Original Article

A BACTERIOLOGICAL STUDY OF DIABETIC FOOT INFECTION IN AN URBAN TERTIARY CARE HOSPITAL OF DHAKA CITY

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Abstract

Identification of organisms and effective antibiotic therapy is an important component of treatment of diabetic foot infections. This study was undertaken to determine the organisms associated with diabetic foot infection (DFI) and their antibiotic sensitivity pattern. A total of 75 patients having type 2 diabetes mellitus with Wagner's grade 1-5 foot ulcers attending BIRDEM hospital were included in the study. Specimens were processed for aerobic culture. The bacteriological isolation and antimicrobial sensitivity tests of the isolates were done by standard microbiological methods. Gram negative bacilli were tested for extended spectrum β lactamase (ESBL) production by double disc diffusion method. Culture was positive in 92% of the cases which yielded 135 pathogens. Of the positive culture, 75.3% had multiple organisms. Polymicrobial infection was more in higher grade of foot ulcers. Gram negative organisms were most frequently isolated (80%) bacteria. *Pseudomonas* (48%) and *Proteus* sp.(33%) was the most common Gram negative organisms isolated. *Staphylococcus aureus* was the most commonly isolated gram positive organism (21.3%). ESBL production was noted in 31.5% Gram negative bacilli and methicillin resistance was noted in 43.8% of *Staphylococcus aureus*. Most of the Gram negative bacilli were resistant to various classes of antibiotics. Imepenem was the most effective agent against Gram negative organisms, while vancomycin was for staphylococcus. The present study has shown that infection with multidrug resistant Gram negative bacilli is the most common cause of DFI in BIRDEM hospital.

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Introduction

Foot ulceration and infections are perhaps the most frequent and serious complication of diabetes mellitus (DM).[1] The annual incidence of leg and foot ulcers is 2, 6.5 and 33 times more common than diabetic coronary disease, stroke and renal failure respectively. About 15% of diabetic patients develop a foot ulcer during their lifetime and 20% suffer from some type of foot infection in their lifetime.[2,3] Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), a central referral hospital in Dhaka city, provides basic diabetes care to a large number of diabetic population. The total number of registered patients in BIRDEM is >3,20,000 and daily turnover is around 2500.[4] A retrospective cohort study from 1980 to1995 among patients in BIRDEM showed a 2.8% prevalence of diabetic foot ulcer.[5] Many studies have reported on the bacteriology of diabetic foot infection over the past 25 years, but the results have varied and have often been contradictory. A number of studies have found that *Staphylococcus aureus* is the main causative pathogen.[6-8] But recent investigations reported a predominance of Gram negative aerobes.[9-11] Several studies have confirmed that chronic lesions or infections receiving prior antibiotic treatment are usually polymicrobial.[12,13]

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A good outcome of DFI depends upon being familiar with the microbiological profile of the infection that can help in selecting the most appropriate antimicrobial therapy.[13] This study was conducted with an aim to attempt determining the microbiological and microbial susceptibility profile of organisms isolated from diabetic foot ulcers of patients attending BIRDEM hospital.

Methods

Study population and grading of foot ulcers

Seventy five diabetic patients with clinically infected foot ulcers attending both Surgery and Orthopedics outpatient and inpatient departments at BIRDEM hospital during the period of June 2008 to October 2008 were studied.

A detailed clinical history was obtained from each patient which included age, sex, type and duration of diabetes, treatment history, and other associated diseases (e.g. hypertension, neuropathy, peripheral vascular disease). Clinical assessment for signs of infection namely swelling, exudates, surrounding cellulitis, odor, tissue necrosis, local crepitation, redness, indurations, pain, warmth and fever were noted. Ulcer size was determined by multiplying the longest and widest diameters and expressed in centimeters squared. Ulcers were graded into following six categories according to the Wagner's Classification system (Fig-1).[14]

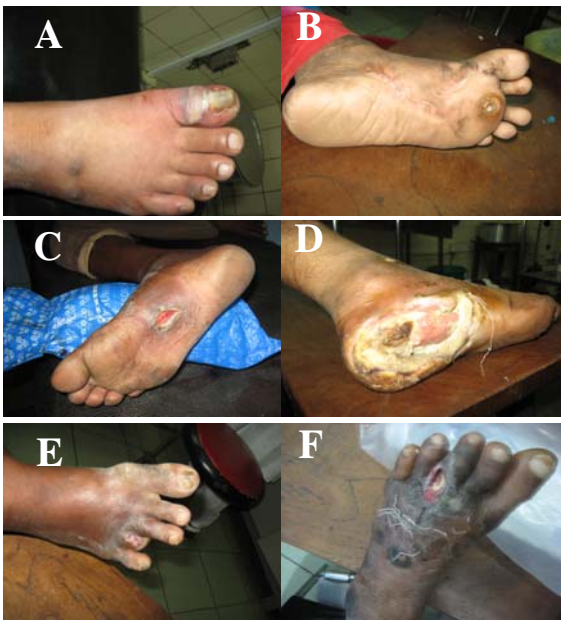

Fig-1: *Different grades of diabetic foot ulcers: A- Grade 0, B- Grade 1, C- Grade 2, D- Grade 3, E- Grade 4, F- Grade-5.*

Grade 0- Preulcer. No open lesions, skin intact; may have deformities, erythemetous areas of pressure or hyperkeratosis.

Grade 1- Superficial ulcer. Disruption of skin without penetration of the subcutaneous fat layer. Superficial infection with or without cellulitis may be present.

Grade 2- Full thickness ulcer. Penetrates through fat to tendon, or joint capsule without deep abscess or osteomyelitis.

Grade 3- Deep ulcer which may or may not probe to bone, with abscess, osteomyelitis, or joint sepsis. Includes deep plantar space infections or abscesses, necrotizing fascitis, and tendon sheath infections.

Grade 4- Denotes gangrene of a geographical portion of the foot such as toes, forefoot or heel. The remainder of the foot is salvageable though it may be infected.

Grade 5- Gangrene or necrosis to the extent that the foot is beyond salvage and will require a major limb or life sparing amputation.

Sample collection procedure

Culture specimens were obtained after the surface of the wound had been washed vigorously by saline and followed by debridement of superficial exudates. The materials used were curettage of the base of the ulcer, needle aspiration of the abscess material and deep wound swab.

Microbiological methods

Each specimen was subject to wet mount microscopy, Gram stain and culture. The specimens were first inoculated onto blood agar and MacConkey agar media. The inoculated plates were incubated aerobically at 35°C for 48 hours. Anaerobic culture was not done. The microorganisms were identified using standard biochemical procedure. The antimicrobial susceptibility of the organisms was performed by disc diffusion method according to the guidelines of the National Committee for the Clinical Laboratory Standards (NCCLS).[15] Gram negative bacilli were tested for extended spectrum β-lactamase (ESBL) production by a double disc diffusion method[16] while

Table-1: *Clinical features of 75 diabetic patients with infected foot ulcer*

Parameters	Number (%)
Sex	
Male	52 (69.3)
Female	23 (30.8)
Diabetic medication	
On insulin	62 (82.7)
On oral hypoglycemic agents	13 (17.3)
Associated Diseases	
Neuropathy	25 (33.3)
Peripheral vascular disease	18 (24.0)
Duration of foot infection	
>1 Month	40 (53.3)
<1 Month	35 (46.7)
Types of ulcers (Wagners's grade)	
Grade 1	3 (4.0)
Grade 2	26 (34.7)
Grade 3	27 (36.0)
Grade 4	13 (17.3)
Grade 5	6 (8.0)
Treatment history	
Received antibiotic before admission	50 (66.7)
Wound debridement before admission	53 (70.7)

Staphylococcus species were tested for methicillin resistance by 1μg oxacillin disc susceptibility testing method.[15]

Table-2: *Bacteriology of diabetic foot ulcer cases (n=75)*

Parameters	Number (%)
Cases with positive culture	69 (92.0)
Cases with negative culture	06 (8.0)
Pattern of organisms isolated from ulcers	
Single organism	17 (24.6)
Two organisms	35 (50.7)
Three organisms	17 (24.6)
Total number of organisms isolated	135

Wagner's grade *	Single infection Number (%)	Polymicrobial infection Number (%)
G-1 (n=3)	2 (66.7)	1 (33.3)
G-2 (n=22)	11 (50.0)	11 (50.0)
G-3 (n=27)	2 (7.4)	25 (92.6)
G-4 (n=12)	3 (25.0)	9 (75.0)
G-5 (n=5)	0	05 (100.0)

Note: *Out of total 69 culture positive cases

Table-3: *Rate of isolation of organism from foot ulcers*

Organism	No. isolated	% of total isolates (n=135)	% of total culture positive cases*** (n=69)
Gram positive cocci	26	19.3	33.3
Staphylococcus aureus	16	11.9	21.3
Coagulase negative *Staphylococcus*	4	2.9	5.3
Others*	6	4.4	8.7
Gram negative bacilli	108	80.0	92.8
Pseudomonas sp.	36	26.7	48.0
Proteus sp.	25	18.5	33.3
Klebsiella sp.	21	15.5	28.0
Escherichia coli	11	8.1	14.7
Acinetobacter sp.	05	3.7	6.7
Others **	11	8.2	15.9
Candida species	01	0.7	1.3

Note: *Other gram positive cocci include *Enterococcus* sp 3, Group B *Streptococcus* 2, *Streptococcus pyogenes* 1; **Other Gram negative bacilli include *Citrobacter* sp 4, *Enterobacter* sp 4, *Providencia* sp 1, *Serratia* sp 1. *** Multiple organisms were isolated per case.

Results

The clinical characteristics of 75 study population are shown in Table 1. Males were predominant (69.3%) and the mean age of the patients was 52.8 $\pm$ 11.7

Table-4: *Antimicrobial resistance pattern of isolated gram negative organisms*

Antimicrobial agents used (µg/ disc)	*Pseudomonas* sp. n=36 N (%)	*Proteus* Sp. n=25 N (%)	*Klebsiella* sp. n= 21 N (%)	*E.coli* n=11 N (%)
Augmantin (30)	27 (75)	19 (76)	13 (61.9)	7(63.6)
Ceftazidime (30)	24 (66.7)	21 (84)	13 (61.9)	8(72.7)
Ceftriaxone (30)	27 (75)	19 (76)	15 (71.4)	8(72.7)
Cefotaxime (30)	25 (69.4)	18 (72)	18 (85.7)	6(54.5)
Cefuroxime (30)	-	-	19 (90.8)	9(81.8)
Cotrimoxazole(25)	35 (97.2)	22 (88)	13 (61.9)	7(63.6)
Ciprofloxacin (5)	24 (66.7)	22 (88)	14 (66.7)	6(54.5)
Tetracycline (30)	29 (80.6)	21 (84)	15 (71.4)	8(72.7)
Gentamicin (10)	20 (55.6)	19 (76)	13 (61.9)	5(45.5)
Amikacin (30)	22 (61.1)	19 (76)	14 (66.7)	7(63.6)
Netilmicin (30)	18 (50)	17 (68)	13 (61.9)	6(54.5)

Note: (-) indicate not done; All the Gram negative bacilli were sensitive to imipenem; 58.3% and 55.6% Pseudomonas were resistant to aztreonam and piperacillin respectively.

Table-5: *Rate of isolation of ESBL producing Gram negative bacilli and ulcer infection rate with ESBL and MRSA*

Parameters	Number (%)
ESBL producing Gram negative bacilli:	
a. *Proteus* sp (n=25)	13 (52.0)
b. *Klebsiella* sp (n=21)	11 (52.4)
c. *Escherichia coli* (n=11)	7 (63.6)
d. Other gram negative bacilli* (n=10)	3 (30.0)
Cases infected with:	
a. ESBL positive Gram negative bacilli	31 (44.9)
b. MRSA	6 (8.7)
c. ESBL and MRSA	1 (1.4)

Note: * Others include *Enterobacter* sp. 4, *Citrobacter* sp. 4, *Providencia* sp. 1, *Serratia* sp. 1.

years. All of them were suffering from type 2 diabetes mellitus (T2DM) and the duration of diabetes ranged between 3-20 years. Among them, 25 (33.3%) had neuropathy and 18 (24%) had peripheral vascular disease. The majority (53.3%) had infected foot ulcer for >1 month and 50 (66.7%) of them had prior antibiotic intake while more than two thirds (70.7%) received surgical treatment prior to admission into BIRDEM hospital. The foot ulcers fell into all the grades (1-5), the most common being grade 3 ulcers (36%).

About 92% ulcers showed growth of organisms (69/75 patients) and a total of 135 organisms were isolated. Of the positive cultures, 52 (75.4%) had multiple microorganisms of which 24.6% wounds had 3 isolates and 52.7% had 2 isolates. Polymicrobial infections were found frequently in Grade- 3, 4 and 5 ulcers as shown in Table 2.

Table 3 shows the frequency of isolation of different organisms from diabetic foot ulcers. Gram negative organisms were most frequently isolated (80%) followed by Gram positive (19.3%) and fungus (0.7%). *Pseudomonas* species (36 isolates) was isolated from 48% cases and accounted for one third of all isolates. Other organisms were *Proteus* sp (33.3%), *Klebsiella* sp (28%), *Esch. coli* ((14.7%), *Acinetobacter* sp (6.6%), *Citrobacter* sp (5.3%), *Serratia* sp (1.3%) and *Providencia* sp (1.3%). *S. aureus* was the most common Gram positive organism and accounted for 21.3% of the infections.

The antimicrobial susceptibility pattern of major organisms is shown in Table 4. Out of 16 *S. aureus* isolated, 43.8% were methicillin resistant or MRSA while all were sensitive to vancomycin. Resistance to cotrimoxazole, ciprofloxacin and tetracycline was 62.5%, 75% and 56.3% respectively. Imipenem was the most effective antimicrobial agent against all the isolated Gram negative bacterial species. Most of the Gram negative bacilli were resistant to various classes of antibiotics. ESBL production was noted in 31.5% Gram negative bacilli and highest producers were *E.coli* (63.3%) followed by *Klebsiella* (52.4%) and *Proteus* (52%). Thus, among 69 culture positive patients, 31 had infection with ESBL producing bacteria while 6 had MRSA (Table 5).

Discussion

Our study was designed to detect the bacteria responsible for diabetic foot infections among patients attending the out and in-patient departments of BIRDEM hospital. Most of our patients had grade 3 ulcers. Our study shows that in chronic, complex and previously treated wounds, infections are generally polymicrobial with mixed Gram positive and Gram negative organisms. We found Gram negative aerobic bacteria as the most frequently isolated organism though previous studies had shown Gram positive aerobes as the predominant organisms in DFI.[9,14,18,19] Thus the major infective organisms in diabetic foot ulcer in our patients appear to be different. The ratio of Gram positive to Gram negative was 1:4. The differences in the age-sex composition and ulcer grades between our study population and those of earlier studies might be the reason for these differences. However, our results are in tune with other studies done in India which also showed that Gram negative bacteria were the most predominant organisms in DFI.[10,11] The role of anaerobic organisms in DFI could not be determined as no attempt was made in this study to isolate the anaerobes.

High levels of resistance to ciprofloxacin, cotrimoxazole, amikacin, gentamicin and cephalosporins were found in all isolated organisms. Only imepenem was the most effective agent against all Gram negative organisms. High rates of antibiotic resistance observed in the present study may be due to the widespread use of broad spectrum antibiotics in the tertiary care hospital leading to survival advantage of resistant pathogens. About 31.5% Gram negative bacteria were ESBL producers and 43.8% of *S. aureus*

were methicillin resistant. The increasing prevalence of ESBL producing organisms and MRSA is disconcerting, because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome.

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