlights the prevalence of aminoglycoside-modifying enzyme genes and virulence determinants among clinical enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. Screening for high-level aminoglycoside resistance against 117 enterococcal clinical isolates was performed using the agar-screening method. Out of the 117 enterococcal isolates, 46 were selected for further detection and determination of the distribution of aminoglycoside-modifying enzyme-encoding genes and virulence determinants using polymerase chain reaction -based methods. *Enterococcus faecium* and *Enterococcus faecalis* were identified as the species of greatest clinical importance. The *aac(6′)-Ie-aph(2″)-Ia* and *ant(6′)-Ia genes* were found to be the most common aminoglycoside-modifying enzyme genes among high-level gentamicin resistance and high-level streptomycin resistance isolates, respectively. Moreover, *gelE* was the most common virulence gene among high-level aminoglycoside resistance isolates. Compared to *Enterococcus faecium*, *Enterococcus faecalis* harbored multiple virulence determinants. The results further indicated no correlation between aminoglycoside-modifying enzyme gene profiles and the distribution of virulence genes among the enterococcal isolates with high-level gentamicin resistance or high-level streptomycin resistance evaluated in our study.

* *Corresponding authors.*
E-mails: btyxyhh@163.com (H. Hu), wang.zhanli@hotmail.com (Z. Wang).
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Introduction

Enterococci have emerged as an important source of hospital-acquired infections, including those related to the surgical site, respiratory tract, urinary tract, skin and soft tissue, and bacteremia.[1] Several studies have indicated increasing enterococci resistance to a broad range of antimicrobial agents via intrinsic and acquired mechanisms.[2] High-level aminoglycoside resistance (HLAR) has been recognized for several decades. Gentamicin and streptomycin are the two main aminoglycosides used in clinical practice. Recently, high-level gentamicin resistance (HLGR) (MIC ≥500 μg/ml) and high-level streptomycin resistance (HLSR) (MIC ≥2000 μg/ml) have been reported worldwide.[3–8] Clinical experience supports the use of aminoglycosides along with cell-wall inhibitors for treating serious enterococcal infections.[9] However, high-level resistance of clinical isolates of *Enterococcus* species to aminoglycosides negates the synergism between cell-wall inhibitors and aminoglycosides, making the treatment of serious enterococcal infections difficult.[10]

In general, enterococci are intrinsically resistant to clinically achievable concentrations of aminoglycosides. However, high-level resistance to aminoglycosides is primarily due to acquisition of genes encoding aminoglycoside-modifying enzymes (AMEs).[11] Over past decades, a great deal of research has been devoted to understanding the mechanisms behind the high-level resistance of enterococci to aminoglycosides. Until now, the following three classes of AMEs have been identified: acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and aminoglycoside nucleotidyltransferase (ANT).[12] The high-level resistance of enterococci to gentamicin is predominantly mediated by *aac(6′)-Ie-aph(2″)-Ia*, which encodes the bifunctional AME, AAC(6′)-APH(2″).[13] Ten years ago, *aph(2″)-Ib*, *aph(2″)-Ic*, and *aph(2″)-Id* were detected as newer AME genes conferring HLGR among enterococci.[14–16] Furthermore, HLSR among enterococci is mediated by *aph(3′)-IIIa* and *ant(6′)-Ia* encoding for APH(3′) and ANT(6′)-Ia, respectively.[3,11]

Additionally, the rise of drug-resistant virulent strains of enterococci is a serious problem in the treatment and control of enterococcal infections. The pathogenicity of enterococci is due to the presence of virulence determinants, such as the *Enterococcus faecalis* (*E. faecalis*) antigen A (*efaA*), adhesion of collagen from *E. faecalis* (*ace*) and products involved in aggregation (*agg*), biosynthesis of an extracellular metalloendopeptidase (*gelE*), biosynthesis of cytolysin (*cylA*) and immune evasion (*esp*).[17] Previous research has shown that clinical isolates of the *Enterococcus* species possess distinctive patterns of virulence factors.[18]

The difficulty in treating enterococcal infections is associated with determinants of virulence and antimicrobial resistance. Therefore, accurate identification of antibiotic susceptibility patterns and virulence determinants is essential for choosing appropriate therapies and means of infection control. The goal of the present study was to investigate the occurrence of HLAR among enterococci in Inner Mongolia, China. Additionally, the prevalence of six AME encoding genes, *aac(6′)-Ie-aph(2″)-Ia*, *aph(2″)-Ib*, *aph(2″)-Ic*, *aph(2″)-Id*, *aph(3′)-IIIa* and *ant(6′)-Ia*, among enterococcal isolates collected from different specimen sources were investigated. Moreover, the presence of virulence determinants, such as *efaA*, *ace*, *agg*, *gelE*, *cylA* and *esp*, was detected.

Experimental

Bacterial strains and identification

A total of 117 clinical isolates of enterococci were collected from four hospitals in Inner Mongolia, China, between May 2012 and May 2014. Duplicate isolates were excluded from the study. Institutional ethical clearance was obtained. The isolates were identified as enterococci by conventional biochemical tests and VITEK 2 Compact (BioMérieux, France). Identifications of *E. faecalis*, *E. faecium* and the other strains were further confirmed via PCR analysis using $ddl_{E.\ faecium}$, $ddl_{E.\ faecalis}$ and *16S rDNA* genes, respectively.[19] The isolates were then stored at −80 °C. Table 1 shows the primers and the product sizes of all the genes analyzed.[19–23]

Detection of HLAR in enterococci

Screening for HLAR was performed using the agar-screening method according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute (CLSI).[24] Briefly, brain heart infusion agar containing gentamicin (500 μg/ml) and streptomycin (2000 μg/ml) was used. Plates were incubated for 24 h at 37 °C. The aminoglycoside-susceptible strain *E. faecalis* ATCC 29212 and the aminoglycoside-resistant strain *E. fecalis* ATCC 51299 were utilized as controls for HLAR detection.

Amplification of AMEs and virulence genes

The total DNA template was extracted from enterococci according to the instruction manuals of commercial DNA extraction kits (Hangzhou BioSci Biotech Co., Ltd, China). A PCR method was used to detect the presence of AME and virulence genes.[20–23] The primer couples, product sizes of the genes and annealing temperatures are shown in Table 1. PCR amplification was performed using 1 μg of the template DNA, 1 μl of each primer (100 pmol), and 25 μl of 2× PCR master mix (Hangzhou BioSci Biotech Co., Ltd, China) in a total volume of 50 μl. A C1000 Touch thermocycler (Bio-Rad, USA) was also employed. PCR conditions were as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing for 30 s and elongation at 72 °C for 1 min, and final extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis on 1% agarose gel following staining with ethidium bromide.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 11.5). A p value <0.05 was considered statistically significant.