International Journal of Medical Science in Clinical Research and Review
Online ISSN: 2581-8945
Available Online at http://www.ijmscrr.in Volume 6|Issue 06 (November-December)|2023 Page: 1140-1145

Original Research Paper

Occurrence, Risk Factors and the Antimicrobial Resistance Pattern of Enterococcal Infections in the University Hospital of Western Maharashtra India

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Article Received: 11-November-2023, Revised: 01-December-2023, Accepted: 21-December-2023

ABSTRACT:

Introduction: Enterococci are gram positive cocci occurring in pairs in majority of the cases, at angles & sometimes can appear as short chains. In the course of the last two decades, enterococci have been recognized as imperative healthcare-associated opportunistic pathogens. The study was planned with the purpose to understand the incidence, risk factors, antimicrobial resistance pattern of enterococcal infections in the University hospital. **Methods**: This retrospective study was conducted in the Microbiology laboratory in a University hospital from January 2021 to June 2022. Identification of enterococci was done by using standard conventional methods. Speciation and antimicrobial susceptibility testing were carried out by using "The BD Phoenix™ M50 automated identification system. **Results**: Total of 7635 clinical samples were received during the study period in the Microbiology laboratory. Of these, 2422 samples [31.72%] showed the pathogen growth. 254 [37.51%] were identified as *E. fecalis* and *E. faecium*. Antimicrobial susceptibility pattern revealed susceptibility of 100% for fosfomycin, tigecycline, and daptomycin. 97.5% isolates showed susceptibility to linezolid, 95% to vancomycin, 93% to teicoplanin,75% isolates were susceptible to imipenem, 66.7% to ampicilin and 63% to amoxiclav. 63% urinary isolates of enterococci were susceptible to nitrofurantoin. 5% enterococcal strains were detected as vancomycin-resistant by phenotypic methods. In present study, the risk factors for acquiring enterococcal infections were prior myocardial infarction, gastrointestinal, and genitourinary infections. **Conclusion**: Sustained investigation dedicated to comprehending the resistance mechanisms in enterococci is crucial for the development of innovative combination treatments or the creation of fresh antimicrobial agents

Keywords: Vancomycin Resistant Enterococci, High Level Aminoglycoside Resistance, Risk factors, Infection Control, Antimicrobial Stewardship

INTRODUCTION:

Enterococci are gram positive cocci occurring in pairs in majority of the cases, at angles & sometimes can appear as short chains. They form smooth, translucent & grey colonies which differ in haemolysis pattern as nonhaemolytic, alpha haemolytic in majority of cases but can also be beta hemolytic. [1,2] They can thrive in environments with temperatures ranging from 10°C to 45°C and can grow in the presence of pyrrolidonylarylamidase the characteristics which distinguish enterococci from Streptococci and related taxonomy. [1] *E.avium, E.faecalis, E.raffinosus, E.malodoratus, E.pseudoavium, E.solitarius, E.gallinarum, E.faecium, E.casseliflavus, E.mundtii, E.durans, E.hirae* are various species of enterococci. Enterococci are among the naturally occurring microorganisms in the human gastrointestinal tract and were earlier considered as pathogens with low virulence. Recently, enterococci are increasingly isolated as opportunistic pathogens both from hospital as well as community acquired infections. This is attributed to increase in the immunocompromised

patient numbers in healthcare systems along with collateral advances in diagnostic techniques as well as the irrational and unrestricted use of broad-spectrum antimicrobials. [3]. Among healthcare-associated infections, enterococci rank as the third most prevalent pathogen, and in intensive care units, they stand as the second most frequent cause of acquired bacteremia. [4-7]

In the course of the last two decades, enterococci have been recognized as imperative healthcare-associated opportunistic pathogens. *Enterococcus faecalis* (*E.fecalis*) and *Enterococcus faecium* (*E. faecium*) can be major sources of a variety of infections including pyelonephritis, cystitis, catheter-associated UTI, endocarditis, intra-abdominal infections and mixed-organism infections of the pelvis. [8-10] Ampicillin and amoxicillin are the antimicrobial agents of empirical choice for susceptible strains. The escalating antibiotic resistance observed in clinical strains of enterococci, characterized by resistance to Vancomycin (VRE), Penicillin, and high-level resistance (HLR) to aminoglycosides, is a significant source of worry. Linezolid, Quinupristin/Dalfopristin, and Daptomycin are antimicrobials used in these enterococcus infections. There are limited treatment options available for Vancomycin resistant enterococcal infection which tend to occur in high frequency among severely ill patients in Intensive Care Units (ICU) .[11] Cell wall active agents such as penicillin and ampicillin are commonly prescribed in combination with aminoglycoside(gentamicin/streptomycin) to treat enterococcal infections even though it is observed that this combination treatment fails to treat these infections due to surfacing of high level aminoglycoside resistant strains (HLAR), beta-lactam antibiotics resistant strains or vancomycin resistance (VRE).[12] The study was planned with the purpose to understand the incidence, risk factors, antimicrobial resistance pattern of enterococcal infections in university Hospital.

METHODS:

The study was conducted in the Microbiology laboratory in the University Hospital. We employed a retrospective study design. The study period was from January 2021 to June 2022. Various clinical samples were received in the Microbiology laboratory as a part of routine investigations after patient admission to the hospital as per hospital investigations protocol depending on the presenting symptoms of each patient. The samples received in the laboratory for culture sensitivity request were processed in the laboratory as a part of routine diagnostic workup of the patient for which hospital has a policy of blanket consent at the admission of the patient to hospital.

Clinical samples were collected with strict adherence to the standard operating procedures and transported to the laboratory without delay. Further the samples were inoculated onto Blood agar and Mac Conkey agar plates using all biosafety precautions. This was followed by incubating the culture plates for the period of 18 hours at 37 degrees Celsius. Identification of enterococci was done by using standard conventional biochemical tests such as catalase test and bile esculin test. Further speciation into *E.fecalis* and *E. faecium* and antimicrobial susceptibility testing of the isolate were carried out by using "The BD Phoenix™ M50 automated identification system.

RESULTS:

Total of 7635 clinical samples were received during the study period in the Microbiology laboratory. Of these, 2422 samples [31.72%] showed the pathogen growth.

Distribution of Pathogens Isolated from Clinical Samples:

Of the total pathogens isolated, 677 [27.95%] were gram-positive cocci. Out of all gram positive isolates, 254 [37.51%] were identified as *E. fecalis* and *E. faecium* by BD Phoenix™ M50 automated identification system.(fig.1)

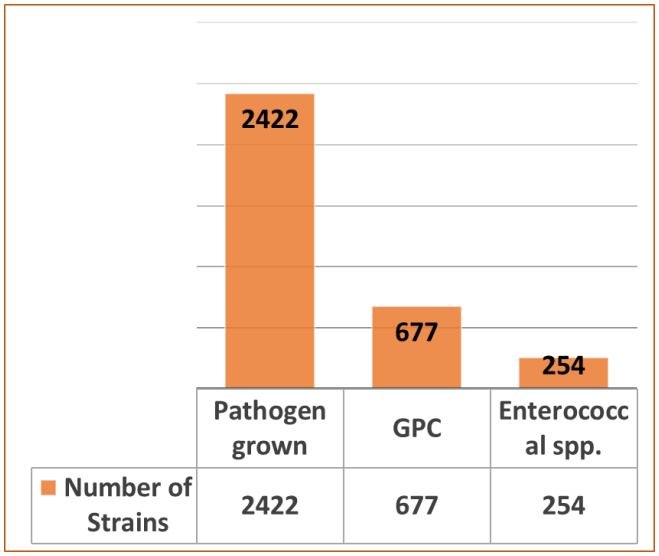

Figure 1: Distribution of pathogens including enterococci

Antimicrobial Susceptibility Pattern-

Antimicrobial susceptibility pattern revealed susceptibility of 100% for fosfomycin, tigecycline, and daptomycin. 97.5% isolates showed susceptibility to linezolid, 95% to vancomycin, 93% to teicoplanin,75% isolates were susceptible to imipenem, 66.7% to ampicilin and 63% to amoxiclav. 63% urinary isolates of enterococci were susceptible to nitrofurantoin. 5% enterococcal strains were detected as vancomycin-resistant by phenotypic methods but we could not conduct diagnostic PCR tests aimed at detecting the presence of resistance genes such as *vanA*, *vanB*, *vanC1*, and *vanC2* PCR. 72 (28%) High-Level Aminoglycoside-Resistant (HLAR) enterococcal strains were isolated in this study. (fig.2)

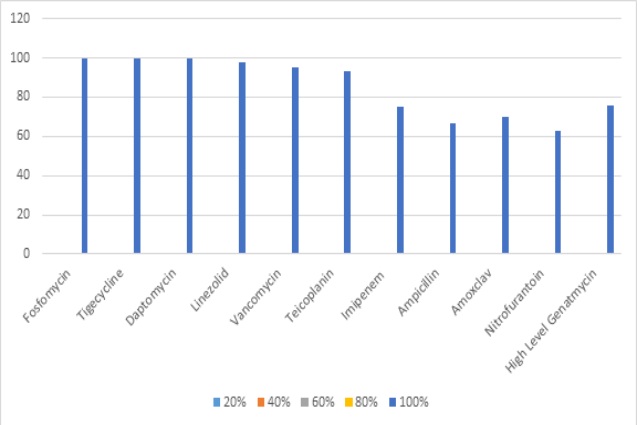

Fig 2 Antimicrobial susceptibility of *Enterococcus spp*

Associated Risk Factors in Enterococcus Infections:

In present study, the risk factors for acquiring enterococcal infections were prior myocardial infarction, gastrointestinal, and genitourinary infections. Chronic kidney disease was associated with increased risk of acquisition, especially among patients receiving renal replacement therapy, diabetes mellitus, heart disease, stroke, rheumatoid arthritis. Peptic ulcer disease, inflammatory bowel disease, and hypertension were also risk factors along with hospital stays longer than one week with device-related infections. The majority of *Enterococcus spp* in this study were from urinary, endovascular and intra-abdominal sources. (fig.3)

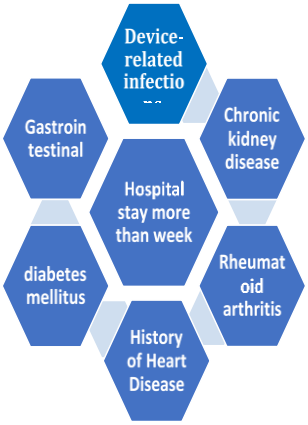

Fig.3 Associated risk factors in *Enterococcus* infections

DISCUSSION:

Majority of the isolates in this study were *E.faecalis* (70.86%) compared to *E.faecium (*29.13%) . This species distribution was similar to other studies done by MM Salem-Bekhit et al in 2012[13] and a study done by Zouain MG, Araj GF in 2001[14] but it contradicts with the reports from studies done in a few countries which reported the higher preponderance of *E. faecium than E. faecalis*. [15] This study reported more number of *E.faecium* isolates as compared with the number of isolates reported in the study by MM Salem-Bekhit et al in 2012.[13] However ,there are a few reports from past studies stating *E. faecium* as the most commonly isolated species.[16] The majority of *Enterococcus species* in this study were from urinary, endovascular and intra-abdominal sources. The findings of our study regarding the occurrence of enterococcal infections and their isolation from various systems align with the work of Osaba et al.who reported similar prevalence rates in their respective studies conducted in different regions.[16] Our identification of risk factors for enterococcal infections in Western part of India, such as prolonged hospitalization and previous antibiotic use, resonates with the conclusions drawn by Bilington et al.in 2014 [22] and Low et al [8] in their study. Nevertheless, it is crucial to acknowledge that unique risk factors, such as environmental factors or specific population demographics, may contribute to the regional differences observed between our study and that of other studies.[16-18] Enterococci are intrinsically resistant to a variety of antimicrobials and can develop resistance to remaining antibiotics after a few days of treatment leading to treatment challenges. [17] Enterococci, with their inherent resistance to a variety of antimicrobials, present a formidable clinical challenge as they can rapidly develop resistance to remaining antibiotics during the early stages of treatment. This fact can create intricate hurdles for effective therapeutic strategies. The reported resistance to routine low end antimicrobials was remarkably lesser in the current study than that a few studies done in countries like Kuwait and Lebanon. [13-16]. These findings suggest a broad range of geographic locations where enterococci with antimicrobial resistance are prevalent. There is a consistent pattern of higher prevalence of antimicrobial resistance in *E. faecium* when compared to *E. faecalis*, as reported in numerous studies [18] contrary to the findings of one study done in Iran in 2018. [19] The incidence of *Enterococci* carrying vancomycin-resistant enterococci (VRE) is on the rise across various global regions, but their epidemiology displays substantial variation in different geographical zones. [20]

In this study, VRE isolates were 5% of the total enterococci and they were identified as *E. faecalis and E. faecium*. The signs & symptoms of these patients were analyzed from the case files . These patients were elderly diabetic and were on oral antidiabetic agents. All the patients were inpatients and had a history of previous hospitalization for some other illness. These findings align with the research conducted by Labibzadeh M and colleagues, where they documented a VRE prevalence of around 7% among the total enterococci isolated at an Iranian hospital. [19].

In this study, there were no instances of linezolid resistance detected in any of the *E. faecium* or *E. faecalis* isolates examined. This is consistent with the findings from study by Salem-Bekhit et al. who

reported only one linezolid resistant isolate among total 206 isolates. [13]. The lesser occurrence of vancomycin-resistant enterococci contributes to the preservation of effective treatment choices for the majority of clinical samples containing enterococcal isolates.

The reported resistance rate to ampicillin was 33.3% in this study which is little higher than the study by Labibzadeh M et al. who reported 22.8% resistance to ampicillin.[19] Resistance rate reported by Mathur et al. in India to ampicillin (66%) is twice higher than resistance rate of enterococcal isolates in this study.[21]. Prior reports have indicated that the production of β-lactamase in enterococci is an uncommon occurrence and the current study reaffirms this observation [17] Comparatively lower resistance rate of enterococcal isolates to ampicillin in this study is a favorable finding as ampicillin continues to be the treatment of choice for uncomplicated enterococcal infections. . 28% of the enterococcal isolates demonstrated high-level resistance to gentamicin in this study which is slightly higher than resistance (20.9%) reported in the study by Salem et al.in Saudi Arabia in 2012. [13] But the high level gentamicin resistance reported in the study by Labizadeh et al in Iran is much higher 82.7% compared to the present study. [19] The detection of high-level gentamicin resistance can be alarming as it may signify the increase in occurrence of such strains in near future. Extended stays in the hospital are linked to the development of drug-resistant strains of enterococci acquired within the healthcare environment. The spread of multidrug-resistant enterococcal infections is approximately tenfold more common than the occurrence of actual infections, often affecting patients with serious underlying medical conditions or those prescribed broad-spectrum antibiotics with activity against anaerobic bacteria. In general, the findings regarding antimicrobial susceptibility in this research indicated a minimal presence of antimicrobial resistance, relatively modest percentages of multidrug resistance, and, most importantly, the retention of susceptibility to the majority of critical and high-importance antimicrobials for human health. Although the proportion of vancomycin resistant enterococcal isolates was low in this study, their existence together with high-level aminoglycoside resistance stains necessitates regular surveillance of health care associated infections along with stringent infection control and antimicrobial stewardship practices. While our study provides a snapshot of enterococcal infections in Western Maharashtra, longitudinal data are lacking. Long-term trends, explored over a decade, may shed light on the evolving nature of enterococcal infections, especially concerning emerging resistance patterns and changes in prevalent strains over time. Though our study provides valuable insights into the antimicrobial resistance patterns, the lack of strain typing and molecular epidemiology data limits our ability to directly compare strain similarities with other studies. Future research efforts, employing advanced molecular techniques, could unravel potential strain transmission dynamics and origin.

Limitations and Generalizability:

It is crucial to acknowledge the limitations of our study, including the retrospective design and potential underreporting of cases. Comparing these limitations with those of other prospective studies, underscores the need for caution when generalizing our findings to broader populations.

CONCLUSION:

In conclusion, our study contributes valuable insights into the occurrence, risk factors, and antimicrobial resistance patterns of enterococcal infections in Western Maharashtra. Sustained investigation dedicated to comprehending the resistance mechanisms in enterococci is crucial for the development of innovative combination treatments or the creation of fresh antimicrobial agents.The comparative analysis with existing literature enhances the contextual understanding of regional variations, emphasizing the need for targeted interventions and ongoing surveillance to address the challenges posed by enterococcal infections on a global scale.

List of Abbreviations:

Enterococcus faecalis (*E.fecalis*), *Enterococcus faecium* (*E. faecium*), *Vancomycin Resistant Enterococci* (VRE), high level aminoglycoside resistant strains (HLAR)

Consent for Publication: This was taken as a part of blanket consent policy of our hospital.

Availability of Data and Material:

Yes. Data can available from the electronic system of hospital records.

Competing Interests:

None

Source of Funding: Research did not receive any external and internal financial support.

Acknowledgements:

Authors acknowledge all the laboratory staff for their help in processing the samples and the clinical departments for sending the investigations to the laboratory.

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Critical revision

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