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Phenotypic Characterization and Antibiotic Susceptibility Pattern of Clinical Isolates of Enterococci with Special Emphasis on Vancomycin Resistance

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Abstract

INTRODUCTION: Antimicrobial resistance is spreading all over the world and becoming a major health concern. To stop the spread of antimicrobial resistance an essential tool is required for the detection of sensitive antibiotics to *Enterococci*. Microbiological surveillance by culture and sensitivity or by PCR methods for gene detection will help the clinicians in choosing the appropriate antibiotic.

MATERIALS AND METHODS: This is a retrospective observational study conducted on 158 various clinical samples. Microbiological processing and find the resistance pattern of isolates was done as per the protocols and CLSI guidelines. Analysis of results was done by counts and percentages using MS Excel, 2007 version.

RESULTS: Out of 52 *Enterococcus* isolates 30 (57.6%) isolated from pus, 15 (30.7%) were from urine, 4 (7.6%) were from blood and 3 (5.7%) were from fluids. 40 (77%) were *E.faecalis*, 12 (23%) *E.faecium.* The *Enterococci* isolates from various clinical samples showed a good sensitivity to vancomycin (96.1%), chloramphenicol (96.1%), high level gentamicin (90.3%), and Amoxicillin/clavulanic acid (86.5%). Out of 12 *E.faecium*, 2 (3.84%) were Vancomycin resistant.

CONCLUSION: Microbiological culture for speciation of bacteria is extremely important in terms of Enterococcus as there is wide difference in resistance to antibiotics by particular enterococcal species. Screening of vancomycin resistant by high sensitivity test takes a crucial step in the management of cases and also to prevent the transfer of VRE among hospitalized patients.

KEYWORDS: Enterococci, Antimicrobial resistance, Clinical samples.

INTRODUCTION

Enterococci are gram positive facultative anerobic opportunistic pathogens that habit the gastrointestinal and biliary tract, as well as the vaginal and male urethra in lesser numbers. The species of *Enterococci* with great clinical importance are *Enterococcus faecalis* and *Enterococcus faecium*. *Enterococcus* species are important agents that cause complicated UTIs, bacterial sepsis, endocarditis, intra-abdominal and pelvic infections, post-operative wound infections and soft tissue infections, neonatal sepsis and rarely meningitis. Nowadays the *Enterococcus* resistance to environmental conditions such as heat and desiccation and to antibiotics over the past three decades resulting in increase in the chance of persistence and spread of this pathogen, these characteristics are also contributing to colonization and infection [1]. *Enterococci* are the third most common pathogen in health care associated infections, the second most common cause of surgical site infections and the second most common cause of bacteremia acquired in intensive care units [2,3].

Enterococci are intrinsically resistant to many commonly used antimicrobial agents including clindamycin, cephalosporins and cotrimoxazole. It is also expressing tolerance to the most common of the beta lactam agents which are first line of treatment; this left the treatment choice of only higher antibiotics such as vancomycin or teicoplanin. However, this tolerance can be overcome by synergistic effect of combination of beta lactam agents and aminoglycosides [4].

Subsequently, the antimicrobial resistance is spreading to vancomycin and other broad-spectrum antimicrobials as the use of these antibiotics is necessary in deep seated infections and immunocompromised patients. Vancomycin Resistant *Enterococcus* first reported in humans in1988, from that time the vancomycin resistance in E.faecium increased from approximately 0% to more than 83% by 2016 [5]. In *Enterococci* six types of vancomycin resistance have been reported (*VanA, VanB, VanC, VanD, VanE, and VanG*), it is an inducible resistance encode by vanA gene cluster, which is carried on transposons [6]. Linezolid is the only currently U.S. Food and Drug Administration (FDA)-approved antibiotic for VRE infections.

Enterococcus faecium is the most common clinical isolate exhibiting the resistance to antimicrobials. It is one of the ESKAPE pathogens highlighted by WHO (World Health Organization) and responsible for the cause of nosocomial infection and antibiotic resistant infections. To stop the spread of antimicrobial resistance an essential tool is required for the detection of sensitive antibiotics to *Enterococci*. Microbiological surveillance by culture and sensitivity or by PCR methods for gene detection will help the clinicians in choosing the appropriate antibiotic.

AIM & OBJECTIVES

1.To isolate and identify the *Enterococci* from various clinical specimens.
2.To determine the Antibiotic susceptibility pattern of the *Enterococci* isolates.

MATERIALS AND METHODS

Study Design & Settings:

This is a retrospective observational study conducted on 158 various clinical samples including urine, pus, fluids, blood received to laboratory of Microbiology department at a government medical college in southern part of India. The ethical committee clearance was obtained before conducting the study. The data of patient details, sample details, culture and sensitivity was collected from the microbiology registers and hospital charts.

Study period: 3 months (April 2024 to June 2024).

Study Procedure:

All the samples which are collected under aseptic precautions were inoculated on to Nutrient agar, Blood agar and Mac Conkey agar and incubated at 37°C for 24 to 48 hrs. Identification of isolate from positive cultures was done using standard microbiological techniques which include motility testing by hanging drop preparation, Gram staining and biochemical reactions such as catalase, coagulase, indole, methyl red, Voges Proskauer, citrate, urease, bile esculin test, phenyl pyruvic acid test and oxidase test. *Enterococci* was identified by its colony features like tiny, non hemolytic and special characteristics gram positive cocci in short chains which are catalase negative, growth in NaCl and black colonies on bile esculin test. E.faecalis and E. faecium were differentiated by Mannitol salt agar growth.

Antibiotic sensitivity testing of all isolates was done by Kirby Bauer's disc diffusion method on Muller Hinton agar and results were interpreted as per CLSI guidelines. Standard antibiotics for *Enterococci* were used like oxacillin (1µg), penicillin (10 units), ampicillin (10 µg), amoxicillin/clavulanic acid (30µg), high level gentamicin (120 µg), erythromycin (15µg), vancomycin (30µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5µg), chloramphenicol (30µg), and nitrofurantoin (300µg). All the culture media, biochemical media and antibiotic discs used were obtained from Hi Media. *Enterococcus* isolates Vancomycin resistance was detected using HiComb E strip method, interpretation of results were reported based on the manufacturer instructions.

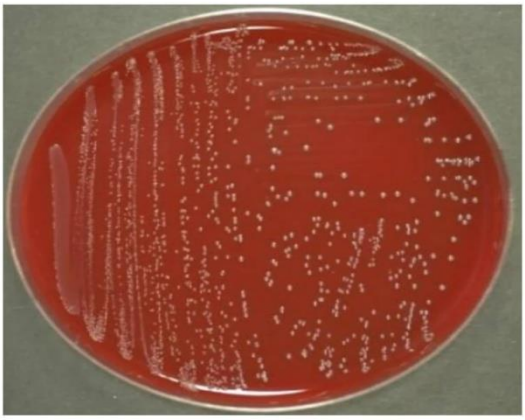
Data Collection:

Analysis of results was done by counts and percentages using MS Excel, 2007 version.

RESULTS

A total of 158 clinical samples were processed in this study period in which 52 *Enterococcus* species were isolated. Out of 52 *Enterococcus* isolates 30 (57.6%) isolated from pus, 15 (30.7%) were from urine, 4 (7.6%) were from blood and 3 (5.7%) were from fluids (Table 1). We have isolated 40 (77%) were *E.faecalis*, 12 (23%) *E.faecium* (Fig 1 & 2).

Fig 1. *Enterococcus* colonies on blood agar



***Enterococcus faecalis* on Blood Agar.**

Fig 2. Bile esculin test

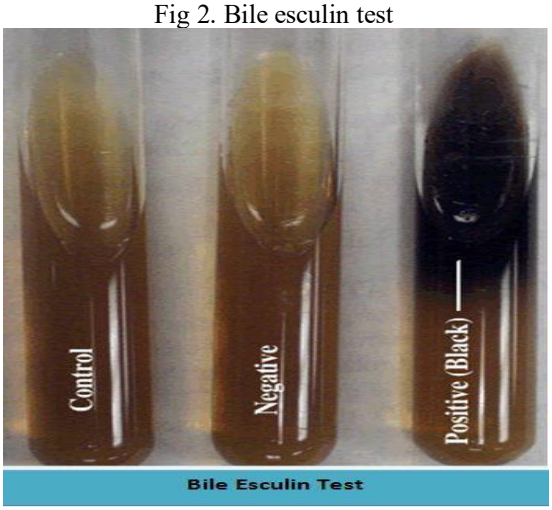
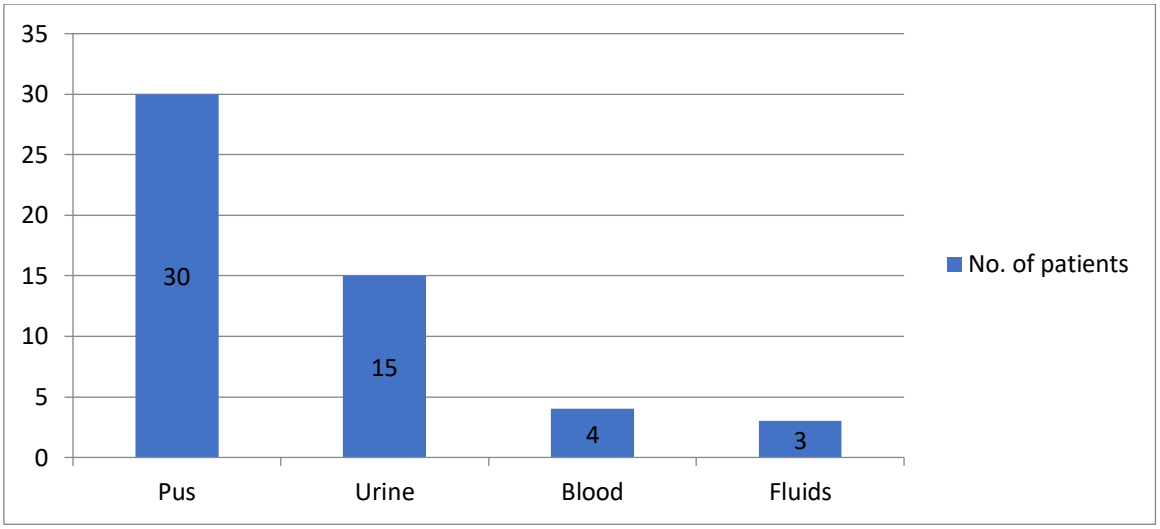


Table 1. Distribution of *Enterococcal* isolates in various clinical samples



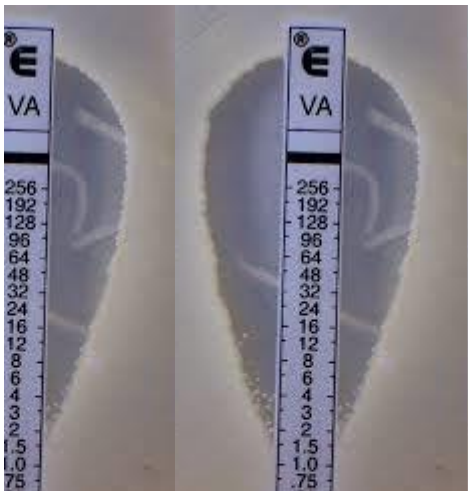
The *Enterococci* isolates from various clinical samples showed a good sensitivity to vancomycin (96.1%), chloramphenicol (96.1%), high level gentamicin (90.3%), and Amoxicillin/clavulanic acid (86.5%). Urine *Enterococcal* isolates showed a good sensitivity to UTI antibiotic i.e., nitrofurantoin which was 84.6% (Table 2).

Table 2. Antibiotic susceptibility pattern of Enterococcal isolates

Antibiotics	No. of patients	Percentage
Penicillin G	4	7.69%
Ampicillin	31	59.6%
Oxacillin	0	0
Amoxicillin/clavulanic acid	45	86.5%
High Level Gentamicin	47	90.3%
Erythromycin	16	30.2%
trimethoprim-sulfamethoxazole	6	11.5%
Vancomycin	50	96.1%
Ciprofloxacin	24	46.1%
Chloramphenicol	50	96.1%
Nitrofurantoin	44	84.6%

Out of 12 *E.faecium*, 2 (3.84%) were Vancomycin resistant (Fig 3). All the isolates of *Enterococcus faecalis* were sensitive to vancomycin and all isolates were sensitive to Linezolid.

Fig 3. Showing the susceptibility pattern of vancomycin E strip test



DISCUSSION

Drug resistance is a major public health problem in the world. Few organisms can acquire drug resistant genes easily by various mechanisms, one among those are Enterococci isolates which can rapidly acquire resistance genes, for instance, they achieved resistance quickly to chloramphenicol, erythromycin and tetracyclines. Ampicillin resistance is high in E.faecium isolates, although it is relatively rare in E.faecalis. Predisposing factors for the emergence of VRE are usage of antimicrobial therapy previously, alteration in bowel flora, patients with immunosuppression or severe underlying illness, long hospital stay, admission to long-term care facilities, extended use of antibiotics, and proximity to other patients with vancomycin-resistant *Enterococcus* [7,8].

Vancomycin resistant is a growing problem, highly prevalent in E.faecium and it is relatively rare in E.faecalis. Future options for vancomycin resistant Enterococci (VRE) are left with very few antibiotics such as quinupristin-dalfopristin, linezolid, daptomycin, tigecycline. Researchers and Pharmaceutical industries are doing focused works to develop newer agents.

A total of 158 clinical samples were processed in this study period in which 52 Enterococcus species were isolated. Out of 52 Enterococcus isolates 30 (57.6%) isolated from pus, 15 (30.7%) were from urine, 4 (7.6%) were from blood and 3 (5.7%) were from fluids. We have isolated 40 (77%) were *E.faecalis*, 12 (23%) *E.faecium*; in similar to this study Yadav RK et al [9] observed a total of 145 *Enterococcus* isolates were obtained; 73 (50.3%) isolates were identified as *Enterococcus faecalis*, 69 (47.5%) *Enterococcus faecium*, 2 (1.3%) *Enterococcus durans* and 1(0.68%) as *Enterococcus gallinarum*. Sood S et al [10] did a study on Enterococcal infections and antimicrobial resistance they noted increase in isolate rate of E.faecalis. Few studies isolated significant number of *Enterococci* in Urine samples [9,11], whereas by Agarwal J & Preeti S studies observed in pus samples in line with this study [12,13]. Hota S et al [11] documented that out of 189 culture-positive *Enterococcal* isolates, the majority were obtained from urine 144 (76%), followed by blood 17 (9%), pus 12 (6%), and a variety of other sources, including tissue samples, vaginal swabs, sputum, bodily fluids, CSF, and catheter tips. They have isolated 57.7% of *E.faecium* and 39.6% of *E.faecalis*.

The *Enterococci* isolates from various clinical samples showed a good sensitivity to vancomycin (96.1%), chloramphenicol (96.1%), high level gentamicin (90.3%), and Amoxicillin/clavulanic acid (86.5%). Urine Enterococcal isolates showed a good sensitivity to UTI antibiotic i.e., nitrofurantoin which was 84.6% in the present study. High-level Gentamicin (HLG) antibiotics resistance were ranged from 12.6% to 100% among *E. faecalis [14]*. Hota S et al [11] observed that the E.faecium is highly resistant to benzylpenicillin (96%) and nitrofurantoin (94%) whereas *E. faecalis* showed higher resistance to high-level gentamicin (80%). *E. gallinarum* (1.6%) showed complete resistance to benzylpenicillin (100%) and moderate resistance to nitrofurantoin (67%), and high-level gentamicin (67%). Conversely, *E. durans* showed complete resistance (100%) to both nitrofurantoin and benzylpenicillin. Said HS and Abdelmegeed ES in 2019, who reported similar rates of resistance in tetracycline (93%), ciprofloxacin (84.5%), levofloxacin (88.7%), and high-level gentamicin (80.3%) [15]. Rana D et al noted significant susceptibility to teicoplanin (91.5%), linezolid (97.2%), and vancomycin (88.6%) [16]. Yadav RK et al [9] showed that 59.3% of isolates were resistant to ampicillin, 60.6% to ciprofloxacin, and 47.5% to high-level gentamicin. A study by Bhatt P et al [17] on emergence of MDR Enterococci shows a drastic increase in the resistance pattern of the commonly used drugs, an increase in the penicillin resistance to 95%, an increase in the ampicillin resistance to 95% and an increase in the HLGR to nearly 50%.

In this study, out of 12 E.faecium, 2 (3.84%) were Vancomycin resistant which were tested by E-strip test. All the isolates of Enterococcus faecalis were sensitive to vancomycin and all isolates were sensitive to Linezolid. India's first VRE case was reported in 1999 by Mathur P et al from New Delhi. Between 1 % and 8.7 % have been reported as VRE prevalence in India between 1999 and 2021 [18]. Hota S et al [11] noted 16 (52%) of Van A and 15 (48%) of Van B phenotype among 31 VRE isolates. Yadav RK [9] and Agarwal L in 2022 [12] reported a prevalence of VanA at 71.4% and VanB at 28.5%. Praharaj I et al [19] and Yadav G et al [20] noted 8.7% and 7% of VRE respectively, Praharaj I *et al* [19] showed the prevalence of Van A and Van B as 90.6% and 6.25% respectively, and Yadav G *et al* [20] reported VanA 78.5% 21.4% van B prevalence.

CONCLUSION

Enterococcus faecalis and Enterococcus faecium are the two common species causing health-associated infections. E. faecalis and E.faecium showed more resistance to antibiotics like Penicillin G, Erythromycin and Ciprofloxacin and good number of isolates were sensitive to Chloramphenicol, High level gentamicin and Vancomycin. Microbiological culture for speciation of bacteria is extremely important in terms of Enterococcus as there is wide difference in resistance to antibiotics by particular enterococcal species. Screening of vancomycin resistant by high sensitivity test takes a crucial step in the management of cases and also to prevent the transfer of VRE among hospitalized patients.

REFERENCES

1. Zirakzadeh A, Patel R. Vancomycin resistant enterococci: colonization, infection, detection, and treatment. Mayo Clin Proc. 2006,81(4):529-36.
2. Brinkwirth S, Ayobami O, Eckmanns T, Markwart R. 2021. Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. Euro Surveill 26:2001628.
3. McCormick JK, Hirt H, Dunny GM, Schlievert PM. Pathogenic mechanisms of Enterococcal endocarditis. Curr Infect Dis. 2000;2(4):315-21.
4. Mohr JF, Friedrich LV, Yankelev S, Lamp KC. Daptomycin for the treatment of enterococcal bacteraemia: results from the Cubicin Outcomes Registry and Experience (CORE). International Journal of Antimicrobial Agents. 2009;33(6):543–548.
5. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, Edwards JR, Sievert DM. 2016. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol 37:1288–1301.
6. Courvalin P. Vancomycin – resistance in gram-positive cocci. Clin Infect Dis. 2006;42 suppl 1:s25-34.
7. Lee T, Pang S, Abraham S, Coombs GW. Antimicrobial-resistant CC17 Enterococcus faecium: The past, the present and the future. J Glob Antimicrob Resist. 2019 Mar;16:36-47.
8. 8.McDermott H, Skally M, O'Rourke J, Humphreys H, Fitzgerald-Hughes D. Near-patient environmental contamination of an intensive care unit with Vancomycin-resistant Enterococci (VRE) and Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae (ESBL-E) before and after the introduction of chlorhexidine bathing for patients. Infect Control Hosp Epidemiol. 2018 Sep;39(9):1131-1132.
9. Yadav RK, Agarwal L. Enterococcal infections in a tertiary care hospital, North India. Ann Afr Med. 2022;21:193–197.
10. 10.Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections and antimicrobial resistance. Indian J Med Res. 2008;128:111–21.
11. Hota S, Patil SR, Mane PM. Antimicrobial Resistance Profile of Enterococcal Isolates From Clinical Specimens at a Tertiary Care Hospital in Western Maharashtra, India. Cureus. 2024 Nov 10;16(11):e73416.
12. Agarwal J, Kalyan R, Singh M. High-level aminoglycoside resistance and beta-lactamase production in enterococci at a tertiary care hospital in India. Jpn J Infect Dis. 2009;62:158–9.
13. Preeti S, Raman M, Nirwan PS, Meeta S, Dahiya SS. Prevalence and antimicrobial susceptibility of Enterococcus Species isolated from different clinical samples in a tertiary care hospital of North India. Natl J Med Res. 2012;3:389–91.
14. Taneja Rani N, Emmanuel PR, Sharma M. Significance of vancomycin resistant enterococci from urinary specimens at a tertiary care centre in northern India. Indian J Med Res. 2004119724.
15. Said HS, Abdelmegeed ES. Emergence of multidrug resistance and extensive drug resistance among enterococcal clinical isolates in Egypt. Infect Drug Resist. 2019;12:1113–1125.

16. Rana D, Sande S. Study of prevalence and antimicrobial susceptibility pattern of enterococci isolated from clinically relevant samples with special reference to high level aminoglycoside resistance (HLAR) in a rural tertiary care hospital. J Evolution Med Dent Sci. 2020;9:2472–2478.
17. Bhatt P, Patel A, Sahni AK, Praharaj AK, Grover N, Chaudhari CN, et al. Emergence of multidrug resistant enterococci at a tertiary care centre. Med J Armed Forces India. 2015;71:139–44.
18. Patel J. Prevalence of Enterococcus species and its vancomycin resistance pattern in a tertiary care hospital, Surat, Gujarat, India: a growing threat. 2023 Aug.
19. Praharaj I, Sujatha S, Parija SC. Phenotypic and genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J Med Res. 2013;138:549–56.
20. Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and vancomycin resistant Enterococci: A therapeutic problem. J Clin Diagn Res. 2017;11:C07–11.