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URINARY TRACT INFECTIONS CAUSED BY VANCOMYCIN RESISTANT ENTEROCOCCUS: AN UPDATE FROM A TERTIARY CARE CENTRE IN NORTH INDIA

Clinical Microbiology

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ABSTRACT

Background: Traditionally considered commensals, the infections due to Enterococcus spp are increasingly becoming clinically significant due to their propensity for developing resistance to high end antimicrobials. Colonization and subsequent infection of the urinary tract with multi-drug resistant strains of Enterococcus often impose therapeutic predicaments owing to an ever narrowing spectrum of available treatment options. **Methodology:** Over a period of two years, all urine samples with pyuria and yielding Enetrococcus spp.in pure and significant numbers were included in the study. Speciation of all isolates was done. Antimicrobial susceptibility testing was done by disc diffusion and E-strip. Conventional Polymerase chain reaction (PCR) was performed on all VRE. **Results:** 250 isolates of Enterococcus were isolated from pyuric urine specimens in pure and significant counts. Prevalence of Vancomycin Resistant Enterococcus(VRE) was 7.6% and 94.7% of the phenotypically detected VRE isolates harbored the vanA gene. **Conclusion:** The isolation of strains resistant to high end antibiotics like vancomycin underscores the evolution high end resistance in gram positive urinary tract infection(UTI). The unscrupulous use of broad spectrum and intrinsically resistant antibiotics, often favor colonization and subsequent infection with these resistant strains. Furthermore the presence of vanA gene in majority of the VRE isolates threatens an impending spread of glycopeptide resistance to other gram positive agents of UTI.

KEYWORDS

Vancomycin, Enterococcus, Risk factors, UTI, genotype

INTRODUCTION:

Enterococcus is a hardy gram positive microrganism, causing a spectrum of infections.[1] Urinary tract infection is the most common enterococcal infection in hospital settings.[2] Among over twenty species of *Enterococcus* that have been isolated till date, 90% of the human infections due to *Enterococcus* are caused by *Enterococcus faecalis* followed by *Enterococcus faecium* (5-10)%. Other *Enterococcus spp.* known to cause human infections include- *Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus raffinosus, Enterococcus mundtii and Enterococcus durans*.[3] Nevertheless, very little information is available regarding these lesser studied *Enterococcus spp.* members with regard to their role in UTI and their antimicrobial sensitivity profile including vancomycin resistance rates.

A combination of penicillin and gentamicin has been the mainstay of treatment of *Enterococcus* infections until the recent past, but with the emergence of high level aminoglycoside resistance (HLAR), vancomycin is probably the only alternative available.[4] The widespread use of glycopeptides in hospitals has lately led to the emergence of Vancomycin Resistant *Enterococcus* (VRE). Now the only efficient treatment modalities for VRE and multi-drug resistant *Enterococcus* are limited to higher antibiotics like quinupristin/ dalfopristin, linezolid, tigecycline, daptomycin. However, these drugs are only approved for specific disease conditions and the resistance even to these drugs has been reported.[5]

VRE has rapidly become one of the leading cause of nosocomial infections. It is a major growing health problem owing to its intrinsic resistance to most of the commonly used antibiotics and its capability to acquire resistance either by mutation or by acquiring foreign genetic elements.[6] Among various genotypes, the principle gene postulated to induce high level glycopeptides resistance in *Enterococcus,* is *vanA*.[7] The possibility of transfer of *vanA* gene to other gram-positive organisms further raises the concern about emergence of vancomycin-resistant *Staphylococcus aureus*.[8]

MATERIALS AND METHOD:

A cross sectional study carried out in a tertiary care centre of north India extending over a duration of two years. All the urine samples coming for routine bacterial culture and sensitivity over the duration of study were cultured semi-quantitatively. Colonies resembling *Enetrococcus* colonies, if significant ($\geq 10^5$ colony forming units /ml) were inoculated on medias like: Bile-esculin media , 6.5% NaCl, potassium tellurite. All isolates that showed blackening on Bile-esculin medium and growth in 6.5% NaCl were further speciated using conventional physiological tests devised by Facklam and Collins.[10] A written informed consent was taken from all 250 patients whose urine samples yielded significant counts of *Enterococcus spp*.

The antimicrobial susceptibility testing of all the *Enterococcus spp.* isolates was performed by modified Kirby-Bauer disc diffusion method as per CLSI guidelines.[11] The MIC of Vancomycin and Teicoplanin was determined by E-strip method and interpreted as per EUCAST criteria.[12]

All the VRE isolates were evaluated for the presence of *vanA* gene by PCR. DNA was extracted using HiPur™ Bacterial Genomic DNA Extraction kit(Himedia, Mumbai, India) as per manufacturer's instructions. The primers for *vanA* gene were synthesized as per previous literature.[13] (Sigma-Aldrich Co., St. Louis, MO, USA) For performing PCR, 25µl reaction mixture comprising of 3µl of extracted genomic bacterial DNA and 1µl of forward and 1µl reverse primers, each at a concentration of 10µmol/µl was prepared in a sterile PCR tube. The conditions for PCR are as follows: 3 minutes of initial denaturation at 95^0C, followed by 35 cycles of amplification at 95^0C for 45 seconds, 47.5^0C for 35 seconds and 72^0C for 35 seconds. This was followed by a final step of extension at 72^0C for 3 minutes.[13] The PCR products were visualized under uv light after gel electrophoresis with 1% agarose and ethidium bromide staining, using a 100bp DNA ladder. A predesigned, semi-structured performa was used for recording the presence of risk factors associated with UTI due to VRE. To assess the risk factors, logistic regression was done and p-value<0.05 was considered significant. All analysis was done by SPSS statistical software version 20.0

RESULTS

Among the 250 isolates of *Enetrococcus spp.* obtained in significant counts, *Enterococcus faecalis* was the predominant species accounting for 62% (155/250) followed by 30.4% (76/250) *Enterococcus faecium*, 3.6% (9/250) *Enterococcus dispar*, 3.2% (8/250) *Enterococcus hirae* and 0.8% (2/250) *Enterococcus raffinosus* .

The distribution of MIC ranges of Enterococcal isolates for Vancomycin by E-test is depicted in Table-1.The prevalence of VRE in cases of urinary tract infection was 7.6% (19/250). Resistance to

vancomycin was most commonly seen among isolates of *Enterococcus faecium* 18.4% (14 /76) followed by *E.faecalis* at 3.2 % (5/155) isolates. All isolates of *E. dispar*, *E. hirae* and *E. raffinosus* were sensitive to vancomycin (Table-1). 31.6% (6/19) isolates with vancomycin MIC ranging between (64 –1000) µg/ml were probably of vanA phenotype and four isolates belonged to vanB phenotype with vancomycin MICs between (4 –1000) µg/ml.[14] The overall prevalence of *vanA* gene among phenotypic VRE isolates was 94.7% (18/19).

All strains that showed beta hemolysis were found to be sensitive to vancomycin, while 73.7% isolates of VRE were alpha-hemolytic and 26.3% showed no hemolysis on 5% sheep blood agar. This variation of hemolysis based on sensitivity to vancomycin was found to be statistically significant ($p<0.05$). Study isolates showed susceptibility to antibiotics by modified Kirby-Bauer disc diffusion as follows: Linezolid (100%), Fosfomycin (96.8%), Teicoplanin (92%), Nitrofurantoin (57.6%), High-level-Aminoglycoside (57.2%), Ciprofloxacin (35.6%), Ampicillin (29.2%) and Tetracycline (12.8%). Table 2 shows the comparison of resistance to antimicrobial agents among VRE and VSE isolates. .

The distribution of risk factors among vancomycin resistant and susceptible study isolates is shown in Table 3. 79% of the patients, from whom VRE was isolated, were exposed to 3rd generation cephalosporins, 42.1% were exposed to aminoglycosides while, 3.7% and 3.2% patients were exposed to vancomycin and carbapenems respectively for ≥3days. Among 19 patients whose urine samples yielded VRE, 63.2% were treated with at least three antibiotics from different classes like 3rd generation cephalosporins, aminoglycosides, carbapenems, glycopeptides and metronidazole for ≥3days.

DISCUSSION

E. faecalis followed by *E. faecium* were the most common *Enterococcus spp*. isolated, which was in line with many other Indian studies.[15,16] Other Indian studies have also established *E.faecalis* as the predominant *Enterococcus spp.* isolated from urine. [17,18] The higher prevalence of *E.faecalis* infections could be attributed to the predominance of *E. faecalis* in the endogenous flora of the body. However, Telkar et al. in their study have reported *E.faecium* as the predominant species.[19]

The prevalence of VRE in UTI cases varied from 1% to 26.1%, among the various provinces of North America with the median prevalence of VRE as (9.2 ± 7.2)%. More recently, a similar study in the UK, showed the prevalence of VRE in urine samples to be 9.8%.[23] Similarly in our study prevalence of VRE associated UTI was 7.6%. According to studies conducted from western to the north eastern province of India the percentage of VRE in urine samples ranged from 3.2% to 21.4% [22] However, other Indian studies conducted by Tripathi et al., Biswas et al., [23] Goel et al. [20] have reported a higher prevalence of 9.3%, 11% and 11.3% respectively among urinary isolates.

E.faecium and *E. faecalis* were the only two *Enterococcus spp*. that comprised all VRE isolates. This was in concordance to the other studies conducted in north India.[9,24] *E. faecium* (73.7%) was the major species isolated among the VRE isolates. Our findings are in line with other studies done from other geographic regions. [9,21 ,24] In the present study none of the other than above mentioned *Enterococcus spp*. were found to be VRE. However, other studies have reported VRE among *E. gallinarum*, *E. durans* and *E.raffinosus*. [20,23]

Concordant to other studies *E.faecalis* was more sensitive to antimicrobial agents in comparison to *E.faecium*.[25,26] The prevalence of HLAR in our study was 42.8% similar to as reported by Goel et al. (39.1%). [20] In the present study we have observed a statistically significant difference in the Nitrofurantoin sensitivity among VSE and VRE isolates ($p=0.004$). To the best of our knowledge, no other research has documented any statistically significant association between resistance to nitrofurantoin and vancomycin. All isolates in our study, including VRE, were found to be uniformly sensitive to Linezolid, which was in agreement with other studies.[20]

31.6% VRE isolates had vancomycin MIC ≥256 µg/ml. However, Maradia et al. have reported 20.5% of VRE isolates with vancomycin MIC ≥256µg/ml. This can be explained on basis of the difference in antibiotic pressure to which these VRE isolates might have been exposed to and the geographic variations in the strains. We could not comment on MIC creep due to the limited research on VRE in the same setting. The *vanA* gene cluster was present in 94.7% of VRE isolates included in our study. NAVRESS 2002 study conducted in North America has reported a *vanA* gene prevalence of 83.8% among VRE isolates.[10] Another study by Bhatt et al. from western India has reported a 100% prevalence of *vanA* gene among VRE isolate.

Traditionally considered as low grade pathogens, *Enterococcus spp*. mainly colonize ulcers, gastrointestinal tract and soft tissue wounds of hospitalized patients. They are increasingly becoming nosocomial pathogens of interest. In our study we have found that 36.8% of the VRE infected patients were from ICU settings. Likewise, data analysis of National Nosocomial Infection Surveillance system demonstrated, ICU as depot of drug resistance, with 28.5% of nosocomial VRE being isolated from ICUs in 300 U.S. hospitals.[10] Similarly in a north Indian study, conducted by Tripathi et al. 34.7% of all nosocomial VRE were isolated from ICU patients.[24] There are ample studies available, concerning the prevalence of associated risk factors for enterococcal UTI, however, there is limited literature regarding the risk factors associated with UTI due to VRE which we have tried to address in the present study. Most pathogens of the urinary tract establish infection by the ascending route, catheter care and early removal of urinary catheters, when no longer needed, should be encouraged. Since a significant number of patients presenting with UTI due to VRE are catheterized, daily meatal hygiene by healthcare staff, emptying of urine bags and hand hygiene practices performed before and after procedures as components of bundle approach can reduce the incidence of catheter associated UTIs. Limiting the duration of hospital stay, empirical broad spectrum antibiotic therapy and vigilant management of comorbid conditions like diabetes, prostatic conditions can reduce the risk of VRE associated UTI.

CONCLUSION

Enterococcus is rapidly emerging as an important pathogen for nosocomial and community acquired infections, especially due to its tendency to acquire and spread resistance to a spectrum of antibiotics. The *vanA* gene, conferring inducible high level resistance to higher antibiotics like vancomycin and teicoplanin, is widely detected in VRE isolates from urine samples. This further accentuates the risk of transferring glycopeptides resistance to other colonizer organisms. Appropriate maintenance of antibiograms and formulation of hospital antibiotic policies is advocated to curb inappropriate use of antibiotics and further emergence of glycopeptides resistance, which continues to pose an ongoing therapeutic challenge.

Conflict of interest:

Authors declare no conflict of interest.

Table 1: Vancomycin MIC ranges of study isolates by E-test

MIC of vancomycin (µg/ml)	E. faecalis n=155 (%)	E. faecium n=76 (%)	E.raffinosus n=2 (%)	E. hirae n=8 (%)	E.dispar n=9 (%)	Total n=250 (%)
≤ 4	150 (96.77)	62 (81.58)	2 (100)	8 (100)	9 (100)	231 (92.4)
5-24	4 (2.58)	5 (6.58)	0(0)	0(0)	0(0)	9 (3.6)
32-128	0 (0)	4 (5.23)	0(0)	0(0)	0(0)	4 (1.6)
≥ 256	1 (0.65)	5 (6.58)	0(0)	0(0)	0(0)	6 (2.4)

Table 2: Comparison of resistance to antimicrobial agents among vancomycin resistant and susceptible study isolates

Antimicrobial agents	VRE n=19 (%)	VSE n=231 (%)	p value
Ciprofloxacin	16 (84.21)	145 (62.77)	0.081
Nitrofurantoin	14 (73.68)	91 (33.93)	0.004
Gentamicin(120µl)	17 (89.47)	88 (38.09)	0.000
Ampicillin	16 (84.21)	161 (64.4)	0.204
Fosfomycin	0 (0)	4 (1.7)	1.000
Teicoplanin	15 (78.94)	5 (2.16)	0.000
Linezolid	0 (0)	0 (0)	-
Tetracycline	17 (89.47)	201 (87.01)	1.000

Table 3: Predisposing factors to UTI caused by vancomycin resistant and susceptible study isolates

Risk factor	VRE n=19(%)	VSE n=231(%)	p value
Female Gender	14(73.69)	152(65.80)	0.67
In-patient departments	17 (89.47)	134 (58)	0.001

Duration of hospital stay	14(73.68)	42(18.18)	0.000
Present medications	19 (100)	169 (73.16)	0.001
Duration of medication	10(52.63)	11(7.53)	0.004
Catheterization	14(73.68)	105(45.45)	0.029
Duration of catheterization	11 (57.89)	59 (25.54)	0.004
Comorbidities	10 (52.63)	44(23.53)	0.000
Previous history of UTI	3(15.79)	22(28.57)	0.291
Surgical intervention	12 (63.12)	70 (30.30)	0.000

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