



PREVALENCE AND RESISTANCE PATTERN OF ENTEROCOCCI ISOLATED FROM VARIOUS SAMPLES OF INDOOR PATIENTS IN A TERTIARY CARE HOSPITAL

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ABSTRACT **INTRODUCTION:** Antimicrobial resistance is becoming a major public health threat affecting humans worldwide. Widespread use and misuse of antibiotics is thought to increase the prevalence and emergence of resistant bacterial strains. *Enterococci* are normal resident flora of the gastrointestinal and biliary tracts and, in lower numbers, of the vagina and male urethra. Vancomycin is an important drug used in treatment of resistant strains of *Enterococci*. Over times, there has been increase in Vancomycin Resistance. And now, VRE have spread rapidly and are encountered worldwide. The aim of this study was to determine the prevalence and resistance of *Enterococci* isolated from various clinical specimens in a tertiary care hospital in North India. **MATERIAL & METHODS:** The present prospective study was conducted in the Department of Microbiology, Government Medical College, Amritsar. The study was conducted for a period of one and a half year i.e. from January 2019 to June 2020. All the samples (pus, urine, blood, body fluids, sputum, etc.) received in Microbiology department of Government Medical College, Amritsar were processed as per standard protocol. **RESULTS:** Out of total clinical samples (26,144), 6,425 (24.6%) were found to be culture positive. Among the culture positive, 254 (3.9%) isolates were identified as *Enterococcus species* comprising of 165 *E.faecalis* (65%) and 89 *E.faecium* (35%). Maximum number of *Enterococci* were isolated from urine samples(54.5%) followed by blood(27.3%) and pus & body fluids(18.2%). 7.9% of *E.faecium* isolates were found to be resistant to vancomycin. All the strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin.

KEYWORDS : *Enterococci*, Prevalence, VRE

INTRODUCTION

Antimicrobial resistance is becoming a major public health threat affecting humans worldwide¹. Widespread use and misuse of antibiotics is thought to increase the prevalence and emergence of resistant bacterial strains. It results in reduced efficacy of antibacterial, antiparasitic, antiviral and antifungal drugs making the treatment of patients difficult, as well as costly².

The genus *Enterococcus* includes the Enterococcal members previously classified with group D Streptococcus³. The genus *Enterococcus* consists of Gram positive cocci, catalase negative, non spore forming, facultative anaerobes that often occurs singly, in pairs or short chains³. *Enterococci* are normal resident flora of the gastrointestinal and biliary tracts and, in lower numbers, of the vagina and male urethra³. This genus comprises of *Enterococcus faecalis*, *E.faecium*, *E.durans*, *E.gallinarum*, *E.avium*, *E.italicus*, etc³. *E.faecalis* is the most common species found in clinical specimens whereas *E.faecium* is more drug resistant⁴.

Antimicrobial resistant *Enterococci* spread from hospitals to environment mostly through water and faeces, secondly, from humans and other sources, increasing their prevalence in environment, humans and animals, and becoming a potential risk for human health⁵. *Enterococci* have become increasingly important over recent years because of their ability to cause serious infections and due to their increasing resistance to different antimicrobials which include β lactam antibiotics, aminoglycosides and most importantly, glycopeptides like vancomycin⁷. The rapid emergence of antimicrobial resistance among *Enterococci* undoubtedly makes them most difficult to treat⁸.

Vancomycin is an important drug used in treatment of resistant strains of *Enterococci*. Over times, there has been increase in Vancomycin Resistance. Vancomycin resistance occurs when the target site is altered to D-alanyl-D-serine or D-alanyl-D-lactate. This altered side chain then have less affinity to bind to glycopeptides⁹. And now, VRE have spread rapidly and are encountered worldwide. Acquisition of resistant genes by *E.faecium* presents serious challenge to clinicians in treating enterococcal infections⁸.

AIMS & OBJECTIVES

To determine the prevalence and resistance of *Enterococci* isolated from various clinical specimens in a tertiary care hospital in North India.

MATERIALS & METHODS

The present prospective study was conducted in the Department of Microbiology, Government Medical College, Amritsar. The study was conducted for a period of one and a half year i.e. from January 2019 to June 2020. All the samples (pus, urine, blood, body fluids, sputum, etc.) from patients of all age groups and both genders admitted in the hospital and received in Microbiology department of Government Medical College, Amritsar were processed as per standard protocol¹⁰.

The samples were processed and cultured on Blood Agar and MacConkey's Agar and incubated for 24 hours aerobically at 37°C. Identification of *Enterococci* were made based on the colony characters, gram staining, motility and by using standard microbiological techniques¹⁰. Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method as per CLSI guidelines¹¹.

Various antibiotics included were:- penicillin (10 μ g), ampicillin (10 μ g), ciprofloxacin (5 μ g), tetracycline (30 μ g), erythromycin (15 μ g), vancomycin (30 μ g), high level gentamycin (120 μ g), high level streptomycin (300 μ g). *Enterococcus* isolates with vancomycin zone size \leq 14mm were further tested with linezolid (30 μ g), teicoplanin (30 μ g) and quinupristin-dalfopristin (15 μ g). Minimum Inhibitory Concentration (MIC) to vancomycin of these isolates was also assessed as per CLSI guidelines¹¹.

RESULTS

During the study period, a total of 26,144 samples were received in Microbiology department of Government Medical College & Hospital, Amritsar from the patients admitted in various wards of Government Medical College & Hospital, Amritsar. Out of total clinical samples, 6,425 were found to be culture positive. Amongst the 6,425 culture positive samples, 2,656 (41.3%) gram positive bacterial isolates were identified while 3,769 (58.7%) gram negative isolates were identified. Among the gram positive isolates, 254 (9.6%) isolates

were identified as *Enterococcus species* [Figure-1] out of which 165 were *E.faecalis* (65%) and 89 were *E.faecium* (35%) [Figure-2]. 54.5% strains of *Enterococcus spp.* were isolated from urine samples followed by blood (27.3%) and pus & body fluids (18.2%) [Figure-3].

Enterococcus faecium was found to be more drug resistant. Maximum resistance was seen to penicillin (80.9% in *E. faecium* & 48.5% in *E. faecalis*), high level gentamycin (65.2% in *E. faecium* & 5.5% in *E. faecalis*), high level streptomycin (65.2% in *E. faecium* & 35.2% in *E. faecalis*) and erythromycin (80.9% in *E. faecium* & 42.4% in *E. faecalis*) [Figure-4].

Vancomycin resistance among *E. faecium* and *E. faecalis* was observed in 7.9% and 2.4% isolates respectively. The screening test was compared to the confirmatory test and it was observed that amongst the isolates which were found to be positive on screening test (14) for the detection of VRE, 78.6% (11) of the isolates were confirmed to be VRE. Maximum number of VRE were isolated from urine (54.6%) followed by blood (27.3%) and pus (18.2%). All Enterococcal strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin (in case of *E. faecium*).

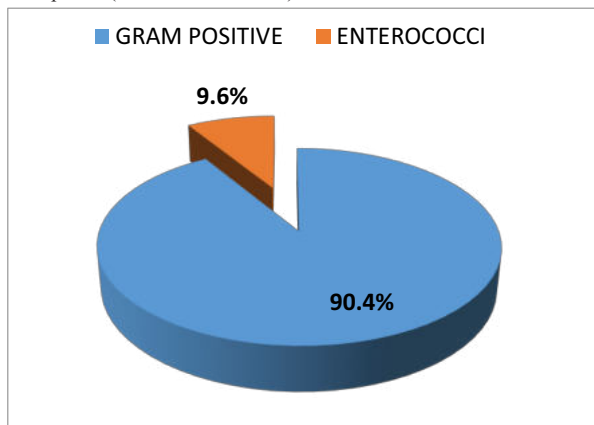


Figure – 1:- Prevalence of Enterococci among gram positive isolates.

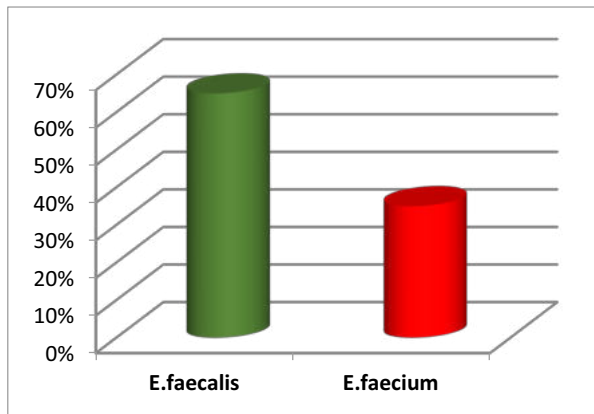


Figure – 2:- Distribution of *E. faecium* & *E. faecalis* isolates.

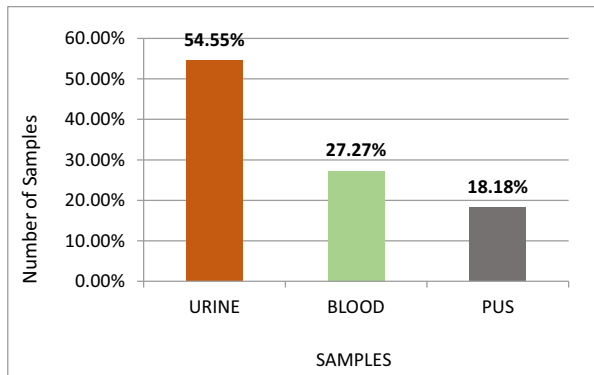


Figure – 3:- Sample wise distribution of Enterococcus

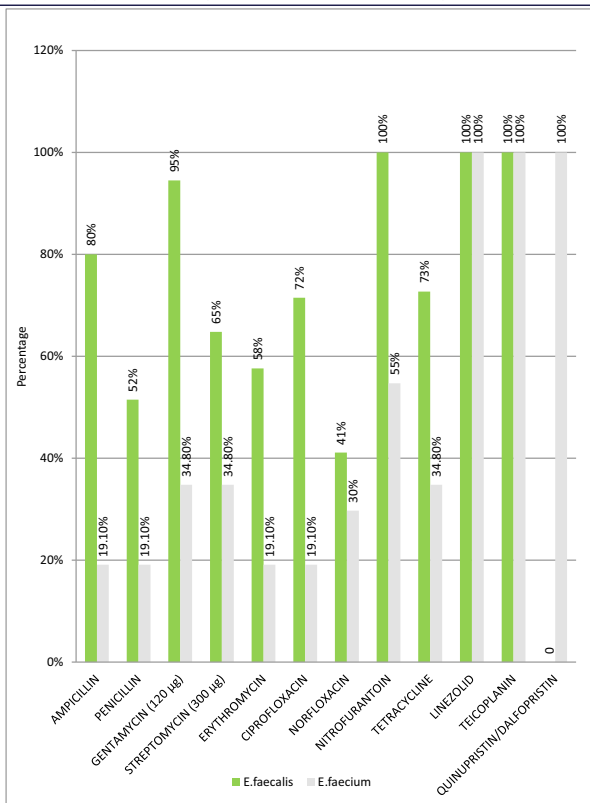


FIGURE 4: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCI

Vancomycin resistance in *E. faecium* was significantly more than in *E. faecalis* [p value=.04]

DISCUSSION

Enterococci are ancient and ubiquitous members of the healthy gastrointestinal tracts¹². Sites less often colonized by these organisms include the oral cavity, genitourinary tract and skin especially in the perineal area¹³. The main sites of colonization in the hospitalized patients are soft tissue wounds, ulcers and the gastrointestinal tract. Enterococci were traditionally regarded as low-grade pathogens, but have emerged as an increasingly important cause of nosocomial infections in recent years¹⁴.

In the antibiotic era, Enterococci emerged as leading cause of drug-resistant and hospital-associated infections¹⁵. Enterococci are intrinsically resistant to a large number of commonly used antibiotics, and they are particularly tolerant to stresses such as desiccation, starvation, and disinfectants¹⁶⁻¹⁸. Additionally, genomic plasticity enables Enterococci to readily acquire mobile genetic elements, and to share drug resistance genes with other pathogens, which further complicates infection treatment and control¹⁹⁻²¹.

Out of 26,144 samples, 6425 (24.6%) were found to be positive on culture. Different studies show different positivity. Higher culture positivity of 52.74% was reported by Raj et al³. Chakraborty reported 7.30% culture positivity in their study of VRE²². A lower culture positivity rate amongst the hospitalized patients can be attributed to prior antimicrobial therapy and the fastidious nature of the organisms²³. Amongst the 6,425 culture positive samples, 2,656 (41.3%) gram positive bacterial isolates were identified while 3,769 (58.7%) gram negative isolates were identified.

Out of these 2,656 gram positive isolates, 254 isolates were found to be *Enterococcus species*. Positivity of Enterococci among gram positive isolates was found to be 9.6% which is in concordance with 9.71% from a study which was held in Eastern India, 2019³ and in comparison to 3.53% from a study conducted in Rajasthan, 2016⁷. In the present study, *E. faecalis* (65%) was predominant isolate as compared to *E. faecium* (35%) which is in concordance with the findings of Moghimbeigi A et al [*E. faecalis* (69%) & *E. faecium* (28%)]²⁴. This finding differs from that of Sattari-Maraji et al who found prevalence of *E. faecalis* to be 35% and of *E. faecium* 57%²⁵.

In the past two decades, *Enterococcus* has emerged as the third leading cause of hospital-acquired infections after *Escherichia coli* and *Staphylococcus*²⁶. Recent literature has reported *Enterococcus* to be the second leading cause of urinary tract infections and the third leading cause of bacteremia worldwide²⁷.

In the present study, 54.5% strains of *Enterococcus* spp. were isolated from urine samples which is similar to study conducted by Chakraborty et al, in which (66.01%) *Enterococcus* species were isolated from urine²². In another study, prevalence of *Enterococcus* in urine samples was found to be 31%²⁸. The prevalence of *Enterococcus* from pus samples in our study (18.2%) correlates well with study done by Mukherjee K et al (16%)⁷. Higher prevalence of 43% was reported by Sreeja S et al²⁸. The prevalence of *Enterococcus* in blood in our study is 27.3%. Various studies have shown prevalence in the range of 4.23% to 20.8% in blood samples^{22,23}.

Enterococcus faecium was found to be more drug resistant as compared to *E. faecalis*. Maximum resistance was seen to penicillin (80.9% in *E. faecium* & 48.5% in *E. faecalis*), high level gentamycin (65.2% in *E. faecium* & 5.5% in *E. faecalis*), high level streptomycin (65.2% in *E. faecium* & 35.2% in *E. faecalis*) and erythromycin (80.9% in *E. faecium* & 42.4% in *E. faecalis*). High level aminoglycoside resistance was mainly seen in *Enterococcus faecium* as compared to *Enterococcus faecalis*. High level aminoglycoside resistant *Enterococci* often have plasmids which carry determinants encoding resistance to other antibiotics, these isolates often become multi-resistant. Finding of the present study has been supported by various similar studies done on *Enterococcus*^{2,29}.

Vancomycin resistance among *E. faecium* and *E. faecalis* was observed in 7.9% and 2.4% isolates respectively which showed similarity to results reported from other studies ranging between 1.7-20% in tertiary care hospitals in other parts of India³. Vancomycin resistance in *E. faecium* is significantly more than in *E. faecalis*. The screening test (Disc diffusion) was compared to the confirmatory test (E-test) and it was observed that amongst the isolates which were found to be positive on screening test for the detection of VRE, 78.6% of the isolates were confirmed to be VRE [Positive predictive value=78.6%]. This finding is well supported by a study conducted by Mathew SK et al in 2018 in Kerala, India which depicted percentage of confirmed VRE to be 79%³⁰.

All *Enterococcal* strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin (in case of *E. faecium*). This is in concordance to various studies performed^{31,32}.

VRE pose particular problems for treatment because the strains which harbor VanA and VanB resistance are also typically resistant to other classes of antibiotics. While only linezolid and quinupristin-dalfopristin have FDA approval for treatment of VRE infections, other antimicrobial agents including daptomycin, tigecycline, fosfomicin, quinolones, tetracyclines and new fifth generation cephalosporins exhibit *in vitro* activity and have been used with success in individual cases. In uncomplicated cases, monotherapy based upon the antibiotic susceptibility profile is appropriate. In complicated cases such as endocarditis, the ideal therapy for VRE has not been determined³.

CONCLUSION:

Vancomycin has long been considered as drug of choice for *Enterococcal* infections but now, due to the imprudent use of vancomycin, colonization pressure and noncompliance with the infection control measures, there has been emergence of Vancomycin Resistant *Enterococci*. To limit the drug-resistant *Enterococcus* prevalence, it is necessary to use vancomycin cautiously. In the present study, Vancomycin resistance was predominantly observed in *E. faecium* isolates (7.9%).

And thus, this emphasizes the need to implement the hospital infection control guidelines to limit the community spread of hospital acquired VRE as it will lead to considerable increase in mortality & morbidity.

Conflict of interest: None

Approval from institutional ethical committee

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