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KEY WORDS: Enterococcus, antibiotic resistance, vancomycin resistance, MIC.

THE ANTIBIOTIC SUSCEPTIBILITY PROFILE OF ENTEROCOCCAL ISOLATES AT A TERTIARY CARE HOSPITAL.

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ABSTRACT

Objectives: The objective of the study was to determine the Antibiotic susceptibility profile of the Enterococcus species isolated from various clinical samples in a tertiary care hospital.

Material and Methods: Enterococcus species isolated from various clinical specimens were identified by the Conventional identification scheme by Faclam and Collins and their susceptibility to various antibiotics were detected by disc diffusion method and vancomycin susceptibility was further confirmed by determining MIC with E-strips.

Results: In this study, 180 Enterococcal isolates were obtained from various clinical samples, from October 2014 to November 2016. Most common isolate was *Enterococcus faecalis (57.2%),* followed by *E. faecium (42.3%)* and one isolate of *E. hirae* (0.5%). Resistance to penicillin was 90.56%. Although vancomycin resistance was low (1.67%) but resistance to high level gentamicin (81.11%) and high level streptomycin (72.22%) is a cause of great concern. None of the isolates were resistant to Linezolid.

Conclusion: The emergence of antibiotic resistance among enterococci, even to drugs like vancomycin is a cause of great concern. Linezolid appears to remain a last resort therapeutic option for enterococcal infections. Hence, the need of hour is to comply with judicious use of antibiotics.

Introduction

Since 1990s enterococci have emerged as pathogens in a growing number of serious nosocomial infections including bacteraemia, intra-abdominal infections and urinary tract infections.[1] The enterococci are intrinsically resistant to a wide range of antibiotics that most notably include beta-lactams and aminoglycosides. In addition, enterococci have the ability to acquire resistance to antimicrobial agents through transfer of plasmids, transposons, chromosomal exchange and mutations.[2] Its antimicrobial resistance poses a great threat as it is not only transferable between species of enterococci but it is also transferable to organisms of other genera, like transfer of vancomycin resistance to *Staphylococcus aureus*.[3] Antibiograms need to be correlated with the species of enterococci and emergence of drug resistant enterococci needs to be monitored. Hence, this study was undertaken.

Material and Methods

The study was carried out in the department of Microbiology, of a tertiary care hospital from October 2014 to October 2016. Total 180 enterococcus strains were isolated from different clinical samples e.g. urine, blood, pus, wound swab, body fluids, urinary catheter tips etc. received from indoor and outdoor patient departments. All the samples were collected and processed by standard Protocol.[4] The isolates were characterised as enterococci according to the conventional identification scheme.[5] Two criteria were used to label an enterococcal isolate as infective, firstly, isolate from urine samples was considered as infective if there was significant bacteriuria.[6] Second, isolates from specimens like blood, wound swab, body fluids like pleural fluid, ascitic fluid etc. were considered as infective when isolated from at least two samples. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method.[7] Isolates were detected to be susceptible, intermediate or resistant for each antimicrobial agent as per the CLSI guidelines.[8] Antibiotic susceptibility test for the following antibiotics were done using antibiotic discs e.g. Ampicillin (10µg), Penicillin G (10 units), Vancomycin (30µg), Linezolid (15µg), Chloramphenicol (30µg), Tetracycline (30µg), Erythromycin (15µg), Quinupristin- dalfopristin (15µg).Also, high level aminoglycoside resistance (HLAR) was detected by using high level streptomycin (HLS) disc (300 µg) and high level gentamicin (HLG) disc (120 µg) as per CLSI guidelines. For isolates from urine and catheter tip only – Norfloxacin (10 µg) and Nitrofurantoin (300 µg) discs were also used. Enterococcal isolates found to be resistant or intermediate resistant to vancomycin by disc diffusion method; were selected for determination of Minimum inhibitory concentration (MIC) by E test.

Observation and Results

Of the 180 isolates obtained, *E. faecalis* accounted for 57.22% and *E. faecium* for 42.22% of the strains. One relatively uncommon enterococcus species, *E. hirae* was also isolated.

Table 1. Antibiotic resistance pattern of the enterococcal isolates from different clinical samples (n=180).

S. No	Antibiotic	No. of resistant isolate			
		E. faecalis n=103 (%)	E. faecium n=76 (%)	E. hirae n=1 (%)	Total n=180 (%)
1.	Penicillin G (10 units)	91 (88.35)	71 (93.42)	1 (100)	163 (90.56)
2.	Ampicillin (10 µg)	68 (66.02)	69 (90.79)	1 (100)	138 (76.67)
3.	Vancomycin (30 µg)	1 (0.97)	2 (2.63)	0	3 (1.67)
4.	Linezolid (15 µg)	0	0	0	0
5.	Gentamicin (120 µg)	79 (76.7)	66 (86.84)	1 (100)	146 (81.11)
6.	Streptomycin (300 µg)	69 (67)	60 (78.95)	1 (100)	130 (72.22)
7.	Chloramphenicol * (30 µg)	8/24 (33.33)	11/28 (39.29)	0/1	19/53 (35.85)
8.	Erythromycin (15 µg)	77 (74.76)	68 (89.47)	1 (100)	146 (81.11)
9.	Tetracycline (30 µg)	73 (70.87)	58 (76.32)	0	131 (72.78)
10.	Quinupristin-dalfopristin (15 µg)	84 (81.55)	11 (14.47)	1 (100)	96 (53.33%)
11.	Norfloxacin** (10 µg)	57/79 (72.15)	45/48 (93.75)	0	102/127 (80.31)
12.	Nitrofurantoin*** (300 µg)	11/79 (13.92)	17/48 (35.42)	0	28/127 (22.05)

*Chloramphenicol have been put for all enterococcal isolates except urine and catheter-tip.

** Norfloxacin and *** Nitrofurantoin have been put for urine and Cather tip samples only, which were 127. Calculations made accordingly.

E. faecium was more resistant to commonly used anti-enterococcal drugs as compared to *E. faecalis*. (Table 1) All 180 enterococcal strains were sensitive to linezolid. Only 3 (1.67%) strains were resistant to vancomycin. There was marked difference in the resistance pattern to ampicillin between the two major species (*E. faecalis* 66.02% strains were resistant and *E. faecium* 90.79% strains were resistant to ampicillin). Notably, *E. faecium* shows good susceptibility to quinopristine-dalfopristine unlike *E. faecalis*. Among the urinary isolates, Nitrofurantoin, displayed good activity against both *E. faecalis* and *E. faecium*.

Out of the total 180 enterococcal strains, 12 (8 *E. faecium* and 4 *E. faecalis*) showed either intermediate or absolute resistance to Vancomycin by disc diffusion method, hence MIC was determined for these 12 enterococcal isolates. 9 enterococcal isolates showed MIC of ≤ 4 µg/ml indicating vancomycin sensitivity. Among remaining 3 enterococcal strains, 2 showed MIC between 8-16 µg/ml, one showed MIC 32 µg/ml (By E- strip). Hence, in the present study, among the 180 enterococcal isolates 3 (1.67%) were vancomycin resistant, of which two were *E. faecium* and one was *E. faecalis*. (Table 2)

Table 2. MIC of Vancomycin in enterococcal species.

S. No.	Enterococcal sp.	No. of isolates with MIC of vancomycin in mcg/ml		
		≤ 4 mcg/ml	8-16 mcg/ml	32 mcg/ml
1.	E. faecalis (4)	3	1	0
2.	E. faecium (8)	6	1	1
	Total (12)	9	2	1

Discussion

Havard et al (1959) had reported 0% penicillin resistance among enterococcal isolates in their study.[9] Thereafter, Penicillin resistance increased markedly. The present study, 90.56% enterococcal isolates were resistant to penicillin, however only 76.67% isolates were resistant to ampicillin. CLSI guidelines claim that enterococci susceptible to penicillin are predictably susceptible to ampicillin. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin, and needs to be tested separately.[8] Rahandale et al 2008 reported 89.43% resistance to penicillin in their study, but 43.90% of their isolates were resistant to both penicillin and ampicillin.[10] Deshpande et al (2013), reported 75.9% resistance to penicillin and 64.9% resistance to ampicillin.[11] Resistance to low level aminoglycoside is an inherent property of enterococci, which is due to low level uptake of these agents.[2] CLSI recommends use of high-content gentamicin disk (120 µg) and high-content streptomycin disk (300 µg) for testing for high-level aminoglycoside among enterococci.[8] In the present study, high-level resistance to gentamicin was 81.11% and high-level resistance to streptomycin was 72.22%. (Table 1). Jain et al (2011) reported resistance to high level gentamicin and high level streptomycin to be 62% and 58% respectively.[12] Deshpande et al (2013) also reported similarly a resistance of 73.5% to high-level gentamycin and 70.8% resistance to high-level streptomycin.[11] 12 (6.67%) isolates were resistant to vancomycin by disc diffusion method. MIC of vancomycin was determined by E-test using E-strip of vancomycin. 9 enterococcal strains were sensitive to vancomycin (MIC: ≤ 4 µg/ml) by MIC determination method. (Table 2) Remaining 3 isolates were resistant to vancomycin by MIC determination method. Thus, indicating that the two methods may not correlate to detect vancomycin resistance among enterococci (Fig:1) Hence, disk-diffusion techniques may fail to recognize those enterococcal strains that have reduced susceptibility to vancomycin. Swenson et al (1989).[13] Karmakar et al (2004), Kapoor et al (2005) and Adhikari et al (2010) too reported poor correlation between the two methods, for determining vancomycin resistance among enterococcal isolates.[14,15,16] Hence, in the present study, vancomycin resistance was found to be 1.67%. Our results were comparable with the studies of Udo et al (2003) and De et al (2009), who reported 2.6% and 1.5% vancomycin resistance among enterococcal isolates in their respective studies.[17,18]

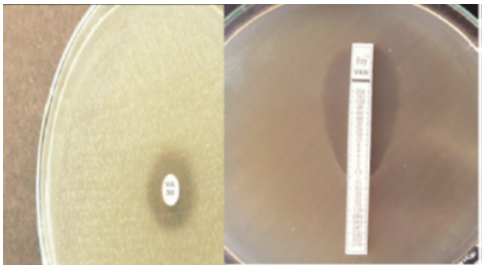

Fig: 1 Vancomycin disc diffusion shows zone of 15mm (intermediate resistance) but vancomycin E-strip shows MIC < 4µg/ml indicating susceptibility.

Unlike our study, Adhikari et al (2010) showed 0% vancomycin resistance.[15] Emergence of vancomycin resistant enterococci has been attributed to imprudent use of cephalosporins, vancomycin and colonization.[16] The present study shows, resistance to Quinupristin-dalfopristin was 81.55% and 14.47% in *E. faecalis* and *E. faecium* respectively. This was comparable to Jia et al (2014), as in their study, the prevalence of Quinupristin-dalfopristin resistance was 81.2% in *E. faecalis*, which was significantly higher than that the 1.8% in *E. faecium*.[19] Quinupristin-dalfopristin has been recommended by CLSI for the treatment of severe vancomycin-resistant infections caused by *E. faecium*.[8] In this study, norfloxacin and nitrofurantoin were tested for enterococcal isolates from urine and catheter tip only. It was found that, resistance to norfloxacin was 80.31% and to nitrofurantoin was 22.05%. In the present study, 0% resistance was reported for linezolid. The results of our study correlated well with Srivastava et al (2013) and Chakraborty et al (2015) ,who also reported 0% resistance to Linezolid in their respective studies.[20,21]

Conclusion: There is emergence of antibiotic resistance among enterococci, particularly to beta lactam antibiotics and high-level aminoglycosides which is a cause of great concern. The prevalence of vancomycin resistance was low (1.67%). None of the enterococcal isolates were resistant to Linezolid, which could probably remain last resort therapeutic option for enterococcal infections. Hence, judicious use of antimicrobials and appropriate infection control practices could prevent emergence of further drug resistant enterococcal infections.

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