

Vancomycin Resistance among Enterococci: Further Limiting Our Therapeutic Options

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ABSTRACT

Introduction: *Enterococci* are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of human. Over recent years, there is increased interest in *Enterococci* not only because of their serious infections but because of their increasing resistance to many antimicrobials. Vancomycin being the only alternative available. But over the time, there has been increase in Vancomycin Resistance which has spread globally. The aim of this study was to determine the prevalence of Vancomycin Resistant *Enterococci* (VRE) isolated from various clinical specimens in a tertiary care hospital in North India.

Material and methods: A cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Amritsar from July 1st, 2018 to June 30th, 2019. All the samples received were processed and identification of *Enterococci* was made by using standard microbiological techniques. Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method as per CLSI guidelines.

Results: Out of total clinical samples (11,098), 3,551 (31.9%) were found to be culture positive. Among the culture positive, 91 (2.56%) isolates were identified as *Enterococcus species* comprising of 37 *E.faecalis* (41%) and 54 *E.faecium* (59%). Maximum number of *Enterococci* were isolated from urine samples (54.92%) followed by pus & body fluids (38.02%) and blood (7.04%). 9.52% of *E.faecium* isolates were found to be resistant to vancomycin. All the strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin.

Conclusion: *Enterococci* have become the major pathogenic bacteria that cause hospital-acquired infections due to multiple-antimicrobial resistance. VRE has emerged as important nosocomial pathogen and pose serious threat to patients. Vancomycin should be cautiously used else we would be left with very few therapeutic options.

Keywords: *Enterococci*, VRE, Prevalence

INTRODUCTION

Enterococci are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of human.¹ The genus *Enterococcus* consists of Gram positive cocci, catalase negative, non spore forming, facultative anaerobes that often occur in pairs or short chains. This genus comprises of *Enterococcus faecalis*, *E.faecium*, *E.durans*, *E.gallinarum*, *E.avium*, *E.italicus*, etc. *E.faecalis* is the most common species found in clinical specimens whereas *E.faecium* is notorious for being drug resistant.²

Over recent years, there is increased interest in *Enterococci* not only because of their serious infections but because of their increasing resistance to many antimicrobials.

Enterococci are multidrug resistant bacteria, which are resistant to penicillins, low level aminoglycosides, sulfonamides, cephalosporins and cotrimoxazole. Combination of penicillin and gentamycin had been the mainstay of treatment till now but with emergence of high level aminoglycosides resistance (HLAR), Vancomycin is the only alternative available. Vancomycin is a glycopeptide which inhibits cell wall synthesis, but over the time, there has been increase in Vancomycin Resistance which has spread globally. Resistance to Vancomycin is because of alteration at the target site i.e. D-alanyl D-alanine side chain of peptidoglycan layer is replaced by D-alanyl D-serine or D-alanyl D-lactate. Most common risk factors associated with Vancomycin Resistant *Enterococci* (VRE) are patients suffering from chronic renal failure, cancer, organ transplant recipients, longer stay in hospital more so in ICU.³ Study aimed to determine the prevalence of VRE isolated from various clinical specimens in a tertiary care hospital in North India.

MATERIAL AND METHODS

A cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Amritsar for a period of one year (July 1st, 2018 to June 30th, 2019). All the samples (pus, urine, blood, body fluids, sputum, etc.) received from the indoor patients of all age groups admitted in Government Medical College & Hospital, Amritsar were included in the study.

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, motility, gram staining and by

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using standard microbiological techniques.⁴ Antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method as per CLSI guidelines.⁵

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

RESULTS

During the study period of one year, a total of 11,098 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, 3,551 (31.9%) were found to be culture positive. Among the culture positive, 91 (2.56%) isolates were identified as *Enterococcus species* [Figure-1] out of which 37 were *E.faecalis* (41%) and 54 were *E.faecium* (59%) [Figure-2]. Maximum number of *Enterococci* were isolated from urine samples(54.92%)

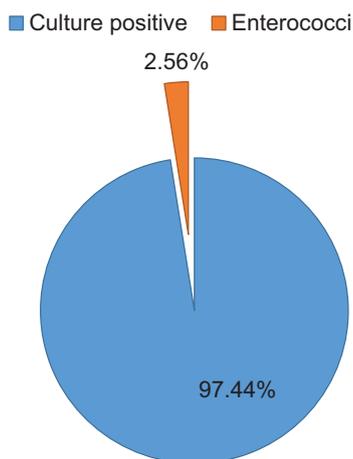


Figure-1: Prevalence of Enterococci among different clinical samples.

followed by pus & body fluids(38.02%) and blood(7.04%) [Figure-3].

Antimicrobial Susceptibility Testing of *Enterococcus faecalis* isolates (n=29) showed maximum resistance to norfloxacin (60%), penicillin (48.27%), high level streptomycin (34.48%) and erythromycin (41.37%). All isolates were 100% sensitive to Vancomycin, Linezolid & Teicoplanin [Table-1]. Among *Enterococcus faecium*(n=42), maximum resistance was seen to ampicillin (80.95%), penicillin (80.95%), high level aminoglycosides (64.28%)

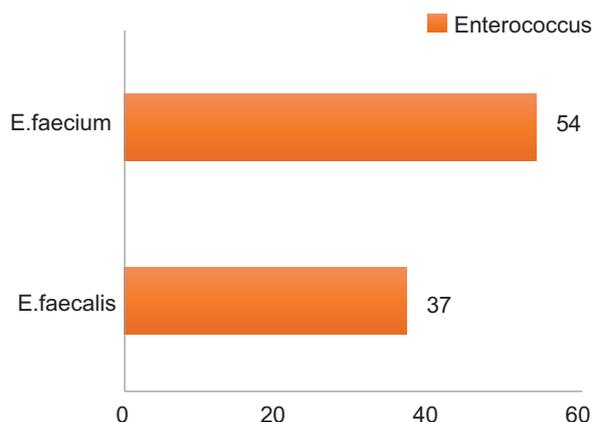


Figure-2: Distribution of *E.faecium* & *E.faecalis* isolates.

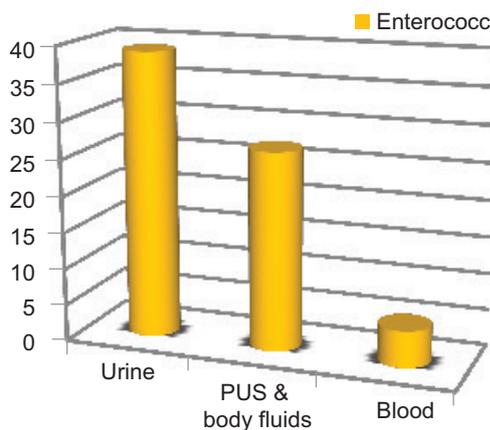


Figure-3: Enterococcus species isolated from various clinical samples

Antimicrobials	<i>E.faecalis</i> [n=29]		<i>E.faecium</i> [n=42]	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	24 (82.75%)	5 (17.24%)	8 (19.04%)	34 (80.95%)
Penicillin	15 (51.72%)	14 (48.27%)	8 (19.04%)	34 (80.95%)
Gentamicin	27 (93.10%)	2 (6.89%)	15 (35.71%)	27 (64.28%)
Streptomycin	19 (65.51%)	10 (34.48%)	15 (35.71%)	27 (64.28%)
Erythromycin	17 (58.62%)	12 (41.37%)	8 (19.04%)	34 (80.95%)
Ciprofloxacin	21 (72.41%)	8 (27.58%)	8 (19.04%)	34 (80.95%)
Norfloxacin [urine isolates]	6, out of 15 (40%)	9, out of 15 (60%)	7, out of 24 (29.17%)	17, out of 24 (70.83%)
Nitrofurantoin [urine isolates]	29, out of 29 (100%)	-	13, out of 24 (54.17%)	11, out of 24 (45.83%)
Tetracycline	21 (72.41%)	8 (27.58%)	15 (35.71%)	27 (64.28%)
Vancomycin	29 (100)	-	38 (90.04%)	4 (9.52%)
Linezolid	29 (100)	-	42 (100)	-
Teicoplanin	29 (100)	-	42 (100)	-
Quinupristin/ dalfopristin [for <i>E.faecium</i>]	-	-	42 (100)	-

Table-1: Antimicrobial susceptibility pattern of the isolates of Enterococci

and erythromycin (80.95%). 9.52% of isolates were found to be resistant to vancomycin. All the strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin [Table-1].

DISCUSSION

In the past two decades, *Enterococcus* was introduced as the third leading cause of hospital-acquired infections after *Escherichia coli* and *Staphylococcus*.⁶ Recently, *Enterococcus* is the second leading cause of urinary tract infections and the third leading cause of bacteremia worldwide.⁷

The prevalence of *Enterococcal* isolates in the present study was 2.56%, in comparison to 3.53% from a study conducted in Rajasthan, 2016⁸ and 9.71% from a study which was held in Eastern India, 2019⁹. In the present study of 11,098 indoor samples, *E. faecalis* was predominant isolate as compared to *E. faecium*. This finding correlates well with the study done by Sattari-Maraji et al (*E. faecalis* 35% and *E. faecium* 57%).¹⁰

More than 50% 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 urine.¹¹ The prevalence of *Enterococcus* in wound infection in our study (38.02%) correlates with study done by Vandamme et al (37.9%)¹² and Amelework et al (25%).¹³ The prevalence of *Enterococcus* in blood was 7.04% as compared to 4.23% and 20.8% reported in different studies.^{11,13}

Enterococcus faecium was found to be more drug resistant. Maximum resistance was seen to penicillin (80.95% in *E. faecium* & 48.27% in *E. faecalis*), high level gentamycin (64.28% in *E. faecium* & 6.89% in *E. faecalis*), high level streptomycin (64.28% in *E. faecium* & 34.48% in *E. faecalis*) and erythromycin (80.95% in *E. faecium* & 41.37% in *E. faecalis*). High level aminoglycoside resistance was mainly seen in *Enterococcus faecium* as compared to *Enterococcus faecalis* [Table-1]. 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*.^{9,14}

Vancomycin resistance among *E. faecium* was observed in 4 (9.52%) isolates which showed similarity to results reported from other studies ranging between 1.7-20% in tertiary care hospitals in other parts of India⁸ [Table-1] whereas Vancomycin resistance was not observed in *E. faecalis* isolates. All enterococcal strains were 100% susceptible to Linezolid, Teicoplanin & Quinupristin-dalfopristin (in case of *E. faecium*) [Table-1]. This is in concordance to various studies performed.^{9,15,16}

The increasing resistance of *Enterococci* to many antimicrobial agents like β lactam antibiotics, aminoglycosides and most importantly glycopeptides like vancomycin have made it a important nosocomial pathogen.¹⁷ Vancomycin has long been considered for treatment of *Enterococcus* infections but now,

its resistance has been increasingly reported from all parts of the world. Thus, for vancomycin-resistant *E. faecium*, we are left behind with few therapeutic options like Linezolid, an oxazolidinone antimicrobial and Quinupristin-dalfopristin.¹⁸ The high prevalence of multidrug resistant *Enterococcal* infection in a tertiary care set up is due to excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from peripheral centres. The emergence of VRE has been attributed to the inprudent use of vancomycin, colonization pressure and noncompliance with the infection control measures.⁸ For long time, *Enterococci* were frequently considered as normal flora. Recently, due to its role in causing variety of infections in hospitalized patients and increasing resistance to different antibiotics has generated interest among researchers to understand the pathogenicity of *Enterococcus*.⁸

CONCLUSION

Increased prevalence of MDR *Enterococci* in hospitalized patients has become a major cause of concern leaving few therapeutic options. To curb the menace of emerging VRE, antimicrobial stewardships, active screening of high risk patients is highly required. In conclusion, early detection of patients infected with VRE is essential component to prevent its nosocomial transmission.

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