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ENTEROCOCCUS UNMASKED: INSIGHTS INTO PREVALANCE AND ANTIMICROBIAL PROFILES IN A TERTIARY CARE CENTRE

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ABSTRACT

Background: Enterococci are part of normal flora of human intestine, biliary tract and lesser extent to vagina and male urethra. The increasing incidence of health care-associated infections (HAI) has been attributed mainly to a group of six pathogens characterized by multidrug resistance (MDR) and virulence and known by the name ESKAPE pathogens. The incidence of enterococcal infections has increased which accounts for the majority of nosocomial infections worldwide.

Materials and Methods: A cross-sectional study was conducted from October 2023 to December 2023 in the Department of Microbiology, Tertiary care hospital among 106 samples. Enterococcus isolates were identified and antimicrobial susceptibility test was done by colony morphology, gram staining and catalase test. VITEK 2 (Biomerieux) using an identification card (ID-GP) and susceptibility card (AST-P628) according to manufacturer's instruction. The results of the antimicrobial susceptibility were interpreted according to CLSI 2024 guidelines. The data collected was entered in MS Excel windowsand the analysis was done through SPSS 16. p value <0.05 is considered as statistically significant.

Results: Majority of the study participants were females 61(57.5%), Enterococcus faecalis 84 (79.24%) was isolated more compared with Enterococcus faecium 22 (20.75%). Out of 106 Enterococcus species isolated majority,80 isolates (75.4%) were Urine cultures, out of 84 E. faecalis only 2% showed resistance for vancomycin. But among the 22 E. faecium 45 % that is 10 isolates were vancomycin resistant.

Conclusion: We concluded our study stating that among the Enterococcus species E. faecalis and E. faecium was the isolated species. E. faecalis was the most common one and it was sensitive to most of the antibiotics but E. faecium was resistant to many antibiotics including Vancomycin.

Keywords: VRE-Vancomycin resistant enterococcus, antimicrobial susceptibility, Multidrug resistance, Health care associated infections.

INTRODUCTION

Enterococci are gram-positive oval cocci arranged in pairs at an angle to each other gives as spectacle shaped appearance. Previously it was grouped under group D Streptococcus but later have been reclassified as a separate genus Enterococcus. Based on the molecular characters now they are placed under a new family Enterococcaceae. Enterococci are part of normal flora of human intestine, biliary tract and lesser extent to vagina and male urethra. (Aburba). Enterococcus genus consists of more than 50 species but only two species are considered to cause the majority of infections, these are Enterococcus faecalis and Enterococcus faecium.

The increasing incidence of health care-associated infections (HAI) has been attributed mainly to a group of six pathogens characterized by multidrug resistance (MDR) and virulence and known by the name ESKAPE pathogens: Enterococcus faecium,

Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.[1] The incidence of enterococcal infections has increased which accounts for the majority of nosocomial infections worldwide. Now Enterococcus species are recognized as third most common cause of healthcare associated infections like nosocomial urinary tract infections, wound infections and bacteremia.

Antimicrobial resistance (AMR) has emerged as one of the most predominant threats to human health in the world today. The increase in antibiotic resistance among enterococci have raised particular concern due to their ability to acquire resistance against many antimicrobial drugs used in clinical practice and to establish severe life-threatening infections in patients living with chronic diseases or cancer,[2-4] (ENTERO-3) the emergence of Vancomycin-resistant Enterococcus (VRE) has increased due to the selective pressure of antibiotics exposure, either by genetic mutation or horizontal gene transfer and increased use of alternative antibiotics, such as daptomycin and linezolid, towards which increasing resistance trends are developing.[5-7] (ENTERO-3) The resistance to Vancomycin and ampicillin is commonly associated with E. faecium than with E. faecalis.

Enterococci are intrinsically resistant to antibiotics like penicillinase susceptible penicillin, penicillinase resistant penicillin, nalidixic acid, cephalosporin, clindamycin and aminoglycoside. E.faecalis is the predominant species implicated in infection followed by E.faecium. 3 Recent evidence suggests that the prevalence Vancomycin-resistant E. faecium is increasing. Speciation of Enterococcus is important because the present study was carried out to know the prevalence, speciation of Enterococci and their antibiotic profiles. So early detection of antibiotic resistance profile may help in choosing optimal alternative treatment and prevent the spread of drug-resistant pathogen.

MATERIALS AND METHODS

Type of Study: Prospective Study
Study Period: 6 Months
Sample Size: 106

Inclusion Criteria

Clinical samples like Urine, Blood, Pus, wound swab Tissue and Body fluids received in our diagnostic Microbiology laboratory for culture and sensitivity.

Exclusion Criteria

Vaginal swab, Sputum, Throat swab and Stool samples.

Clinical samples received for bacterial culture and sensitivity in the diagnostic Microbiology laboratory at our tertiary care hospital were included in the study. Samples inclusive were urine, blood, pus and body fluids, Whereas vaginal swab, sputum, throat swab and stool samples were not included in the study as Enterococcus spp forms a part of normal flora in these sites, Culture and Identification of the isolates were done by as per the standard protocol. The isolates were identified by colony morphology, Gram staining and Catalase test.

Those isolates had tiny non-hemolytic colonies on blood agar were subjected for gram staining.Gram-positive oval cocci arranged in pairs at an angle to each other in Gram staining and catalase test negative colonies were taken for this study. The further identification of the colonies and antimicrobial susceptibility testing was performed with VITEK 2 (Biomerieux) using an identification card (ID-GP) and susceptibility card (AST-P628) according to manufacturer's instruction. The results of the antimicrobial susceptibility were interpreted according to CLSI 2024 guidelines.

RESULTS

Table 1: Characteristics of the study population

Characteristics of study population	No.of samples
Inpatients samples	74(69.8%)
Outpatient samples	32(30.2%)
Total	106 (100%)

Out of 106 samples received 74 samples were from inpatients and 32 were from out patients.

Gender wise distribution

Table 2: Gender

Sex	Frequency
Male	45 (42.5%)
Female	61 (57.5%)
Total	106 (100%)

Out of 106 samples 45 (42.5%) samples from Male patients and 61(57.5%) from Female patients.

Table 3: Samples distribution.

Samples	Frequency
Urine culture	80 (75.4%)
Pus culture	20 (18.8%)
Blood culture	5 (4.71%)
Bone culture	1 (0.9%)
Total	106 (100%)

Out of 106 Enterococcus species isolated 80 isolates (75.4%) were Urine cultures, Pus cultures were 20 (18.8%), 5 (4.71%) were blood culture and one isolate (0.9%) from bone culture.

Table 4: Enterococcus species wise distribution.

Enterococcus species	Frequency
Entero. faecalis	84 (79.24%)
Entero. faecium	22 (20.75%)
Samples	Frequency
Urine culture	80 (75.4%)
Pus culture	20 (18.8%)
Blood culture	5 (4.71%)
Bone culture	1 (0.9%)
Total	106 (100%)
Samples	Frequency
Urine culture	80 (75.4%)
Pus culture	20 (18.8%)
Blood culture	5 (4.71%)
Bone culture	1 (0.9%)
Total	106 (100%)

Table 5: Enterococcus species distribution among clinical samples

Samples	E.faecalis	E.faecium
Urine culture	66 (62%)	14 (13%)
Pus culture	13 (12%)	7 (7%)
Blood culture	4 (4%)	1(1%)
Bone culture	1(1%)	0
Total	84 (79%)	22 (21%)

Out of 80 enterococcus spp positive urine samples 66 (62%) E. faecalis was isolated and 14 (13%) E.faecium was isolated. In 20 (19%) of pus samples E. faecalis was 13 (12%), E. faecium was 7 %. Among 5 blood culture positive enterococcus 4 (4%) was E. faecalis and 1(1%) was E. faecium. Only one bone cuture had a growth of E. faecalis.

Table 6: Enterococcus species distribution among clinical samples

Antibiotics	Entero.faecalis		Entero.faecium	
	Sensitive	Resistant	Sensitive	Resistant
Benzylpenicillin	35(42%)	49 (58%)	6 (27%)	16 (73%)
High level Gentamycin	42 (50%)	42 (50%)	8 (36%)	14 (64%)
Ciprofloxacin	35 (42%)	49 (58%)	4 (18%)	18 (82%)
Levofloxacin	38 (45%)	46 (55%)	8 (36%)	14 (64%)
Erythromycin	30 (36%)	54 (64%)	3 (14%)	19 (86%)
Linezolid	84 (100%)	0 (0%)	22 (100%)	0 (0%)
Daptomycin	83 (99%)	1(1%)	12 (54.5%)	10 (45.5%)
Teicoplanin	84 (100%)	0 (0%)	19 (86%)	3 (14%)
Vancomycin	82 (98%)	2 (2%)	12 (54.5%)	10 (45.5%)
Tigecycline	84 (100%)	0 (0%)	22 (100%)	0 (0%)

Among the 84 Enterococcus faecalis, 98 % and 99% of the E.faecalis were sensitive to vancomycin, and teicoplanin. They were showed 100% sensitivity to tigecycline and linezolid. 64% of E. faecalis were resistant to Erythromycin and 58% were resistant to ciprofloxacin and benzyl penicillin. Out of 84 E, faecalis 50% was sensitive and 50% showed resistance to HLG.

Out of 22 enterococcus faecium, all 22 species (100%) sensitive for linezolid. Only 14% of them were resistant to teicoplanin. 45.5% of isolated E.faecium was resistant to vancomycin and daptomycin. 86% and 82% of the E. faecium were resistant to erythromycin and ciprofloxacin. 64 % were resistant to HLG and levofloxacin.

Table 7: Distribution of Vancomycin resistant Enterococcus species

Enterococcus species	Vancomycin resistant
Entero faecalis	2
Entero faecium	10

Out of 84 E. faecalis only 2% showed resistance for vancomycin. But among the 22 E. faecium 45 % that is 10 isolates were vancomycin resistant.

DISCUSSION

Enterococcus is the normal bacterial microbiota of the gastrointestinal tract. Though they are low virulence, due to the decreased intestinal defence mechanisms we develop endocarditis, bacteremia and peritonitis. The major nosocomial pathogen which is responsible for the hospital infections is 10% to 20%. It is the major cause of hospital acquired infections,[8] In our present study Enterococcus species isolated 80 isolates (75.4%) were Urine culture, Pus cultures were 20 (18.8%), 5 (4.71%)

were blood culture and one isolate (0.9%) from bone culture. Similar results was also observed in Yadav et al,[9] study where the Enterococcus was isolated from urine culture was more 125(86.2%).In contrast to our results in some studies they isolated from pus.[10,11] In our present study, out of 106 Enterococcus species, 79.24% E. faecalis (84 strains) and 20.75 % E. faecium (22 strains) were isolated from the clinical samples. In Yadav et al study also E.fecalis was present in 80% to 90% of the samples whereas E faecium was observed in 5% to 10%.However in recent studies the E faecium was tend to increase in the incidence which indicates that there exist an intrinsic resistance which leads to treatment failure.[12,13]

More common are infections located in the urinary tract (75%) and soft tissue infections (19%). According to a study, the maximum number of isolates was obtained from urine (46.6%), followed by pus cultures (19.4%).[14] These data highlight the prevalence of enterococci in urinary tract infections (UTIs).

The major problem in the enterococcus is the increased resistance to the antimicrobial agents. In our study Out of 84 E. faecalis only 2% showed resistance for vancomycin. But among the 22 E. faecium 45 % that is 10 isolates were vancomycin resistant. In Iancu et al,[15] study the vancomycin resistance for E.Faecalis was found to be 5.8% which is more than our results and E faecium showed 15.1% of resistance to vancomycin which is lesser than our results. This variations in trend is limited to the bloodstream infections spread.

In our study among the 84 Enterococcus faecalis, 98 % and 99% of the E.faecalis were sensitive to vancomycin, and teicoplanin. They were showed 100% sensitivity to tigecycline and linezolid. 64% of E. faecalis were resistant to Erythromycin and 58% were resistant to ciprofloxacin and benzyl penicillin. Out of 84 E, faecalis 50% was sensitive and 50% showed resistance to HLG. Out of 22 enterococcus faecium, all 22 species (100%) sensitive for linezolid. Only 14% of them were resistant to teicoplanin. 45.5% of isolated E.faecium was resistant to vancomycin and daptomycin. 86% and 82% of the E. faecium were resistant to erythromycin and ciprofloxacin. 64 % were resistant to HLG and levofloxacin.In yadav et al study E fecalis shows sensitive to teicoplanin (73) and linezolid (71) and E facaium shows sensitive to Teicoplanin (59) and Linezolid (63),Thus the rapid spread of VRE leads to requirement of new antibiotics like linezolid and teicoplanin. Similarly resistance was also developing rapidly to the drugs like vancomycin, linezolid and higher level aminoglycosides which is making difficulty for the clinician to treat the infections.[16,17]

CONCLUSION

Now a days, Enterococcus spp. has been of particular concern due to their increased incidence and and their antibiotic resistance. The aim of our study was to understand not only the prevalence of Enterococcus spp. in clinical samples but also the pattern of antibiotic profiles in order to help to identify the most effective pharmacological treatments against these microorganisms and therefore limit their spread. The monitoring of antibiotic resistance could provide valuable assistance to clinics in the selection of effective empirical therapy. the studies of antibiotic sensitivity pattern of enterococcus are an important tool that aim to provide the antimicrobial susceptibility patterns information for improving empirical therapy, accelerating time to cure, and reducing hospitalization time there by we can reduce the health care associated infections. We concluded our study stating that among the Enterococcus species E. faecalis and E.faecium was the isolated species. E. faecalis was the most common one and it was sensitive to most of the antibiotics but E. faecium was resistant to many antibiotics including Vancomycin

Limitations: The sample size was small.VRE subtypes cannot be confirmed by molecular method due to the financial constraints.The study was done in a single centre so the results cannot be generalized.

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