SPECIES PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROCOCCI ISOLATED IN A TERTIARY CARE HOSPITAL OF NORTH INDIA

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Abstract. The present prospective study was carried out to determine the species distribution and antimicrobial susceptibilities of enterococci isolated from clinical samples in a tertiary care hospital of North India. *Enterococcus* species isolated from blood, urine, pus, sterile fluids and the hospital environment from October 2003 to January 2004 were identified by standard biochemical tests. Antimicrobial susceptibility testing was performed by the disk diffusion method as per NCCLS guidelines. Out of a total of 105 *Enterococcus* species recovered during the study period, *E. faecium* (42.90%) and *E. faecalis* (40.00%) constituted the predominant isolates. *Enterococcus faecium* was the commonest blood culture isolate while *E. faecalis* predominated pus and urine samples. Other species isolated were *E. mundtii*, *E. dispar*, *E. durans*, *E. avium*, *E. raffinosus* and *E. gallinarum*. High-level aminoglycoside resistance was detected in 73.3% of isolates. Resistance to vancomycin, teicoplanin and linezolid was not detected. Prevalence of a wide variety of *Enterococcus* species in clinical samples together with their variable antimicrobial susceptibility patterns emphasizes the need for routinely carrying out detailed speciation and *in vitro* susceptibility testing of enterococcal isolates in the clinical bacteriology laboratory.

INTRODUCTION

The enterococci have emerged over the last decade as one of the most important nosocomial pathogens worldwide, being responsible for an increasing number of episodes of bacteremia, endocarditis, meningitis, urinary tract and soft tissue infections (Facklam and Teixeira, 1998; Huycke *et al*, 1998; Cetinkaya *et al*, 2000). Traditionally, of the 19 species of enterococci recognized so far, *E. faecalis* has accounted for approximately 80-90% of clinical isolates, while *E. faecium* was isolated in the remaining 5-15% of cases, which was also the experience in earlier Indian studies (Cherian *et al*, 1995; Bhat *et al*, 1998; Facklam and Teixeira, 1998). However, in recent times, a shifting spectrum of enterococcal infections is being reported from different parts of the world with an increasing proportion being caused by *E. faecium*. This finding is important since *E. faecium* strains display a higher degree of drug resistance (Marcus *et al,* 1997; Nelson *et al,* 2000). Furthermore, it is essential to identify species like *E. gallinarum* and *E. casseliflavus* which are intrinsically resistant to vancomycin, in order to avoid inappropriate treatment with vancomycin in these cases (Ratanasuwan *et al,* 1999).

In order to look for the species distribution of enterococci isolated from clinical samples in our hospital, we carried out the present prospective study. This will help us to study the epidemiology of enterococcal infections and define their antimicrobial susceptibility patterns enabling important therapeutic decisions to be made depending on these findings.

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MATERIALS AND METHODS

Enterococcus isolates

All consecutive strains of *Enterococcus* isolated from clinical samples over a 4 - month period between October 2003 and January 2004 in the clinical bacteriology laboratory, Department of Microbiology, at the All India Institute of Medical Sciences, New Delhi, were included in the study. The strains were isolated from blood cultures in cases of blood stream infections

(n=38), urine (present in pure culture as $\geq 10^5$ cfu/ml, n=37), pus (n=19), and sterile fluids (n=7). In addition, isolates from the hospital environment during the same time were also included (n=4).

Identification

The isolates were identified to the genus and species level by cultural characteristics, Gram's stain, motility testing and conventional biochemical tests using standard microbiological techniques (Facklam and Collins, 1989; Facklam and Teixeira, 1998). These included catalase negativity, growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride, tellurite reduction, pigment production, arginine dihydrolase reaction and the generation of acid from mannitol, arabinose, sorbitol, and raffinose. The carbohydrate fermentation reactions were performed in brain heart infusion broth containing 1% carbohydrate with bromocresol purple as an indicator (Facklam and Collins, 1989; Facklam and Teixeira, 1998).

The performance and reading of the tests were quality controlled using the reference strains *E. faecalis* ATCC 29212, *E. faecium* WHO 3 and *E. gallinarum* WHO 11.

Susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (Hi-Media, India) by the standard disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The following antibiotics were tested: penicillin (10 units), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (120 μg), vancomycin (30 μg), and teicoplanin (30 μg). In addition, the isolates were tested for their susceptibility to linezolid (30 μg), a new oxazolidinone antibacterial that has been reported to have activity against gram-positive cocci, including methicillin-resistant *Staphylococcus aureus* and vancomycin resistant enterococci (Udo *et al,* 2003). The diameter of the zone of inhibition of growth was recorded and interpreted as susceptible or resistant by the criteria of NCCLS (NCCLS, 2002). Organisms with "intermediate" levels of resistance were included in the percentage of resistant organisms for final analysis.

Detection of high-level aminoglycoside resistance (HLAR) was performed with disks containing gentamicin (120 μg). Results were read after incubation at 35ºC overnight. Vancomycin resistance was detected by vancomycin disk (30μg) and a screen agar containing vancomycin 6 μg/ml. *Enterococcus faecalis* strain ATCC 29212 was used as a sensitive control. *Enterococcus faecium* WHO3 and *Enterococcus gallinarum* WHO11 were used as resistant controls.

Statistical analysis

Statistical analysis was carried out using the chi-square test with a 0.05 significance level.

RESULTS

Bacterial isolates

A total of 105 *Enterococcus* strains were isolated from various clinical specimens during the study period. Table 1 displays the sources and species identities of the 105 clinical enterococcal isolates. These included 45 (42.9%) strains of *E. faecium*, 42 (40%) strains of *E. faecalis* and 18 (17.1%) belonging to other species. *Enterococcus faecium* was the predominant isolate from blood stream infections and the hospital environment while *E. faecalis* was predominant in urine and pus samples. The predominant species of the 18 non-*E. faecalis*, non-*E. faecium* isolates was *E. munditi* (10/18, 55.6%).

Antimicrobial susceptibility testing

The results of the susceptibility tests are shown in Table 2. A large number of the isolates were resistant to the tested antibiotics. Compared with *E. faecalis*, antibiotic resistance was more common among *E. faecium* isolates though this was not found to be statistically significant. None of the enterococci were resistant to vancomycin, teicoplanin or linezolid. However, a high proportion (77/105, 73.3%) of isolates exhibited HLAR. A statistically significant difference was observed in the resistance pattern of HLAR strains compared to the sensitive strains as follows: ampicillin resistance (96.0% *vs* 67.8%, $p<0.001$), ciprofloxacin resistance (97.4% *vs* 67.8%, $p<0.001$), and erythromycin resistance (94.3% *vs* 64.3%, $p<0.001$).

DISCUSSION

In the present study, we determined the species distribution and the antimicrobial sus-

Table 1
Distribution and species identities of *Enterococci* from clinical specimens.

Specimen type	Number of isolates									Total no. of isolates
	E. faecalis	*E. faecium*	*E. mundtii*	*E. dispar*	*E. durans*	*E. avium*	*E. raffinosus*	*E. gallinarum*	Unidentified	
Blood	7	24	3	-	1	-	1	1	1	38
Urine	20	13	3	1	-	-	-	-	-	37
Pus	13	3	1	-	1	1	-	-	-	19
Sterile fluids	1	2	3	1	-	-	-	-	-	7
Hospital environment	1	3	-	-	-	-	-	-	-	4
Total	42 (40.0%)	45 (42.9%)	10 (9.5%)	2 (1.9%)	2 (1.9%)	1 (0.9%)	1 (0.9%)	1 (0.9%)	1 (0.9%)	105

Table 2
Antimicrobial resistance profile among enterococcal clinical isolates.

Antimicrobial agent	No. (%) of resistant strains			
	E. faecalis (n=42)	*E. faecium* (n=45)	Other enterococci (n=18)	Total (n=105)
Penicillin	32 (76.2)	41 (91.1)[a]	18 (100)	91 (86.6)
Ciprofloxacin	35 (83.3)	41 (91.1)[a]	18 (100)	94 (89.5)
Erythromycin	35 (83.3)	39 (86.6)[a]	18 (100)	92 (87.6)
Gentamicin (HLAR)	29 (69.0)	33 (73.3)[a]	15 (83.33)	77 (73.3)
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Teicoplanin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a]p>0.05 (=not significant) for difference in resistance between *E. faecalis* and *E. faecium* by chi-square test.

ceptibility profiles of clinical enterococcal isolates in a tertiary care hospital of North India. Recent longitudinal studies have indicated an increasing incidence of enterococcal infections in tertiary care teaching hospitals, often accompanied by a high proportion of *E. faecium* isolates (Marcus *et al,* 1997; Huycke *et al*, 1998; Nelson *et al,* 2000). For example, some workers reported a change in the ratio of *E. faecalis* to *E. faecium* from 3.7:1 in 1996 to 1.9:1 in 1999 for blood isolates (Edwards 2000). Other researchers (Gray *et al,* 1991; Simonsen *et al,* 2003) found a greater proportion of *E. faecium* in blood cultures and *E. faecalis* in cultures of samples from other sites. The relatively high proportion of *E. faecium* among hospital isolates together with their greater isolation from blood cultures observed in our study is thus consistent with those reported in other studies. Changes in the hospital patient population and antimicrobial use patterns coupled with a greater antibiotic resistant nature of *E. faecium* probably confers a greater selective survival advantage compared to *E. faecalis* and explains the emergence of *E. faecium* bloodstream infections. Thus, the traditional outnumbering of *E. faecium* by *E. faecalis* by 10:1 in clinical specimens no longer seems to be valid for hospital isolates. This further supports the fact that we should study the distribution of enterococcal species to reflect the local situation.

An interesting point of note was that *E. mundtii* was the commonest non-*E. faecalis* non-*E. faecium* isolate in the present study. *Enterococcus mundtii* has been isolated only rarely in previous studies (Osornio *et al,* 1996; Van Horn and Rodney, 1998). In a Mexican tertiary care center (Osornio *et al,* 1996), it was found in only one out of 407 enterococcal isolates. In regard to motile enterococci, such as *E. gallinarum* or

E. casseliflavus, worldwide their proportion remains low, *ie* <2% (Ratanusuwan *et al,* 1999) which is consistent with our findings.

In the current study, there was a high prevalence of HLAR with a statistically significant difference observed in the resistance pattern of HLAR strains compared to the sensitive strains. Such a finding is of concern since HLAR abrogates the synergistic antienterococcal effect of beta-lactams and aminoglycosides, the therapy of choice for enterococcal infections, thus limiting therapeutic options. Overall, the *E. faecium* strains were observed to be more resistant to the tested antimicrobials similar to studies from India and outside (Bhat *et al,* 1998; Huycke *et al,* 1998; Cetinkaya *et al,* 2000) as compared to *E. faecalis,* though this was not found to be statistically significant in our study. Fortunately, none of the enterococci were resistant to vancomycin or teicoplanin. Nonetheless, there is a need for constant monitoring as sporadic isolates have recently been reported as vancomycin resistant (Mathur *et al,* 2003). Linezolid also demonstrated good antienterococcal activity and may be kept as a second line drug for vancomycin resistant strains.

In conclusion, this study illustrates the changing epidemiology of enterococcal infections encountered in clinical practice and thus a need for routine speciation of isolates. We further emphasize the need for constant monitoring of antibiotic susceptibility profiles in defined geographical areas which will be helpful in formulating local guidelines.

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