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Original Research Article

Characterization and antimicrobial profile of enterococcal species from various clinical samples in a tertiary care centre

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ABSTRACT

Background and Rationale: *Enterococci* have long been recognised as low virulence bacteria occurring as commensals in the human intestine. However in the last two decades they emerged as one of the leading causes of nosocomial infections with the development of resistance to antibiotics. So appropriate identification and characterization and antimicrobial susceptibility testing of Enterococcal species is necessary for management and prevention of these infections.
Materials and Methods: 150 isolates of Enterococcal species were obtained from various clinical samples. Characterisation was done by standard Microbiological methods and antibiotic susceptibility testing was done by Kirby- Bauer disc diffusion method and Vancomycin MIC tested by E- test.
Results: Out of 150 isolates from various clinical samples like urine 93(62%), pus 45(30%), blood 7(4.6%) and other body fluids 5(3%), *E.faecalis* 131(87.3%) was the predominant isolate followed by *E.faecium* 14(9.3%), *E.avium* 2(1.3%), *E.raffinosus* 2(1.3%) and *E.durans* 1(0.6%). All isolates were sensitive to Vancomycin, Teicoplanin and Linezolid. Sensitivity to High level Gentamicin was 92%. Rate of resistance to Penicillin 150(100%), Tetracycline 95(63.3%), Ciprofloxacin 103(68.6%) and Ampicillin 67(44.6%).
Conclusions: Even though no Vancomycin resistant strains were isolated from our study, there is incidence of Vancomycin resistant *Enterococci* are emerging as potent pathogen. So methods for characterization, antimicrobial susceptibility testing and MIC of Vancomycin should be done routinely for Enterococcal species.

1. Introduction

Enterococci, an indigenous flora of the intestinal tract, oral cavity and genitourinary tract of humans and animals, are known to be relatively avirulent in healthy individuals.[1,2] However over the last two decades they have emerged as a serious pathogen causing infections like endocarditis, bacteraemia, intra – abdominal and urinary tract infections. They have posed major therapeautic challenges, including the need for synergistic combinations of antibiotics to treat enterococcal infections.[3] The genus *Enterococcus* includes five groups with 28 species, only a few causing clinical infections in humans. *Enterococcus faecalis* is the most common isolate, being associated with 80 – 90% of human enterococcal infections. *Enterococcus* faecium ranks second and is isolated from 10 – 15% of infections.[3,4]

Infections by *Enterococci* have been treated with cell wall active agents like Penicillins, in combination with an Aminoglycoside.[4] Isolation of *Enterococci* resistant to multiple antibiotics has become increasingly common.[2,3] *Enterococci* have intrinsic resistance to Cephalosporins, Cotrimoxazole, Lincosamide, low level Penicillin and low

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level Aminoglycosides. They have also developed resistance to many other antibiotic groups via transmission of genetic material or via mutation. Vancomycin has been used as the drug of choice in many infections caused by resistant strains. Then there was an emergence of *Vancomycin resistant Enterococci* (VRE) causing serious superinfections among patients receiving broad spectrum antimicrobial chemotherapy. The organism can horizontally transfer this resistant determinant to Vancomycin susceptible Staphylococcus aureus.[5]

Vancomycin resistant Enterococci (VRE) was first notified in England in 1988. Infection with VRE is associated with increased mortality, prolonged hospital stay, admission to the ICU, surgical procedures and high cost.[6] Such strains pose therapeutic dilemmas for clinicians.[7,8] Thus there is a need in the tertiary care hospitals to identify, isolate and speciate *Enterococci* for the better understanding of their role in infections.[8] Monitoring the antibiotic resistance of *Enterococci* isolated from clinical specimens is a useful tool to get information about VRE and other resistance patterns which may arise.[8]

2. Materials and Methods

Present study was conducted over a period of 3 months at the Department of Microbiology, Sree Gokulam Medical College and Research Foundation, Venjaramoodu, Thiruvananthapuram district, Kerala.,. The isolates were obtained from clinical samples like pus, urine, blood and other body fluids. On receiving the sample in the laboratory, macroscopic appearance of thesample was recorded. Direct examination using Gram stain was done and the smear was examined. The colour, shape and appearance of the microorganism was recorded along with the presence of pus cells.

Culture – The samples were inoculated onto Blood agar and Mac Conkey agar. All plates were incubated aerobically at 37° C and growth was observed after 24 hours and 48 hours. The colonies were further processed according to standard guidelines, Gram staining, Detection of motility, Catalase test, Bile Aesculin test, PYR test, Growth in the presence of 6.5% Sodium chloride, Growth at 45°C and 60°C, Fermentation of sugars – 1% Glucose, Sucrose, Lactose, Mannitol, Arabinose and Raffinose, Arginine hydrolysis, Tellurite reduction, Production of Hydrogen sulphide, and Pigment production. All the biochemical reagents were procured from HiMedia.

The sensitivity test was performed by Kirby-bauer disc diffusion method using commercially available discs (Himedia). The results were interpreted as per the CLSI 2014 guideline. Additionally for Vancomycin (<2 we did E strip test (Biomeriux). All the isolates were confirmed using Vitek 2 test.

3. Results

Sample	Urine	Pus	Fluids	Blood
Nos	93 (62%)	45 (30%)	5 (3%)	7 (4.6%)

Majority of the Enterococcal isolates in our study were from the urine samples (62%). Out of 93 samples, 7 were from catheterized patients. Pus samples were from surgical wound sites, diabetic wounds, burns, abdominal abscess and A-V fistula site. *Enterococci* isolated from blood 7 (4.6%). 3% of the clinical samples were from infected body fluids like ascitic fluid (2), knee joint aspirate (1) and from bile duct drainage (1).

Among 5132 clinical samples, *Enterococcus spp* were isolated from 150 samples accounting for an isolation rate of 2.9%. enterococcal isolates, the main species is *E.faecalis* 87.3% (131), followed by *E.faecium* 9.3% (14). *E.avium* and *E.raffinosus* 1.3% (2) and *E.durans* 0.6% (1).

Out of 150 isolates, 85 (57%) from males and 65 (43%) from females.

Out of 45 pus samples, 30 were poly-microbial. Along with *Enterococci*, *Proteus spp*. 30% (9), *Pseudomonas spp*. 23% (7), *Klebsiella spp*. 17% (5), *Escherichia coli* 10% (3), Citrobacter spp. 7% (2) and other NFGNB 13% (4).

Out of 150 isolates, all were sensitive to Vancomycin, Teicoplanin and Linezolid. 138 (92%) isolates were sensitive to High level Gentamicin. Isolates resistant to Penicillin 150(100%), Erythromycin 150(100%), Tetracycline 95(63.3%), Ciprofloxacin 103 (68.6%) and Ampicillin 67(44.6%).

Out of 93 urine samples, 10 were mixed growth of Enterococci and *Escherichia coli* 50% (5), *Pseudomonas spp*. 30% (3) and Yeast 20% (2).

Predisposing factors include Diabetes mellitus (39.4%), chronic kidney disease (31.5%), benign prostate hypertrophy (4%), renal calculi (3.8%), malignancy (5.2%), trauma (5.2%) and chronic liver disease (5.2%). Out of 31.5% of chronic kidney disease patients, 18% were on haemodialysis.

Antibiotic tested	Nos of sensitive strains	Nos of resistant strains	Percentage of resistance
Ampicillin	83	67	44.6
Tetracycline	55	95	63.3
Vancomycin	150	0	0
Teicoplanin	150	0	0
Linezolid	150	0	0
Ciprofloxacin	47	103	68.6
High Level Gentamicin	138	12	8
Erythromycin	0	150	100

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sensitive to High level Gentamicin. Isolates resistant to Penicillin 150(100%), Erythromycin 150(100%), Tetracycline 95(63.3%), Ciprofloxacin 103(68.6%) and Ampicillin 67(44.6%) High level Gentamicin was tested using 120mcg disk. Out of 150 isolates, 12(8%) showed HLGR. *E.faecalis* 7(5.34%), *E.faecium* 4(28.57%) and *E.avium* 1(50%). No HLGR detected in *E.raffinosus* and *E.durans*. The sensitivity pattern of High level Gentamicin was statistically significant (p=0.05).

Vancomycin MIC was tested using E-strip. None of the enterococcal isolates in our study was resistant to Vancomycin.

4. Discussion

Developing resistance to multiple antibiotics allows *Enterococci* to survive and proliferate in patients receiving broad spectrum antibiotics. So, there is a need to isolate, identify and speciate *Enterococci* from clinical samples, study their antimicrobial susceptibility pattern and detect the presence of virulence factors. In a study conducted in Saudi Arabia by MM Salem – Bekhit et al in 2011, 10.8% from urine, 8.8% from pus, 12.1% from blood and 2.9% from ascitic fluid.[6] This is comparable to our study. The present study was similar to that of study conducted in 2011 in Saudi Arabia by MM Salem-Bekhit et al.[6] Most of the infections were caused by *E.faecalis* followed by *E.faecium*. Now there is an increasing trend that *E.faecium* emerging as a multidrug resistant nosocomial pathogen than *E.faecalis*.[1] In a study by M Mathur et al, *E.faecium* was the predominant isolate.[2] Occasional infections caused by *E.avium*, *E.raffinosus* and other species also have been reported.

Predisposing factors include diabetes mellitus (39.4%), chronic kidney disease (31.5%), benign prostate hypertrophy (4%), renal calculi (3.8%), malignancy (5.2%), trauma (5.2%) and chronic liver disease (5.2%). Out of 31.5% of chronic kidney disease patients, 18% were on haemodialysis. *Enterococci* isolated from various samples like pus from A-V fistula site and blood of these patients. Factors which causes infection in a haemodialysis unit includes cross transmission of pathogens, presence of co- morbid conditions, frequent use of broad spectrum antibiotics and numerous hospitalization during the course of the disease. All isolates were sensitive to Vancomycin, Linezolid and Teicoplanin. Out of 150 isolates tested, rate of resistance of isolates were Penicillin 150 (100%), Erythromycin 150 (100%), Ampicillin 67 (44.6%), Tetracycline 95 (63.3%), Ciprofloxacin 103 (68.6%) and High level Gentamicin 12 (8%). In a study done by Latika Shah et al[2] at Surat in 2012, the rate of resistance among 92 isolates were Penicillin 46%, Ampicillin 40%, High level Gentamicin 40%, Ciprofloxacin 62% and Vancomycin 8%. All strains were sensitive to Teicoplanin and Linezolid. The study done by Saraswathy et al.[6] in 2013 at Tamil Nadu, the rate of resistance among 112 isolates of *Enterococci* was Ampicillin 35%, High level Gentamicin 29%, Ciprofloxacin 58%, Tetracycline 62%, and Vancomycin 1%.

Mendiratta DK et al[8] in 2004 at Maharashtra showed resistance against High level Gentamicin was more in *E.faecium* (81.8%) than in *E.faecalis* (22.6%). In 2003, study conducted at AIIMS, New Delhi by Mathur P et al[9] the rate of resistance was 26% in *E.faecalis* and no resistance was reported in *E.faecium*. Study conducted by Rahangdale VA et al[14] in 2007 at Nagpur, the rate was 47.96% in both *E.faecalis* and in *E.faecium*. In 2011 study conducted in Saudi Arabia by MM Salem – Bekhit et al[4] showed 22.3% and 18.5% was the rate of resistance in *E.faecalis* and in *E.faecium* respectively. *E.faecium* showed 28.57% resistance which was in accordance with the study done by Saraswathy et al.[7] Study conducted by Karmarkar et al showed high rate of resistance to high level Gentamicin.

In 2000- 2001 study conducted in PGI, Chandigarh by Taneja et al[10] showed the rate of resistance to Vancomycin by *E.faecalis* and *E.faecium* was 5.5%. In 2003, study done by Mathur P et al[9] in AIIMS, New Delhi showed only 1% resistance in both *E.faecalis* and in *E.faecium*. Karmarkar MG et al in 2004 at Mumbai showed that the resistance in *E.faecalis* was 10% and in *E.faecium* 28.57%. Rahangdale VA et al[8] in 2007 at Nagpur showed both *E.faecalis* and *E.faecium* had 11.38% resistant strains. The study done by Saraswathy et al in 2013 at Tamil Nadu showed only 0.89% resistance among *E.faecalis* and in *E.faecium*. No Vancomycin resistant strains was isolated in our study. 0.89% was reported by Saraswathy et al[7] and 1% resistance was reported by Mathur P et al.[9]

5. Conclusion

Enterococci was a low virulence organism initially. In the recent years, they emerged as a pathogen causing plethora of infections mainly urinary tract infections, blood stream infections, endocarditis, skin and soft tissue infections and intraabdominal and intra pelvic abscesses. Use of broad spectrum antibiotics for underlying diseases like chronic kidney disease on dialysis, patients within travascular devices, chronic liver disease with peritonitis leads to development of resistance in *Enterococci* which is a coloniser of the gastrointestinal tract. Characterization of *Enterococci* is important due to difference in the antibiotic susceptibility pattern exhibited by different species. All strains of *Enterococci* isolated in our study was resistance to Penicillin. *E.faecium* showed increased rate of resistance to Ampicillin and High level Gentamicin compared to *E.faecalis*. Minimum inhibitory concentration of Vancomycin was tested by E-test and all strains were found to be sensitive. Vancomycin resistant *Enterococci* is now emerging as a potent nosocomial pathogen. VRE can colonize the gastrointestinal tract. So it is important to define risk factors for acquisition and to evaluate

the effect of interventions on rates of colonisation and infection. Prompt isolation, accurate identification and antibiotic susceptibility testing of *Enterococci* will help in the early identification of antibiotic resistant strains, especially Vancomycin resistant *Enterococci* which help us to control their spread.

6. Summary

In this study, *E.faecalis* remains the predominant isolate. *E.faecium* showed high rate of resistance to antimicrobials when compared with *E.faecalis*. All strains were sensitive to Vancomycin. Appropriate methods should be used routinely in laboratory for detection of antibiotic resistance. Vancomycin resistant *Enterococci* can colonise the gastrointestinal tract and the risk for developing a subsequent blood -stream infection with the same VRE colonizing strain is high in patients with underlying comorbidities. Periodic surveillance programmes should be done inorder to check the emergence of Vancomycin resistant *Enterococci*.

7. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

8. Source of Funding

None.

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