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"PHENOTYPIC CHARACTERISATION OF ENTEROCOCCI AT A TERTIARY CARE CENTRE OF SOUTH INDIA".

Microbiology

KEYWORDS: Enterococci, High level glycopeptide resistance,High level aminoglycoside resistance,E test

Reena Rajan	Senior Lecturer, Department of Microbiology, Penang International Dental College, Vinayaka Mission 's University ,Salem,Tamil Nadu .
C.Amirtha	Intern, Department of Medicine, Rajah Muthiah Medical College, Annamalai University, Chidambaram,Tamil Nadu.
K.M.Mohana Soundaram	Professor, Department of Microbiology, IRT Perundurai Medical College, Erode, Tamil Nadu
V.Anandi	Professor, Department of Microbiology, Vinayaka Mission' s Medical college . Karaikkal, Tamil Nadu.

ABSTRACT

Enterococci have evolved from being commensal gut flora of little clinical significance to becoming an increasingly important cause of nosocomial infection in the last decade .The present study was undertaken to identify the species prevalence , detect vancomycin resistance among enterococci and to determine antimicrobial resistance profile.A total of 134 isolates obtained from various clinical samples were speciated by Facklam and Collins scheme.Resistance to glycopeptides were determined by agar dilution and Minimum Inhibitory Concentration(MIC) was determined by E test.Out of 134 isolates studied, 127 (94.78%) were Enterococcus faecalis and 7(5.22%) were Enterococcus faecium. . Out of 107 Enterococcus faecalis studied from urine sample, two isolates were with Minimum Inhibitory Concentration of ≥ 32 g/ml for both vancomycin and teicoplanin by Agar dilution method and E test .Among the Enterococcus faecium isolates ,5 isolates from urine were with MIC range of 0.5 to 2 µg/ml for vancomycin by E test.

INTRODUCTION:

Enterococci ,although harmless commensals in the gastrointestinal tract has become formidable pathogens since last two decades.These organisms has been recognised as a important causative agent of hospital acquired urinary tract infection , followed by wound infection,and blood stream infection. Infection caused by these organism presents a great challenge as they exhibit resistance to variety of antimicrobial agents.[1]

The major reasons for the emergence of multidrug resistance among Enterococci are base line resistance to several antimicrobial agents ,acquired resistance via mobility of resistance genes on plasmids and transposons,chromosomal exchange and transferability of resistance.

[2] Enterococci showing resistance to vancomycin have been reported from many parts of the world.[3] Vancomycin Resistant Enterococci (VRE) are genetically diverse and are phenotypically and genotypically heterogenous.[3,4] The emergence and spread of glycopeptides resistance has made it difficult to manage serious infection caused by this pathogen .Resistance to high level aminoglycoside is most often due to aminoglycoside modifying enzyme or mutation or due to the production of 3 'phosphotransferase. [5,6]

The most serious infection due to Vancomycin Resistant Enterococci occurs in severely ill immunocompromised patients. The various risk factors associated with VRE colonisation or infection are stool carriage of organisms by patients infected with Vancomycin Resistant Enterococci ,hospitalisation in critical care unit and particularly exposure to antimicrobial agents including vancomycin ,broad spectrum cephalosporins, carbapenems and antibiotics with activity against anaerobes including metronidazole and clindamycin. Asymptomatic carriage of Vancomycin Resistant Enterococci by hospital staff serves as a reservoir for the dissemination of these strains to other patients.[7] Hence accurate identification of VRE is important for the management of infected patients,to select appropriate antimicrobial agent for treatment,and to implement infection control measures. The present study was undertaken to identify the species prevalence of Enterococci , their antibiotic resistance pattern and to detect the Minimum Inhibitory Concentration of vancomycin and teicoplanin by Agar dilution method and E test.

METHODOLOGY:

A total of 134 Enterococcal isolates obtained from various clinical samples (urine,pus,blood,body fluids etc) during march 2015 to July 2016 were included in this study.The samples were inoculated on to Blood agar and Mac Conkey agar and incubated at 37^{0}C for 24 to 48 hrs.After incubation the colonies were identified by gram staining, catalase test,bile esculin agar,6.5% Nacl,heat tolerance test. Speciation of enterococcal isolates were performed according to Facklam and Collin 's scheme using 1% solution of glucose, lactose,raffinose,arabinose ,sorbose ,sorbitol,sucrose, pyruvate utilisation test ,arginine dihydrolase, motility and pigment production.[8]

Antibiotic susceptibility testing was done on Muller Hinton agar by Kirby Bauer disk diffusion method and the results were interpreted as per CLSI guidelines.[9] E.faecalis 29212 was used as quality control .The following antibiotics were used :Amoxicillin/clavulanate(25 µg/ 10µg), Ampicillin (10 µg),High level gentamicin(120 µg), Amikacin(30µg), Cotrimoxazole (25µg), Teicoplanin (30µg),Nitrofurantoin (300µg),Erythromycin (15µg), Ciprofloxacin(5 µg).

Vancomycin screen agar test was done on Brain heart infusion agar with 6 µg/ml vancomycin.[6,10] Vancomycin sensitive strain ATCC 29212 E.faecalis was used as negative control and Vanomycin resistant strain ATCC 51299 E.faecalis was used as positive control.
Minimum Inhibitory Concentration for vancomycin and teicoplanin was determined by agar dilution method on Brain heart infusion agar supplemented with 2,4,8,16,32 µg/ml of antibiotics .The plates were incubated at 37^0 C for 24 hrs and examined for growth. High level aminoglycoside resistance was determined by screen agar containing 500 µg /ml gentamicin.[6]. Minimum Inhibitory Concentration for Gentamicin was determined by agar dilution method (500,1000,2000 µg/ml of Gentamicin.) and E test.

E test was performed to determine Minimum Inhibitory Concentration of vancomycin and teicoplanin .Enterococci isolates with MIC >32 µg/ml were considered as resistant ;8-16 µg/ml as intermediate resistant and 4 µg/ml as susceptible to vancomycin Isolates with MIC ≤ 8 µg/ml were considered as susceptible, 16

µg/ml as intermediate resistant and > 32 µg/ml as resistant for teicoplanin[3].Enterococcus.faecalis ATCC 29212 and Staphylococcus aureus 25923 were used as control strains.

RESULTS:

A total of 134 isolates were obtained from various clinical samples, 112 (83.58 %) were from urine, 16 (11.94%) were from pus ,4(2.99%) from blood ,2(1.49%) from body fluids.The most predominant species obtained were Enterococcus faecalis (94.78%) followed by E faecium(5.22%) **(Fig:I)**

Among the urinary isolates 95.53% were Enterococcus faecalis ,and 4.47% were Enterococcus faecium **(Table :I ,II & V).** Of these E.faecalis isolates,31(28.97%) were resistant to Ampicillin, 79(73.83%) to Erythromycin , 84(78.50%) to Ciprofloxacin, and 55(51.40%) to teicoplanin **(Table:I).** Out of 107 isolates of urinary isolates of Enterococci ,2 isolates were with MIC range of 32µg/ml for vancomycin and teicoplanin by agar dilution**(Table: III & IV)** and E test(Fig: II & III). 58 isolates were resistant to High level gentamicin(≥500µg/ml) by agar screen method.Out of these, 57 isolates showed high level resistance(≥2000µg/ml) to gentamicin by agar dilution **(Table:VI).**

Among the pus samples studied,93.75 % were Enterococcus faecalis, out of these 8(53.33%)were resistant to Ciprofloxacin , 2(13.33%) to Ampicillin and 5(33.33%) to High Level Gentamicin.Out of 15 E.faecalis isolates from pus sample , one isolate showed high level resistance to Gentamicin(2000 µg/ml) by Agar dilution and E test.

3 out of 4 blood isolates were E.faecalis ,out of which 2 isolates were resistant to Ampicillin and Ciprofloxacin and 1 isolate to High Level Gentamicin.

Figure I

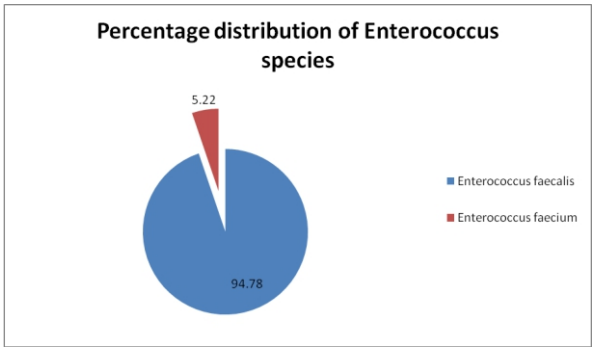

Table I :Antibiotic resistant pattern of Enterococcus faecalis

Antibiotic	Urine (107)	Pus (15)	Blood (3)	Body fluids (2)
Amoxyclav	22(20.56%)	0(0.00%)	1(33.33%)	2(100.00%)
Ampicillin	31(28.97%)	2(13.33%)	2(66.67%)	2(100.00%)
Erythromycin	79(73.83%)	7(46.67%)	2(66.67%)	2(100.00%)
Teicoplanin	55(51.40%)	4(26.67%)	1(33.33%)	0(0.00%)
Cotrimoxazole	62(57.94%)	9(60.00%)	3(100.00%)	1(50.00%)
Amikacin	70(65.42%)	9(60.00%)	0(0.00%)	2(100.00%)
High Level Gentamicin	60(56.07%)	5(33.33%)	1(33.33%)	2(100.00%)
Ciprofloxacin	84(78.50%)	8(53.33%)	2(66.67%)	2(100.00)

Table II :Antibiotic resistant pattern of Enterococcus faecium

Antibiotics	Urine (5)	Pus (1)	Blood (1)
Amoxyclav	1(20.00%)	0(0.00%)	1(100.00%)
Ampicillin	1(20.00%)	1(100.00%)	1(100.00%)
Erythromycin	2(40.00%)	1(100.00%)	1(100.00%)
Teicoplanin	0(0.00%)	1(100.00%)	1(100.00%)
Cotrimoxazole	5(100.00%)	1(100.00%)	1(100.00%)
Amikacin	5(100.00%)	0(0.00%)	1(100.00%)
High Level Gentamicin	1(20.00%)	1(100.00%)	1(100.00%)
Ciprofloxacin	2(40.00%)	1(100.00%)	1(100.00%)

Table III:MIC range of vancomycin for Enterococcus faecalis by agar dilution

Samples	2µg/ml	4µg/ml	8µg/ml	16µg/ml	32µg/ml
Urine(107)	60	7	2	2	2
Pus(15)	6	-	-	-	-
Blood(3)	1	-	-	-	-
Body fluids(2)	1	-	-	-	-

Table IV:MIC range of Teicoplanin for Enterococcus faecalis by agar dilution

Samples	2µg/ml	4µg/ml	8µg/ml	16µg/ml	32µg/ml
Urine(107)	9	2	2	2	2
Pus(15)	1	-	-	-	-
Blood(3)	-	-	-	-	-
Body fluids(2)	-	-	-	-	-

Table V:MIC range of Vancomycin for Enterococcus faecium by agar dilution

Samples	2µg/ml	4µg/ml	8µg/ml	16µg/ml	32µg/ml
Urine(5)	2	-	-	-	-
Pus(1)	1	-	-	-	-
Blood(1)	1	-	-	-	-

Fig II:MIC range (0.5-32µg) of vancomycin for Enterococcus faecalis by E test

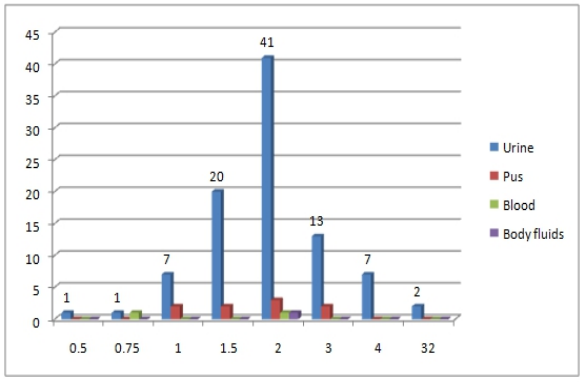

Fig III:MIC range (0.19-32µg)of Teicoplanin for Enterococcus faecalis by E test

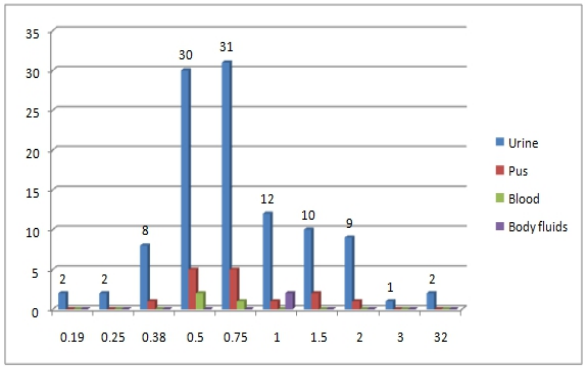

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Table VI:MIC range of High Level Gentamicin for Enterococcus faecalis by agar dilution

Samples	500 µg/ml	1000 µg/ml	2000 µg/ml
Urine(107)	58	57	57
Pus(15)	1	1	1
Blood(3)	1	1	1
Body fluids(2)	1		

DISCUSSION:

The incidence of nosocomial colonisation and infection due to Enterococcus spp has increased steadily during 1980' s. Enterococcus faecalis and Enterococcus faecium have been frequently isolated from clinical cases accounting 80-90% and 5-10% respectively. Enterococcus faecium has gained importance in recent years ,probably due its greater antibiotic resistance.[11]The most common clinical impact of VRE is intestinal colonisation which serves as a reservoir for VRE transmission to other patients.Skin contamination with VRE increases the risk of contamination of blood cultures. Nosocomial UTI caused by VRE includes cystitis, pyelonephritis, prostatitis and perinephric abscess and are associated with urinary instrumentation.[7] In the present study, 112 (83.58 %) isolates were obtained from urine sample and 4(2.99%) were from blood samples. A study conducted by Taneja et al [12] have reported Enterococcus faecalis (55%) as predominant isolate from urine sample.

The second most common infection caused by enterococci are intraabdominal and pelvic sepsis and surgical wound infection. [13] In our study, 16(11.94%) were from pus samples Enterococci exhibit decreased susceptibility to penicillins and ampicillins as a result of expression of low level Penicillin Binding Proteins (PBPs).[14] In the present study, ampicillin resistance was found to be 29.85%.In an Indian study by Atray et al [15], ampicillin resistance was reported to be 62%. Ampicillin resistance was found to be 70% in a similar study by Mukherjee et al [16]

Among the aminoglycoside studied, 52.99 % were resistant to high level gentamicin, 64.93 % to amikacin by disk diffusion method .75.37 % Enterococci isolates were resistant to ciprofloxacin and 70.15 % to erythromycin . In a North Indian study by Mathur et al [17] 66% strains were resistant to Ampicillin,26% to high level aminoglycoside and 88% to ciprofloxacin In the present study,29.13% E.faecalis isolates were resistant to Ampicillin,53.54% to High level Gentamicin,75.59% to Ciprofloxacin,47.24% to teicoplanin and 59.06% to Cotrimoxazole by disk diffusion method.

In a similar study from Mumbai,reported by Despande et al [18] 73.6% E.faecium isolates resistant to Ampicillin and 77% were found to be resistant to high level gentamicin. Among the Enterococcus faecalis isolates, 61.3% and 72.1% were found to be resistant to ampicillin and high level gentamicin respectively.In a North Indian study Gupta et al [19]

In a North Indian study by Gupta et al [19],96 % Enterococci isolates were found to be resistant to gentamicin by disc diffusion method.

96% Enterococci isolates were found to be resistant to gentamicin by disc diffusion method In our study 2 Enterococcus faecalis isolates from urine showed MIC of ≥32µg/ml to vancomycin and teicoplanin agar dilution method and E test .Ghosh etal [20] have reported 11 isolates with vancomycin MIC >6µg/ml and 2 isolates with MIC >8µg/ml by agar dilution method.Out of 11 isolates, 9 were identified as Van B and 2 isolates as Van A by E test.

In a study conducted by Kamarkar etal [21]among various clinical samples ,12 isolates were found to be Van B phenotype. In a study by Mathur et al [17], four isolates of Enterocccus faecalis were of Van A phenotype. Revathy Sharma et al [22] have reported 11 isolates as Van A and 12 isolates as Van B among enterococci studied In a study by Gupta V et al [19] among blood and urinary isolates have reported a single strain of E.faecalis with a MIC value of 512µg/ml.

CONCLUSION:

Our study showed prevalence of glycopeptide resistance among Enterococci isolates In the present study, two E.faecalis isolates were with an MIC value of ≥ 32µg/ml for glycopeptides This study emphasize regular screening and detection of vancomycin resistance to recognise VRE colonisation and infection. Emergence of vancomycin resistance among enterococci threaten to compromise the effective treatment of infection caused by multidrug resistant enterococci in seriously ill patients .

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