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Prevalence and Risk Factors for Vancomycin Resistant Enterococci Isolated from Clinical Samples in Kashmir, North India: A Hospital Based Study

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Authors' contributions

The work has been carried out in collaboration between all the authors. Author JA carried out the study. Author DK managed the literature searches. Author NB wrote the protocol, managed the literature searches, analyzed the results and wrote the initial and final draft of the paper. Authors SL, HB and BF helped in literature searches. All the authors read and approved the final draft of the manuscript.

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Original Research Article

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ABSTRACT

Aims: To find out the prevalence and risk factors for vancomycin resistant Enterococci in a leading tertiary care center of north India.
Design: Cross sectional study.
Place and Duration of Study: Sher-I-Kashmir Institute of Medical Sciences, Srinagar. Kashmir. One year study.
Methodology: A total of 400 isolates of Enterococci from patients admitted to our hospital were recovered using standard microbiological procedures, during a period of one year. Antimicrobial

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susceptibility of these isolates to various antibiotics was performed according to Clinical Laboratory Standard Institute (CLSI) guidelines. Minimum inhibitory concentration (MIC) of isolates found to be resistant to vancomycin on disc diffusion was done by microbroth dilution method. Various risk factors like placement of IV line catheter, urinary catheter, hospital stay and prior use of antimicrobial agents was noted for all the patients.

Results: A total of 25 (6.3%) isolates of Enterococci were found to be vancomycin resistant, most of them recovered from the blood samples. *E. faecium* 16 (64%) was the predominant VRE isolated followed by *E. faecalis* 9 (36%). Factors like stay in an ICU, prior use of antimicrobials, placement of IV line and urinary catheter were associated with vancomycin resistant Enterococci (VRE) acquisition.

Conclusion: VRE were recovered from our hospital and strict adherence to infection control guidelines needs to be followed to control their dissemination.

Keywords: Enterococci; vancomycin; VRE.

1. INTRODUCTION

Enterococci especially *E. faecium* and *E. faecalis* have emerged as important nosocomial pathogens in the last few decades due to increasing antimicrobial resistance seen in them [1]. These organisms have been implicated in causing bacteremia, urinary tract infections, peritonitis, surgical site infections, etc., in the hospital settings worldwide. In the Indian scenario, Enterococci are emerging nosocomial pathogens that are increasingly being isolated from a variety of clinical conditions like urinary tract infections and bacteremia [2].

The emergence of vancomycin resistance in Enterococci (VRE) represents a worst case scenario for clinicians as their ability to treat infections caused by these strains is compromised. Furthermore nosocomial spread of these pathogens may create a reservoir of mobile resistance genes for other more virulent organisms like *S. aureus* [3]. According to the National Nosocomial Infections Surveillance (NNIS) data from United States more than 28 per cent of all nosocomial enterococcal strains are vancomycin resistant thus making it a major problem in most of the western world [4].

While there is ample data on the prevalence of vancomycin resistance in *Enterococcus* spp, from other parts of the country there is no report that has assessed the magnitude of vancomycin resistance in clinical isolates of Enterococci from our state. This study was thus undertaken to ascertain the extent to which vancomycin resistance is a problem in Enterococci isolates and access some of the risk factors associated with acquisition of infection due to these bacteria from a leading tertiary care institute in a temperate north Indian state that attracts huge numbers of visitors from across the globe and as such is not only of major public health importance but also assumes significance in light of the health of people travelling to this state.

2. MATERIALS AND METHODS

A total of 400 enterococcal isolates were recovered from clinical samples (from patients of all age groups) like blood, pus and other body fluids, sputum and urine in the Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, over a period of one year from 1st Dec 2012 to 30th Nov 2013. The study was approved by the institute's ethical committee bearing clearance number SIMS 131/IEC-SKIMS/2014.

Blood samples were processed by BacT/ALERT 3 D (BioMerieux Inc. USA) automated system, semi-quantitative analysis of urine samples was done using Hichrome UTI agar and blood agar plates whereas pus other body fluids were plated onto blood agar and MacConkey agar plates with a backup in Robertson's cooked meat broth. All the culture plates were incubated at 37ºC for 24 hrs. Gram positive, catalase negative cocci were classified as Enterococci on the basis of growth in the presence of 40% bile and subsequent hydrolysis of esculin and growth in 6.5% NaCl. Species level identification of *Enterococcus* isolates was done as per the Facklam and Collin's phenotypic characterization scheme [5].

Isolates of Enterococci were preserved in brain heart infusion broth with 10% glycerol and stored at -70ºC; fresh cultures being prepared from these stock cultures whenever required.

Antimicrobial susceptibility testing was done on Muller Hinton agar plates by Kirby-Bauer disc diffusion method as per the CLSI guidelines [6].

Antibiotic discs used included penicillin (10 units), ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg) vancomycin (30 µg) and linezolid (30 µg). Also urinary isolates were tested with nitrofurantoin (300 µg) discs. In addition to these, all the isolates were screened for high level gentamicin (HLGR) and high level streptomycin (HLSR) resistance, using gentamicin (120 µg) and streptomycin (300 µg) discs. Zone of inhibition around the discs was interpreted as sensitive, intermediate or resistant as per CLSI guidelines [6]. *Staphylococcus aureus* ATCC 25923 was used as a quality control strain for disc diffusion method.

Minimum inhibitory concentration (MIC) of vancomycin was done by microbroth dilution method in brain heart infusion broth. Concentration of vancomycin used was in the range of 64-0.06 µg/ml. Fifty microlitre of bacterial suspension (turbidity 0.5 McFarland standards) was dispensed into the wells of a microtitre plate which was incubated at 35ºC for 24 hours and results read the other day. *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212 strains were used as positive and negative control respectively. MIC endpoint was read as the lowest concentration of antibiotic at which there was no visible growth. MIC interpretive criteria for vancomycin was; ≤4 µg/ml: susceptible; 8-16 µg/ml: intermediate and ≥32 µg/ml: resistant. MIC of linezolid for isolates found resistant to it on disc diffusion test was done by E-test method.

Several risk factors like presence of any intravenous catheter, urinary catheter, prior use of antibiotics (including vancomycin) and length of hospital stay were studied in the patients from whom VRE were isolated.

All the discs, media, antibiotic powder and control strains were procured from Himedia Laboratories Pvt. Ltd., Mumbai.

2.1 Statistical Analysis

Z- test for difference between two proportions was employed for statistical analysis of the results. Two sided p-values were reported. Analysis was done using Primer of Biostatistics software (Primer of Biostatistics, version 5.0, Mc-Graw Hill Global Education Holdings LLC, Columbus, OH).

3. RESULTS

Twenty five (6.3%) out of the 400 isolates of Enterococci were found to be vancomycin resistant (VRE) whereas 375 (93.7%) were sensitive to this glycopeptide (VSE). Majority of VSE were *E. faecalis* 272 (72.5%) with 101 (26.9%) isolates being *E. faecium* and 2 (0.5%) being *E. durans*; whereas a significant number of VRE were *E. faecium* 16 (64%) with only 9 (36%) isolates being *E. faecalis*; (p<0.001). VRE were isolated more from male patients (76%), than female patients (24%) in contrast to VSE that were isolated more from female patients, (57.1%) than male patients (42.9%). Significantly higher isolation of VRE (p=0.040) was seen in patients belonging to the age group of ≥60 years (40%).

Both VSE (56.8%) as well as VRE (60%) were isolated more from urine samples. Specimens received from patients housed in the SICCU yielded maximum number of VRE isolates (28%) in contrast to VSE that were recovered more form the OPD samples (35.3%); (p=0.001). Most of the patient's from whose samples VSE were isolated had UTI (n=183; 48.8%); whereas VRE were isolated more from patients of septicemia (n=8; 32%).

Isolation of VRE was seen more from patients having peripheral IV line catheters (n=25, 100%), indwelling urinary catheters (n=19, 76%) and those with history of hospital stay >10 days (n=17, 68%). Prior use of β-lactam antibiotics, cephalosporins, fluoroquinolones and vancomycin were noted more often in patients from whom VRE were isolated than from whom VSE were isolated Table 1.

For 19 (76%) VRE isolates the MIC of vancomycin was 64, whereas for 6 (24%) it was 32. All the *E. faecium* isolates; 100% and 12% *E. faecalis* isolates had high level vancomycin resistance (MIC ≥64 µg/ml).

Of the 375 VSE isolates 97.9% were resistant to penicillin, 72.3% to ampicillin, 60.8% to chloramphenicol and 69.6% to tetracycline. In comparison all isolates of VRE were resistant to penicillin; 100%, with 68% showing resistance to ampicillin. Chloramphenicol resistance was seen in 56% isolates and 64% VRE were resistant to tetracycline. Nitrofurantoin resistance was seen in 54.7% VSE and 46.7% VRE isolates recovered from urine.

HLGR was seen in 24% VRE isolates whereas for VSE, HLGR was seen in 18.9% of the isolates. HLSR was seen in 32% VRE isolates with 22.9% VSE isolates exhibiting resistance to this aminoglycoside. Two isolates (0.5%) of Enterococci were found to be resistant to

Table 1. Association of vancomycin resistance with some risk factors

Risk factors	VSE (N=375)	VRE (N=25)	p value*	95% CI**
	N (%)	N (%)		
β-lactam \ intake	268 (71.5)	23 (92)	0.026 (S)	0.0248 to 0.3852
Cephalosporin intake	172 (45.9)	18 (72)	0.011 (S)	0.0588 to 0.4632
Quinolone intake	215 (57.3)	16 (64)	0.511 (NS)	- 0.133 to 0.267
Vancomycin intake	87 (23.2)	12 (48)	0.005 (S)	0.0733 to 0.4227
IV line catheter	223 (59.5)	25 (100)	<0.001 (S)	0.2085 to 0.6015
Urinary catheter	158 (42.1)	19 (76)	0.001 (S)	0.1379 to 0.5401
Hospital stay >10 days	228 (60.8)	17 (68)	0.474 (NS)	- 0.1252 to 0.2692

**Z-Test (for difference between two proportions) ** 95% CI for difference between proportions*

linezolid in addition to being resistant to vancomycin (LRVRE). MIC of both the isolates for linezolid, confirmed by E-test method was >8 µg/ml and both were *E. faecium*.

4. DISCUSSION

The mainstay of treatment of serious enterococcal infections is a combination of penicillin/ampicillin or vancomycin and an aminoglycoside. However, high level resistance to these antibiotics has been reported increasingly in recent years fueled in part due to the inadvertent use and misuse of these agents [7-9]. An understanding of the local ecology of drug resistant bacteria and an insight into factors that contribute to their acquisition and subsequent infection can help reduce morbidity and mortality due to them.

The prevalence of VRE in our study was 6.3%, lower than what has been reported from different parts of the country. Praharaj I et al. in their study found the prevalence of VRE to be 8.7% in isolates of Enterococci recovered from clinical samples [9]. Like wise Shah L et al. reported 8% enterococcal strains to be resistant to vancomycin [7]. Gangurde N et al. in their study of 180 enterococcal isolates recovered from clinical samples found that the prevalence of VRE was 8.3% [10]. Higher isolation of VRE, 19.6% was reported by Deshpande VR et al. in their study on the prevalence of multidrug resistant Enterococci from a tertiary care hospital [11].

E. faecalis was the most common species isolated (70.2%) followed by *E. faecium* (29.3%), consistent with many studies where a greater isolation of *E. faecalis* from clinical specimens was seen [7,8,11,12]. The predominance of *E. faecalis* in the endogenous flora of the body could be the reason behind its high proportion among hospital isolates. Among the two species, vancomycin resistance was seen more in *E. faecium* isolates; 64%, with only 36% of *E. faecalis* isolates being resistant to the said glycopeptide, a finding corroborated by many earlier studies [10,11,13,14]. However Shah L et al found *E. faecalis* to be more resistant to vancomycin [8].

Commonest enterococcal infections include the ones involving the urinary tract, hepatobiliary sepsis, endocarditis, surgical site infection, bacteremia and neonatal sepsis [15]. We found that majority of the patients from whom VSE was isolated had UTI, however a significantly higher isolation of VRE was seen from patients who had septicemia. Interplay of a number of factors like advanced age, placement of intravenous lines, and admission in an intensive care unit in these patients could have been the reason behind the greater isolation of VRE in them. Maximum VRE were recovered from urine, followed by blood samples. Similar results where a higher isolation of VRE was seen from urine and blood have been reported in the past [8,13,16].

Risk factors known for acquiring infection due to VRE include prolonged hospitalization, immune-suppression, stay in SICCU, oncology or transplant wards, surgical procedures and previous treatment with vancomycin and other antimicrobials. Also, VRE are now being isolated with increasing frequency from patients with CRF, cancer, organ transplant and those with placement of urinary catheters or central intravenous catheters for prolonged periods [17].

In this study factors like presence of peripheral IV line catheters, indwelling urinary catheters and admission in SICCU were found to have significant association with the VRE isolation. Also VRE were isolated more from the samples of patients with a >10 days hospital stay although the difference was not significant form those whose samples yielded VSE.

Prior use of β-lactam antibiotics, cephalosporins and vancomycin were found to be significantly higher in patients from whom VRE were isolated. Parenteral as well as oral vancomycin and third-generation cephalosporins [18,19,20] have been cited as risk factors for colonization or infection with VRE [21]. Vancomycin, by inhibiting the growth of the normal Gram-positive bowel flora and providing a selective advantage for VRE that may be present in small numbers in the individual's bowel is thought to predispose patients to colonization and infection with these bacteria [17].

High level resistance to various anti-enterococcal antibiotics like penicillin, ampicillin, tetracycline and chloramphenicol was seen in our study with two isolates of Enterococci being resistant to linezolid; one of which belonged to a patient suffering from carcinoma lung and the other was from a patient of septicemia. Recovered from the blood of these patients both the isolates were phenotypically characterized as *E. faecium* and exhibited high level resistance to vancomycin (>64 µg/ml). MIC of linezolid for both the isolates was >8 µg/ml.

The use of linezolid has increased in response to increase in methicillin resistant *S. aureus* (MRSA) infection in this hospital [22] and also due to its good bioavailability and no requirement for blood-level or renal-dose adjustment. Prior MRSA infection has been cited as a strong risk factor for acquisition of linezolid resistant Enterococci (LRE) [23]. However since none of the patients from whom LRE were recovered had an evidence of prior MRSA infection; a possibility of patient to patient transmission of LRE cannot be ruled out in this situation. Outbreaks due to linezolid resistant Enterococci, and cases of linezolid resistant vancomycin resistant *E. faecium* infection have been reported in the past [24-26].

High level resistance to aminoglycosides (HLAR) is also of great clinical concern, since it eliminates synergy with cell wall active antibiotics. In our study 24% VRE isolates were found to have HLGR with 32% having HLSR. This is lower than what has been reported from other parts of the country [27,11].

Small numbers of LRE and LRVRE isolates in our study limit our understanding of the contribution of various risk factors for vancomycin resistance; additional studies are required in this respect. Also, the need to go beyond the phenotypic predictors and look for the genetic basis of resistance in VRE can not be over emphasized.

5. CONCLUSION

In conclusion, although VRE are prevalent in our hospital in numbers smaller than those reported from other parts of the country, their presence should be viewed as a serious concern as they carry transferable vancomycin resistance markers. Moreover isolation of LRVRE and strains with high resistance to aminoglycosides complicates the scenario further as treatment options are limited. Continued surveillance and close monitoring of these isolates is necessary in order to keep tract of any changes in their susceptibility profiles. It is a well-known fact that the solution to the most complex problems is often very simple; thus strict adherence to the current infection control guidelines, judicious use of the available antimicrobial agents, and targeted evidence based therapy is the need of the hour.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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