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Research Article

PREVALENCE AND ANTIMICROBIAL RESISTANCE IN ENTEROCOCCUS SPECIES

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ABSTRACT

Objectives: The study was conducted to determine the prevalence of vancomycin-resistant *Enterococci* (VRE) isolated from various clinical samples received from the indoor patients of all age groups admitted in Government Medical College and Hospital, Amritsar.

Methods: A prospective cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Amritsar, for a period of 4 years (July 1, 2018–June 30, 2022). All the samples (pus, urine, blood, body fluids, sputum, etc.) received from the indoor patients of all age groups admitted in Government Medical College and Hospital, Amritsar, were included in the study.

Results: During the study period of 4 years, among the culture positive samples, 1815 (6.62%) isolates were identified as Enterococcus species. Among 1815 isolates, 1089 isolates were *Enterococcus faecalis* (60%) and 726 were *Enterococcus faecium* (40%). Both *E. faecalis* and *E. faecium* isolates showed the maximum resistance to ciprofloxacin while linezolid, teicoplanin, and quinupristin/dalfopristin showed the maximum sensitivity.

Conclusion: Our study reports the prevalence of *Enterococci* isolates as well of VRE isolates. To reduce the VRE prevalence worldwide, appropriate use of antibiotics according to antimicrobial susceptibility testing should be encouraged. Efforts should be made to reduce the transmission of VRE isolates.

Keywords: Enterococcus, Vancomycin-resistant *Enterococci*, Prevalence.

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INTRODUCTION

Enterococci are Gram-positive bacteria which are normal inhabitants of the intestinal flora and generally cause infections when host immunity is disrupted. These are Gram-positive bacteria, facultative anerobic oval cocci arranged singly, in pairs or in short chains. They have the ability to grow at 10°C as well as 45°C, at 9.6 pH, in 6.5% NaCl and also survive at 60°C for 30 min [1]. The genus *Enterococci* includes *Enterococcus faecalis*, *Enterococcus faecium*, *E. durans, E. gallinarum*, *E. italicus*, and *E. avium.* The most common species found worldwide among various clinical samples is *E. faecalis* followed by *E. faecium* which also accounts for being the most drug resistant.

The name "enterocoque" was first used by Thiercelin in a study from France published in 1899; the name was proposed to emphasize the intestinal origin of this new Gram-positive diplococcus. In the same year, MacCallum and Hastings reported a case of endocarditis caused by an organism; they called Micrococcus zymogenes; later studies suggest that this organism was actually a hemolytic Enterococcus. In 1937, Sherman proposed a classification scheme which separated streptococci into four divisions: pyogenic, viridans, lactic, and Enterococcus. The latter term was used for organisms that grew at 10 and 45°C in 6.5% NaCl, at pH 9.6 and which survived 60°C for 30 min; the ability to split esculin was also noted. Sherman's classification scheme also correlated with the serological scheme originated by Lancefield in the early 1930s. In that system, the Enterococci reacted with Group D antisera, while the pyogenic streptococci reacted with Groups A, B, C, E, F, or G and the viridans streptococci were nongroupable. The proposal to transfer Enterococci to a new genus named Enterococcus had been previously suggested, and it was this genus name that was proposed by Schleifer and Kilpper-Balz. Shortly thereafter, Collins, Jones, and Farrow, working with Klipper-Bilz and Schleifer, used similar methodology to show that strains called *Streptococcus avium, Streptococcus casseliflavus, Streptococcus durans, Streptococcus faecalis* subspecies malodoratus, and *Streptococcus gallinarum* were sufficiently closely related to other members of the genus Enterococcus to be transferred to this genus but sufficiently distinct to be considered separate species. The names proposed were *Enterococcus avium*, *E. casseliflavus, E. durans, E. malodoratus, and E. gallinarum* [2].

Nosocomial infections, also called as Hospital Acquired Infections (HAIs), are the infections which are acquired in health-care setting which first appear at 48 h or more after hospital admission or within 30 days after the discharge of the patient [3]. ESKAPE (*E. faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) pathogens are majorly responsible for nosocomial infections. *Enterococci* have become the major cause of HAIs which include nosocomial urinary tract, wound infections, and bacteremia [1]. They also cause endocarditis, neonatal sepsis, and in rare cases, meningitis as well.

Over the past two decades, Enterococci have been identified as the agents of nosocomial infection with increasing frequency. Enterococci are primarily opportunistic pathogens. The use of various intravascular access devices, implanted prosthetic devices, cytotoxic chemotherapy, and immunosuppression has magnified the impact of organisms of relatively low virulence, such as Enterococci and intensive use of broad-spectrum antibiotics in the hospital, which provides selective pressure favoring the growth of intrinsically drug-resistant commensal organisms such as Enterococci. These organisms have survived in the hospital environment due to their intrinsic resistance to several commonly used antibiotics and more importantly their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons [4].

Another reason of *Enterococci* emergence in the past two decades is due to their resistance to many frequently used antimicrobial agents such as aminoglycosides, cephalosporins, aztreonam, semisynthetic penicillins, trimethoprim-sulfamethoxazole and along with this, their ability to attain and transfer-resistant genes, thus giving rise to *Enterococci* with high-level aminoglycoside resistance (HLAR),

β-lactamase production [5]. After the emergence of resistance to these many antibiotics, vancomycin was the main drug of choice in various nosocomial infections caused by *Enterococci*.

However, due to over and misuse of vancomycin in *Enterococcal* infections, vancomycin-resistant *Enterococci* (VRE) were first reported in 1986 in Europe [6]. Nowadays, VRE is well-known cause of multidrug-resistant *Enterococcus* spp. in health-care settings. Mechanism of VRE is due to acquisition of van gene clusters which occurs probably from the environmental organisms. VanA being the most frequent gene cluster is usually located in a Tn3-family transposon (Tn1546) which is found in conjugative and non-conjugative plasmids [6].

Objectives

The objectives of the study are to determine the prevalence of VRE isolated from various clinical samples received from the indoor patients of all age groups admitted in Government Medical College and Hospital, Amritsar, North India.

METHODS

A prospective cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Amritsar, for a period of 4 years (July 1st, 2018 to June 30th, 2022). All the samples (pus, urine, blood, body fluids, sputum, etc.) received from the indoor patients of all age groups admitted in Government Medical College and Hospital, Amritsar, were included in the study.

The samples were then inoculated on Blood Agar and MacConkey's Agar and incubated for 24 h aerobically at 37°C. *Enterococci* were identified based on the colony characteristics, gram staining, motility and by using standard microbiological techniques [7]. Kirby–Bauer disc diffusion method was performed after inoculum for antimicrobial susceptibility testing was standardized to 0.5 McFarland standards for various *Enterococci* isolates as per the CLSI guidelines [8].

The antibiotics which were tested were penicillin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), erythromycin (15 µg), vancomycin (30 µg), high-level gentamycin (120 µg), and high-level streptomycin (300µg). *Enterococci* isolates with vancomycin zone size ≤14 mm were further tested with linezolid (30 µg), teicoplanin (30 µg), and quinupristin-dalfopristin (15 µg). Minimum inhibitory concentration to vancomycin of these isolates was also assessed as per the CLSI guidelines [9].

The study was conducted after the approval from the institutional ethical committee which stated. An informed consent as well as official permission was obtained from the hospital as well as the participating subjects of the present study. The confidentiality of the information was maintained.

RESULTS

During the study period of 4 years, a total of 68,575 samples were received in Department of Microbiology, Government Medical College and Hospital, Amritsar, from various indoor patients admitted in various wards of Government Medical College and Hospital, Amritsar. Out of total clinical samples, 27,430 (40%) were found to be culture positive. Among the culture-positive samples, 1815 (6.62%) isolates were identified as *Enterococcus species* (Fig. 1).

Amongst 1815 isolates, 1089 isolates were *E. faecalis* (60%) and 726 were *E. faecium* (40%) (Fig. 2).

Enterococci isolates were maximum isolated from urine samples followed by pus and body fluids and blood as shown in the Fig. 3.

The results of antimicrobial susceptibility testing of *E. faecalis* and *E. faecium* isolates are depicted in the Table 1 below. Both *E. faecalis* and *E. faecium* isolates showed the maximum resistance to ciprofloxacin while linezolid, teicoplanin, and quinupristin/dalfopristin showed the maximum sensitivity. The prevalence of vancomycin resistance in *E. faecalis is* 2.94% while in *E. faecium* is 29.89% with overall prevalence of 13.72% (Table 1 and Fig. 4).

DISCUSSION

The rapid emergence of antimicrobial resistance is an important public health issue which has gained importance worldwide. Assessing the

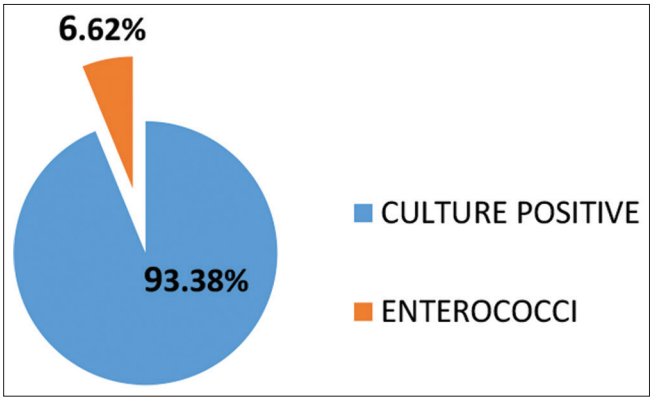

Fig. 1: Prevalence of *Enterococci* isolates among different clinical samples

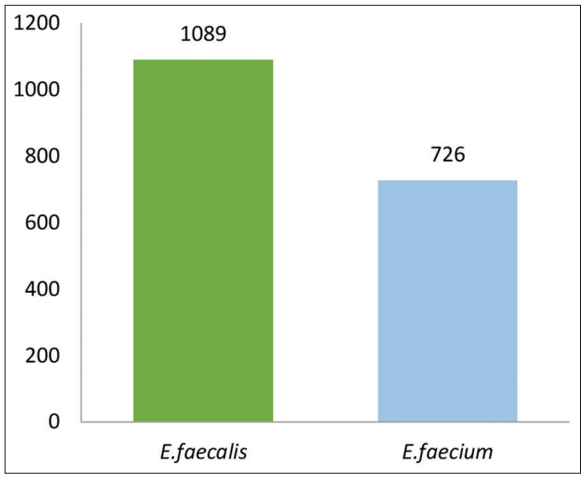

Fig. 2: Distribution of *Enterococcus faecalis* and *Enterococcus faecium* isolates

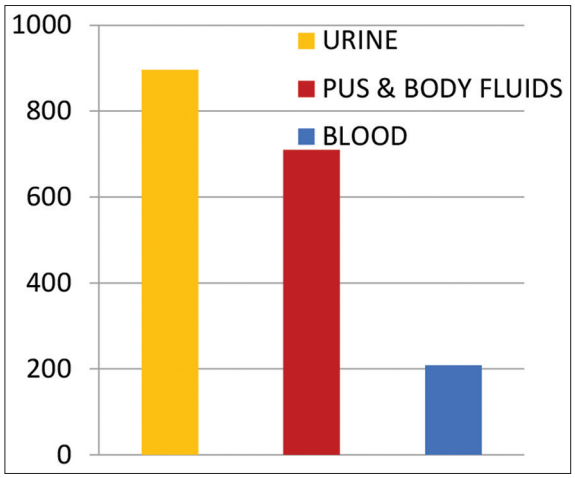

Fig. 3: Specimen-wise distribution of isolated *Enterococcus species*

Table 1: Resistance pattern toward different antimicrobials

Antimicrobials	*E. faecalis* (n=1089), n (%)	*E. faecium* (n=726), n (%)	Total isolates (n=1815), n (%)
Antimicrobials	Resistant	Resistant	Resistant
Ampicillin	562 (51.61)	512 (70.52)	1074 (59.17)
Penicillin	562 (51.61)	512 (70.52)	1074 (59.17)
Gentamicin (high dose)	560 (51.42)	522 (71.90)	1082 (59.61)
Streptomycin (high dose)	560 (51.42)	522 (71.90)	1082 (59.61)
Erythromycin	326 (29.94)	468 (64.46)	794 (43.75)
Ciprofloxacin	774 (71.07)	621 (85.54)	1395 (76.86)
Norfloxacin (urine isolates)	101, out of 144 (70.14)	51, out of 56 (91.07)	152 (76)
Nitrofurantoin (urine isolates)	0	13, out of 56 (23.21)	13 (6.50)
Tetracycline	444 (40.77)	601 (82.78)	1045 (57.58)
Vancomycin	32 (2.94)	217 (29.89)	249 (13.72)
Linezolid	0	0	0
Teicoplanin	0	0	0
Quinupristin/dalfopristin (for *E. faecium)*	NA	0	0

NA: Not available, *E. faecalis: Enterococcus faecalis, E. faecium: Enterococcus faecium*

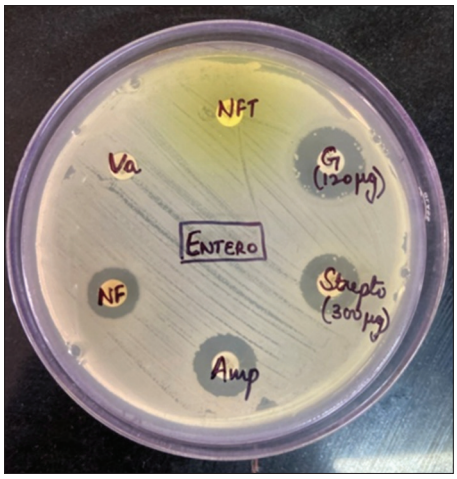

Fig. 4: Antibiotic susceptibility test (Kirby–Bauer disk diffusion method) showing vancomycin resistance

prevalence of antibiotic resistance has become an important step during the formulation of interventions to reduce the emergence and transmission of resistant pathogens [10]. An increase in VRE infections has been recently reported worldwide.

The prevalence of *Enterococci* isolates in our study accounts for 6.62% as compared to 5.5% which was in an another study conducted by Toru *et al*. in South West Ethiopia in the year 2018 [11] and 9.71% in other study conducted by Hirak *et al.* in Eastern India in 2019 [12]. Among the 1815 *Enterococci* isolates, *E. faecalis* (60%) was the predominant species isolated followed by *E. faecium* (40%). Our finding is in concordance with an another study conducted by Boccella *et al*. in the year 2021 [13].

Enterococci isolates were majorly isolated from urine cultures (49.37%) followed by pus and body fluids (39.12%) and blood cultures (11.52%). Identical results were noted in a study conducted by Salem-Bekhit *et al*. in the year 2012 [14] and in an another study conducted by Bhatt *et al*. in Armed Forces India in 2015 [15]. Enterococcal urinary tract infections are more likely to be acquired in hospital or long-term care settings and account for 15% of health-care-associated urinary tract infections [16].

In our study, 59.17% isolates showed resistance to ampicillin and penicillin, which are similar to study conducted by Arif *et al*. in Uttar Pradesh in 2019 [17] and by Mathur *et al*. in North India in 2003 [18]. 59.61% isolates in our study were resistant to high-level gentamicin and high-level streptomycin. Similar results were observed in a study conducted by Bhatt *et al*. in Armed Forces India in 2015 [15]. 43.75% and 57.58% isolates were resistant to erythromycin and tetracycline, respectively, in our study which is in contrast to a study conducted by Nisarta in Gujarat in 2016 [19] which showed 96.87% resistance to erythromycin and 28.10% resistance to tetracycline. In urinary isolates, 76% resistance was shown to norfloxacin and 6.50% in case of nitrofurantoin. Maximum resistance in our study was shown to ciprofloxacin, i.e., 76.86% which is in concordance to another study conducted by Arif *et al*. in Uttar Pradesh in 2019 [17] in which maximum resistance was too observed in case of ciprofloxacin (84.90%). Furthermore, overall, *E. faecium* was found to be more drug resistant as compared to *E. faecalis* in our study (Table 1).

Vancomycin resistance in *E. faecalis* was observed to be 2.94% and 29.89% in case of *E. faecium* with overall VRE to be 13.72% in our study in contrast to 30.1% VRE in a study conducted in 2019 [17] and 0.64% in a study conducted by Nisarta in Gujarat in 2016 [19]. Similar results were found to be in a study conducted by Shrestha *et al*. in 2021 [20]. In our study, there was 100% susceptibility to linezolid, teicoplanin, and quinupristin/dalfopristin. Similar results were found to be in a study conducted by Mukherjee in Kolkata in 2013 [21] and Chitnis *et al*. in Central India in 2013 [22].

In our study, as depicted, the high prevalence and multidrug resistance rate of *Enterococci* isolates is a matter of concern to the physicians as it has become a major therapeutic challenge to treat multidrug-resistant *Enterococci*. Resistance to vancomycin in *Enterococci* isolates has remained at a constant level of 8%–15% [23] in recent years. Earlier vancomycin was a last resort in treating various *Enterococci* infections but now many alternative drugs such as linezolid, teicoplanin, and quinupristin/dalfopristin are being used to treat VRE infections.

Our study reports the prevalence of *Enterococci* isolates as well of VRE isolates. To reduce the VRE prevalence worldwide, appropriate use of antibiotics according to antimicrobial susceptibility testing should be encouraged. Efforts should be made to reduce the transmission of VRE isolates. Delayed identification of VRE carriers leads to increase in nosocomial transmission of VRE. Strict infection contact precautions should be taken to effectively decrease nosocomial transmission.

CONFLICT OF INTEREST

None.

SOURCE OF FUNDING

Nil.

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