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Clinico Microbiological Spectrum of Diabetic Foot Infections

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Abstract: Diabetic foot infections are a common cause of morbidity and mortality. This study was undertaken to study the microbiological flora of diabetic foot ulcers and to assess the in vitro antibacterial susceptibility of the causative bacteria. This was a prospective observational study conducted in which 247 wound swabs collected from patients with diabetic foot infection. All collected specimens processed using standard microbiological techniques. Disc diffusion method was used to find out the susceptibility of bacterial agents. 209 specimens yielded microbial growth on culture media. Monomicrobial growth accounted for 74.06%. Staphylococcus aureus was the predominant organism isolated in which MRSA was 27%. Second common pathogen was Pseudomonas aeruginosa. All Gram positive strains were found sensitive to linizolide. Imipenem showed good susceptibility against Gram negative bacilli. Candida was the only fungi isolated. Staphylococcus aureus was isolated predominantly from diabetic foot infections. Monomicrobial growth was seen in 177 patients and accounted for 74.06%. Polymicrobial growth was seen in 62 patients and accounted for 25.94%. The most common combination was S. aureus and Candida species. Majority of Gram positive and Gram negative isolates were susceptible to linezolide and imipenem respectively.

Keywords: Diabetic foot infections, Staphylococcus aureus, Linezolide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and target organ damage.

Diabetes is a fairly common disease seen in India with a prevalence of almost 12% - 17% in the Indian urban population as per a study in 2001 with a prevalence of 2.5% in the rural population [1]. The Indian diabetic population is expected to increase to 57 million by the year 2025 [2]. Various Co morbidities with diabetes mellitus are cardiovascular disease, retinopathy, nephropathy, neurological, peripheral vascular diseases and infections. Approximately 15% of patients with DM develop foot infections which eventually progress to osteomyelitis and amputation [3].

A commonly accepted definition of foot infection is the presence of systemic signs of infection (e.g., fever and leukocytosis) or purulent secretions or two or more local symptoms or signs (redness, warmth, indurations, pain, or tenderness) [4]. Different studies have reported on microbiological spectrum of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory [5]. S.aureus, Enterococci, E.coli, Proteus and Pseudomonas spp, are the most frequent pathogens which are cultured from diabetic foot ulcers. However, the etiology of wound infection differs from country to country and from hospital to hospital even within the same region [6]. Many of these microorganisms are developing resistance to commonly used antibiotics largely due to their inappropriate use.

Hence, this study was undertaken to study the microbiological flora of diabetic foot ulcers and to assess the in vitro antibacterial susceptibility of the causative bacteria.

MATERIALS AND METHODS

This was a prospective observational study in which 247 consecutive samples from diabetic patients with foot ulcers attending outpatient and in patient department of general surgery of vinayaka mission's medical college and hospital over a period of six months i.e from July 2017 to December 2017 were included, after getting the informed consent from the study group. All the patients underwent detailed history and clinical examination. Demographical data that included age, sex, duration of diabetes, duration of

diabetic foot, location of foot ulcer, and Wagner's grade were recorded for every case.

Sample collection

Wound beds were prepared before specimen collection, where the wound immediate surface exudates and contaminants were cleansed off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with non-bacteriostatic sterile normal saline after removing the dressing. Aseptically the end of a sterile cotton-tipped applicator was rotated over 1 cm2 area for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue. Two wound swabs were taken from each wound at a point in time to reduce the chance of occurrence of false-negative cultures and to increase the chance of recovering bacterial pathogens. It is also indicative of contamination in that if the two swab samples differ in types of organisms during presumptive test [7].

The specimens were subjected to gram staining 10% KOH and they were inoculated onto bacteriological medium (Blood agar, Mac Conkey's agar, chocolate agar) and mycological medium (SDA) for the isolation of aerobic bacteria and fungi respectively. The isolates were identified by the standard biochemical tests. Antibiotic sensitivity testing was performed by Kirby Bauer-Disk Diffusion Method [8].

Inclusion/Exclusion criteria: Patients who had received systemic antibiotic therapy for more than 24 h within the previous 72 h and those who had undergone amputation were excluded from the study.

RESULTS

A total of 247 patients who had diabetic foot infections were enrolled in the study. 209 specimens yielded microbial growth on culture media. Monomicrobial growth was seen in 177 patients and accounted for 74.06%. Polymicrobial growth was seen in 62 patients and accounted for 25.94%.Demographic details of patients shown in Table-1.

Table-1: Demographic details of patients with diabetic foot infections

Demographic data	NUMBER (%)
Age	
Sex	
Male	140 (66.99%)
Female	69 (33.01%)
Duration of diabetes	
< 1 year	50 (23.92%)
> 1 year	159 (76.08%)
Duration of Ulcer	
< 1 month	119 (56.94%)
>1 month	90((43.06%)
Grading of ulcer	
Wagner's classification	
Grade 2	57 (27.27%)
Grade 3	78 (37.32%)
Grade 4	43 (20.57%)
Grade 5	31 (14.83%)

Staphylococcus aureus (32.22%) was the predominant organism isolated from diabetic foot. Pseudomonas aeruginosa (24.68%) was the second common pathogen isolated followed by Enterococci (12.55%) and Klebsiella species (12.13%). In the present study, Candida species (6.69%) was the only fungi isolated from diabetic foot infections (Table-2)

Table-2: Organisms isolated from diabetic foot

Organism	Number (%)
Stapylococcus aureus	77 (32.22%)
Pseudomonas aeruginosa	59 (24.68%)
Enterococci	30 (12.55%)
Klebsiella species	29 (12.13%)
E.coli	21 (8.79%)
Proteus species	5 (2.09%)
CONS	2 (0.84%)
Candida species	16 (6.69%)
Total	239(100%)

CONS: Coagulase negative staphylococci.

In our study, Methicillin resistant Staphylococcus aureus (MRSA) was isolated and accounted for 27%. All MRSA strains were susceptible to linezolide (100%) followed by piperacillin/tazobactum (95%). Ceftazidime (9%) and Ceftriaxone (4%) were found to least susceptible. All Gram positive cocci were found to be susceptible to linezolide (100%) (Table-3).

Table-3: Susceptibility pattern of Gram positive cocci

Antibiotic	MRSA(n=21)	MSSA(n=46)	Enterococci(n=30)	CONS(n=2)
Ampicillin	0(0)	9(19%)	10(33%)	0(0)
Amikacin	11(52%)	41(89%)	NT	2(100%)
Gentamicin	5(23%)	21(45%)	NT	2(100%)
Ciprofloxacin	5(23%)	30(65%)	8(26%)	1(50%)
Pipracillin/Tazobac	20(95%)	44(95%)	17(56%)	2(100%)
Cefeperazone/Sulbact	11(52%)	35(76%)	15(50%)	2(100%)
Ceftazidime	2((9%)	18(39%)	11(36%)	1(50%)
Ceftriazone	1(4%)	17(36%)	10(33%)	1(50%)
Linezolide	21(100%)	46(100%)	30(100%)	2(100%)

MRSA: Methicillin resistant Staphylococcus aureus
MSSA: Methicillin sensitive Staphylococcus aureus
CONS: Coagulase negative Stahylococci.
NT: Not tested

Imipenem was most susceptible antibiotic to majority of Gram negative strains, except Pseudomonas aeruginosa (94%) and E.coli (95%). Piperacillin/tazobactum and Cefeperazone/sulbactum showed good susceptibility (93%-100%).

Table-3: Susceptibility pattern of Gram negative bacilli ()

Antibiotic	Pseudomonas(n=59)	Klebsiella (n=29)	E.coli(n=21)	Proteus(n=5)
Amikacin	50(84%)	28(96%)	21(100%)	5(100%)
Gentamicin	25(42%)	11(37%)	17(80%)	5(100%)
Ciprofloxacin	21(35%)	19(65%)	15(71%)	3(60%)
Pipracillin/Tazobac	55(93%)	28(96%)	20(95%)	5(100%)
Cefeperazone/Sulbact	51(86%)	28(96%)	20(95%)	5(100%)
Ceftazidime	31(52%)	12(41%)	9(42%)	4(80%)
Ceftriazone	19(32%)	14(48%)	3(14%)	2(40%)
Imipenam	56(94%)	29(100%)	20(95%)	5(100%)

DISCUSSION

Diabetic foot pathologies are common in diabetics and pose serious health problems for developing countries. In the present study, males (66.99%) were predominantly developed diabetic foot infections. Similar findings were observed in other studies that male sex has been purported to be a risk factor for the development of diabetic foot lesions [9]. But there is one study by Bose [10] reporting female patients to constitute the majority of the patients. The mean age of patients in the present study is 55.35 + 17.5. While the study of Benedicto *et al.*, showed an incidence of diabetic foot ulcer with mean age 68 + 5.9 [11].

In the present study, diabetic foot infections were predominantly developed among diabetic patients who had diabetes more than one year (76.08%). In our study, majority of diabetic patients who developed foot infections were grade 3(37.32%). According to the study conducted by Priyadarshini *et al*., maximum number of patients with infected diabetic foot ulcers belonged to Wagner grade 3 and 4 [12]. Monomicrobial growth was observed in 74% patients. But as per Priyadarshini *et al*., [12] monomicrobial growth was seen in 50% patients. Mohd Zubair *et al*., [13] Anandi *et al*., [14] Rama Kant *et al*., [15] Pappu K *et al*., [16] and Citron *et al*., [5] have reported 56.6%, 19%, 23 %, 92% and 16.2 % monomicrobial infections and 33%, 67%, 66%, 7.7% and 83 % of polymicrobial infections respectively. The most common combination was found to be Staphylococcus aureus and Candida species. According to the study conducted by Saravanan Sanniyasi *et al*., [17] commonest bacterial organism found in fungal positive patients was Pseudomonas followed by Enterococcus compared to the study by Emilija *et al*., [18] where Enterobacter followed by Pseudomonas were the commonest organisms.

In our study, S.aureus (32%) was the predominant pathogen isolated. This is similar to the studies conducted by Citron *et al*., [5] Mohammed

Zubair *et al*., [13], and Alavi SM *et al*., [19] reported *Staphyloccus aureus* as the predominant pathogen, which comprised 57.2%, 28% and 26.2% of their isolates respectively. In our study, Enterococci was accounted for 12%. But as per Citron *et al*., [5] and Mohammed Zubair *et al.*, [13] *Streptococcus pyogenes* was isolated in 10% and 15% respectively. In contrast, Pappu K *et al*., [16], who reported that 76% of the organisms which were isolated were gram negative bacilli, Pseudomonas being the predominant pathogen (23%), followed by *Staphylococcus aureus* (21%). Mohammed Zubair *et al*., [13] reported *Escherichia coli* (26.6%) and *Pseudomonas aeruginosa* (10.6 %) as the predominant gram negative isolates.

In the present study, 16 specimens from diabetic foot infections yielded the growth of Candida species and accounted for 6% which is lower compared to Bansal E et al (20) 9% (9 out of 103 patients) and higher compared to Emilija *et al.*, [18] 4.5% (23 out of 509 patients). Emilija *et al.*, [18] grew only Candida species which is similar to the present study. But other studies reported Aspergillus (30%) [21].

In our study, no anerobic bacterial culture was performed. Involvement of anaerobic bacteria in diabetic foot infections is not clear and few studies reported minor role of anaerobic bacteria [22]. While other studies reported preponderance of anaerobic bacteria [23].

In this study, 27% MRSA were isolated. But Umadevi *et al*., [24] reported 65% MRSA from diabetic foot infections. While other studies on diabetic foot infections which have reported 10–44% MRSA [9]. MRSA are more often isolated from patients who have been previously hospitalized or reside in a chronic care facility, who have recently received antibiotic therapy or who have had a previous amputation. The isolation of MRSA in DFIs would be associated with more severe infections [24].

CONCLUSION

Staphylococcus aureus was isolated predominantly from diabetic foot infections. Monomicrobial growth was seen in 177 patients and accounted for 74.06%. Polymicrobial growth was seen in 62 patients and accounted for 25.94%. The most common combination was S.aureus and Candida species. Linezolide was found to be sensitive to all Gram positive cocci including MRSA. Majority of Gram positive and Gram negative isolates susceptible to Piperacillin /tazobactum and amikacin.

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