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Original Article

Characterization and In Vitro Susceptibility Pattern of Enterococci

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Abstract

Background: Enterococci has assumed a great clinical importance because of their increasing resistance to various group of antibiotics. Thus, knowledge about its species and antibiogram is of utmost importance in formulating an effective antibiotic policy to manage such infections and to reduce the resulting morbidity and mortality. **Aims:** To assess the antimicrobial susceptibility pattern of enterococci and to determine the prevalence of multidrug resistance among them. **Materials and Methods:** Clinical samples of patients in a referral hospital was studied over a period of one year and non-repetitive clinical isolates of enterococci were included. Speciation and antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method and minimum inhibitory concentration(MIC) of vancomycin was determined by agar dilution method. **Results:** A total of 98 Enterococcal isolates from various clinical samples were obtained. E.faecalis (77.5%), E.faecium (15.3%), E.durans (4%), E.raffinosus (2%) and E.gallinarum (1%) species were isolated in order of frequency. Varying levels of resistance was seen to different antibiotics. All the isolates were resistant to penicillin and cefotaxime and none of the strains showed resistance to vancomycin and nitrofurantoin. **Conclusion:** Emergence and prevalence of multidrug resistant enterococci emphasises the need for routine speciation and in vitro antimicrobial susceptibility testing of enterococcus in various clinical samples.

Keywords: Enterococcus spp., Antimicrobial susceptibility testing, Antibiotic resistance

Introduction

Enterococci are facultative anaerobes that are part of normal intestinal flora in humans. They are commonly implicated in urinary tract infections, pelvic infections, endocarditis with or without bacteremia, neonatal sepsis and surgical wound infection.[1] They are considered to be the second leading cause of nosocomial infections and third among the most common cause of bacteremia. Enterococci have intrinsic résistance and ability to acquire resistance to several broad spectrum antibiotics. This may cause super infections in patients already receiving antimicrobial therapy. Enterococci have acquired resistance to several classes of antibiotics either by mutation or by receipt of foreign genetic materials through the transfer of plasmid and transposons.[2] Isolation of vancomycin resistant Enterococci (VRE), has limited the therapeutic options available for clinicians. The transfer potential of vancomycin resistant gene from Enterococci to Staphylococcus aureus has been achieved invitro, but not reported in clinical settings. This increases the importance of finding ways to limit the spread of vancomycin resistant Enterococci. High level aminoglycoside resistant Enterococci (HLAR) often have plasmids which carry determinant encoding resistance to other antibiotics, besides limiting the option of using a combination of cell wall active antibiotic and aminoglycoside.[3] This drug combination depends on the synergistic bactericidal activity between the two high occurrence of high level aminoglyco-

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sides resistance has necessitated routine testing of Enterococci isolates.

Material and Methods

Following the approval from institutional ethics committee, clinical samples were collected over a period of one year at a tertiary care hospital. The clinical samples of urine, wound swab/pus, high vaginal swab and blood were inoculated on to blood agar and Macconkey agar. These isolates were identified at the species level with the help of conventional phenotypic methods which included Gram's stain, colony morphology, catalase test, bile esculin test, growth in 6.5% NaCl, mannitol fermentation, arginine dihydrolase test, arabinose fermentation, lactose fermentation and sucrose fermentation.

Antibiotic susceptibility of enterococcus species

The isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method as per CLSI recommendations using commercially available 6mm disks (HIMEDIA, Mumbai, India). The disks used were Ampicillin(30µg), Cefotaxime (30µg), ciprofloxacin (5µg), erythromycin (15µg), Amikacin(30ug), vancomycin (30µg). For high level gentamicin resistance (HLGR) detection, gentamicin (120µg) disk was used.

Table 1. Distribution of enterococccal species in the studied clinical specimen

Clinical samples	No. of specimen	E. faecalis (n= 76)	E. faecium (n=15)	E. durans (n=04)	E. raffinosus (n=02)	E. gallinarum (n=01)
Urine	72	61	09	-	-	-
Pus	10	08	01	01	-	-
Stool	07	04	02	01	-	-
Sputum	05	01	01	01	01	01
Tracheal aspirate	04	01	01	01	01	-
Blood	02	01	01	-	-	-

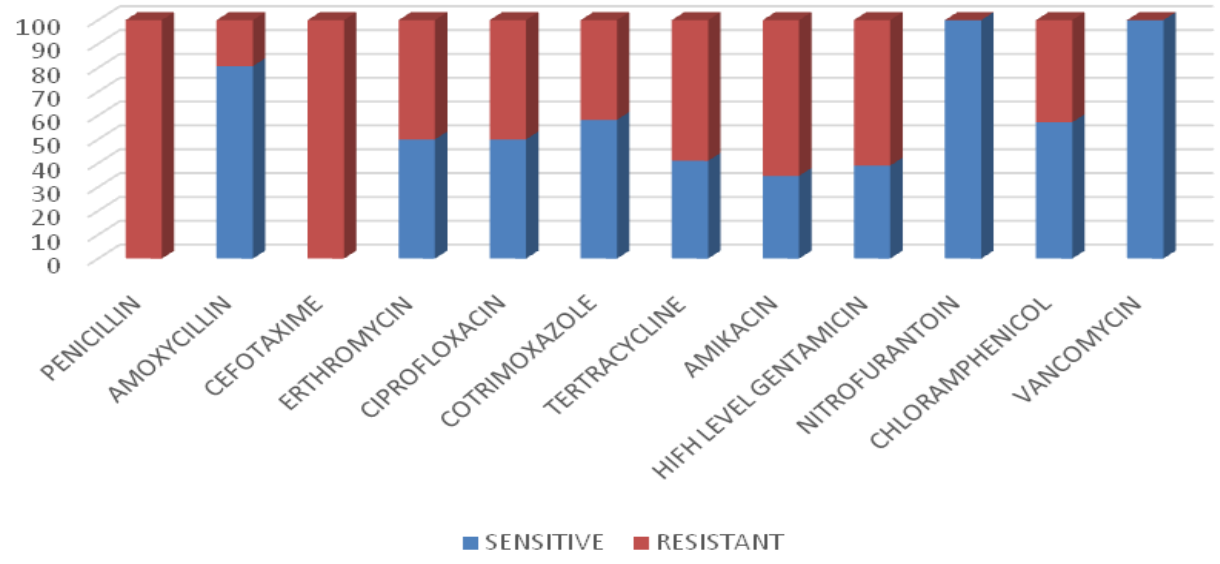

Fig 1. Antibiotic susceptibility pattern of Enterococcal isolates

The inoculated plates were incubated for 18h at 35 ºC. The diameter of zone of inhibition of each antibiotic was measured and interpreted as sensitive, intermediate and resistant according to CLSI criteria. For HLGR resistance was indicated by no zone and susceptibility by zone of diameter ≥10mm.E.faecalis ATCC 29212 and E. faecium ATCC 51299 were used as the susceptible and resistant quality control strains.[4]

Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) of vancomycin were determined by agar dilution method. Brain heart infusion agar (Hi Media, Mumbai) was supplemented with different concentrations of vancomycin. The test organism was grown in broth and the turbidity matched with McFarland 0.5 standard (approximately 1.5×10^8 cfu/ml). Spot inoculation of the agar medium was done using 10 µl of bacterial culture. The plates were incubated at 37ºC for 24 hours and examined. The MIC of vancomycin that inhibited bacterial growth was considered MIC.

Results

A total of 98 Enterococcal isolates from various clinical samples of the studied patients were obtained. Of the 98 isolates 72 were recovered from urine, 10 from pus, 07 from stool, 05 from sputum, 04 from tracheal aspirate and 2 from blood. Five different species were identified of which E. faecalis was the most common. Out of 98 enterococcal species 76(77.5%) were identified as E. faecalis, 15 (15.3%) E. faecium, 04(4%) E. durans, 02(2%) as E. raffinosus and 01(1%) E. gallinarum (Table 1). Males 75 (76.5%) were found to be more prone to enterococcal infection as compared to females 23(23.4%). High prevalence of enterococcal infection was seen in the age group 21-40 years (50%) followed by 41-60, 60-80 and >20 years of age. All the isolates (100%) were resistant to penicillin and cefotaxime. β-lactamase production was shown by 49(50%) isolates. Resistance to ciprofloxacin was seen in 49(50%) isolates and 41(41.8%) were resistant to cotrimaxozole. Resistance to tetracycline was shown by 58(59.1%) isolates. Forty two (42.8%) isolates exhibited resistance to chloramphenicol, 49(50%) to erythromycin and 64(65.3%) to amikacin. High level gentamicin resistance was observed in 06 isolates. Of 06, five strains showed MIC between 256µg/ml and 512 µg/ml and one isolate >2048 µg/ml. None of the Enterococcal strains isolated showed resistance to vancomycin and nitrofurantoin (Fig. 1).

Discussion

This study determined the incidence of enterococcal infections and the pattern of antibiotic resistance in a large referral hospital. Ninety eight enterococcal isolates were recovered from the clinical samples of urine, pus, stool, sputum, tracheal aspirate and blood. In our study highest prevalence of enterococcal infection was seen in the age group 21-40 years. The male and female ratio of 2:1 in our study is comparable to the study carried out by Adhikari et al.[5] Majority of the isolates were obtained from urine(73.4%), followed by pus 12.2%, stool(7.1%), sputum (5.1%), tracheal aspirate (4%) and blood (2%).

Most of the isolates were from urine which correlates with two Indian studies where the isolation rates are 50-63%.[3,6] On the contrary in another study maximum isolates of enterococcus were from pus.[7] E. faecalis (77.5%) was found to be the most predominant enterococci followed by E. faecium (15.3%), E. durans (4%), E. raffinosus (2%) and E. gallinarum (1%) which is in agreement with other studies, but it is disagreement with report from few studies where E. faecium was predominant over E. faecalis.[8,9] All the isolates were resistant to penicillin and cefotaxime. β-lactamase production was shown by 49(50%) of the isolates which correlates with one of the study.[6] A study showed 57% of enterococcal isolates were resistant to penicillin and 54% showed β-lactamase producers.[10] In contrast to our study Bhatet al stated that 17% of the isolates were resistant to penicillin and none of the strains produced β-lactamase.[11] A study at Mumbai observed that all the enterococcal isolates were sensitive to ciprofloxacin, while we observed that around 41.8% of the

isolates were resistant to it. All the species were sensitive to nitrofurantoin whereas Butch et al in 2011 reported that more than 60% were sensitive to it.[12] Many studies conducted in India show the prevalence of HLGR to be around 7.8 to 26%. The HLGR prevalence was found to be 6.1% in our study and was more common in E. faecalis than E. faecium species.[13,14] As all the isolates were found to be sensitive to vancomycin the therapeutic efficacy against the majority of enterococci isolated from patients in this referral hospital is retained.

Conclusion

Multidrug resistant E. faecalis is common in this referral hospital and poses a serious therapeutic challenge. Hence there is a constant need to monitor the antibiotic susceptibility pattern of defined geographical areas which are helpful in formulating local antibiotic policy.

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