Study of Bacterial Spectrum in Diabetic Foot Ulcers

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Abstract

Introduction: Diabetic foot ulcerations, infections and their sequelae are one of the leading causes of mortality and morbidity, especially in the developing countries. It is essential to assess the magnitude of bacterial infection in the lesions to avoid further complications and save the diabetic foot. **Aims and Objectives:** This study was carried out to determine the bacterial profiles of infected ulcers and the antibiotic resistance pattern of the isolates. **Methods and Material:** Eighty six diabetic foot patients underwent detailed history, clinical examination, and laboratory investigations including parameters of systemic infections. Microbial culture and sensitivity were performed at the time of presentation. **Results:** Among 86 cases, 37.2% had mono-microbial infection, 54.6% had poly-microbial infections, and 8.1% had sterile culture. Gram negative aerobes were the most frequently isolated bacteria constituting 84 isolates (64.1%), followed by gram-positive aerobes 45 isolates (34.8%).The most frequently isolated aerobic organisms were *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Antimicrobial sensitivity pattern of the isolates were done in which Imepenem was found to be effective against Gram Negative Infections. **Conclusion:** There is a growing trend of isolating gram negative bacteria in these naïve lesions of the diabetic foot. The need for adequate gram negative antibacterial coverage at the commencement of diabetic foot therapy is essential to prevent and treat limb/life threatening infections.

Key Words: *Diabetic Foot Infections, Empirical Antimicrobial therapy, Imepenem, Piperacillin tazobactum*

Introduction

Diabetes mellitus is a chronic endocrine disorder that affects large segment of population and is a major public health problem.(1) The Indian diabetic population is expected to increase to 57 million by the year 2025. (2) Diabetes and foot problems are almost synchronous, the impaired micro-vascular circulation in patients with a diabetic foot limits the access of phagocytes, thus favouring the development of an infection, the individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes. (1,2)

A diabetic foot infection is simply defined as any infra-malleolar infection in a person with diabetes mellitus.(3) Diabetic foot ulcer (DFU) is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration, and infection with or without osteomyelitis, leading to the development of gangrene and it is one of the chronic wound infection which leads to non-traumatic lower limb amputation.[2,3]

Majority of the diabetic foot lesions are initially treated empirically based on the prevalence of the microbial pattern in the locality and the hospital.[4] Many studies have reported on the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory, these discrepancies could partly have been due to the differences in the causative organisms, which had

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occurred over time, geographical variations, or the type and the severity of the infection. [2]

Mostly, the diabetic foot infections are mixed bacterial infections and the proper management of these infections requires an appropriate antibiotic selection, based on the culture and the antimicrobial susceptibility testing results, in addition to administering regular glycemic control, wound care, surgical debridement, pressure offloading, and maintaining adequate blood supply.[2,5] As there is a growing trend of isolating gram negative bacteria in these naïve lesions of diabetic foot, the empirical antimicrobial therapy should be comprised of antibiotics to cover both gram negative and gram positive antibiotics. [4, 6, 7]

Patterns of microbes infecting diabetic foot wounds have been studied widely. Bacterial profiles have been reported from various regions indicating area-specific studies to be conducted for assessing the problem of DFI (diabetic foot infection) and instituting effective treatment. Within the same context, the current study was undertaken as an attempt to examine the major populations of bacteria which were associated with the bio burden of infected diabetic foot ulcers.[2]

Materials and Methods

A prospective study was carried out on 86 patients with foot ulcer from surgery unit, Sri Lakshminarayana Institute of Medical Sciences, Pondicherry during the period of December 2017 to February 2019. The criteria for inclusion in this study were presence of foot infection in diabetics of grade 1 and above. A clinical history was elicited with regards to the duration of diabetes, the type of treatment which was received and the presence of other systemic illnesses. (*Table:1,2,3*)

Samples were collected from the deeper portion of the ulcers by using sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. All the samples collected were immediately brought to the laboratory and then processed. Two swabs were collected, one swab was used for Gram staining and the other was used for culture. A direct Gram stained smear of the specimen was examined. The specimens were inoculated on to blood agar and Mac Conkey's agar. The inoculated plates were incubated at 37°C overnight and the plates were examined for growth, the next day. The further processing was done according to the nature of the isolate, as was determined by Gram staining and the colony morphology. The organisms were identified on the basis of their Gram staining properties and their biochemical reactions. The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method, as per the CLSI guidelines, 2011.[8]

The antimicrobial discs which were used were those of Ampicillin (10µg), Gentamicin (10µg), Amikacin (30µg), Amoxy+clavum (20/10µg), Ciprofloxacin (5µg), Ceftazidime/Clavulanicacid (30/10µg), Ticarcillin Clavum(75/10µg), Cefotaxime (30µg), Ceftriaxone(30µg), Cefoperazone/sulbactam (75/10µg), Piperacillin/tazobactam (100/10µg), Imipenem (10µg), for the Gram negative bacilli.

Penicillin (10 units), Ampicillin (10µg), Cefoxitin (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Clindamycin (2µg), Erythromycin (15µg), Oxacillin (1µg), Vancomycin (30µg), Ciprofloxacin (5µg), Linezolid (15µg), Cotrimoxazole(25µg), Rifampicin (5µg), and Tetracycline (30µg) were used to study the susceptibility patterns of the Gram positive cocci.

Results

Of the total 86 diabetic foot patients studied 59 were males and 27 were females, the male:female ratio being 2:1. Their ages ranged from 39 years to 83 years. The maximum number of patients having diabetic foot infections was in the age group of 58-67 years, the cases had diabetes mellitus for more than a decade. Among the 86 cases 47 (54.6%) were polymicrobial, 32 (37.2%) were mono microbial and 7 (8.1%) were sterile. Altogether 129 organisms were isolated from 86 cultures. Among the 129 organisms, 84 (65.1%) were gram negative and 45 (34.8%) were gram positive. *Pseudomonas aeruginosa* 24 (18.6%) was the most common isolate followed by *Staphylococcus aureus* 20 (15.5%), *Escherichia coli* 18 (13.9%), *Proteus* 12(9.3%), *Streptococci* 12 (9.3%), *Citrobacter sp* 10 (7.7%), *Klebsiella* 9(6.9%), Coagulase negative Staphyloccus 9(6.9%), *Enterococcus sp* 4 (3.1%), *Providencia* 2(1.5%), *Acinetobacter* sp.6(4.6%), *Enterobacter* sp. 2(0.7%) and *Candida* 1(0.7%).(*Table:4*)

Among the 32 cases of mono microbial infection, Gram-negative infection was seen in 19 (59.3%) cases, whereas both Gram-positive and Gram-negative were high 27(57.4%) in cases with poly-microbial infection 20(42.5%). Isolated bacteria showed differential sensitivity patterns against commonly used antibiotics. The majority of the isolates were resistant to several antibiotics that are usually prescribed on an empirical basis. Antibiotic sensitivity of the isolated microbes showed highest sensitivity for Imepenem, Cefeperazone sulbactum and Piperacillin/tazobactum for gram negative organisms. Linezolid and Vancomycin showed good sensitivity against Gram positive organisms. (*Table: 5,6*)

Table 1: Characters, Frequency and Percentage

Characters	Frequency	Percentage
Type of diabetes mellitus		
Type- 1	1	1.1%
Type-II	85	98.8%
Duration of diabetes Mellitus		
1-5 yrs	29	33.7%
5-10 yrs	23	26.7%
More than 10 yrs	13	15.1%
Not known	21	24.4%
Random Blood Sugar at the time of admission		
Less than 200mg/dl	18	20.9%
Greater than 200mg/dl	68	79%
Co-morbid conditions among type II Diabetic patients (n=85)		
Osteomyelitis	14	16.4%
Neuropathy	58	68.2%
Nephropathy	12	14.1%
Septicaemia	2	2.3%
Gangrene	19	22.3%
Retinopathy	1	1.1%
Peripheral Vascular disease	21	24.7%
Hypertension	49	57.6%
History of Amputation		
Performed	32	37.6%
Not performed	54	63.5%

Table 2: Age and Gender distribution

Age	Male	Female	No of patients
Less than 40	2	1	3
41-50	11	3	14
51-60	19	9	28
61-70	21	12	33
71-80	4	2	6
81-90	2	0	2

Table 3: Wagner's Grade

Wagner's Grade	No. of Patients	Flora		
		Sterile	Mono	Poly
Grade-1	3	1	1	1
Grade-2	17	2	6	9
Grade-3	32	4	13	15
Grade-4	29	0	11	18
Grade-5	5	0	1	4
Total	82	7	32	47

Table: 4: Antimicrobial susceptibility pattern of Gram Positive bacterial isolates from infected foot ulcers in diabetic patients (*n* =)

Antibiotic	S.aureus n=20	CONS n=9	Streptococci n=12	Enterococci n=4
Methicillin Sensitive	12(60%)	7(77.7%)	-	-
Methicillin Resistant	8(40%)	2(22.2%)	-	-
Penicillin	-	-	4	-
Amikacin	8(40%)	8(88.8%)	-	-
Ampicillin	16(80%)	-	6	2(50%)

Cont ... **Table: 4: Antimicrobial susceptibility pattern of Gram Positive bacterial isolates from infected foot ulcers in diabetic patients (*n* =)**

Erythromycin	16(80%)	7(77.7%)	6	4(100%)
Tetracycline	-	-	0	3(75%)
Clindamycin	3(15%)	1(11.1%)	3	-
Cotrimoxazole	-	4(44.4%)	-	2(50%)
Ciprofloxacin	5(25%)	3(33.3%)	-	3(75%)
Chloramphenicol	-	2(22.2%)	5	2(50%)
Rifampicin	5(25%)	3(33.3%)	-	2(50%)
Vancomycin	1(5%)	0	0	1(25%)
Linezolid	2(10%)	3(33.3%)	0	0

Table: 5: Antimicrobial resistance pattern of Gram negative bacterial isolates from infected foot ulcers in diabetic patients (*n* =)

Antibiotics	Pseudomonas n=24	E.coli n= 18	Proteus n= 12	Citrobacter n=10	Acinetobacter n=6	Klebsiella n=9	Providencia n=2	Enterobacter n=2
Ampicillin	-	16(88.8%)	12 (100%)	8(80%)	5(83.3%)	6(66.6%)	2(100%)	2(100%)
Amikacin	5(20.8%)	8(44.4%)	1(8.3%)	0	3(50%)	6(66.6%)	1(50%)	1(50%)
Amoxy+ clavum	6(25%)	9(50%)	6(50%)	1(10%)	4(66.6%)	6(66.6%)	0	1(50%)
Ciprofloxacin	8(33.3%)	8(44.4%)	2(16.6%)	0	2(33.3%)	5(55.5%)	1(50%)	2(100%)
Cefotaxime	7(29.1%)	9(50%)	7(58.3%)	6(60%)	3(50%)	5(55.5%)	1(50%)	1(50%)
Ceftazidime/ Clavulanic acid	6(25%)	10(55.5%)	1(8.3%)	3(30%)	1(16.6%)	2(22.2%)	0	1(50%)
Cefaperazone Sulbactum	5(20.8%)	10(55.5%)	1(8.3%)	3(30%)	4(66.6%)	0	1(50%)	1(50%)
Piperacillin tazobactum	4(16.6%)	9(50%)	5(41.6%)	2(20%)	2(33.3%)	5(55.5%)	0	1(50%)
Imepenem	2(8.3%)	0	1(8.3%)	0	0	0	0	0
Gentamicin	8(33.3%)	12(66.6%)	2(16.6%)	-	4(66.6%)	7(77.7%)	1(50%)	-
Ticarcillin-Clavum	4(16.6%)	6(33.3%)	2(16.6%)	-	4(66.6%)	6(66.6%)	1(50%)	-

Discussion

The prevalence of diabetic foot ulceration was found to be 68.6% in males and 31.3% in females. The most common risk factors for diabetic foot ulceration include neuropathy, poor glycaemic control, ischaemia and infection. Diabetic neuropathy was seen in 68.2% of our patients with diabetic ulcers. The detection of neuropathy before the development of its complications is the best way to prevent diabetic foot infections [9].

In the present study the maximum number of patients with infected diabetic foot ulcers belonged to Wagner grade 3 and 4. Severe diabetic foot infections usually yield polymicrobial isolates, whereas mild infections are frequently monomicrobial. In cases of severe diabetic foot infection, three to five organisms may be cultured.[6] Polymicrobial nature of diabetic foot infections have been observed in various studies in subcontinent and abroad.[2,10,11,12] We observed that Gram-negative infections were more common in the studied population as recent studies reported a predominance of gram negative aerobes1,2,3,7,20. In previous reports, researchers have shown the predominance of Gram-positive infections in their regions.[9,10,13,14,6,15,16,17,18]

In our study, *Pseudomonas aeruginosa* (18.6%) was the predominant pathogen isolated, followed by *Staphylococcus aureus* (15.5%) *Escherichia coli* (13.9%), which was in accordance with the findings of the study which was conducted by Ekta Bansal et.al.[12] Priyadarshini Shanmugam et.al and Pappu AK et.al. also made similar observation and found *Pseudomonas aeruginosa*16%, *Escherichia coli* 14.6% and *Staphylococcus aureus* 13.3% and *Pseudomonas aeruginosa* 23%, *Staphylococcus aureus* 21% and *Escherichia coli* 12% as the most common pathogens. Our results are similar to many studies in the southern parts of our country the reason could be the similar geographical locations.

As regards to antibiotic sensitivity pattern of Gram negative bacilli more than 60% strains were sensitive to cephalosporins, Imepenem showed more than 95% sensitivity similar to the studies of J.Vimalin Hena et.al. [10]

Pseudomonas is 91.7% sensitive to Imepenam, 16.6% resistance to Piperacillin tazobactum, 20.8% resistance to Amikacin, and 33.3% resistance to Ciprofloxacin almost similar findings were seen with G.S.Banashankari.et.al.[4], where *Pseudomonas* is 100% sensitive to Imepenem, 25% resistance to Piperacillin tazobactum, 25% resistance to Amikacin, and 38% resistance to Ciprofloxacin, in similar E.coli showed 100% sensitive to Imepenem, Amikacin 44.4% and Ciprofloxacin 44.4% and the findings with G.S.Banashankari.et.al. for E.coli are Imepenem 100% Amikacin 45%, Ciprofloxacin 45%.

Proteus exhibited 100% resistance to Ampicillin, 50% resistance to Amoxy+Clavum and 8.3% resistance to Cefeperazone sulbactum; similar to the studies of Ekta Bansal et.al., which showed 100% resistance to Ampicillin, 50% resistance to Amoxy+Clavum and 100% sensitive to Cefeperazone sulbactum.

Imepenem is the drug of choice in infection caused by *Pseudomonas*, *E.coli*, *Proteus*, *Acinetobacter* and *Klebsiella* which exhibited more than 90% sensitivity, similar to the findings of G.S.Banashankari et.al., and Ekta Bansal et.al.

Pertaining to S.aureus 40% strains were Methicillin resistant and 25% of the isolated *Enterococcus* strains were resistant to Vancomycin. *Enterococci* and Gram-negative *Citrobacter*, *Providencia* are considered commensals with low virulence whereas they may cause severe tissue damage in diabetic patients. [19]

Imipenem, Piperacillin/tazobactum and Cefeperazone / sulbactum were the agents which were most effective against gram negative organisms, while Linezolid and Vancomycin were effective against the gram positive organisms.

Conclusion

Both Gram positive cocci and Gram negative bacilli caused diabetic foot infections and this study showed a preponderance of Gram negative bacilli. There is a growing trend of isolating gram negative bacteria in these naïve lesions of the diabetic foot. The need for adequate gram negative antibacterial coverage at the commencement of diabetic foot therapy is essential to prevent and treat limb/life threatening infections. Knowledge on the antibiotic susceptibility pattern of the isolates from diabetic foot infections is crucial for

planning the appropriate treatment of these cases, prior to getting the susceptibility reports from the laboratory. Since there is an increasing rate of multidrug resistant organisms, there is a need for continuous surveillance to provide the basis of the empirical therapy and to reduce the risk of the complications. The inadvertent use of broad spectrum antibiotics should be discouraged. The selection of the antibiotic treatment should be based on the predominant organisms which are isolated and their antimicrobial susceptibility patterns. This will improve the overall antibiotic utilization and reduce the emergence of multidrug resistant organisms. The detection of neuropathy before the development of its complication is the best way to prevent the diabetic ulcer complications.

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