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Short Communication

A STUDY OF VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATED FROM URINARY TRACT INFECTIONS

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ABSTRACT

Objective: Vancomycin resistant enterococci (VRE) are becoming a major emergence problem concern in urinary tract infection (UTI). This study provides accurate and complete description of antimicrobial susceptibility pattern and to know the prevalence of VRE in this area.

Methods: A total of 3400 urine samples were collected and processed bacteriologically. The enterococci was isolated and identified by biochemical tests and Vitek 2. VRE was determined by disc diffusion, agar dilution and Vitek 2 automated machine. Statistical analysis was done by Graph Pad InStat Software.

Results: The 143 (4.2%) enterococci were isolated from UTI patients. The incidence was higher in young females and old males. E. faecalis (78%) is the most common isolate followed by *E. faecium* (15%). The rare species (9%) like *E. durans, E. avium, E. gallinarum* and *E. hirae* were also isolated. Fosfomycin (96.5%) and nitrofurantoin (93%) was the drug of choice for enterococcal UTI while linezolid (98.6%) also can be used to treat other enterococcal infections. Among the UTI 98.6% enterococci were susceptible to vancomycin.

Conclusion: Empirical therapy for enterococcal infections should be guided by local patterns of drug resistance. Linezolid, fosfomycin or nitrofurantoin may be considered to treat the patients with VRE.

Keywords: Enterococci, Urinary Tract Infection, Vancomycin Resistance.

Enterococci are Gram positive cocci arranged in angulated pairs. They are the normal flora of the human gastrointestinal tract and are also important nosocomial pathogens [1]. The genus *Enterococcus* includes more than 29 species. According to recent studies 80% of clinical isolates are *Enterococcus faecalis* and *is* followed by *E. faecium* (10-15%). *E. durans, E. avium, E. raffinosus, E. gallinarum, E. casseliflavus,* and *E. hirae* are the rare species reported in India [1, 2].

Enterococci are isolated from various infections. They have an ability to cause a variety of infections like urinary tract infection (UTI), abdominal and pelvic abscesses, peritonitis, bacteraemia, sepsis, intravascular catheter infection, infection of wounds, and other rare infections [3]. The Center for Disease Control and Prevention's National Nosocomial Surveillance Survey listed enterococci as the second most common cause of nosocomial UTI [4].

There is also an emergence of acquired resistance to vancomycin, which has been increasingly reported from all parts of the world. Very limited numbers of antibiotic are available for treating enterococcal infections and currently there is no ideal antibiotic regimen with bactericidal activity for serious infections caused by vancomycin resistant enterococci (VRE) [5]. It is crucial to provide accurate and complete description of antimicrobial susceptibility pattern and current possibility for treatment of enterococcal urinary tract infections. Studies are required to clarify epidemiology of VRE infection in these areas and this is possible by an investigation of VRE among patients. The present study is a prospective and cross sectional study conducted from Oct. 2008 to Sept. 2012 in the Dept. of Microbiology, at Krishna Institute of Medical Sciences, Karad, Maharashtra. Informed consent was obtained from all the patients. The study was approved by our institutional ethical committee. The 3, 400 midstream or catheterised urine samples were collected from suspected urinary tract infection patients attending outpatient department or admitted at BLDEU's Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijaypur, Karnataka and processed bacteriologically. The urine specimens were inoculated on Mac-Conkey agar and Cysteine Lysine Electrolyte Deficient (CLED) agar for isolation. The suspected colonies (more than 10^5 colony forming unit/ml) of enterococci were identified and classified by conventional scheme of Facklam and Collins by using Gram stain, cultural characteristics and various biochemical tests such as sugar fermentation and arginine hydrolysis [6]. The rare and doubtful 23 isolates were identified by Vitek 2 automated machine. All enterococcal isolates were subjected for antibiotic sensitivity testing for commonly used antibiotics including vancomycin by Kirby Bauer's disc (from Hi-Media, Mumbai) diffusion method. Resistance to vancomycin and teicoplanin was determined by agar screen method (6 µg/ml) [7] and confirmed by agar dilution method and Vitek 2 (0.25 to 128 µg/ml) automated machine at MicroPath Laboratory, Kolhapur. *Staphylococcus aureus* (ATCC 25923), vancomycin susceptible *Enterococcus faecalis* (ATCC 29212) and vancomycin resistant *Enterococcus faecalis* (ATCC 51299) strains were used as control for antibiotic susceptibility. Statistical analysis was done by Graph Pad In Stat Software. Data was analyzed using Mean(SD), Median and Chisquare test.

In the present study, out of total 3400 urine samples, 1236 specimens yielded growth and of these 143 (4.2%) were identified as enterococci. The highest incidence was seen in the age group of 21 to 40 years comprising 35.7% each. There was no significant difference incidence of enterococcal infections among males (52%) and females (48%) (table 1). Among 143 isolates 19 (13%) isolates were from UTI in pregnant women, 14 (10%) from catheterized patients and 13 (9%) from hypertension patients and 17 (12%) were with other complications while remaining 80 (56%) patients with symptomatic bacteriuria and did not have any other complication.

Enterococcus faecalis was the major species isolated i. e. 112 (78.32%) followed by *Enterococcus faecium* i. e. 22 (15.38%). The rare species like *E. durans* (3), *E. avium* (2), *E. gallinarum* and *E. hirae* (one each) were also isolated. We could not identify two Enterococcus isolates. *E. faecium* was more resistant than *E. faecalis* and other species to most of the antibiotics but *E. faecalis* was more resistant than *E. faecium* to tetracycline (table 2).

Table 1: Distribution of patients with respect to age and sex

Age (years)	1-20	21-40	41-60	61-80	> 81	Total	%	Mean (SD)	Median
Male	9	14	22	26	3	74	52	46 (27)	52
Female	6	37	15	10	1	69	48	40 (21)	40
Total	15	51	37	36	4	143	100	--	--
%	10.5	35.7	26	25	2.8	100	---	--	--

Table 2: Antibiotic resistance pattern of Enterococci among UTI

Antibiotics	Ciproflo xacin	Erythro mycin	Tetracyc line	Penicil lin	Amoxyc lav	Rifampi cin	Nitrofuran toin	Fosfomy cin	Vancom ycin	Teicopla nin	Linezo lid
Resistant (n=143)	118	115	105	70	56	52	10	5	2	2	2
Percentage	82.5	80.4	73.4	49	39	36.4	7	3.5	1.4	1.4	1.4

The enterococcal infections was commonly seen in the age group of 50-59 and mean age of the patients was around 60 years [8], while in the present study mean and median is around 45 years (table 1). Obstructive uropathies with catheterization is more frequent in old age patients.

The incidence was higher in females belonging to the sexually active age group (21 to 40 years). During intercourse and after bowel movement (cleaning), there is the possibility of entry of intestinal or vaginal enterococci (normal commensals) in to a urinary tract due to proximity of urethral, vaginal and anal openings. The higher prevalence was noted in females in different studies [8]. In the present study, the incidence of enterococcal urinary tract infection is significantly higher in males belonging to old (61 to 90 years) age group.

The incidence (4.23%) rate in the present study is nearer to that of studies conducted by Parvathi S *et al.* [2] (4.48%). It is lesser than that reported by PJ Desai *et al.* [3] (28.57%) and Miskeen *et al.* [9] (7.4%). Sonal Saxena *et al.* [1] compared hospitalized and non-hospitalized patients and reported that enterococci were known to have been an increasing role in nosocomial infections.

Jaylaxmi et al. [10] has reported 2.12% incidence of enterococci in asymptomatic bacteriuria in pregnant women. Their physiological, hormonal and mechanical changes make them more susceptible to UTI. The urinary statics, vesicourethral reflux and difficulty with hygiene due to distended pregnant belly increase the risk for UTI. Catheter-related UTI occurs because enterococci may gain entry in to the bladder during insertion of the catheter, during manipulation of the catheter or drainage system, around the catheter and after removal. It also promotes colonization by providing surface for bacterial adhesion and causing mucosal irritation [11]. Krishna KS et al. [12] from Lucknow, has reported that the most common form of enterococcal urinary tract infection was seen in renal transplant recipients.

Enterococci are the most common causative agents of urinary tract infection. The rate of urinary tract colonization and infection by enterococci rises among hospitalized patients, who have been instrumented [8], received antibiotic therapy (particularly cephalosporin) [3], having structural abnormalities and / or recurrent urinary tract infections [2]. Now there is twenty-fold increase enterococcal incidence in nosocomial UTI [1].

All together 9 (6.3%) isolates were identified as non- *E. Faecalis* non-*E. faecium* in the present study. The same species were reported by most of the workers from India [1, 2]. Enterococci have adequate intrinsic and acquired resistance to many antibiotics [5]. They are intrinsically resistant to penicillinase resistant penicillins and cephalosporins; acquired resistance to chloramphenicol, erythromycin and high level resistant to tetracycline, aminoglycosides, penicillin, fluoroquinolone and vancomycin [13]. Antimicrobial resistant enterococci are being reported with increasing frequency in an United States and other parts of the world [14]. Careful review of in vitro susceptibility data is required to treat infections caused by multi-drug resistant *E. faecium.*

In the present study, 49% of enterococcal isolates were resistant to penicillin [table 2], which is in between the study conducted by Parvati S *et al.* [2] (43%) and Bhat KG *et al.* [15] (55%). But it is more than [9] (23%). The highest resistance was observed against ciprofloxacin, erythromycin and tetracycline [16] reported 63%, 61% and 40% isolates were resistant to erythromycin, tetracycline and ciprofloxacin respectively. Lowest resistance was observed against linezolid, fosfomycin and nitrofurantoin. Fosfomycin (96.5%) and nitrofurantoin (93%) is the drug of choice [9] for enterococcal UTI while linezolid (98.6%) also can be used to treat other enterococcal infections.

Vancomycin Resistant Enterococci (VRE) has been increasingly reported from all parts of the world [1]. But in the present study, more than 98.6% enterococcal isolates were susceptible to vancomycin and teicoplanin, showing MICs below 4 µg / ml. There are various phenotypes (Van A, B, C, D, E, G and Vancomycin Dependent Eenterococci) of glycopeptide resistance in enterococci [4, 17]. Van A phenotype is more widely distributed and thus the predominant type of resistance reported. Moreover, vancomycin resistance has appeared preferably in *E. faecium,* which is inherently more resistant to multiple drugs making therapy extremely problematic [5]. In the present study also both the VRE (1.4%) isolates showed high level (>1024 µg/ml) resistant to vancomycin as well as teicoplanin (>128 µg/ml) and belonged to Van A phenotype, of which one belonged to *E. faecium* and another to *E. durans*.

In the present study, *E. faecalis* isolates were 100% susceptible to vancomycin and teicoplanin but among *E. faecium* isolates 96% were susceptible to vancomycin and teicoplanin. This correlates with the study conducted by Bhat KG *et al.*[15]. The majority of VRE are encountered in *E. faecium*, but of late strains of *E. gallinarium* and *E. faecalis* resistant to vancomycin have also been reported [1]. Emergence of vancomycin resistance was reported in few more studies [2, 3]. Gordon *et al.* [8], Udo EE *et al.* [16] (99.6%) and Miskeen PA *et al.* [9] (100%), reported that many isolates were susceptible to both the glycopeptides and the organisms were inhibited at concentration ranging from 0.5 µg/ml to 4 µg/ml.

There is dramatic increase in vancomycin resistance among enterococci. They also have an ability to transfer the *vanA* and *vanB* gene to self- transferable (with in genus-to other enterococci) [17] as well as other Gram positive organisms like staphylococci, streptococci [18] and listeria [19]. CDC Hospital Infection Control Practices Advisory Committee (HICPAC) had published recommendations to control the nosocomial transmission of VRE [4, 20]. Aim of this recommendation is to minimize nosocomial transmission of VRE; hospitals must use a multidisciplinary approach that requires participation by a variety of departments and personnel.

The present study concludes that the overall incidence of enterococci among urinary tract infections is 4.23% in this region. Among the genus *Enterococcus, E. faecalis* is most common isolate followed by *E. faecium*. Antibacterial susceptibility pattern reveals that *E. faecium* isolates was significantly more resistant to most of the antibiotics except tetracycline than *E. faecalis*. Vancomycin resistance is less (1.4%) in our hospital. Linezolid, fosfomycin or nitrofurantoin may be considered to treat the patients with VRE. The use of vancomycin is acceptable only for life threatening illnesses unless there is no other choice. We have focused on the emergence

of vancomycin resistant enterococci, which are most often found in *E. faecium*. Empirical therapy for enterococcal infections should be guided by local patterns of drug resistance.

CONFLICT OF INTERESTS

Declared None

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