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Vancomycin Resistant Enterococci and Trend of Antimicrobial Susceptibility in Urine Cultures

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ABSTRACT

Background: Enterococci are emerging nosocomial pathogen showing resistance to broad spectrum antibiotics. As reported by the center for disease control and prevention, enterococci (13.9%) are the second leading cause of urinary tract infection, after *Escherichia coli*. After the first report of vancomycin-resistant enterococci in England (1988), India reported its first vancomycin resistant enterococci isolate from New Delhi (1999). The prevalence of vancomycin resistant enterococci ranges from 1-9%, in India. To evaluate the prevalence of vancomycin resistant enterococci among urinary isolates and to check their antimicrobial resistance pattern.

Methods: A retrospective observational study conducted at a tertiary care hospital. Data were analysed from November, 2017 to October, 2018. A total of 12,129 urine samples were received and subjected to culture on HiCrome UTI agar (Hi-Media laboratories, Mumbai) and incubated at 37 °C for a period of 18-24 hours. Antimicrobial susceptibility was performed using Kirby–Bauer disc diffusion method. Identification was done on the basis of colony colour and species identification was done by using biochemical test as per standard laboratory protocol. In one case automated siemens microscan walkaway identification system was used for confirmation.

Results: Enterococci were isolated in 4.06% from urine cultures out of which 7.52% were vancomycin resistant. Most of the vancomycin resistant enterococci isolate (86.49%) were multi drug resistant.

Conclusion: In vancomycin resistant enterococci related urinary tract infection, nitrofurantoin can be used as an appropriate first choice in uncomplicated cases and linezolid should be reserved for serious and complicated urinary tract infections.

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Introduction

Enterococcus species are commensal flora of human intestinal tract, the oral cavity, and the vagina. Enterococci although less virulent, are emerging multi-drug resistant nosocomial pathogen (1). They are the second most common organisms isolated from the health-care associated urinary tract infections (2). Enterococci (11%) are the second most common causative agent following uropathogenic Escherichia coli associated with complicated UTIs. While, amongst uncomplicated UTI patients, prevalence of enterococci are around 5% (3). According to the National Institute of Health, 80% of infections are related to biofilm-forming microbes (4). Biofilm protects enterococci from host immune response and antibiotics. Biofilm-producing enterococci can cause recurrent, chronic, and antibiotic-resistant infections (5, 6). Apart from biofilm-forming ability, *Enterococcus* spp. are known to produce various virulence factors such as aggregation substances, surface proteins, pilin gene clusters, collagen binding protein, TcpF, and gelatinase (7). A high mortality rate of enterococcal infections is due to increasing resistance of the organism to β-lactam antibiotics, aminoglycosides, and glycopeptides and inadequate response to the treatment (8, 9). Enterococci show intrinsic resistance towards broad spectrum antibiotics which is the major reason for survival of this organism in the hospital environment. They have ability to acquire resistance through mutation, plasmid or through transposons (10). Vancomycin, a glycopeptide antibiotic, is used for enterococcal infections in case of resistance to high level beta-lactam agents or hypersensitivity to beta-lactam agents (11). After the first report of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in England by Uttley et al. (12) in 1988, vancomycin resistant enterococci (VRE) isolates have also been reported from United Kingdom, France (13) and the United States (2) within a short period of time. The National Healthcare Safety Network at the Centers for Disease Control and Prevention has reported that among enterococci causing UTIs, vancomycin resistance has been seen in 81% of *E. faecium* and 6% of *E. faecalis* (14). In 1999, India reported its first case of VRE at New Delhi (15). Its prevalence at Indian subcontinent varies from 1- 9% (16).

The main objective of this study was to evaluate the prevalence of VRE among urinary isolates and to determine their antimicrobial resistance pattern as there are limited published data available on VRE from the study region.

Materials and Methods

This is a retrospective observational study conducted in the Department of Microbiology at a tertiary care hospital from November, 2017 to October, 2018. A total of 12129 urine samples were received from various outpatient departments, in patient departments and intensive care units for aerobic culture in Bacteriology laboratory during the study period. Specimens were subjected to culture on HiCrome UTI agar (Hi-Media laboratories, Mumbai) and incubated at 37 °C for a period of 18-24 hours. *Enterococcus* species isolated from the urine specimen were analyzed for antimicrobial sensitivity as well as relation with age and sex. Identification of *Enterococcus* was done on the basis of colour of the colony on agar plate as per the manufacturer's instruction. Species identification was done on the basis of biochemical test as per standard laboratory protocol and in one case Automated Siemens Microscan Walkaway identification system was used for confirmation. Significant bacteriuria was defined as ≥105 colony-forming units (CFU)/mL except for samples from extreme age groups and patients who were already on antibiotics for which a cut off value of $\geq 10^3$ CFU/mL was considered. In case of suprapubic aspirate any colony count was considered significant. Antimicrobial susceptibility testing was performed by using Kirby–Bauer disc diffusion method and

interpretation was done as per Clinical and Laboratory Standards Institute (CLSI) guideline, 2018. *Enterococcus* species were tested against ampicillin, high level gentamicin, norfolxacin, erythromycin, nitrofurantoin, vancomycin, teicoplanin and linezolid. Minimum inhibitory concentration (MIC) of vancomycin was detected by using E-strip method (Hi-Media Laboratories, Mumbai). *E. faecalis* ATCC 29212 was used as a quality control strain for performing antimicrobial tests. This study was approved by hospital ethics committee with reference number AIIMS/IEC/2019/1072.

Statistical Analysis

The interpretation and analysis of the data were done by using Microsoft Excel. The quantitative data were expressed as numbers and percentages in tabular form and figures.

Results

Out of total 12,129 urine samples received during the study period, 4.06% (492/12129) isolates was *Enterococcus* species of which 7.52% (37/492) was vancomycin resistant *Enterococcus* (VRE). Maximum numbers of isolates were from female patients 51.42% (253/492) with male and female ratio 1:1.06. The age distribution was ranged from 5 day to 93 years with maximum number of isolates were from >60 years age group 28.25% (139/492) followed by 21 to 40 years 27.64% (136/492). Age and sex wise distribution of culture positive cases was shown in Table 1. Alarmingly, 2.24% (11/492) isolates were from infants. Age and sex wise distribution of VRE isolates was shown in Figure 1. Antimicrobial resistance profile of VRE isolates showed maximum resistance towards teicoplanin 100% (37/37) which is another glycopeptide followed by ampicillin (89.19%). Resistance pattern of VRE isolates towards various antimicrobials was shown in Figure 2.

Minimum inhibitory concentration (MIC) of vancomycin in all VRE isolates was ≥ 256 µg/mL, determined by epsilometer test. Out of total 37 VRE isolates, 24 (64.86%) were *Enterococcus faecalis*, 12 (32.43%) were *Enterococcus faecium* and one was *Enterococcus raffinosus* (by Microscan Walkaway system).

Table 1. Age and sex wise distribution of *Enterococcus* species, isolated in urine culture.

Age group	Culture positive Females, n (%)	Culture positive Males, n (%)	Total culture positive, n (%)
<1 year	4 (0.82)	7 (1.42)	11 (2.24)
1-20 year	35 (7.11)	46 (9.35)	81 (16.46)
21-40 year	105 (21.34)	31 (6.30)	136 (27.64)
41-60 year	64 (13.01)	61 (12.40)	125 (25.41)
>60 year	45 (9.14)	94 (19.11)	139 (28.25)
Total, n (%)	253 (51.42)	239 (48.58)	492 (100)

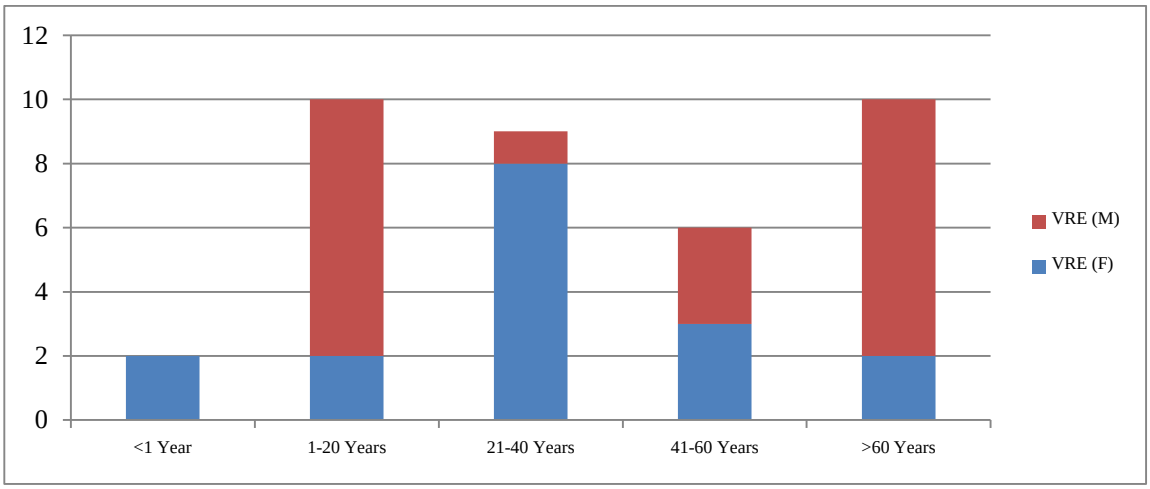

Fig 2. Age and Sex wise distribution of VRE isolates.

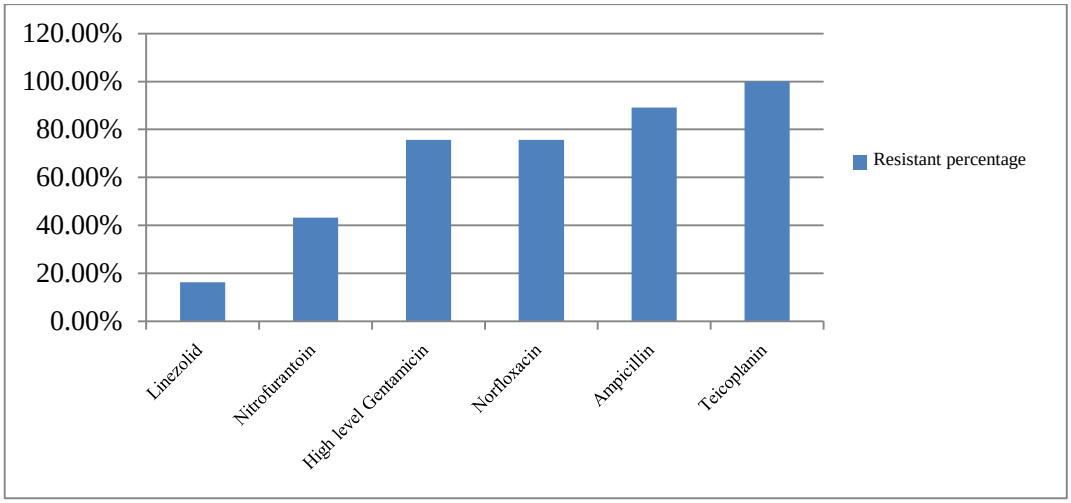

Fig 2. Resistant pattern of VRE isolates toward other commonly used drugs.

Discussion

Enterococci are Gram-positive cocci, facultative anaerobe and appear as pair and short chains under microscope (17). Their ability to tolerate the effect of various antimicrobials which limits therapeutic options and associated mortality and morbidity gains their importance. This may be attributed to various factors like empirical use of antimicrobials, prolong stay in hospital, use of immunosuppressive medications and invasive procedures etc (18). Another important concern is their ability to transfer antibiotic-resistant gene determinants to other enterococcal species and to other bacteria like *Staphylococcus aureus* (19). In last two decades infections due to Vancomycin

resistant enterococcus (VRE) have increased significantly by around 20-folds globally (20). In this study out of total 4.06% (492/12129) *Enterococcus* species, 7.52% (37/492) were VRE. A study from central India by Wavare SM et al. (21) showed similar rate (4.2%) of isolation of enterococcus species from urinary tract infections (UTI) but isolation of VRE in that study was very less (1.4%) as compared to the present study. This may be attributed to various factors but one important factor may be because of the study was three years older than the present study. A ten year retrospective study from United Kingdom by Toner L et al. (22) showed 9.8% isolation of VRE from urine culture which was slightly higher than the present study. A recent study from the same region by Meena M et al. (23) showed 3.3% isolation of *Enterococcus* species from patients having UTI which was slightly lower than the present study. However the sample size in that study was less as compared to the present study. Another older study from the same study region showed higher rate (9.24%) of isolation of *Enterococcus* species with much lower rate (1.82%) of VRE as compared to the present study (24). In the present study maximum number of *Enterococcus* species (51.42%) were from female patients of as compared to male and maximum number (28.42%) of isolates were from >60 years age group followed by 21 to 40 years (27.64%) of age group. In accordance to the present study other Indian as well as studies from different country also showed almost similar findings (21, 22, 24). In this study isolation of VRE was almost equal in both male (20/37) and female (17/37). Age group mostly affected were 1-20 years and >60 years for male patient and 21-40 years in case of female patient in this study. Male patients with genitourinary malformation and on urinary catheter were responsible mostly in the below 20 years of age group. Elderly (>60 years) males usually have higher incidence of UTI as compared to female patient of this group probably because of age related prostate enlargement and neurogenic bladder (25). In present study also *Enterococcus* species including VRE was mostly isolated from male patients of this age group. Some other studies also showed similar results (21, 24). In this study, out of total 37 VRE isolates, 24 (64.86%) were *Enterococcus faecalis*, 12 (32.43%) were *Enterococcus faecium* and one was *Enterococcus raffinosus*. Almost similar findings regarding isolation of *Enterococcus faecalis* and *Enterococcus faecium* were also observed by other Indian studies (26, 27). However one study from north India observed different finding (28). Isolation of VRE was very high (37%) in that study and *Enterococcus faecium* (96%) to be the most predominant species. The reason for this they have mentioned was all the isolates were from hospitalized patients only.

All the VRE isolates in this study were also resistant to teicoplanin which is another glycopeptide. Apart from glycopeptides most of the VRE isolates 89.19% (33/37) were resistant to ampicillin. Other important findings were 75.68% (28/37) resistance towards high level gentamicin and 16.22% (6/37) towards linezolid. Resistance towards nitrofurantoin was observed in 43.24% (16/37) isolates. Multi drug resistance (MDR) VRE isolates were 86.49% (32/37) in this study. MDR isolates were identified according to the criteria recommended by international expert committee of the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention (29). Isolates resistant to at least one antimicrobial from three different groups of drugs tested was considered as MDR. Analysis of resistance could not be done towards fosfomycin, ciprofloxacin and tetracycline due to unavailability of data of some of the isolates. In accordance to the present study, some other studies also showed high resistance towards ampicillin and low resistance towards linezolid and nitrofurantoin (22, 30). However in this study linezolid resistance was high as compared to some Indian studies in which no isolates were resistant towards linezolid which is

alarming as limited options are available to treat VRE infections (27, 28). In those studies resistance towards nitrofurantoin was also lower (~20%) than the present study. The frequencies of high level gentamicin resistance were also almost similar to those Indian studies. A study on high level gentamicin resistance from 27 European countries observed 1-49% resistance among enterococci isolates (31).

Regarding treatment of VRE causing UTI, various review articles have suggested treatment protocols. Ampicillin or amoxicillin was recommended by Heintz et al. (32) as first-line treatment for uncomplicated VRE cystitis. However in the present study resistance towards ampicillin was much higher among VRE isolates. Nitrofurantoin can be considered as first-line option on the basis of resistance profile which agrees with recommendations by other authors in uncomplicated adult VRE cystitis (33, 34). The low level of resistance towards linezolid observed in this study supports recommendations to treat VRE causing UTI in complicated cases (30, 32, 35). Source control is important step along with specific antimicrobials after performing antimicrobial susceptibility test.

This was a retrospective study in which we couldn't able to look into the significant determinants such as type of infection whether hospital acquired or community acquired, the length of hospital stay, and clinical outcome of the patients. Present study included all positive urine cultures and it was not possible to distinguish between symptomatic or asymptomatic bacteriuria. Present study results were from a single center which may not reflect the antibiogram of VRE isolates of other hospitals in the study region. Some of the data regarding types of antibiotics tested by the laboratory were missing because of which analysis of some important drugs like fosfomycin could not be done. This study was based on characterization of VRE isolates based on phenotypic and automated methods only. Molecular characterization regarding type of resistance gene would have generated more useful epidemiological results. However the minimum inhibitory concentration of vancomycin in this study was >256µg/mL for all the VRE isolates which is generally associated with van A or van B gene.

Conclusion

Enterococci were isolated in 4.06% from urine cultures out of which 7.52% was VRE in this study. Most of the VRE isolates (86.49%) were multi drug resistant in this study. For uncomplicated urinary tract infection caused by VRE, nitrofurantoin can be considered as first line treatment. For good antimicrobial stewardship and to prevent emergence of resistance, linezolid should be reserved for serious, complicated and treatment resistant urinary tract infections.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

None.

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