Antimicrobial Resistance and Species Prevalence of Enterococcal Isolates in Srinagarind Hospital, Northeastern Thailand

การดื้อต่อยาต้านจุลชีพและความชุกของสปีชีส์ของเชื้อเอนเทอโรคอคคัส ที่แยกได้ที่โรงพยาบาลศรีนครินทร์ ภาคตะวันออกเฉียงเหนือ ประเทศไทย

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ABSTRACT

A total of 300 clinical isolates of enterococci, collected from patients admitted at Srinagarind hospital, were investigated for antimicrobial resistance and species distribution. Antimicrobial susceptibility to 10 antimicrobial agents was performed by disc diffusion method. Screening for vancomycin resistance was performed by the agar plate method and minimal inhibitory concentration of vancomycin was determined for vancomycin resistant strains by microbroth dilution method. *Enterococcus faecalis* was found to be predominant species (58.7%) followed by *E. faecium* (35.7%) and the rest species (5.7%) including *E. hirae*, *E. gallinarum*, *E. durans* and *E. dispar*. The isolates were resistant to penicillin (51.3%), ampicillin (43.3%), high level gentamicin (57.7%), azithromycin (100%), chloramphenicol (16.3%), doxycycline (48%), quinupristin/dalfopristin (53%) and linezolid (8%). None of the isolates were resistant to teicoplanin. Five (1.7%) strains were resistant to vancomycin. The minimal inhibitory concentrations of vancomycin for vancomycin-resistant enterococci strains were 16μg/ml. *E. faecium* was more resistant to penicillin, ampicillin and high level gentamicin than *E. faecalis*. Two hundred and seventy four (91.3%) strains showed multidrug resistance. Our study showed low prevalence of vancomycin resistance. However, there is a need to carry-out regular surveillance of antimicrobial resistance of enterococci to monitor changes in their patterns to prevent the spreading of resistant isolates.

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บทคัดย่อ

เชื้อแบคทีเรียเอนเทอโรคอคคัสซึ่งแยกได้จากผู้ป่วยโรงพยาบาลศรีนครินทร์ จำนวน 300 สายพันธุ์ ได้ถูกนำมาทำการศึกษาการดื้อต่อยาต้านจุลชีพ และการกระจายสปีชีส์ของเชื้อในสิ่งส่งตรวจ การทดสอบความไวต่อยาต้านจุลชีพ 10 ชนิด ทำโดยวิธี disc diffusion การตรวจคัดกรองเชื้อที่ดื้อต่อยา vancomycin ทำโดยใช้อาหารแข็ง และนำเชื้อที่ดื้อ vancomycin มาหาค่าความเข้มข้นต่ำที่สุดของยา vancomycin ที่สามารถยับยั้งการเจริญของเชื้อ โดยวิธี microbroth dilution ผลการศึกษาพบเชื้อ *Enterococcus faecalis* มากที่สุด (ร้อยละ 58.7) รองลงมาได้แก่ *E. faecium* (ร้อยละ 35.7) ส่วนที่เหลืออีก ร้อยละ 5.7 ได้แก่ *E. hirae*, *E. gallinarum*, *E. durans* และ *E. dispar* สำหรับการดื้อต่อยาต้านจุลชีพ พบว่าเชื้อที่แยกได้ดื้อต่อยาต้านจุลชีพต่าง ๆ ดังนี้ penicillin (ร้อยละ 51.3) ampicillin(ร้อยละ 43.3) gentamicinขนาดสูง (ร้อยละ 57.7) azithromycin (ร้อยละ 100) chloramphenical (ร้อยละ 16.3) doxycycline (ร้อยละ 48) quinupristin/dalfopristin (ร้อยละ 53) และ linezolid (ร้อยละ 8) ไม่พบการดื้อต่อยา teicoplanin เชื้อจำนวน 5 สายพันธุ์ (ร้อยละ 1.7) ดื้อต่อยา vancomycin โดยมีค่าความเข้มข้นต่ำที่สุดของยาที่สามารถยับยั้งการเจริญเติบโตของเชื้อเท่ากับ 16 ไมโครกรัมต่อมิลลิลิตร เชื้อ *E. faecium* มีอัตราการดื้อต่อยากลุ่ม penicillin ampicillin และ gentamicin ขนาดสูงมากกว่าเชื้อ *E. faecalis* นอกจากนี้ยังพบว่าเชื้อจำนวน 274 สายพันธุ์ (ร้อยละ 91.3) ดื้อต่อยาต้านจุลชีพหลายชนิด ถึงแม้การศึกษาครั้งนี้พบ ความชุกของเชื้อที่ดื้อต่อยา vancomycin ในระดับต่ำ แต่การสำรวจการดื้อต่อยาต้านจุลชีพควรทำอย่างสม่ำเสมอเพื่อติดตามการเปลี่ยนแปลงรูปแบบการดื้อต่อยาของเชื้อเอนเตอโรคอคคัสทั้งนี้เพื่อป้องกันการแพร่กระจายของสายพันธุ์ที่ดื้อยา

Key Words: Antimicrobial resistance, *Enterococcus* species
คำสำคัญ : การดื้อต่อยาต้านจุลชีพ เชื้อเอนเทอโรคอคคัส

Introduction

Enterococci are gram positive coccus and facultative anaerobes that remain as normal flora in human intestines (Murray, 1997). However, enterococci are one of the focussed bacteria today in causing nosocomial infections; urinary tract infection, bacteriamia, intraabdominal infection, wound infection, and endocarditis (Murray, 1997; Moellering, 1992). *E. faecalis* is the most predominant species implicated in human infections and followed by *E. faecium*. Nevertheless, the increasing frequency of latter is a matter of concern as it is highly resistant to most of the available antibiotics especially in clinical settings (Liassine et al., 1998; Nelson et al., 2000; Sahm et al., 1997).

The intrinsic and acquired antimicrobial resistant properties of enterococci, to several antibiotics, have enabled them to survive in clinical environment (Arthur and Courvalin, 1993). Enterococci acquire resistance to several available antimicrobial agents by either mutation or by receiving the foreign resistant determinants through plasmids and transposons (Murray, 1990). The combination of beta-lactams (penicillin or ampicillin) with an aminoglycoside (gentamicin or streptomycin) is used to obtain a synergistic bactericidal effect for treatment of enterococcal

infections. In the last two decades, enterococci have emerged with an increasing frequency of multidrug resistance including high-level resistance to aminoglycosides and ampicillin (Huycke et al., 1998; Grayson et al., 1991), thereby, limiting the treatment options for enterococcal infections. The glycopeptides (e.g. vancomycin) were the drug of choice for treating infections caused by multidrug-resistant enterococci. Vancomycin resistant enterococci (VRE) have increased commonly in Europe and North America (Jones et al., 1997; Perlada et al., 1997; Schouten et al., 2000). The problem is further concern by the fact that resistance genes can potentially be transfer to other pathogenic organisms such as *Staphylococcus aureus* (Weigel et al., 2007).

However, the occurrence of enterococcal infections and species prevalence in Thailand is not thoroughly investigated. Recently low prevalence of VRE has been reported at hospitals in central and southern Thailand (Nilgate et al., 2003; Chayakul et al., 2007).

The present study was undertaken to investigate species distribution and antimicrobial susceptibility of enterococci isolated from clinical specimens at Srinagarind hospital, a university hospital, in Northeastern Thailand.

Materials and Methods

1. Bacterial isolates

A total of three hundred enterococci were isolated from patients admitted at Srinagarind Hospital, Faculty of Medicine, Khon Kaen, Thailand. The bacterial strains were isolated from pus, body fluids, urine, and blood collected from June 2005 to July 2007. The bacterial strains were stored in BHI broth containing 20% glycerol at - 80° C until the time of analysis.

2. Identification of enterococcal species

The genus enterococcus was identified by the following physiological and biochemical characteristics; Gram positive cocci, catalase-negative, tolerance to bile esculin, hydrolysis of L-pyrrolidonyl-b-napthylamide (PYR test) and growth in 6.5% sodium chloride. The conventional method (Facklam and Collins, 1989) was followed for the species identification of the isolates based on the sugar fermentation (arabinose, raffinose, lactose, mannitol, sorbose, sucrose, sorbitol), pigmentation and motility. Arginine hydrolysis and pyruvate utilization were also included among the various reactions. Some of the strains were further identified by API 20 Strep kit and the soft ware supplied by the manufacturer (Biomerieux).

3. Antimicrobial susceptibility test

Disc diffusion method using Mueller-Hinton agar was used to detect antimicrobial susceptibility pattern of the isolates. Isolates were grown overnight on 5% sheep blood agar plate at 37° C. Inocula were prepared by suspending the organisms in sterile normal saline solution adjusted to a 0.5 McFarland standard (approximately 108 CFU/ml) and were directly inoculated onto Mueller Hinton agar plate. The following antibiotic discs were used; ampicillin (10μg), penicillin (10 units), azithromycin (15μg), doxycycline (30μg), chloramphenicol (30μg), vancomycin (5μg), teicoplanin (30μg), quinupristin/dalfopristin (15μg), linezolide (10μg) and gentamicin (120μg). High-level aminoglycoside resistance was

determined by disc diffusion method using 120 μg of gentamicin. The inhibition zone diameters were recorded and interpreted by using the criteria of British Society for Antimicrobial and Chemotherapy (BSAC) (Andrew, 2007) and Clinical and Laboratory Standards Institute (CLSI 2006).

In this study, multiple drug resistance was defined as resistance to three or more antibiotics (Hujer et al., 2005).

The control strains for sensitive and resistant to vancomycin were *E. faecalis* ATCC 29212 and *E. faecalis* KU 1857, respectively.

4. VRE detection

Vancomycin resistance was detected by a screening agar containing vancomycin 6μg/ml (Swenson et al., 1994). The inoculum, grown in BHI broth at 37 °C for 6 hours, was compared with 0.5 Mc Farland standard and 10 μl of the inoculum was used to spot on Brain heart infusion agar containing 6μg/ml vancomycin and incubated at 37 °C for 24 hours. Minimal inhibitory concentrations (MIC) of vancomycin were determined for VRE by microbroth dilution method using Mueller Hinton broth. The MIC breakpoint of ≥ 8μg/ml for vancomycin resistance was adopted according to BSAC (2007). *E. faecalis* KU 1857 (vanB) and *E. faecalis* ATCC 29212 were used as control strains.

5. Statistical Analysis

Chi-square was used to compare the differences in resistance to antibiotics among the enterococcal species. A P value of <0.05 was used to indicate significant differences.

Results

Species distribution

The distribution of isolates among all the clinical specimens is given in Table 1. Of all the 300 isolates: 180 (60%) strains were isolated from urine; 57 (19.3%) from pus; 45 (15%) from body fluids; and 17 (5.7%) from blood (Table 1). *E. faecalis* (58.7%) was the most common species isolated from the clinical samples followed by *E. faecium* (35.7%). The other enterococcus species (5.7%) comprise of *E. hirae* (3%), *E. gallinarum* (1.7%), *E. durans* (0.3%) and *E. dispar* (0.7%). The 17 isolates of the unusual species were from various clinical specimens including blood (5.9%), body fluid (11.1%), pus (5.1%) and urine (4.4%)

Antimicrobial resistance

Antimicrobial resistance patterns of the isolates are showed in Table 2. One hundred and fifty four (51.3%) and 130 (43.3%) of the isolates were resistant to penicillin and ampicillin, respectively. Resistance to penicillin and ampicillin among *E. faecium* isolates was significantly higher (P value <0.05) than *E. faecalis* isolates. The rates of resistance to penicillin and ampicillin were different in 24 enterococcal isolates including 19 *E. faecalis*, 3 *E. faecium* and 2 *E. dispar*. All such isolates were resistant to penicillin but susceptible to ampicillin.

High-level gentamicin resistance (HLGR) was detected in 173 (57.7%) of the isolates (Table 2). *E. faecium* showed higher resistance rate to high-level gentamicin (76.6%) than *E. faecalis* (50%). Other enterococci accounted only 17.6%.

The resistance patterns of HLGR strains are showed in Table 3. Out of 173 high-level gentamicin resistant strains, 72.3% and 61.8% were found to be resistant to penicillin and ampicillin respectively. *E. faecium* with HLGR showed higher resistance to penicillin and ampicillin than *E. faecalis*, while *E. faecalis* isolates were higher resistance to chloramphenicol, linezolid, doxycycline and quinupristin/dalfopristin than *E. faecium* (Table3). The high-level gentamicin resistant strains were mostly isolated from urine (69.9%). The data is not shown. All HLGR isolates were found to be multi-drug resistance. The most multiple-drug resistant profile was the resistance to penicillin, ampicillin, azithromycin and doxycycline (46.8%). The data is not shown.

All the isolates were resistant to azithromycin (100%) followed by quinupristin/dalfopristin (53%) and to doxycycline (48%). Only 16.3% of the isolates were resistant to chloramphenicol. No isolates were found resistant to teicoplanin.

Thirteen isolates were resistant to vancomycin according to criteria of BSAC for detection by disc diffusion method but only five vancomycin resistant strains grew in the screening media containing vancomycin 6μg/ml. Vancomycin MICs of all five strains were 16μg/ml. The rest eight strains, detected by disc diffusion method, exhibited MICs lower than 4μg/ml (Table 4). Therefore, only 5 isolates were considered to be VRE. All VRE strains were *E. gallinarum*. Four VRE strains were isolated from body fluids and one from blood (Table 4). All the VRE isolates were susceptible to teicoplanin, chloramphenicol, penicillin, ampicillin and high-level gentamicin. They were resistant to azithromycin. Four, out of five vancomycin resistant isolates, were intermediately resistant to combination of quinupristin and dalfopristin, and one was fully resistant to this drug. Similarly, two out of five VRE were intermediately resistant to doxycycline and rest was sensitive.

The isolates were also tested for their susceptibility to linezolid, a relatively new drug that has been reported to have activity against gram-positive cocci, including methicillin-resistant *S. aureus* and VRE. Eight percent of the isolates were detected resistant to linezolid.

Two hundred and seventy four (91.3%) strains showed multidrug resistance to at least three classes of antibiotics.

Table 1 Distribution and species identities of Enterococci from clinical specimens

Specimen	Number (%) of isolates						
	E. faecalis	*E. faecium*	*E. hirae*	*E. gallinarum*	*E. durans*	*E. dispar*	Total
Blood	11 (64.7)	5 (29.4)	0	1(5.9)	0	0	17 (5.7)
Body fluids	28 (62.2)	12 (26.7)	1 (2.2)	4 (8.9)	0	0	45 (15)
Pus	47 (81)	8 (13.8)	2 (3.4)	0	1 (1.7)	0	58 (19.3)
Urine	90 (50)	82 (45.6)	6 (3.3)	0	0	2 (1.1)	180 (60)
Total	176 (58.7)	107 (35.7)	9 (3)	5 (1.7)	1 (0.3)	2 (0.7)	300 (100)

Table 2 Antimicrobial resistance patterms of Enterococcus species tested by Disc diffusion method

Antimicrobial agents	No (%) of resistant strains			
	E. faecalis (n=176)	*E. faecium* (n=107)	*Other enterococci** (n=17)	Total (N=300)
Penicillin	47 (26.7)	104 (97.2)	3 (17.6)	154 (51.3)
Ampicillin	28 (15.9)	101 (94.4)	1 (5.9)	130 (43.3)
Gentamicin(HLR)	88 (50)	82 (76.6)	3 (17.6)	173 (57.7)
Chloramphenicol	43 (24.4)	5 (4.7)	1 (5.9)	49 (16.3)
Azithromycin	176 (100)	107 (100)	17 (100)	300 (100)
Doxycycline	92 (52.3)	44 (41.1)	8 (47.1)	144 (48)
Vancomycin	7 (4)	1 (0.9)	5(4.7)	13 (4.3)
Teicoplanin	0	0	0	0
Quinupristin/Dalfopristin	143 (81.3)	13 (12.1)	3 (17.6)	159 (53)
Linezolid	18 (10.2)	4 (3.7)	2 (1.9)	24 (8)

HLR: High-level resistance

*Include *E. hirae* (9), *E. gallinarum* (5), *E, durans* (1) and *E. dispar* (2)

Table 3 Resistance profile of high-level gentamicin resistant (HLGR) *Enterococcus*

Antimicrobial agents	No (%) of HLGR Strains			
	E. faecalis (n=95)	*E. faecium* (n=73)	Other enterococci* (n=5)	Total (N=173)
Penicillin	49 (51.5)	73 (100.0)	3 (60.0)	125 (72.3)
Ampicillin	35 (36.8)	71 (97.2)	1 (20.0)	107 (61.8)
Chloramphenicol	27 (28.4)	3 (4.1)	0	30 (17.3)
Azithromycin	95 (100.0)	73 (100.0)	5 (100.0)	173 (100.0)
Vancomycin	4 (4.2)	0	0	4 (2.3)
Teicoplanin	0	0	0	0
Linezolid	9 (9.4)	2 (1.3)	0	11 (6.3)
Doxycycline	57 (60.0)	29 (39.7)	2 (40.0)	88 (50.8)
Quinupristin/Dalfopristin	59 (62.1)	6 (8.2)	3 (60.0)	68 (39.3)

*Include *E. hirae (3) and E. dispar (2)*

Table 4 VRE resistant profile, species distribution and vancomycin MICs

Species	Resistance	Vancomycin MIC (㎍/ml)	Sources
E. gallinarum	Azi, Dox*, Q/dal*	16	Body fluid
E. gallinarum	Azi, Q/dal*	16	Body fluid
E. gallinarum	Azi, Q/dal*	16	Blood
E. gallinarum	Azi, Q/dal*	16	Body fluid
E. gallinarum	Azi, Dox*, Q/dal	16	Body fluid

*Intermediate resistance

Azi, azithromycin; Dox, doxycycline; Q/dal; quinupristin/dalfopristin

Discussion

Enterococci are the gram positive cocci that have been considered normal flora in human gastrointestinal tracts. However, several recent studies have reported increasing enterococcal associated nosocomial infections (Moellering, 1992; Jones et al., 1997; Nelson et al., 2000). Therefore, it is important for a hospital setting to monitor such infections and to determine their species and antimicrobial resistance patterns. This study investigated the species prevalence and antibacterial-resistance patterns of clinical enterococcal isolates in a university hospital.

The most frequent source of enterococcal isolations in this study was urine (60%), which is consistent with the previous reports (Huycke et al., 1998; Kacmaz and Aksoy, 2005). The majority of the clinical isolates were *E. faecalis* (58.7%), followed by *E. faecium* (35.7%), while other *Enterococcus* spp. accounted for 5.7% of isolates, comparable to the distribution of species in previous studies (Perlada et al., 1997; Udo et al., 2003; Kacmaz and Aksoy, 2005; Aleyasin et al., 2007). However, unlike those studies, our proportion of *E. faecium* strains was higher than those reported previously. The prevalence of *E. faecium* in our study (35.7%) was comparable to the prevalence of *E. faecium* in Siriraj Hospital (38.8%) (Srifuengfung et al., 2004) but higher than the prevalence of *E. faecium* reported in Songklanakarind Hospital (12.2%) (Chayakul et al., 2007). This finding is of clinical importance since *E. faecium* is often more resistant than *E. faecalis*, thus limiting the therapeutic options. Previous studies have indicated an increasing incidence of enterococcal infections in tertiary care teaching hospitals, often accompanied by a high proportion of *E. faecium* isolates. (Huycke et al., 1998; Nelson et al., 2000; Vindigni et al., 2007)

Penicillin or ampicillin along with gentamicin is the drug of choice for treatment of enterococcal infections. Therefore, resistance of Enterococci against these antibiotics has important clinical implications. The decreased affinity of penicillin-binding proteins and some strains with plasmid-mediated β-lactamases have been responsible for the resistance to penicillin or ampicillin (Kak and Chow, 2002). Overall, in the present study, *Enterococcus* species were resistant to penicillin and ampicillin, 51.3% and 43.3%,

respectively. Twenty four isolates (8%) were resistant to penicillin but susceptible to ampicillin similar to the data obtained in the earlier study (Miskeen and Deodhar, 2002). In this study, *E. faecium* had higher ($p<0.05$) frequency of resistance to penicillin (97.9%) and ampicillin (94.7%) than *E. faecalis* (30.4%, 21.2%, respectively). Our results are consistent with other studies (Busani et al., 2004; Srifuengfung et al., 2004; Kacmaz and Aksoy, 2005; Vindigni et al., 2007).

High-level-gentamicin resistant enterococci were first reported in 1979 (Herman and Gerding, 1991). Ribosomal resistance and aminoglycoside modifying enzymes are the two mechanisms responsible for the aminoglycoside resistance by enterococci (Eliopoulos et al., 1993). Efflux pumps have been held responsible for resistance against some drugs like chloramphenicol, tetracycline and norfloxacin (Lynch et al., 1997). Subsequently, multidrug resistance efflux pumps have been suggested to be responsible for multidrug resistance in *E. faecalis* (Jonas et al., 2001). In addition, impairment in gentamicin uptake has been experimentally identified as one of the mechanisms of gentamicin resistance in *Enterococci* (Aslangul et al., 2006)

In our study, *E. faecalis* accounted 50% whereas *E. faecium* revealed 76.6% resistance to high-level gentamicin. This was lower than in India, where 100% and 85.7 % for *E. faecalis* and *E. faecium* respectively were reported (Karmarker et al., 2004). Our result is also contrary to the one reported in Turkey (Kacmaz, Aksoy, 2005), where *E. faecalis* accounted 16% resistance and *E. faecium* accounted 88%. Moreover, 61.8% of the total high-level gentamicin resistant strains showed resistance to both penicillin and ampicillin signifying poor efficacy of combination therapy at least in this hospital, and this is a serious concern in the treatment of enterococcal infections.

All the HLGR strains were found to be multi-drug resistant. The most multiple-drug resistant profile was the resistance to penicillin, ampicillin, azithromycin and doxycycline. Multidrug resistance efflux pumps may have a role in multidrug resistance, particularly in high-level gentamicin resistant enterococci, in our study.

Chloramphenicol resistance was found only 16.3%, suggesting its possible role in VRE infection (Lautenbach et al, 1998).

Among all the drugs tested in this study, teicoplanin and linezolid demonstrated good anti-enterococcal activities.

All the strains in this study were resistant to azithromycin. Busani and his colleagues also reported 100% resistance by *E. faecium* and 79% by *E. faecalis* to macrolide in their study in Italy (Busani et al., 2004). Ribosomal modification is the most common resistance mechanism (Jensen et al., 1999) to macrolide. Multidrug resistance efflux pumps, ABC16 (Jonas et al., 2001) have been reported as another resistance mechanism. In this study, the later mechanism seems to be the most plausible way of resistance since all the strains exhibited resistance to other drugs along with azithromycin.

Glycopeptides, especially vancomycin and teicoplanin, are the last resort drugs for the multiple-resistant enterococcal infections. Although resistance against these drugs have been reported in many parts of the world (Jones et al., 1997; Perlada et al., 1997; Schouten et al., 2000), it is still not frequently isolated in Thailand (Nilgate et al., 2003; Chayakul et al., 2007).

In our study, only 1.7% strains were resistant to vancomycin and this was similar to previous studies (Kacmaz and Aksoy, 2005; Udo et al., 2003; Karmarkar et al., 2004) but, contrary to the situation in most hospitals in the USA (Jones et al., 1997; Perlada et al., 1997) and Europe (Nelson et al., 2000; Schouten et al., 2000) where high prevalence of vancomycin resistance is common. Majority of VRE strains were isolated from body fluids and only one from blood. All the VRE strains in this study showed low level vancomycin resistance and were not resistant to teicoplanin. They were susceptible to chloramphenicol, teicoplanin, high-level gentamicin, penicillin and ampicillin. This implies that the combination therapy of high-level gentamicin and beta-lactam agents remains effective against these VRE infections. The low prevalence of VRE in our study indicates that vancomycin retains its therapeutic efficacy against the majority of enterococci in this hospital.

Quinupristin-dalfopristin is almost ineffective to *E. faecalis* (Gilmore, 2002; Maschieto et al., 2004). In our study, 81.3% of *E. faecalis* and 12.1% of *E faecium* showed resistance to this agent. This result was in concordance with the one reported from Korea (Oh et al., 2005). Our result indicates that these drugs do not assure their efficacy against enterococcal infections in this hospital, where most of enterococcal isolates were *E. faecalis*.

Linezolid, being the newer drug, our study showed 8% of strains resistant to this drug. *E. faecalis* (10.2%) was found to be more resistant than *E. faecium* (3.7%). This result was contrary to the one reported in Iran, (Aleyasin et al., 2007) where all the isolates tested were susceptible to linezolid.

Since most of the strains revealed multidrug resistance, intensive selective pressure from antimicrobial usage in the hospital has been proposed to be the major causative mechanism for developing resistance. Interestingly, all these strains were susceptible to vancomycin. *E. faecium* was more resistant to commonly used antibiotics than other enterococcal species.

Overall, this study revealed *E. faecalis* as the most common species among the clinical isolates of enterococci in Srinagarind hospital. *E. faecium* was isolated considerably in higher percentage than other studies. Teicoplanin was found to be the most effective amongst the drugs tested for the enterococcal infections in this study followed by vancomycin, linezolid and chloramphenicol. This study also showed low prevalence of vancomycin-resistant enterococci but high prevalence of multidrug resistant strains and high-level gentamicin resistant strains. Therefore, there is a need for development of effective strategies to address the problem of multidrug resistance. However, determination of MIC of every drug for each strain is needed to reconfirm and compare the susceptibility patterns. Speciation of *Enterococcus* needs to be carried out in clinical diagnostic microbiology laboratory of this hospital in order to determine species-specific drug resistance profile. Eventually, the information derived from this analysis would be helpful in formulation local guideline for early treatment of enterococcal infections. It is also equally essential to conduct periodical surveillance of enterococci to monitor changes in the antimicrobial resistance patterns and to prevent spread of resistant isolates.

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References

Aleyasin, A., Mobarez, AM., Sadeghizadeh, M., Doust, RH., Khoramabadi, N. 2007. Resistance to vancomycin in Enterococcus *faecium* and *faecalis* clinical isolates. Pak J Med Sci. June; 23(3):390-393.

Andrews, JM. 2007. BSAC standardized disc susceptibility testing method (version 6). J Antimicrob Chemother. Jul; 60(1):20-41.

Arthur, M., Courvalin, P. 1993. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob Agents Chemother. Aug; 37(8):1563-1571.

Aslangul, E., Massias, L., Meulemans, A., Chau, F., Andremont, A., Courvalin, P, et al. 2006. Acquired gentamicin resistance by permeability impairment in Enterococcus *faecalis*. Antimicrobial Agents and Chemotherapy. Nov; 50(11):3615-3621.

Busani, L., Del GM., Paladini, C., Graziani, C., Pantosti, A., Biavasco, F., et al. 2004. Antimicrobial susceptibility of vancomy cin-susceptible and -resistant enterococci isolated in Italy from raw meat products, farm animals, and human infections. Int J Food Microbiol. Dec 1; 97(1):17-22.

Chayakul, P., Hortiwakul, R., Ingviya, N.,Chayakul, V. 2007. Species distribution and antimicrobial susceptibility of Enterococci in hospitalized patients in Southern Thailand J Infect Dis Antimicrob Agents. ; 24:49 - 54.

Clinical and Laboratory Standard Institute, 2006. Performance standard for antimicrobial disk susceptibility testing. Sixteenth Informational SupplementM2-A9 and M7-A7 2006 Jan; 26(1).

Eliopoulos, GM., Klimm, K., Eliopoulos, CT., Ferraro, MJ., Moellering, RJ. 1993. In vitro activity of CP-99,219, a new fluoroquinolone, against clinical isolates of gram-positive bacteria. Antimicrob Agents Chemother. Feb; 37(2):366-370.

Facklam, RR., Collins, MD. 1989. Identification of Enterococcus species isolated from human infections by a conventional test scheme. J Clin Microbiol. Apr; 27 (4) : 731-734.

Gilmore, MS., 2002. The Enterococci: Pathogenesis, Molecular biology and Antibiotic resistance. Washington DC: ASM Press;

Grayson, ML., Eliopoulos, GM., Wennersten, CB., Ruoff, KL., De Girolami, PC., Ferraro, MJ., et al.1991. Increasing resistance to beta-lactam antibiotics among clinical isolates of Enterococcus faecium: a 22-year review at one institution. Antimicrob Agents Chemother. Nov ; 35 (11):2180-2184.

Herman, DJ., Gerding, DN. 1991. Antimicrobial resistance among enterococci. Antimicrob Agents Chemother. January;35(1):1-4.

Hujer, KM., Hujer, AM., Hulten, EA., Bajaksouzian, S., Adams, JM., Donskey, CJ., et al. 2005. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrobial Agents and Chemotherapy. Decr; 50 (12): 4114-4123.

Huycke, MM., Sahm, DF., Gilmore, MS. 1998. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. Emerg Infect Dis. Apr-Jun; 4(2):239-249.

Jensen , LB., Frimodt-Moller, N., Aarestrup, FM. 1999. Presence of *erm* gene classes in gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol Lett. Jan 1; 170(1):151-158.

Jones, RN., Marshall, SA., Pfaller, MA., Wilke,WW., Hollis, RJ., Erwin, ME., et al.1997. Nosocomial enterococcal blood stream infections in the SCOPE Program: antimicrobial resistance, species occurrence, molecular testing results, and laboratory testing accuracy. SCOPE hospital study group. Diagn Microbiol Infect Dis. Oct; 29(2):95-102.

Kacmaz, B., Aksoy, A. 2005. Antimicrobial resistance of enterococci in Turkey. Int J Antimicrob Agents. Jun; 25(6):535-538.

Kak, V., Chow, JW. 2002. Acquired antibiotic resistances in *Enterococci*. In: Gilmore MS, editor. The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic resistance. Washington, DC: ASM Press;

Karmarkar, MG., Gershom, ES., Mehta, PR. 2004. Enterococcal infections with special reference to phenotypic characterization & drug resistance. Indian J Med Res. May; 119 Suppl:22-25.

Kropec, A., Frank, U., Jonas, D., Thriene, W., Schmidt-Eisenlohr, E., Daschner, FD. 2001. In vitro susceptibility to everninomycin of gram-positive nosocomial pathogens isolated from intensive care units in Germany. Chemotherapy. Jan-Feb; 47(1):15-18.

Liassine, N., Frei, R., Jan, I., Auckenthaler, R. 1998. Characterization of glycopeptide-resistant enterococci from a Swiss hospital. J Clin Microbiol. Jul;36(7):1853-1858.

Lynch, C., Courvalin, P., Nikaido, H. 1997. Active Efflux of antimicrobial agents in wild-type strains of Enterococci. Antimicrob Agents Chemother. April; 41(4):869-871.

Maschieto, A., Martinez, R., Palazzo, IC., Darini, AL. 2004. Antimicrobial resistance of *Enterococcus* sp. isolated from the intestinal tract of patients from a university hospital in Brazil. Mem Inst Oswaldo Cruz. Nov; 99(7):763-767.

Miskeen, PA., Deodhar, L. 2002. Antimicrobial susceptibility pattern of *Enterococcus* species from urinary tract infections. J Assoc Physicians India. Mar; 50:378-381.

Moellering, RJ. 1992. Emergence of *Enterococcus* as a significant pathogen. Clin Infect Dis. June; 14(6):1173-1176.

Murray, BE. 1990. The life and times of the *Enterococcus*. Clin Microbiol Rev. Jan; 3(1):46-65.

Murray, BE. 1997. Vancomycin-resistant enterococci. Am J Med. Mar; 102 (3): 284-293.

Nelson, RR., McGregor, KF., Brown, AR., Amyes, SG., Young, H. 2000. Isolation and characterization of glycopeptide-resistant enterococci from hospitalized patients over a 30-month period. J Clin Microbiol. Jun; 38(6):2112-2116.

Nilgate, S., Nunthapisud, P., Chongthaleong, A. 2003. Vancomycin-resistant enterococci in King Chulalongkorn Memorial Hospital: a 5-year study. J Med Assoc Thai. Jun; 86 Suppl 2:S224-229.

Oh, WS., Ko, KS., Song, J-H., Lee, MY., Park, S., Peck, KR., et al. 2005. High Rate of Resistance to Quinupristin-Dalfopristin in Enterococcus faecium Clinical Isolates from Korea. Antimicrob Agents Chemother. Dec; 49(12):5176-5178.

Perlada, DE., Smulian, AG., Cushion, MT. 1997. Molecular epidemiology and antibiotic susceptibility of enterococci in Cincinnati, Ohio: a prospective citywide survey. J Clin Microbiol. Sep;35(9):2342-2347.

Sahm, DF., Free, L., Smith, C., Eveland, M., Mundy, LM. 1997. Rapid characterization schemes for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol. Aug; 35(8):2026-2030.

Schouten, MA., Hoogkamp-Korstanje, JA., Meis, JF., Voss, A. 2000. Prevalence of vancomycin-resistant enterococci in Europe. Eur J Clin Microbiol Infect Dis. Nov; 19(11):816-822.

Srifuengfung, S., Techachaiwiwat, W., Sanpanya, S., Dhiraputra, C. 2004. The antibiotic susceptibility of *Enterococcus* species in Siriraj Hospital during 1994 and 2001. J Infect Dis Antimicrob Agents. May-August;2:47-52.

Swenson, JM., Clark, NC., Ferraro, MJ., Sahm, DF., Doern, G., Pfaller, MA., et al. 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J Clin Microbiol. Jul; 32(7):1700-1704.

Udo, EE., Al-Sweih, N., Phillips, OA., Chugh, TD. 2003. Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. J Med Microbiol. Feb; 52(Pt 2):163-168.

Vindigni, SM., Srijan, A., Wongstitwilairoong, B., Marcus, R., Meek, J., Riley, PL., et al. 2007. Prevalence of foodborne microorganisms in retail foods in Thailand. Foodborne Pathog Dis. Summer; 4(2): 208-215.

Weigel, LM., Donlan, RM., Shin, DH., Jensen, B., Clark, NC., McDougal, LK., et al. 2007. High-level vancomycin-resistant *Staphylococcus* aureus isolates associated with a polymicrobial biofilm. Antimicrob Agents Chemother. Jan; 51(1):231-238.