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ORIGINAL ARTICLE

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Isolation, identification, and antibiogram of enterococci isolated from patients with urinary tract infection

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Abstract

Background/objectives: Enterococci, though commensals in adult feces are important nosocomial pathogens. The most common nosocomial infection caused by these organisms is urinary tract infection. Objectives: (1) To isolate and speciate enterococci from cases of urinary tract infection. (2) To know antibiotic susceptibility pattern of the isolates. (3) To determine high level aminoglycoside resistance (HLAR) among the isolates.

Methods: Identification and speciation of the isolates were done by the standard conventional methods. Antibacterial susceptibility pattern was determined by standard disc diffusion method and HLAR by using gentamicin (120 µg) and streptomycin (300 µg) discs.

Results: A total of 150 strains of enterococci were isolated from a total of 2520 urine samples. Out of 150 strains, 95 (63.3%) were *Enterococcus faecalis*, 55 (36.7%) were *E. faecium*. A total of 102 (68%) isolates showed high level resistance to gentamicin and/or streptomycin by high content disc diffusion.

Conclusion: Antibiotic sensitivity pattern revealed presence of multidrug resistance in *E. faecium* as well as *E. faecalis* and resistance among *E. faecium* isolates was higher than *E. faecalis*. HLAR among enterococcal isolates was high in our institute.

Abstract in French

Contexte/objectifs: Entérocoques, bien que commensaux dans les excréments de l'adultes est pathogènes nosocomiaux importants. La plus courante infection nosocomiale causée par ces organismes est une infection des voies urinaires.

Objectifs: (1) à isoler et speciate entérocoques provenant de cas d'infection urinaire. (2) De connaître le motif de la sensibilité aux antibiotiques des souches. (3) Pour déterminer les aminosides niveau haute résistance (HLAR) parmi les isolats.

Méthodes: Identification et spéciation des isolats ont été réalisées par les méthodes conventionnelles standards. Modèle antibactérien susceptibilité a été déterminée par la méthode de diffusion des disques standard et HLAR en utilisant des disques de streptomycine (300 µg) et de la gentamicine (120 µg).

Résultats: Un total de 150 souches d'entérocoques ont été isolés sur un total de 2520 échantillons d'urine. Des 150

souches, 95 (63,3 %) étaient des *Enterococcus faecalis*, 55 (36,7 %) *E. faecium*. Un total de 102 (68 %) des isolats ont montré à haut niveau résistance à la gentamicine ou streptomycine par diffusion du contenu du disque haute.

Conclusion: Modèle de sensibilité aux antibiotiques a révélé la présence d'une pharmacorésistance dans *E. faecium* ainsi que *E. faecalis* et résistance chez *E. isolats* était supérieure à *E. faecalis*. HLAR parmi les isolats d'entérocoques était élevé dans notre Institut.

Mots-clés: Entérocoques, aminosides niveau haute résistance, infection des voies urinaires

Keywords: Enterococci, high level aminoglycoside resistance, urinary tract infection

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Introduction



The genus *Enterococcus* consists of Gram-positive, facultatively anaerobic organisms that are ovoid in shape and may appear on smear in short chains, in pairs or as single cells. Enterococci, though commensals in adult feces are important nosocomial pathogens. Enterococcal infections may be due to at least 12 species, including *Enterococcus avium*, *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, *E. raffinosus*, and *E. solitarius*. Most clinical infections are due to either *E. faecalis* or *E. faecium*. Among enterococcal species, *E. faecalis* and *E. faecium* are the two major human pathogens accounting for 85-89% and 10-15% of all enterococcal infections, respectively. [1],[2],[3],[4]

Prior to the 1990s also, enterococci have been recognized as an important cause of bacterial endocarditis for almost a century. [3] However, more recently they have been recognized as a cause of nosocomial infection and "superinfection" in patients receiving antimicrobial agents. The most common enterococci-associated nosocomial infections are infections of the urinary tract, followed by surgical wound infections and bacteremia. Enterococci are often present in intraabdominal and pelvic infections, although not all patients with such infections require specific antienterococcal therapy. Other enterococcal infections include infections (including meningitis and bacteremia) in very ill neonates; central nervous system infections in adults, typically with a history of central nervous system surgery or intrathecal chemotherapy; and rarely, osteomyelitis and pulmonary infections. [5] Enterococci account for more than 9% of bacteremia in US and Canada (rates are lower in Latin America). The highest detected rate of enterococcal urinary tract infections (UTI) was in Canada (16.8%), followed by the US (12.5%), and Europe (11.7%). [3]

Species identification of enterococci may be useful both as an epidemiologic tool in the investigation of outbreaks of nosocomial infections and for clinical decisions about therapy because antimicrobial susceptibility may vary by species, especially *E. faecium* and other species tend to be more resistant than *E. faecalis* to several commonly used antimicrobial agents. [6]

The intrinsic antibiotic resistance of enterococci, coupled with their promiscuity in acquisition and dissemination of genetically mobile antibiotic resistance elements, presents serious challenges to the treatment of enterococcal infections. Infections by enterococci have traditionally been treated with cell wall active agents (e.g., penicillin or ampicillin) in combination with an aminoglycoside (streptomycin/gentamicin), however, emergence of high level aminoglycoside resistance (HLAR), β lactam antibiotics and to vancomycin by some strains has led to failure of synergistic effects of combination therapy. [7],[8]

This study was undertaken to provide accurate antimicrobial resistance patterns for enterococci so that effective therapy and infection control measures can be initiated, to isolate and speciate enterococci from cases of urinary tract infection and to determine HLAR among the isolates.

Materials and Methods



Study population, design, and setting

The present study was a prospective cross sectional study conducted in the department of Microbiology in our medical college, over a 2 year period from November 2007 to October 2009. Our hospital primarily caters to the semi urban population of southern India.

Patient evaluation

A total of 2520 urine specimens from both outpatients and inpatients of our hospital having one or more urinary symptoms, like burning during micturition, fever, pyuria, frequency of urine, dysuria, hematuria, flank pain, suprapubic

discomfort, etc., were processed. Patients from whom *Enterococcus* species were isolated during routine diagnostic testing were included in the study.

Sample collection

Mid-stream urine sample in early morning was collected in wide mouth sterile container. Female patients were instructed to cleanse the area around the urethral opening with soap and water, dry the area, and collect the urine with the labia held apart. Male patients were instructed to cleanse the glans penis with soap and water, dry the area, and collect the urine with foreskin retracted.

Identification and speciation

All urine samples were examined by routine microscopic examination by wet mount of urine sediment after centrifuging urine for 10 minutes at 1000 revolution per minute (rpm). Presence of pus cells, red blood cells (RBCs), epithelial cells, casts, and crystals were noted as supportive findings of urinary infection. Simultaneously all urine samples were cultured over routine culture media; MacConkey agar and Cysteine lactose electrolyte deficient (CLED) agar with a sterile standard loop. These plates were incubated at 37°C for 2 consecutive days.

Enterococci were identified on the basis of appearance on gram stain, growth in 6.5% NaCl, catalase-negative, growth on bile esculin medium. Other tests such as bacitracin resistance and positive Voges-Proskauer test were also used for the confirmation of isolates as enterococci. Speciation of the isolates were carried out by using conventional tests devised by Facklam and Collins [9] which are based on carbohydrate fermentation using 1% solution of glucose, mannitol, rabinose, raffinose, sorbitol, sucrose, lactose, trehalose, and inulin; by pyruvate utilization test; arginine decarboxylation; hippurate hydrolysis; motility test; starch hydrolysis; polysaccharide production and gelatin liquefaction.

Antibiotic susceptibility testing [10]

Antimicrobial susceptibility testing and interpretation was carried out on Mueller-Hinton agar (HiMedia laboratories, India) by standard disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines using discs of standard concentration. Standard strains of *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were used as controls. [10]

The antibiotics tested were (concentration in µg) as follows: Ampicillin (10), chloramphenicol (30), penicillin (10U), tetracycline (30), erythromycin (15), ciprofloxacin (5), vancomycin (30), teicoplanin (30), and linezolid (30). These discs were obtained from HiMedia laboratories, India.

Detection of HLAR

Isolates were tested for HLAR by standard disc diffusion method as per NCCLS guidelines using high content gentamicin (120 µg) and high content streptomycin (300 µg) discs. Resistance was indicated by no zone and susceptibility by zone of >10 mm.

Statistical analysis

Data collected were analyzed by

1. Diagrammatic representation
2. Using Chi square test with a 0.05 significance level.

Results



A total of 2520 urine samples were studied from patients with suspected signs and symptoms of urinary tract infection, out of which 1233 specimens were positive in culture. Enterococci were isolated in pure cultures in clinically significant numbers in 150 (12.1%) specimens. Of the 150 persons from whom these isolates were obtained, 103 (68.7%) were males, 47 (31.3%) were females. The male:female ratio was 2:1 and 79% were inpatients, and 32% were between 40 and 60 years of age. The mean age of the patients was 65 years (range: 9 months to 91 years) [Table 1].

Parameter	Value	Number (n=150)	Percentage
Sex	Male	103	68.7
	Female	47	31.3
Age group	0-20	27	18
	21-40	30	20
	41-60	49	32.7
	>61	44	29.3

Table 1: Distribution of isolates in relation to patient's age and sex

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Only two species of enterococci were isolated. Out of these two, *E. faecalis* (63.3%) was the predominant species followed by *E. faecium* (36.7%) [Table 2]. Enterococci were highly resistant to ciprofloxacin (93%), tetracycline (90.7%), erythromycin (90%) and sensitive to linezolid (98%), chloramphenicol (69.3%), vancomycin (64%), teicoplanin (59.3%). Sensitivity was much higher to linezolid compared with other antibiotics and the difference is statistically significant ($P < 0.05$).

Enterococcus spp.	No. of isolates	Percentage
<i>E. faecalis</i>	95	63.4
<i>E. faecium</i>	55	36.6
Total	150	100

Table 2: Different *Enterococcus* spp. isolated

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E. faecalis isolates were resistant to tetracycline (90.6%), ciprofloxacin (89.5%), erythromycin (85.3%) and sensitive to linezolid (98.9%), ampicillin (90.5%), penicillin (84%) chloramphenicol (69.5%). *E. faecium* isolates were 100% resistant to ciprofloxacin, 98% to penicillin, ampicillin and sensitive to linezolid (96.4%), vancomycin (89.1%) and chloramphenicol (69.1%). Penicillin and ampicillin resistant was significantly more in *E. faecium* than in *E. faecalis* (for penicillin, 54 of 55 *E. faecium* isolates [98%] versus 15 of 95 [16%] *E. faecalis* isolates ($P < 0.05$), for ampicillin, 54 of 55 *E. faecium* isolates [98%] versus 9 of 95 [9.5%] *E. faecalis* isolates ($P < 0.05$)) [Table 3].

Antibiotic	<i>E. faecium</i>	<i>E. faecalis</i>
Linezolid	54/55 (98.2%)	93/95 (97.9%)
Ampicillin	54/55 (98.2%)	9/95 (9.5%)
Penicillin	54/55 (98.2%)	15/95 (15.8%)
Chloramphenicol	48/55 (87.3%)	66/95 (69.5%)
Erythromycin	47/55 (85.5%)	81/95 (85.3%)
Ciprofloxacin	55/55 (100%)	85/95 (89.5%)
Tetracycline	50/55 (90.9%)	86/95 (90.6%)
Vancomycin	49/55 (89.1%)	88/95 (92.6%)

Table 3: Antibiogram of enterococcal isolates

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[Table 4] shows HLAR pattern of *Enterococcus* species against gentamicin and streptomycin. HLAR was detected in 68% (102/150) of the isolates. HLAR among *E. faecium* isolates (98.6%) was significantly higher ($P < 0.05$) than *E. faecalis* (52.3%). High level resistance to gentamicin and streptomycin among *E. faecalis* strains were 48.4% and 43.1%, respectively, and high level resistance to gentamicin and streptomycin among *E. faecium* strains were 92.7% and 67.3%, respectively. Combined resistance to both the aminoglycosides was slightly higher in *E. faecium* (56.4%) as compared with *E. faecalis* (44.2%). There was a higher percentage positivity of the isolates having high level gentamicin resistance (64.7%) in comparison to the isolates having high level streptomycin resistance (52%).

Antibiotic	<i>E. faecium</i>	<i>E. faecalis</i>
Gentamicin	92/95 (96.7%)	48/95 (50.5%)
Streptomycin	67/95 (70.5%)	43/95 (45.3%)
Combined	56/95 (58.9%)	44/95 (46.3%)

Table 4: High level aminoglycoside resistance (HLAR) pattern of *Enterococcus* spp.

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Discussion



Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. [8] Urinary tract infections are the most common cause of infectious disease produced by enterococci, both within and outside hospital settings. The reported frequency of enterococcal urinary tract infection is variable in the different studies. Miskeen *et al.* [11] isolated these organisms in 7.4% of the patients. Cornia *et al.* [12] investigated the causative agents of bacteriuria in elderly male inpatients and outpatients and identified *Enterococcus* as the most frequent uropathogen, this pathogen was isolated in 22.5% of the patients. Enterococci was reported as the third most frequent uropathogen in intensive care unit-acquired urinary tract infections after *Escherichia coli* and *Pseudomonas aeruginosa* by studies conducted by Bagshaw *et al.* [13]

In the current study, enterococcal urinary tract infection was less frequent than has been reported in other studies. This difference may be related to settings under which the studies were carried out. Our study was conducted in a medical college hospital in which children as well as adults were treated both as inpatients and outpatients. It is well-known that enterococcal caused urinary tract infections are more frequent after 60 years of age when obstructive uropathy are more frequent. [14] In our study, the highest prevalence was seen in elderly patients. The mean age of patients with enterococcal infection was 65 years. The male:female ratio was 2:1 in our study, which is comparable to the study carried out by Adhikari *et al.* [15]

Two species of enterococci were identified in the current study, *E. faecalis* was found to be the predominant isolates, followed by *E. faecium*. Both these species are long known to be significantly associated with the clinical disease hence their isolation is a cause of serious concern. [1] Species identification of isolates also enabled us to assess species-specific antimicrobial resistance characteristics. *E. faecium* isolates were more resistant to penicillin, ampicillin, and ciprofloxacin than the isolates of *E. faecalis*. This finding is consistent with previous reports of enterococcal resistance. [6] Our observations on the incidence of various enterococcal species are similar to those of other authors [8],[11] who noted, that the majority (63-89%) of clinical isolates were *E. faecalis*, followed by *E. faecium* (11-37%).

In our study majority of the enterococcal isolates (more than 90%) were resistant to tetracycline, erythromycin, and ciprofloxacin, which is comparable to the study conducted by Mendiratta *et al.* [8] Overall, resistance to penicillin, ampicillin, ciprofloxacin, and erythromycin among strains of *E. faecium* was higher than among strains of *E. faecalis*. For all other antibiotics there was no significant difference between resistance pattern of *E. faecalis* and *E. faecium*. Gordon *et al.* [6] reported that *E. faecium* was found more resistant to commonly used antibiotics as compared with *E. faecalis*. Fifty-eight percent (58%) (87/150) and 54% (81/150) of enterococci were sensitive to ampicillin and penicillin, respectively. Penicillin and ampicillin resistance was significantly more ($P < 0.05$) in *E. faecium* than in *E. faecalis*. NCCLS [10] designates penicillin and ampicillin as comparable agents that need not be duplicated in antibiotic sensitivity testing. In the present study, there were a total of 69 isolates resistant to penicillin, of which 63 isolates were resistant to both penicillin and ampicillin, therefore we endorse the NCCLS guidelines with our observation that penicillin and ampicillin need not be tested separately.

In our study, 98% of enterococci were sensitive to linezolid, 69.3% to chloramphenicol and 59.3% to teicoplanin. Sensitivity was much higher to linezolid compared with other antibiotics and the difference is statistically significant (P

<0.05). This drug may be of utmost utility for multidrug-resistant strains. It should not be used indiscriminately and also it has some potentially important drug-drug interactions, therefore careful review of the patient's medical regimen, in consultation with a pharmacist, is recommended before it is prescribed. [2]

The most recent and disturbing resistance reported in enterococci is vancomycin resistance. It has been increasingly reported from all parts of the world. The majority of vancomycin-resistant enterococci (VRE) encountered to date have been *E. faecium*. [16] In our study 34% of the isolates were resistant to vancomycin. In a study by Karmarkar *et al.*, [17] 24% of the isolates were resistant to vancomycin.

HLAR enterococci were first reported in France in 1979 and since then have been isolated from all the continents. [18] Schouten *et al.* reported prevalence rate of high level gentamicin resistance in enterococci varying from 1% to 49% in the 27 European countries studied. [19] Another study reported a prevalence of 55% of high level gentamicin resistance in enterococci in an US center. [20]

The reason for higher prevalence in our hospitals ascribed to the source of the isolates being from a tertiary care set up where chronic cases are prevalent, excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from peripheral centers. The overall prevalence of high level resistance to any aminoglycoside among the study isolates was 69%. The frequencies of high level resistance to the individual aminoglycosides were similar to those recently reported for enterococci isolated from patients in Nagpur and Sevagram, India. [8],[21] High level resistance usually occurred in combinations of more than one aminoglycoside.

There is usually little need to test aminoglycosides other than streptomycin or gentamicin since these are the agents for which there are the most clinical data. To date, all strains with high level resistance (HLR) to gentamicin have also had resistance to synergism and/or HLR to tobramycin, sisomicin, netilmicin, kanamycin, and amikacin by virtue of the 2"-APH-6'-AAC. This enzyme is not active against streptomycin so that gentamicin-resistant strains are not necessarily resistant to streptomycin; in other words, a variable percentage of strains will have HLR to gentamicin while lacking HLR to streptomycin. [1] A total of 19 (12.7%) of the 150 isolates with high level gentamicin resistance were not resistant to high level streptomycin; therefore, cell-wall inhibitors in combination with streptomycin may be useful in the treatment of serious infections due to these organisms.

In our study, HLAR among *E. faecium* isolates was significantly higher ($P < 0.05$) than *E. faecalis*. In a study conducted by Mendiratta *et al.*, [8] HLAR among *E. faecium* isolates (95.5%) was significantly higher than *E. faecalis* (37.5%), which is comparable to our study. In our study combined resistance to both the aminoglycosides was slightly higher in *E. faecium* as compared with *E. faecalis*, similarly in a study conducted by Mendiratta *et al.*, [8] combined resistance to both the aminoglycosides was much higher in *E. faecium* as compared with *E. faecalis*.

This study permitted the determination of the prevalence of antibiotic resistance among enterococci causing a wide spectrum of diseases. Identification of enterococcal isolates to the species level in the clinical microbiology laboratory is useful because it can help predict patterns of antimicrobial susceptibility, particularly to penicillin. The present study also highlighted the importance of high occurrence of HLAR enterococci in our set up. This would necessitate routine testing of the isolates for HLAR.

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