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Research Article

Prevalence of enterococci and its antibiotic resistance in various clinical samples at tertiary care hospital in Southern Rajasthan, India

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ABSTRACT

Background: Enterococci has been emerging as a major cause of infection and gaining increasing clinical importance due to developing resistance to various antibiotics. Emergence of vancomycin resistant enterococci (VRE) in the recent past has been a concern. Aim of the study was to determine the prevalence of enterococci in tertiary care hospital in Southern Rajasthan, India and observe its antibiotic resistance pattern.
Methods: Enterococci strains were isolated from various clinical samples by culture and biochemical methods and its antibiotic susceptibility testing was seen by Kirby Bauer method as per CLSI guidelines.
Results: Out of 100 samples, 50% were isolated from urine samples followed by pus and blood. Resistance to all the drugs was observed with least towards Vancomycin (11%), followed by doxycycline (14%).
Conclusions: The prevalence of multiple drug resistance enterococci with 11% VRE is observed in present study. The study emphasizes on invitro antibiotic susceptibility testing for clinical samples and also rational drug usage.

Keywords: Enterococci, Vancomycin, Antibiotic resistance

INTRODUCTION

Enterococci have emerged as important nosocomial pathogens over the past decade, ranking only second to staphylococci as a leading cause of nosocomial infections.[1] The clinical importance of the genus Enterococcus is directly related to its antibiotic resistance, which contributes to the risk of colonization and infection. The species of the greatest clinical importance are Enterococcus faecalis and *Enterococcus faecium*.[2]

Enterococci are 'tough bugs' that can survive for long periods on environmental surfaces, including medical equipment, bed rails and doorknobs. They are tolerant to heat, chlorine and some alcohol preparations, which may help explain why these organisms are widely disseminated in the hospital setting.[3]

The most common nosocomial infections caused by enterococci are urinary tract infections followed by surgical site infections. Prolonged stay in hospital, empirical use of antibiotics and lack of sufficient information and programs to control rapid spread of enterococci has led to increased mortality caused by enterococcal infections.[4]

Several proteins that are secreted into the extracellular medium have been implicated in enterococcal virulence like Haemolysin–cytolysin, extracellular serine proteinase and protease gelatinase.[3]

Serious enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of amino glycoside and vancomycin. Enterococci with high level resistance to amino glycosides (HLAR), beta lactamase production and glycopeptides resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions.[5] Relative to the streptococci, enterococci are intrinsically resistant to many commonly used antimicrobial agents. All enterococci exhibit decreased susceptibility to penicillin and ampicillin, as well as high-level resistance to most cephalosporin and all semi-synthetic penicillins, as the result of expression of low-affinity penicillin-binding proteins.[2]

First report of glycopeptides resistance was observed in 1980s. Three phenotypes of glycopeptides resistance have been observed in enterococci. Van A with high level inducible resistance to Vancomycin and Teicoplanin, Van B with variable resistance to Vancomycin and sensitive to Teicoplanin and Van C with intrinsic low level resistance to Vancomycin and susceptible to Teicoplanin. Van A and Van B are usually associated with *E. faecalis* and *E.faecium*.[6]

VRE is making the treatment of various infections extremely difficult and pose a great challenge to clinicians.[7] So the present study was conducted to detect the prevalence of enterococci in various clinical samples at a tertiary care hospital and its resistance to various antibiotics.

Aims and objectives

To study the prevalence of enterococci and its antibiotic resistance pattern at a tertiary care hospital in southern Rajasthan from January 2016 to May 2016.

METHODS

The present study was conducted in tertiary care hospital from January 2016 to May 2016. Total 100 samples of Enterococcus were isolated from various clinical isolates like urine, pus, blood, wound swab, vaginal swab, sputum by inoculating the sample on blood agar at 37˚C for 24hours. Furthermore, following biochemical tests were performed for confirmation of gram positive catalase negative cocci. The tests were:

Bile esculin agar test, potassium tellurite reduction, arginine dihydrolase test and sugar fermentation test such as of glucose, mannitol and sucrose. Antibiotic susceptibility testing was done by Kirby Bauer method as per the CLSI guidelines using commercially available discs from HiMedia, Mumbai, Maharashtra, India.

Colonies were inoculated into peptone water and turbidity was adjusted at 0.5 McFarland standard. Broth culture was spread on the plate to make lawn culture on Mueller Hilton agar. Discs were applied on surface of agar and plates were incubated overnight at 30-35˚C in ambient air. Result was interpreted using CLSI guidelines.[8] ATCC *S. aureus* 25923 was used as control.

The following antibiotics were tested-vancomycin (30µg), tetracycline (30µg), doxycycline (30µg), amoxicillin (30µg), ampicillin (10µg), ciprofloxacin (5µg), gentamycin (10µg), norfloxacin (10µg), nitrofurantoin (300µg).

RESULTS

In present study total 100 samples of enterococci were isolated from various clinical samples which were urine (50), pus (20), blood (10), sputum (5), stool (5), vaginal swab (6), and wound swab (4) as shown in Table 1.

Table 1: Number of isolates from various samples.

Sample	No. of isolates	% isolates (n=100)
Urine	50	50%
Pus	20	20%
Blood	10	10%
Sputum	5	5%
Stool	5	5%
Vaginal swab	6	6%
Wound swab	4	4%

Table 2: Antibiotic resistance pattern of enterococci.

Antibiotics	No.of resistant isolates	No. of sensitive isolates
Vancomycin	11 (11%)	89 (89%)
Doxycycline	14 (14%)	86 (86%)
Tetracycline	20 (20%)	80 (80%
Amoxicillin	56 (56%)	44 (44%)
Ampicillin	62 (62%)	38 (38%)
Ciprofloxacin	46 (46%)	54 (54%)
Gentamycin	25 (25%)	75 (75%)
For urine isolates (n=50)		
Norfloxacin	34 (68%)	16 (32%)
Nitrofurantoin	10 (20%)	40 (80%)

Table 3: Number of VRE in various clinical isolates.

Sample	Number of VRE
Urine	5
Pus	2
Blood	2
Sputum	1
Stool	0
Vaginal swab	1
Wound swab	0

Sensitivity to vancomycin (89%) was observed maximum followed by doxycycline (86%) and tetracycline (80%) as shown in Table 2. However high sensitivity was observed to nitrofurantoin (80%) in urine isolates.

Minimum sensitivity was observed for ampicillin (38%), amoxicillin (44%) and ciprofloxacin (54%). Minimum sensitivity was observed to norfloxacin (32%) among urine isolates.

Maximum vancomycin resistant enterococci were seen in urine isolates i.e.5/11 followed by pus and blood isolates as shown in Table 3.

DISCUSSION

Recent attention has focused on enterococci not only because of their increasing role in nosocomial infections, but also because of their remarkable and increasing resistance to antimicrobial agents.[9] In the present study 100 isolates of enterococcus were studied from various clinical samples. Out of those, 50% were isolated from urine sample followed by pus (20%) and blood (10%). Chakraborty et alconducted a study in Kolkata which also showed similar results with maximum isolates from urine (66%), followed by wound swab (19.6%) and blood (8.5%) which correlate to present study.[10] Similarly study conducted by Karmarkar et al in Mumbai showed maximum isolates from urine (50%) followed by blood and pus swabs.[11]

Antimicrobial resistance is one of the major public health problems especially in developing countries where relatively easy availability and higher consumption of medicines have led to disproportionately higher incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries.[12] In present study, vancomycin resistant enterococci (VRE) were found to be in 11% of isolates. Out of that 5/11 were found to be in urine isolates followed by pus and blood. Least resistance after vancomycin was seen in doxycycline (14%), tetracycline (20%) and nitrofurantoin (20%) in urinary isolates. In present study, higher resistance was seen in gentamycin, penicillins, ciprofloxacin and norfloxacin

Shrivastava et al observed similar results showing more no. of VRE in urine isolates with 27% resistance to vancomycin in urine enterococci with along with multiple drug resistance.[13] Shah et al also observed 8% Vancomycin resistant enterococci in various clinical isolates with drug resistance to other antibiotics.[7] Similar study conducted by Bhatt et al observed 14% resistance to vancomycin in enterococci. However Chakraborty et al observed no resistance to vancomycin which contradicts present study but has high resistance in other antibiotics.[10] All the studies including ours showed high level resistance to penicillins and ciprofloxacin.

CONCLUSION

The present study concludes the occurrence of multiple drug resistance in enterococci in various clinical samples with emerging VRE strains hereby limiting the use of these drugs for therapeutic use. So in-vitro antibiotic susceptibility for clinical samples should be done. This study also emphasizes on the proper infection control in clinical practice and empirical use of drugs like Vancomycin to avoid further resistance.

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