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Full Length Research Paper

Resistant phenotypes and genotypes of clinical isolates of multidrug-resistant *Enterococcus faecium* in a teaching hospital in Shantou, China

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This study is the first to report on the resistant phenotypes and genotypes of *Enterococcus faecium* clinical isolates in Shantou, China. A total of 39 *E. faecium* clinical isolates were collected from January 2004 to January 2006 and analyzed for their resistance to 10 antibiotics and for genes coding for resistance to the associated antibiotics. *E. faecium* isolates were resistant to 5 or more antibiotics, and most showed high minimal inhibitory concentrations to many antibiotics as well. Resistance to erythromycin, ciprofloxacin, levofloxacin, and penicillin was 100.0, 100.0, 97.4, and 92.3%, respectively. The mean resistance to ampicillin, chloramphenicol, tetracycline, and high levels of gentamicin was 80% or greater for each. Neither β-lactamase-producing nor vancomycin-resistant isolates were found. Genes such as *aph(3')-Ⅲ,ermB, aac(6')/aph2", ant(6)-Ⅰ,gyrA, TetM, ParC,* and *pbp5* coding for resistance to the associated antibiotics were present at 79.5, 71.8, 92.3, 71.8, 100.0, 38.5, 23.1 and 69.2% respectively. *E. faecium* isolates showing multidrug resistance (MDR) were prevalent in Shantou. A total of 32 strains carried at least 5 resistance genes. The gene profile of *E. faecium* isolates (*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/Pbp5*) indicated that most of the strains carried MDR in different regions. The high occurrence of MDR suggested maintenance of selective pressure by use of different antibiotics. A rapid increase in antibiotic resistance is the result of incorrect antibiotics.

Key words: *Enterococcus faecium,* multidrug-resistance, phenotype, genotype.

INTRODUCTION

The two enterococcal species most often isolated from clinical infections are *Enterococcus faecalis* and *E. faecium*, the former being responsible for most of the infection, and the latter showing predominant multidrug resistance (MDR)(Mundy et al., 2000). *E. faecium* isolates are known opportunistic nosocomial pathogens capable of causing life-threating infections such as endocarditis and bacteremia, largely in immune-compromised patients (Maki and Agger, 1988; Mundy et al., 2000), although it was normally considered an avirulent bacterium that infected only people with special predispositions (Murray and Weinstock, 1999).

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The infections caused by *E. faecium* have been increasing in frequency in recent years, probably owing to the broad spectrum of intrinsic and acquired antibiotic resistance, as well as presence of virulence genes (Rice et al., 2003). This scenario differs greatly for the anti-biotics used with respect to the time separating the initial drug marketing and emergence of resistance (Walsh, 2000). The China Bacterial Resistance Surveillance Study Group revealed that enterococci accounted for 24.3% of the bacterial infection isolates, with *E. faeciums* accounting for 9.18% of the isolates in China from 2006 to 2007(Xiao et al., 2008). Since the infection is resistant to multiple antibacterial drugs, only limited options for effective therapy and prophylasis exist for serious infections. However, improper therapeutic usage of anti-biotics still invariably led to the emergence of resistance

under selective pressure, and resistance to this bacterium was greatly increased.

E. faeciums has now emerged as an important pathogen in China (Deng et al., 2003; Huang et al., 2006; Mi, 2004; Zhu and Fei., 2004; Liu et al., 2002; Tong et al., 2006). Therefore, it becomes necessary to know the prevalence of antibiotic resistant *E. faeciums* and their current resistant phenotypes and genotypes for monitoring antimicrobial resistance and the selection of appropriate treatment. This study was designed to determine the resistant phenotypes and genotypes of clinical isolates of multidrug-resistant *E. faecium* from a teaching hospital in Shantou, China.

MATERIALS AND METHODS

Bacteria isolates

E. faecium strains in this study were clinical isolates collected from the First Affiliated Hospital of Shantou University Medical College (Shantou, China), a 1200-bed teaching hospital from January 2004 to January 2006. The strains were derived from a variety of sources, including wound secretion (9), sputum (7), blood (5), drained abdominal fluid (5), cervical secretion (3), urine (3), stools (3), prostatic fluid (2) and urethral secretion (2). All isolates were identified by use of the AMS Vitek-60 instrument (Automatic Microbiological Analysis Systems, Bio-Meriex, France).

Susceptibility tests

Isolates were cultured at 37℃ on blood agar plates. Minimal inhibitory concentrations (MICs) to 10 antibiotics, including penicillin (Pen), ciprofloxacin (Cip), levofloxacin (Lev), erythromycin (Ery), vancomycin (Van), ampicillin (Amp), chloramphenicol (Chl), tetracycline (Tet), high levels of gentamicin (HLG) and high levels of streptomycin (HLS), including susceptibility tests, were assessed by the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2006). *E. faecalis* ATCC29212 and *E. faecalis* ATCC51299 were used as control strains. Activity of β-lactamase was determined by the nitrocefin method, and *Staphylococcus aureus* ATCC29213 was used as a control strain[14].

PCR amplification of genes associated with antibiotic resistance

DNA was extracted as described by Huang et al. (2006). PCR detection of 13 genes coding for resistance, including *TEM* of β-lactamase, *aac(6')/aph(2")*, *aph (3')-Ⅲ, ant(2")-Ⅰ, ant(6)-Ⅰ* of aminoglycoside, *tetM* of Tets, *ermB* and *mefA* of macrolides, *gyrA* and *ParC* of fluoroquinolones, *pbp5* of penicillins, and *vanA* and *vanC* of glycopeptides was as described (Huang et al., 2006; Mi et al., 2004; Wisplinghoff et al., 2003). PCR products were analyzed by gel electrophoresis with 1% agarose gel.

RESULTS

Detection of β-lactamase activity

No β-lactamase-producing strain was found.

Susceptibility tests

Resistance of the antibiotics to *E. faecium* isolates is shown in Table 1.

PCR amplification of resistance genes

The genes *aph(3')-Ⅲ, ermB, aac(6')/aph2", ant(6)-Ⅰ, gyrA, TetM, ParC,* and *pbp5* in isolates that code for resistance to the associated antibiotics were present at 79.5, 71.8, 92.3, 71.8, 100.0%, 38.5, 23.1 and 69.2%, respectively. However, *ant(2 ")* was absent. Resistant phenotypes and genotypes of the 39 *E. faecium* isolates are in Table 2. PCR products of the genes positive in isolates are shown in Figure 1.

DISCUSSION

E. faecium has recently evolved from a generally avirulent commensal into an MDR healthcare-associated pathogen causing difficult-to-treat infections. Therefore, studies of *E. faecium* resistance have increased. Determination of β-lactamase activity has a significant role in guiding antibiotic usage. In this experiment, we found no *E. faecium* strain with β-lactamase activity and did not find *TEM* as the most common gene. This phenomenon had been reported in China previously (Zhu and Fei, 2004; Liu et al., 2002; Tong et al., 2006.) However, the level of *pbp5* in isolates was 69.2%, which indicated that Amp-resistant *E. faecium* infection is related not to β-lactamase activity but, rather, to PBPs. Amp resistance was strongly associated with *pbp5* (level 71.2%), which suggested that the *pbp5* gene plays an important role in the Amp-resistant *E. faecium*.

Most of the isolates (80%) were HLG-resistant phenotypes, which were strongly associated with the *aac(6 ')-aph(2 ")* bifunctional enzyme gene and *aph(3 ')-III*. HLG-resistant (MIC≥500 mg/l) strains rather than non-HLA-resistant strains showed higher resistant to Pen, Cip, Ery and Chl.

Resistance to Tet was 87.2%, which was higher than that to other antibiotics such as Ery, Cip, Lev and Pen. Otherwise, *tetM* seemed to play an important role in Tet resistance to *E. faecium* because it was detected in 38.5% of strains. That all Cip-resistant isolates carried *gyrA* but only 9 isolates carried *parC,* confirmed that *gyrA* was the main gene coding Cip resistance in *E. faecium*. Recent studies showed that Amp resistance was associated with a high level of Cip resistance; Cip resistance appeared to be associated with Amp resistance in genotypically related *E. faecium* isolates from Norway (Torell, et al., 2003) and Spain (Coque, et al., 2002). We found 32 Amp-resistant isolates (MIC≥128 mg/l) associated with Cip resistance (MIC≥64 μg/ml), which suggests that Cip had no therapeutic effect on *E. faecium* infection because Cip resistance was 100%.

Table 1. Antibiotic susceptibility of 39 *E. faecium* isolates.

Antibiotic class	Antibiotics	Equivalent MIC (μg/ml)			MIC range (μg/ml)	Resistance isolates (n)	Resistance rate (%)
		R	*I*	*S*			
Penicillins	Pen	≥16	-[a]	≤8	8 - 256	36	92.3
	Amp	≥16	-[a]	≤8	2->256	35	89.7
Fluoroquinolones	Cip	≥4	2	≤1	8->128	39	100.0
	Lev	≥8	4	≤2	1->128	38	97.4
Macrolides	Ery	≥8	1-4	≤0.5	>256	39	100.0
Tetracyclines	Tet	≥16	8	≤4	2 - 256	33	84.6
Glycopeptides	Van	≥32	8-16	≤4	≤0.125 - 2	0	0.00
	Chl	≥32	16	≤8	≤0.5 - 32	17	43.6
Aminoglycoside	HLG	>500				31	79.5
	HLS	>2000				8	20.5

R, resistant; I, intermediate; S, susceptible; Pen, penicillin; Amp, ampicillin; Cip, ciprofloxacin; Lev, levofloxacin; Ery, erythromycin; Van, vancomycin; Tet, tetracycline; Chl, chloramphenicol; HLG, high levels of gentamicin; HLS, high levels of streptomycin. a: The CLSI reference not available.

Table 2. Resistant phenotypes and genotypes of 39 *E. faecium* isolates.

Resistance phenotypes	Resistance profiles	Gene profiles	Isolates (n)
a	Pen/Amp/Cip/Lev/Ery/Tet/Chl/HLG/HLS	*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/Pbp5*	3
Ib		*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/ TetM/Pbp5*	1
Ic		*ermB/aac(6')/aph2"/gyrA/ TetM/ ParC*	1
Id		*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/ TetM/ParC*	1
IIa	Pen/Amp/Cip/Lev/Ery/Tet/Chl/HLG	*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/Pbp5*	8
IIb		*aac(6')/aph2/gyrA/TetM/Pbp5*	3
IIc		*gyrA/ ParC*	2
IId		*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/ TetM*	1
IIe		*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ /gyrA/ Pbp5*	1
IIf		*aac(6')/aph2/gyrA/TetM/Pbp5*	1
IIIa	Pen/Amp/Cip/Lev/Ery/Tet/Chl/HLS	*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/Pbp5*	1
IIIb		*aac(6')/aph2/gyrA/TetM/Pbp5*	1
IV	Pen/Amp/Cip/Lev/Ery/Chl/HLG/HLS	*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)-Ⅰ/gyrA/Pbp5*	1
V	Pen/Amp/Cip/Lev/Ery/Chl/HLG	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/Pbp5*	3
VIa	Pen/Amp/Cip/Lev/Ery/Tet/Chl	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/ TetM*	1
VIb		*aac(6')/aph2"/aph(3')-III/ gyrA/TetM/ ParC*	1
VII	Pen/Amp/Cip/Lev/Ery/Tet/HLG	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/Pbp5*	1
VII	Pen/Amp/Cip/Ery/Tet/Chl/HLG	*aac(6')/aph2"/aph(3')-III/ gyrA/TetM/ Pbp5*	1
IX	Pen/Amp/Cip/Lev/Ery/HLG/HLS	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)- I /gyrA/Pbp5*	1
X	Cip/Lev/Ery/Tet/Chl/HLG/HLS	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/ TetM/ParC*	1
XI	Pen/Cip/Lev/Ery/Tet/Chl	*aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/ TetM*	1
XII	Pen/Amp/Cip/Lev/Ery/Tet	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/ TetM/ParC*	1
X III	Pen/Amp/Cip/Lev/Ery/HLG	*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/Pbp5*	1
X IVa	Cip/Lev/Ery/Tet/Chl	*ermB/aac(6')/aph2"/ gyrA/ TetM/ ParC*	1
XIVb		*ermB/aac(6')/aph2"/aph(3')-III/ant(6)-I/gyrA/TetM/ ParC*	1

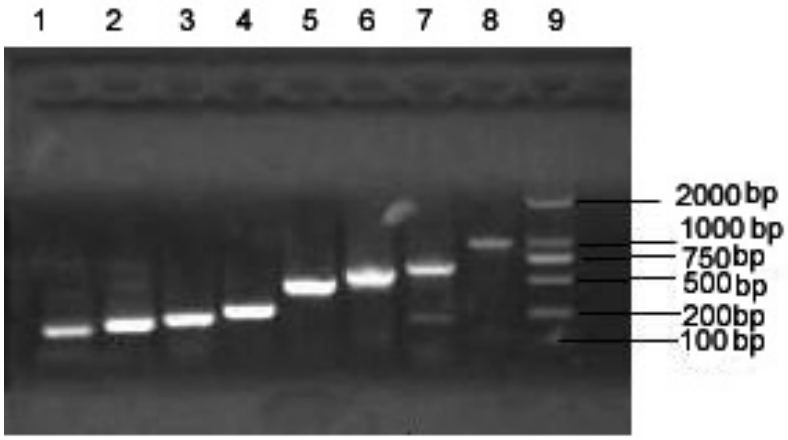

Figure 1. PCR amplification of genes associated with antibiotic resistance. Bands from 1 to 8 show the PCR products of ParC (191 bp), aac(6')/aph2″(220 bp), gyrA (241 bp), aph(3')-Ⅲ(292 bp), TetM (501 bp), ant(6)- Ⅰ (597 bp), ermB (616 bp), and pbp5 (962 bp); Lane 9: DNA marker.

Resistance to Chl was 43.6%, but its intermediate rate of 46.2% indicated that its sensitivity was reduced, so the antibiotic would not be the first choice in the treatment of *E. faecium* infection. Nevertheless, Chl could act as an alternative drug for infection with Vancomycin-resistant enterococci (VRE).

All Ery-resistant *E. faecium* carried *ermB*, considered the most prevalent macrolide-resistance gene in enterococci. Similar to a domestic report (Huang et al., 2006), *ermB* was detected in 75% but *mefA* in 0% of isolates while the *ermB* rate was significantly higher than that reported abroad (Udo et al., 2002).

Genotyping results showed that 32 strains contained at least 5 resistance genes. The gene profile (*ermB/aac(6')/aph2"/aph(3')-Ⅲ/ant(6)- Ⅰ/gyrA/Pbp5*) indicated that most of the isolates among the different regions carried mutiple resistance genes.

In China, VRE prevailed at 4.9% from 2006 to 2007 (Xiao, et al., 2008) without outbreak. *VanA, vanB* and *vanC* genes were detected in domestic clinical isolates from the VRE strains (Fortún et al., 2002). No VRE strain with *vanA* and *vanC* genes was found in our experiment indicating that vancomycin is still the drug of choice for *E. faecium* infection in Shantou.

In summary, the prevalence of MDR *E. faecium* isolates is high in Shantou. A rapid increase in antibiotic resistance and widespread MDR infection contributed to the incorrect utilization of antibiotics. Therefore, prudent antibiotic utilization practices must be incorporated into an institutional strategy to limit the burden of resistant pathogens. The high occurrence of MDR strains suggests the maintenance of selective pressure with the use of different antibiotics. The relatively high number of wide-spread strains might reflect particular environmental/host-adapted enterococcal strains that could contribute to the persistence of antibiotic resistance, for a reservoir of non-susceptible isolates that are insensitive to intervention.

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