

Species Distribution and Antimicrobial Susceptibility of Enterococci in Hospitalized Patients in Southern Thailand

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ABSTRACT

This study was carried out to determine the species distribution and antimicrobial susceptibilities of enterococci isolated from clinical specimens in a tertiary care hospital in southern Thailand. Throughout 2003, ninety-eight consecutive clinical specimens of enterococci were identified. *Enterococcus* species were determined using standard biochemical tests. Susceptibility testing of the identified enterococci to vancomycin, fosfomycin, imipenem, and nitrofurantoin was performed using the Etest, and to penicillin using the disc diffusion method. Only *E. faecalis* (87.8%), and *E. faecium* (12.2%) were identified. The percentage of enterococcal isolates susceptible to fosfomycin, nitrofurantoin, penicillin, and vancomycin was 94.8 percent, 79.6 percent, 100 percent, 70.4 percent, and 98.8 percent, respectively. Only one isolate of vancomycin-resistant *E. faecalis* was found which was solely resistant to vancomycin. The results showed that fosfomycin and nitrofurantoin could be used instead of vancomycin in the treatment of enterococcal infections in certain clinical situations, e.g. urinary tract infections. Using fosfomycin and nitrofurantoin more in these situations could reduce the development of enterococci resistant to vancomycin and newer drugs. (*J Infect Dis Antimicrob Agents* 2007;24:49-54.)

INTRODUCTION

The treatment of enterococcal infection is complicated as a result of the limited number of effective antibiotics and the increasing antibiotic resistance of

enterococci.^{1,2} There has also been an increase in the incidence of nosocomial enterococcal infections.^{3,4} The main antibiotics used in the treatment for enterococcal infections are either penicillin or ampicillin, with the

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addition of aminoglycoside when bactericidal activity is needed. Once enterococci have developed resistance to penicillin, vancomycin is the usual drug of choice. However, vancomycin-resistant enterococci (VRE) have become increasingly prevalent in western countries.^{3,4}

The objectives of this study were to prospectively determine the species distribution and antimicrobial susceptibilities of enterococci isolated from clinical specimens at Songklanagarind Hospital, Songkhla, southern Thailand. Vancomycin was chosen in order to evaluate the prevalence of VRE infection. There was also a need to determine the appropriateness of certain antibiotics against enterococci. Fosfomycin is a drug that is active against the resistant Gram-positive cocci, i.e. methicillin-resistant *Staphylococcus aureus* (MRSA)⁵ and penicillin-resistant *Streptococcus pneumoniae* (PRSP)⁶, and has been proven to have an in vitro activity against enterococci including VRE in western countries.⁷⁻¹⁰ Nitrofurantoin, another broad-spectrum antimicrobial drug used exclusively in urinary tract infection¹¹, has also been proven to be active against VRE.¹² Imipenem was chosen because it is frequently used as an empirical therapy in nosocomial

infections and is often aimed at covering enterococci as well.

The second part of the study was to retrospectively analyze the trends in susceptibility of enterococcal isolates using routine disc diffusion test at Songklanagarind Hospital between 2001 and 2006.

MATERIALS AND METHODS

Consecutive enterococcal isolates were obtained from patients hospitalized at Songklanagarind Hospital in 2003. Enterococci were identified using standard microbiological methods, and species were all identified by conventional biochemical methods.¹³

The isolates were tested for susceptibility to fosfomycin, imipenem, nitrofurantoin, and vancomycin using the Etest[®] (Biodisk, Solna, Sweden). *S. aureus* strain ATCC 29213 and *Enterococcus faecalis* ATCC strain 29212 were used as the quality-control organisms. The breakpoint criteria to determine susceptibility were based on the National Committee for Clinical Laboratory Standards (NCCLS) 2003 guidelines.¹⁴ Specific minimal inhibitory concentration (MIC) breakpoints (in mg/L) of each drug are shown in Table 1.

Table 1. In vitro susceptibility of vancomycin, fosfomycin, imipenem, and nitrofurantoin against *Enterococcus* species.

Antimicrobial agents	MIC (mg/L)			Percentage of susceptibility	MIC breakpoints*
	Range	MIC ₅₀	MIC ₉₀		
Nitrofurantoin	0.38 - 6	1.5	3	98.9	< 4
Vancomycin	8 - 96	32	64	94.8	< 64
Fosfomycin	0.125 - 32	1	32	79.6	< 4
Imipenem	0.25 - 24	3	8	100	< 32

MIC: minimal inhibitory concentration

*Reference number¹⁴

RESULTS

Enterococcal isolates were obtained from 98 different infected patients. The distribution and species identities of the enterococci from the clinical specimens are presented in Table 2. The only enterococcal species identified were *E. faecalis* and *E. faecium*. The disc diffusion method showed that 68 isolates (70.4%) were susceptible to penicillin. The overall percentages of antibiotic susceptibility for isolates of *Enterococcus* spp. using the Etest are shown in Table 1. The MIC values showed that the most active drugs were nitrofurantoin and vancomycin, followed by fosfomycin and imipenem with 0 percent, 1.1 percent, 5.2 percent, and 20.4 percent resistance, respectively. The sole vancomycin-resistant isolate was an *E. faecalis* with the MICs to vancomycin, fosfomycin, imipenem, and nitrofurantoin of 6, 32, 0.75 and 1.5 mg/L, respectively. Table 3 shows a comparison of the antibiotic susceptibility of the two enterococcal species isolated.

Imipenem was shown to be much less active against *E. faecium* than against *E. faecalis*.

The susceptibility of enterococcal isolates to the tested antibiotics by routine disc diffusion tests at Songklanagarind Hospital between 2001 and 2006 is shown in Table 4. Nitrofurantoin was tested for susceptibility because it was not included in the hospital formulary. Vancomycin susceptibility has remained at 100 percent and fosfomycin has also remained very active, while enterococci have been becoming increasingly resistant to ampicillin, penicillin, and imipenem.

DISCUSSION

It is well known that *E. faecium* is more resistant to various antibiotics including vancomycin than *E. faecalis*.^{1,2,4} This study showed that the most common species of enterococci in our hospital was *E. faecalis* (87.8%), with the remaining 12.2 percent *E. faecium*

Table 2. Distribution and species identities of enterococci from clinical specimens.

Specimen type	Number of isolates						Total number of isolates
	Urine	Body fluid	Tissue	Pus	Sputum	Blood	
<i>E. faecalis</i> (87.8%)	36	21	11	9	5	4	86
<i>E. faecium</i> (12.2%)	4	3	2	1	1	1	12
Total	40	24	13	10	6	5	98

Table 3. Antibiotic susceptibility of each *Enterococcus* species using the Etest except penicillin susceptibility which is determined by the disk diffusion test.

Species (number)	Antibiotic susceptibility (%)				
	Fosfomycin	Imipenem	Nitrofurantoin	Vancomycin	Penicillin
<i>E. faecalis</i> (86)	94.1	84.7	100	98.8	77.9
<i>E. faecium</i> (12)	100	50.0	100	100	16.7

Table 4. The percentage of susceptibility of *Enterococcus* species to five antibiotics by the disc diffusion test at Songklanagarind Hospital from 2001 to 2006.

Antibiotics	Year					
	2001 (N=789)	2002 (N=754)	2003 (N=650)	2004 (N=956)	2005 (N=1,172)	2006 (N=1,013)
Ampicillin	80	76	77	77	74	74
Fosfomycin	90	82	94	93	91	94
Imipenem	81	76	77	77	76	75
Penicillin	77	70	71	65	60	63
Vancomycin	100	100	100	100	100	100

(in a proportion of 7:1). This is consistent with the traditional ratio of *E. faecalis* to *E. faecium* of 10:1 in clinical specimens.^{1,2} Published data from Kuwait has revealed a prevalence of 85.3 percent of *E. faecalis* and 7.7 percent of *E. faecium*.¹⁵ Recent longitudinal studies have indicated an increasing incidence of enterococcal infections in tertiary care teaching hospitals, often accompanied by a higher proportion of *E. faecium* isolates especially those isolated from the blood (29% in Cincinnati, Ohio, USA¹⁶, 42.9% in North India¹⁷, and 38.8% in Siriraj Hospital¹⁸). A change in the ratio of *E. faecalis* to *E. faecium* from 3.7:1 in 1996 to 1.9:1 in 1999 in blood cultures has been reported¹⁹, and also a greater proportion of *E. faecium* in blood cultures. In addition, *E. faecalis* has been found in cultures of samples from other sites.^{20,21} Changes in the hospital patient population and in antimicrobial use patterns coupled with the more antibiotic-resistant nature of *E. faecium* probably confer a greater selective survival advantage, compared to *E. faecalis* and could explain the emergence of *E. faecium* bloodstream infections.

In this study, 98.9 percent of isolates were susceptible to vancomycin. This finding was compatible with the routine susceptibility data of our hospital

showing that vancomycin susceptibility had been 100 percent in the past six years. The very low prevalence of vancomycin resistance among enterococci (1% in 2001) has also been found at Siriraj Hospital, Bangkok.¹⁸ Such a low prevalence is contrary to the situation in most hospitals in the United States in which the prevalence of vancomycin resistance has been increasing in the past 10 years.^{1,4} Approximately 70 percent of all vancomycin-resistant isolates of *E. faecium* and *E. faecalis* in the United States exhibit the *vanA* phenotype which is frequently associated with a multidrug-resistant phenotype.^{1,2}

This study showed that imipenem was still more active against enterococci than penicillin (79.6% and 70.4%, respectively). A disadvantage of imipenem is that it is less effective against *E. faecium*. However, our results should be interpreted with caution since the NCCLS recommends ampicillin susceptibility must be used to determine imipenem susceptibility providing the species is confirmed to be *E. faecalis*.

Fosfomycin susceptibility in enterococci was high in our study (94.8%), while it was low (23%) in the study from Siriraj Hospital.¹⁸ Again, our results should also be interpreted with caution since the NCCLS approves fosfomycin susceptibility testing by agar

dilution method. And agar media must be supplemented with 5 microgram/mL of glucose-6-phosphate. Etest with the strip containing fosfomycin mixed with glucose-6-phosphate was used to determine the MIC in this study. Our result was consistent with the findings of other studies, published in 2002 and 2003 from the United States which showed that an in vitro activity against vancomycin-resistant *E. faecium* of fosfomycin approached 100 percent.^{8,9} No apparent association was noted between the presence of *vanA* or *vanB* genes and fosfomycin susceptibility.⁹ At present, there was still limited information on the treatment of enterococcal infections with fosfomycin, but there was a report published in 2005 of a successfully treated complicated VRE urinary tract infection with fosfomycin trometamol.¹⁰ Fosfomycin has been available in Thailand for many years as a parenteral compound, fosfomycin disodium, and has been mainly used in treating MRSA infections.⁵

Nitrofurantoin was active against *E. faecalis*, and *E. faecium* and also retained its activity against *vanA*- and *vanB*-positive isolates.¹² All 98 enterococcal isolates were susceptible to nitrofurantoin. A study from Pakistan revealed 88-percent susceptibility to nitrofurantoin of 144 enterococci causing urinary tract infection.²² In the United States, 78.7 percent of 75 isolates of vancomycin-resistant *E. faecium* were found to be susceptible to nitrofurantoin.¹² Nitrofurantoin is available only in an oral form, and has a single indication for acute cystitis due to its very low serum concentrations and adequate concentration only in kidney tissue and urine.^{11,23,24}

When penicillin-resistant enterococci are the causative agent of uncomplicated cystitis, the majority of clinicians often choose to use vancomycin as though it was the last resort. Fosfomycin and nitrofurantoin are significantly cheaper than vancomycin. The use of these two drugs to treat enterococcal urinary tract

infections will reduce vancomycin usage, and this should delay the development of VRE. Also, there should be less need for newer antimicrobial drugs like linezolid or tigecycline.

CONCLUSION

This study showed that *E. faecalis* was the most common species isolated from clinical specimens at Songklanagarind Hospital, and revealed a very low prevalence of vancomycin resistance among *Enterococcus* spp. Nitrofurantoin was active against all clinical isolates, and fosfomycin was also active against both *E. faecalis*, and *E. faecium*. Our in vitro data are consistent with the very limited clinical studies which suggests that fosfomycin and nitrofurantoin are effective in the treatment of enterococcal urinary tract infections.

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