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ORIGINAL RESEARCH

Antibiogram of Enterococcus species among Diabetic foot ulcer Patients

[1]Dr. Rohit Kumar, [2]Sapre Rohit Rajendra

[1]Associate Professor, [2]PhD Research Scholar, Department of Microbiology, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, MP, India

Correspondence:
Sapre Rohit Rajendra
PhD Research Scholar, Department of Microbiology, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, MP, India

ABSTRACT

Introduction: *Enterococci* are normal flora of oral cavity, gut, and female genital tract of humans and are known to cause nosocomial infections. *E. faecalis* is responsible for 80-90 percent and *E. faecium* 5-10 percent of the human enterococcal infections. Most frequent infections caused by *Enterococcus* spp. are urinary tract infections followed by intra-abdominal abscesses and bloodstream infections.

Materials and Methods: This is a prospective, descriptive and observational study conducted in the Department of Microbiology, Index Medical College, Hospital and Research center Indore from January 2019 December 2021. All isolates of Enterococcus species during the study period will be included. All patients over 18 years of age having chronic diabetic foot ulcers where ulcer duration is greater than three months were included in the study

Results: A total number of 72 isolates of E. faecalis, 42 (58.33%) were isolated from males whereas 30 (41.66%) from female patients of diabetic foot ulcers. In case of E. faecium, 34 (68%) were isolated from males and 16 (32%) isolated from females. In our study, Among E. faecalis organism, few strains were 100% sensitive to Amikacin, Lenizolid and Teicoplanin respectively. Rate of resistance to Penicillin G 57 (79.16%), Tetracycline 62 (86.11%), Gentamycin 53 (73.61%), Clindamycin 48(66.66%), Amoxy-clav 48 (66.66%), Cefoxitin 43(59.72%) and Ciprofloxacin 57 (79.16%). Moreover, Among E. faecium isolates shows maximum susceptibility to vancomycin. Clindamycin (100%), Amikacin (50%) and Lenizolid (76%) respectively. Rate of resistance to Penicillin G: 38 (76%), Tetracycline 19 (38%), Gentamycin 19 (38%), Clindamycin 38(76%), Amoxy-clav 19 (38%), Cefoxitin 32 (64%) and Ciprofloxacin 38 (76%) in table 4.

Conclusion: Multiple drug resistant strains of E. faecalis and E. faecium are progressively related to health care associated infections. This study emphasises the need to screen for HLGR and Vancomycin in clinical isolates, active surveillance and the prompt reporting of resistance by the laboratories to prevent injudicious use of antibiotics.

Keywords: Antibiogram, Enterococcus, Diabetic foot ulcer Patients

INTRODUCTION

Bacteria within biofilms are sheltered from various stresses, including immune responses and antimicrobial agents. The biofilm-forming ability of bacteria has been associated with increased antibiotic resistance and chronic recurrent infections. [1] Enterococci are described as vital hospital-associated pathogens and have thus been reported to withhold lots of

virulence potentials considered significantly essential in exacerbating ailments caused by them. [2]

Enterococci are normal flora of oral cavity, gut, and female genital tract of humans and are known to cause nosocomial infections. [3] *E. faecalis* is responsible for 80-90 percent and *E. faecium* 5-10 percent of the human enterococcal infections. Most frequent infections caused by *Enterococcus* spp. are urinary tract infections followed by intra-abdominal abscesses and bloodstream infections. [4]

Enterococcus strains of clinical origin have been described extensively in literature with limited information of the phenotypic virulence factors coupled with its genetic structure. [5] Furthermore, enterococci have demonstrated intrinsic antimicrobial resistance to numerous antibiotic agents and can adapt to obtain resistance to antimicrobials from the environment. [6]

A high mortality rate of enterococcal infections is due to increasing resistance of the organism to *β*-lactam antibiotics, aminoglycosides, and glycopeptides and inadequate response to the treatment. [7] Pandemic spread of vancomycin-resistant *Enterococci* (VRE) and acquisition of resistance to newer antimicrobials warrant continued surveillance and early detection of VRE along with Minimum Inhibitory Concentrations (MIC). [8]

Diabetic foot ulcers (DFU) have a lifetime prevalence of 15–25%. Infection is the most common, severe and costly. DFU complication with high risk of mortality and morbidity associated with lower limb amputation. [9] The diagnosis of diabetic foot infection (DFI) is often difficult, leading to the inappropriate use of antibiotics. The bacterial organization in DFU and the involvement of multidrug-resistant (MDR) bacteria require new antimicrobial solutions. [10]

Biofilm formation is a multistep process whereby heterogeneous communities of microorganisms (bacteria and/or fungi) are embedded into an extracellular polymeric substance (EPS) matrix that contains proteins, deoxyribonucleic acid (DNA), glycoproteins and polysaccharides, and confers the ability to adhere to biotic or abiotic surfaces. [11] In DFU, the biofilm architectural structure differs among patients due to the variability of the involved bacterial genera and species. Conversely, the multistep formation process is similar. [12]

MATERIALS AND METHODS

This is a prospective, descriptive and observational study conducted in the Department of Microbiology, Index Medical College, Hospital and Research center Indore from January 2019 December 2021. All isolates of Enterococcus species during the study period will be included.

INCLUSION CRITERIA

- All patients over 18 years of age having chronic diabetic foot ulcers where ulcer duration is greater than three months were included in the study
- Persons willing to give consent.

EXCLUSION CRITERIA

- Children (<18 years) was excluded.
- Pregnant women
- Patients with other comorbid conditions like chronic venous insufficiency, and osteomyelitis
- Persons not willing to give consent.

Enterococci isolated from clinical specimens like pus, wound swab and aspirates etc. received in Microbiology Department.

All samples were processed by standard bacteriological procedures. Gram staining was done for pus, wound swab and aspirates and findings were recorded. Culture was done on 5%

sheep blood agar and MacConkey agar. Inoculated plates were incubated at 37°C for 18- 24 hours. Preliminary identification of Enterococci was done by standard bacteriological techniques including colony morphology, gram staining and catalase test. Farther specification was done by Bile Aesculine test, Pyrrolidonyl Arylamidase (PYR) test, growth in presence of 6.5% Sodium chloride, growth at 10°C and 60°C and heat resistance test.

RESULTS

A total number of 122 Enterococcus species isolates were included in in the present study. Among this 72 (59.01%) were E. faecalis and 50 (42.98%) were E faecium. (Table 1).

Table 1: Distribution of Enterococcus species isolated from Diabetic foot Ulcers

Total isolates of Enterococcus species	E. faecalis	E. faecium
122	72 (59.01%)	50(42.98%)

Of the 23 Enterococcus isolates, the main species isolated was E faecalis 59.01% (72), followed by E. faecium 42.98% (50) in graph 1.

Table 2: Gender wise distribution of the study subjects

Gender	E. faecalis		E. faecium	
	No. of isolates	%	No. of isolates	%
Male	42	58.33	34	68
Female	30	41.66	16	32
Total	72	100	50	100

A total number of 72 isolates of E. faecalis, 42 (58.33%) were isolated from males whereas 30 (41.66%) from female patients of diabetic foot ulcers. In case of E. faecium, 34 (68%) were isolated from males and 16 (32%) isolated from females. In present study, males [76 (62.29%)] predominance was noted over females [46 (37.70%)]. This revealed that diabetic foot ulcer was prevalent in the male population in our study. (Table no. 2)

Table 3: Frequency of Age distribution among the study subjects

Age	No. of individuals	Percentage
31-40 years	25	20.49
41-50 Years	28	22.95
51-60 Years	39	31.96
61-70 Years	30	24.59
Total	122	100.0

According to age wise distribution of the study subjects, the majority of the patients (39 patients) belonged to the age group 51-60 years. Followed by 30 patients in the age group of 61-70 years. The age and gender wise distribution of the study subjects is shown in the following table no.3.

Table 4: Antibiotic susceptibility pattern of Enterococcus species

	E. facalis		E. faecium		Total sensitive	Total resistant
	Sensitive	Resistant	Sensitive	Resistant		
Cefoxitin	29(40.27%)	43(59.72%)	18(36%)	32 (64%)	47	75
Clindamycin	24(33.33%)	48(66.66%)	12 (24%)	38 (76%)	36	86
Penicillin G	15(20.83%)	57(79.16%)	12 (24%)	38 (76%)	27	84
Amikacin	72(100%)	0	25 (50%)	25 (50%)	97	25
Ciprofloxacin	15(20.83%)	57(79.16%)	12 (24%)	38 (76%)	27	95
Linezolid	72(100%)	0	38 (76%)	12 (24%)	110	12

Gentamycin	19(26.38%)	53(73.61%)	31 (62%)	19 (38%)	50	72
Amoxy-clav	24(33.33%)	48(66.66%)	31 (62%)	19 (38%)	55	67
Teicoplanin	72(100%)	0	31 (62%)	19 (38%)	103	19
Vancomycin	67(93.05%)	5(6.9%)	50(100%)	00	117	5
Tetracycline	10(13.88%)	62(86.11%)	31 (62%)	19 (38%)	41	81

In our study, Among E. faecalis organism, few strains were 100% sensitive to Amikacin, Lenizolid and Teicoplanin respectively. Rate of resistance to Penicillin G 57 (79.16%), Tetracycline 62 (86.11%), Gentamycin 53 (73.61%), Clindamycin 48(66.66%), Amoxy-clav 48 (66.66%), Cefoxitin 43(59.72%) and Ciprofloxacin 57 (79.16%). Moreover, Among E. faecium isolates shows maximum susceptibility to vancomycin. Clindamycin (100%), Amikacin (50%) and Lenizolid (76%) respectively. Rate of resistance to Penicillin G: 38 (76%), Tetracycline 19 (38%), Gentamycin 19 (38%), Clindamycin 38(76%), Amoxy-clav 19 (38%), Cefoxitin 32 (64%) and Ciprofloxacin 38 (76%) in table 4.

DISCUSSION

Diabetic foot is defined as infection, ulceration or destruction of deep tissues associated with neurological abnormalities and various degrees of peripheral vascular diseases of lower limb (based on WHO). [13] In the modern world, today diabetic is the problem as metabolic, vascular and neuropathic components are interrelated. [14] Metabolic syndrome is due to alteration of carbohydrate, fat and protein metabolism secondary to absent or markedly diminished secretion or ineffective action of Insulin. [15] Vascular system consists of abnormalities in both large vessels (macroangiopathy) and small vessels (microangiopathy), lastly a variety of abnormalities occur in peripheral nervous system. [16] These neuropathic changes are due to metabolic alteration as well as vascular cause. [17]

Defective production and action of insulin is the underlying cause. It is a long-term disease with variable manifestations and progression. [17] India has a diabetic population of about 50.8 million which is expected to increase to 87 million by 2030. Foot infections account for 20% of hospitalization of diabetic patients yearly. [18]

In our study, of the 190 samples collected from patients with diabetic foot ulcers, it is found in the present study that the male to female ratio is 2.6:1 (121 males as compared to 69 females. [137] Male incidence is higher and the possible reasons may be males are exposed more to trauma during heavy manual work. [18] Smoking habits are higher in males, may cause peripheral arterial disease that may coexist with diabetes which flare up the lesions. This was almost similar to study conducted by M Madan et al where 70% of males were affected as compared to 30% of females. [19] Even study conducted by Vinod kumar et al showed males were affected more than females (M:F=1.6:1) and also male to female ratio was similar to our study in a tertiary hospital in Nigeria which was 2.3:1. [20]

In our study, the maximum number of infections was found in patients aged 51-60 years. In the literature, the maximum number of infections was reported in patients aged 51-60 years by Ibrahim et al. [21] and in patients aged 60-65 years by Shanmugam et al . [22] This may be attributed to the high prevalence of comorbid conditions in this age group. When compared with the recent study of M Madan et al, age difference was almost similar with the present study conducted on hundred patients. [23]

In our study, Gram negative bacteria were more prevalent (74.21%) than gram positive bacteria (25.78%). In the study of Benwan et al., [24] which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than gram-positive pathogens (32.3%). The findings of this study correlate with Zubairs study. These findings correlated well with those of Pappu K et al., [25] who reported that 76% of the organisms which were isolated were gram negative bacilli. The reason could be the similar geographical locations where the 2 studies were conducted. [26]

In the present study, Staphylococcus Aureus (25.78%) was the commonest organism found in majority of cases of lesions on culture. Other organisms isolated were E. Coli (22.63%), Pseudomonas (19.47%), *Citrobacter sp* (15.26%), *Enterococcus sp.* (12.10%) and *Proteus sp* (4.7%). Even in a study conducted by Amir AH et al, Staphylococcus aureus was commonest pathogen on pus culture which was found in 46% of patients, followed by E. Coli 10%, Pseudomonas 5% and Proteus 5%. [27] In a study by Madan M et al, Staphylococcus aureus was the commonest organism (32%), followed by Pseudomonas and E. coli with 20% and 17% respectively and Streptococci being the least with 2%. [28] Even Staphylococcus aureus was the commonest pathogen found in study conducted by other researchers like Mummidi et al, Tanveer et al and Shah JV et al with the percentage being 30%, 41.66% and 42.5% respectively in those studies. [29]

In our study, the *Enterococcus* showed 60.86% resistance to Cefoxitin, 78.26% resistance to Erythromycin, 65.21% resistance to Clindamycin, 82.60% resistance to Penicillin G, 30.43% resistance to Ciprofloxacin, 73.91% resistance to Gentamicin, 61.61% resistance to Amoxy-clav. These findings did correlate with our study findings. Shanker et al., has reported that *Enterococcus* isolates were drug resistant. [30]

CONCLUSION

The emergence of gram-positive organisms in 1990s, as the important cause of both hospital- and community-acquired infections has warranted a revaluation of public research priorities. Multiple drug resistant strains of E. faecalis and E. faecium are progressively related to health care associated infections. This study emphasises the need to screen for HLGR and Vancomycin in clinical isolates, active surveillance and the prompt reporting of resistance by the laboratories to prevent injudicious use of antibiotics. The efforts of the various departments of the hospital by educating the staff, rationale use of antibiotics, early detection and reporting by laboratories and implementation of appropriate infection control measures can help in prevention and control of the spread of multi drug resistant Enterococcal infections.

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