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Original Research Article

Study of Antimicrobial Resistance Pattern in Clinical Isolates of Enterococci in a Tertiary Care Hospital of Bikaner Region of Rajasthan with Special Reference to Vancomycin

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ABSTRACT

Objectives: To determine the antibiogram of Enterococci, and to detect Vancomycin resistance by various methods, viz. Kirby Bauer disk diffusion, Vancomycin screening agar, and E test.

Materials and Methods: This study includes all the Enterococci isolated from various samples. Antibiotic testing was done by Kirby Bauer's Disc Diffusion method. Additional testing for vancomycin resistance was done with BHI agar containing 6 µg/ml vancomycin, and the Epsilometer test (E-Test).

Results: A total of 194 isolates of Enterococci were obtained within the study period, of which 88.66% were E. faecalis, and 11.34% were E. faecium. By the Kirby Bauer Disk Diffusion method, 88.65% of the isolates were resistant to penicillin, 57.73% to ampicillin, 70.10% to erythromycin, 22.68% to high level gentamicin, 13.40% to vancomycin, and 5.15% to linezolid. 15.46% of the isolates indicated resistance by the BHI agar screening method. The E test showed 13.40% resistance vancomycin, and 2.06% intermediate sensitivity.

Conclusion: The rising incidence of resistance among enterococci should prompt clinicians to adhere to the antibiogram provided by the microbiologists. Screening for vancomycin resistance by BHI agar containing vancomycin is an effective and cheap method. The E test may be used as a confirmatory test or to obtain the MIC.

Keywords: Enterococci, Vancomycin-Resistant Enterococci, Antibiotic Resistance, Emerging Drug Resistance.

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INTRODUCTION

Resistant bacteria dramatically reduce the possibilities of treating infectious diseases effectively and multiply the risk of complications. In addition, antibiotic resistance jeopardizes advanced medical procedures such as organ transplantations and implants of prosthesis, where antibiotics are crucial for patient safety and to avoid complications.[1] There has been a great deal of concern in recent years about the growing menace of antimicrobial-resistant organisms.[2]

Resistance is a natural biological outcome of antibiotic use. The more we use these drugs, the more we increase the speed of emergence and selection of resistant bacteria. The relationship between antibiotic use and resistance is complex. Underuse, through lack of access to antibiotics, inadequate dosing and poor adherence to therapy may play as important role in driving resistance as over-use.[1]

Arguably the most impressive accomplishment of bacteria to date in this arena has been the development of Vancomycin resistance in Enterococci.[3] Moreover, in contrast to coliforms and other intestinal bacteria, the Enterococci are rather tough and can survive for long periods of time in soil and water, and thus re-enter the food chain.[4]

Apart from Enterococcus faecalis and Enterococcus faecium which are predominately isolated from clinical samples, the much less commonly isolated species include E. gallinarum, E. casseliflavus, E. durans, E. avium and E. raffinosus.[5]

The most frequent infections caused by Enterococci are UTI. The second most frequent infections are intra-abdominal and pelvic sepsis and surgical wound infections, in which Enterococci are almost always a part of a mixed flora of colonic organisms. The third most frequent infections are bacteraemia, including both primary bacteraemia i.e. from source in the GIT and secondary to the urinary tract and intra-abdominal infections or from the use of intra vascular devices. Although Enterococcal endocarditis is relatively rare, it is difficult to treat because of the relative

resistance of Enterococci to antimicrobial agents. Meningitis represents an even rarer but potentially serious infection caused by Enterococci.[6]

Currently there is no ideal therapy which yields bactericidal activity for serious infections caused by VRE. Above all, assessing the efficacy of therapy remains difficult because VRE is often associated with severe underline illness and can be a part of polymicrobial infections. Prudent use of Vancomycin and a proper surveillance for VRE may permit early recognition and containment of spread of this emerging pathogen in our country.

Even when a single agent or a combination of agents show in vitro activity against a particular VRE strain, overall therapeutic efficacy may be <70%.[7]

AIMS AND OBJECTIVES

1. To isolate Enterococci from various clinical samples of PBM and associated group of hospital, Bikaner upto species level.
2. Determine the antibiogram of the isolated Enterococci.
3. To detect Vancomycin resistance among the isolates by the various methods :

MATERIALS AND METHODS

Study Centre

The present study was carried out on 194 isolates in the Department of Microbiology, S.P.M.C. Bikaner from Nov 2013 to December 2014. It was a descriptive type of observational study.

Inclusion Criteria

All the enterococcal isolates from clinical samples such as blood, urine, pus, wound swab, catheter tip and other body fluid were included.

Exclusion Criteria

All commensal enterococcal isolates from anatomical sites like the gastrointestinal tract, female genital tract, and oral cavity were excluded.

Permission and Ethical Consideration

Permission for this study was obtained from the Institutional Ethics Committee.

Identification of Enterococci was done by various methods such as Gram's staining & colony characteristics were studied on Blood agar & MacConkey's agar. Speciation was done by various biochemical tests as per standard guidelines.

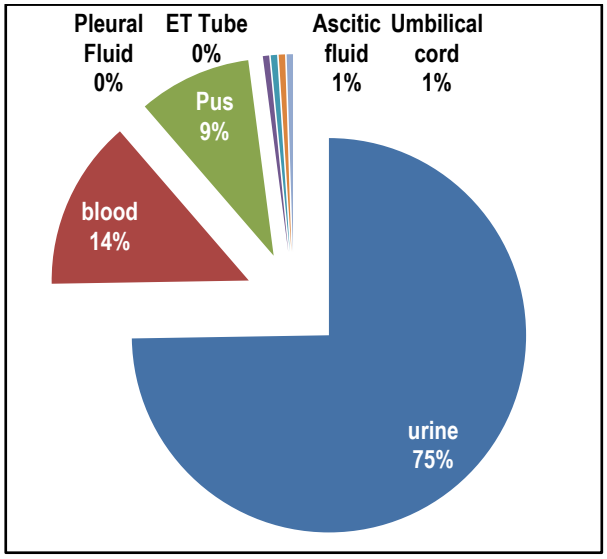


Fig 1: Enterococci Isolated from Various Samples

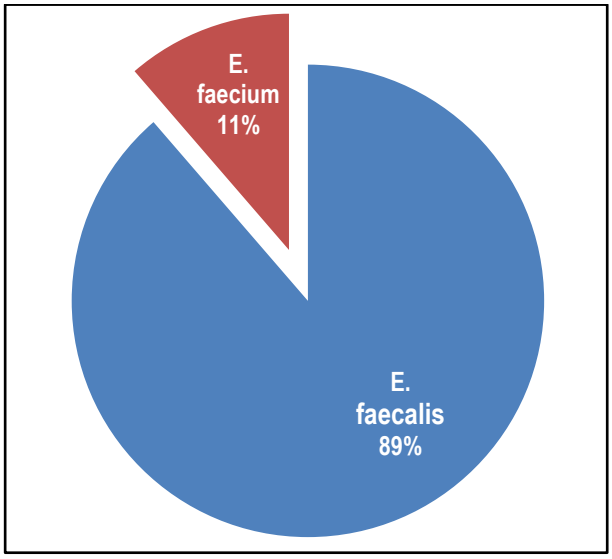
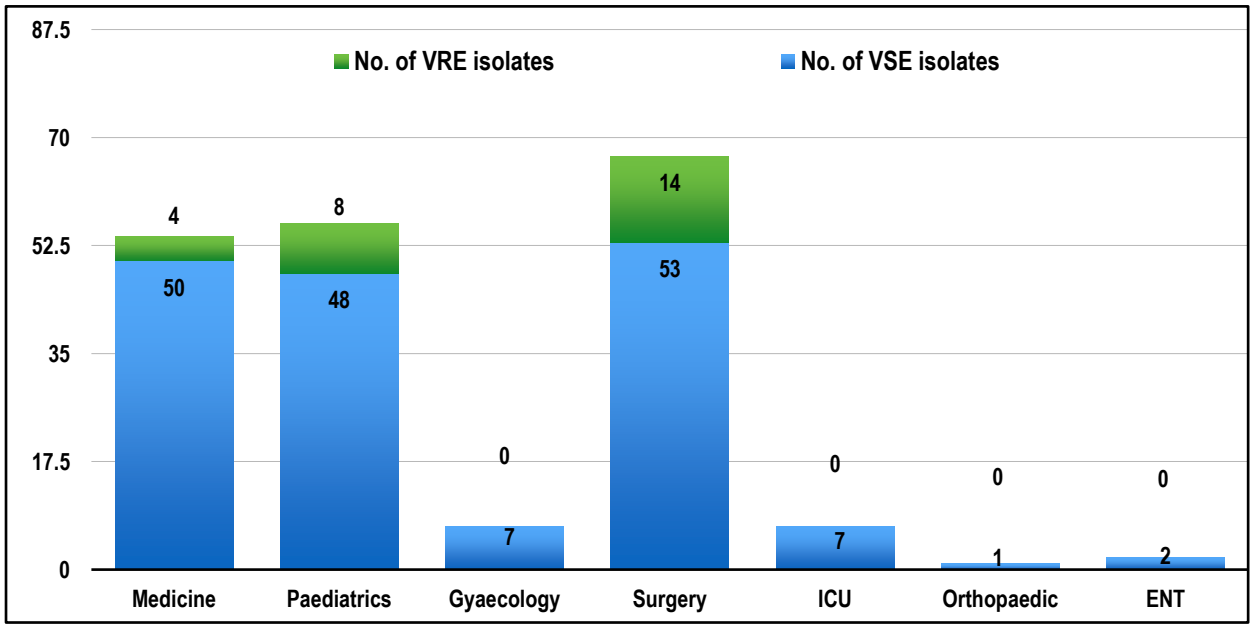


Fig 2: Entercocci species in clinical samples

Fig 3: Ward-wise distribution of vancomycin-sensitive and vancomycin resistant enterococcal isolates



RESULTS

During the study period, a total of 194 samples yielded isolates of enterococci. A majority of the isolates were from urine samples (74.74%) followed by blood cultures (13.91%) and pus samples (9.27%). Equal numbers of isolates (0.52%) were isolated from endotracheal tube aspirate, pleural fluid, ascitic fluid and umbilical cord. (Fig.1)

172 (88.66%) of isolates were identified as *E. faecalis*, and 22 (11.34%) were *E. faecium*.(Fig.2)

34.54% of the enterococci were isolated from patients admitted in the surgery ward, followed by the Paediatric (28.87%) and Medicine (27.84%) wards. 3.6% isolates were from patients admitted in the ICU.

The Surgery (53.84%) accounted for more than half of the VRE isolates, followed by Paediatric (30.77%) and Medicine (15.39%). There were no VRE isolates accounted from Gynaecology, ICU, Orthopaedic and ENT. (Fig.3)

Among the enterococcal isolates, maximum resistance was noted to penicillin (88.65%), erythromycin (70.10%), ciprofloxacin (68.56%), ampicillin (57.73%), and amikacin (48.97%). 22.68% of the isolates were resistant to high-level gentamycin (120 µg). The enterococci were more sensitive to linezolid (94.85%) and vancomycin (86.60%).

The vancomycin-resistant strains of *E. faecalis* and *E. faecium* showed significant resistance to all the other antibiotics tested, except to linezolid. Vancomycin-resistant *E. faecalis* showed total resistance to penicillin. (Table.1)

Resistance to vancomycin was observed by 3 methods. Kirby Bauer disk diffusion indicated 14.43% resistance among enterococci to vancomycin, while BHI screening agar containing 6ug/ml vancomycin showed growth indicating resistance in 15.46% of isolates. The E-test showed intermediate sensitivity in 2.06% isolates and resistance in 13.40%. (Fig.4)

Table 1: Comparison of antibiotic resistance between VR *E. faecalis* and VR *E. faecium*

Antibiotics	VR E. faecalis (n=22)		VR E. faecium (n=4)	
	Resistance (number)	Resistance (%)	Resistance (number)	Resistance (%)
Penicillin	22	100	4	100
Ampicillin	15	68.18	3	75
Erythromycin	16	72.73	3	100
Amikacin	9	40.90	2	50
Ciprofloxacin	17	77.27	4	100
Gentamycin (HL)	10	45.45	2	50
Linezolid	8	36.36	2	50

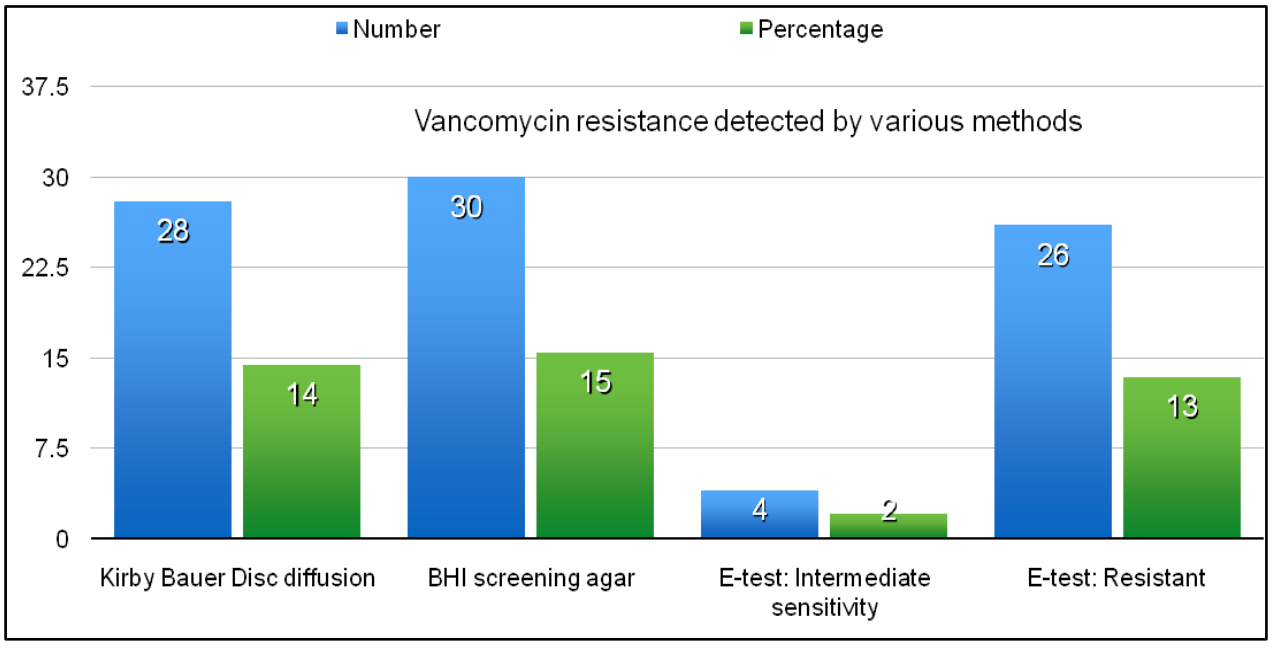


Fig 4: Vancomycin resistance detected by various methods.

DISCUSSION

Glycopeptides-resistant enterococci have become a major threat to hospitalized patients. Like methicillin-resistant *Staphylococcus aureus*, VRE can cause important nosocomial epidemics and can increase morbidity, mortality, and costs related to admission in hospitals. The emergence of VRE has resulted in an increase in the incidence of infections that are caused by these organisms and that cannot be treated with currently available antimicrobial agents, and have caused serious concern to both, Physicians and health authorities.[8] Enterococci have become the second most common cause of nosocomial infections in the United States, and are responsible for approximately 8% of all nosocomial bloodstream infections.[9]

The purpose of this study is to generate data on the occurrence of enterococcal infections in a tertiary care hospital, to identify the isolates to the species level, to assess the antibiotic resistance pattern to widely prescribed antibiotics with a focus on vancomycin resistance, and to compare the detection of vancomycin resistance by various methods.

In this study, the maximum number of samples (30.41%) was isolated from patients in 0-10 year age group. It should be noted that out of the 59 patients in this age group, 21(35.59%) were less than one year old. The mean age of incidence of enterococcal infections was 31.53 years. This in contrast to a study by Carmeli *et al.*[10] where the average age was 62 years, and one by Gordon *et al.*[11] where 96% of the patients were greater than 18 years of age.

The prevalence of enterococcal infections was found to be lower in female patients (40.72%). Other studies have shown the prevalence to be 46%[10], and 49.5%[11] in females.

Urinary tract infections (UTIs) are considered to be the most common bacterial infection. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations. Women are significantly more likely to experience UTI than men. Nearly 1 in 3 women will have had at least 1 episode of UTI requiring antimicrobial therapy by the age of 24 years. Almost half of all women will experience 1 UTI during their lifetime.[12] UTIs usually result from the intraluminal ascent of faecal bacteria which normally contaminate the distal urethra, which is shorter in women than in men.[13] The higher prevalence of enterococcal infections in women in this study, and the finding that a majority of the isolates were from urine samples could be attributed to these observations.

194 isolates of enterococci were obtained from various clinical samples. (88.66%) were identified as *E. faecalis*, and (11.34%) were *E. faecium*. This is similar to the study by Ruoff *et al.* who observed that 87.7% of strains were *E. faecalis,* while *E. faecium* accounted for 8.6%. Other species (*E. avium, E. durans, E. casseliflavus, E. gallinarum, E. hirae* and *E. raffinosus*) accounted for only 3.6% of the isolates.[14]

Out of 705 enterococcal isolates studied by Gordon *et al.*, 90% were *E. faecalis*, 8% were *E. faecium*, and 2% were other *Enterococcus* species. (Five were *E. gallinarum*, four were *E. avium*, three were *E. casseliflavus*, one was *E. raffinosus*, one was *E. hirae*, and one was a biochemical variant of *E. faecalis*).[11]

The predominance of infections by *E. faecalis* can be related to the fact that many studies have reported *E. faecalis* is more common and is found in higher numbers than *E. faecium* and other species in the faeces of the most healthy adults.[15-18]

Bryce *et al.* had identified only three species of enterococci, viz. *E. faecalis, E. faecium,* and *E. casseliflavus* among the 14 strains isolated.[19] Some studies have reported a higher incidence of non-*faecalis*, non-*faecium* species. Desai *et al.* reported that 49.5% of the isolates in their study were *E. faecalis*, 35.64% *E. faecium*, 9.4% *E. avium*, 2.47% *E. hirae*, 1.19% *E. raffinosus*, 0.49% *E. gallimarum*, 0.49% *E. casseliflavus*.[20]

Udo *et al.* isolated enterococci from 415 samples, out of which 85.3% were reported to be *E.faecalis*, 7.7% were *E. faecium*, 4% were *E. casseliflavus*, 1.2% was *E. avium*, and 1% was *E. durans*. 0.5% were *E. gallinarum*, and 0.2% were *E. bovis*.[21]

A study by Prakash *et al*. reported 19% (46 out of 242) of the enterococcal isolates belonging to non-*faecalis* and non-*faecium* species with *E. gallinarum* accounting for 6.2%, *E. avium* 4.1%, *E. raffinosus* 2.5%, *E. hirae* 2.5%, *E. mundtii* 1.7%, *E. casseliflavus* 1.2%, and *E. durans* 0.8%.[22]

CONCLUSION

A patient cannot have VRE infection if VRE is not first spread to the patient. Originally classified as enteric gram positive cocci, enterococci have been identified as an important cause of a wide variety of infections, particularly urinary tract and wound infections, endocarditis. The surgery ward, accounted for more than half the isolates of VRE followed by pediatrics and medicine.

The E.faecium strains showed a higher percentage of resistance to all the antibiotics tested, as compared to the E.faecalis strains. The problem of treatment and control of enterococcal infections is underscored by the high prevalence of nosocomial isolates and their ability to acquire resistance to the limited number of useful antimicrobial agents available in the treatment of enterococcal infections.

Keeping in mind the difficulty that disk diffusion has with detecting truly intermediate strains, we conclude that BHI agar containing 6µg/ml of vancomycin, with the inoculated media incubated for 24 h, can be used as a reliable screen for detecting vancomycin resistance in enterococci, and the E test can be used to confirm resistance.

The prescription of antibiotics, in particular, glycopeptides, should probably be dramatically restricted in order to avoid the selection of VRE, which in some cases are already part of the human micro flora. Periodic surveillance programmes to identify patients colonized with VRE, and to monitor the occurrence of VRE in high risk patients should be undertaken.

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