Bacteriological Profile and Antibiotic Sensitivity Pattern in Infected Diabetic Foot Ulcers

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Abstract

Objectives: To identify the common bacterial pathogens responsible for infection in diabetic foot ulcer and their sensitivity pattern to different antibiotics.

Methods: This prospective observational study was conducted from 24th June 2019 to 27th December 2019 at Services Institute of Medical Sciences Lahore. Specimens of discharge from diabetic foot ulcers (DFU) were received from Diabetes Management Centre (DMC). Cultures were put up and bacteria isolated were identified by standard methods. Antibiotic sensitivity was determined by Kirby-Bauer disc diffusion method.

Results: Samples from DFU of 50 patients were processed. Thirty-six (72%) samples were from males and 14(28%) were from females; mean age of patients was 53±9.5 years. Forty-nine (98%) patients had unsatisfactory glycemic control. Forty three (86%) samples were growth positive while 07(14%) were bacteriologically sterile. Monomicrobial infection was observed in 38 (76%) cases while polymicrobial infection was seen in 12 cases (24%). The most common isolates were Staphylococcus aureus 14(28%), Pseudomonas species 11 (22%) and Proteus species 10 (20%). Forty three percent of Staphylococcus aureus, were methicillin resistant (MRSA). All MRSA remained sensitive to vancomycin and linezolid. In Pseudomonas species, resistance to third generation cephalosporins, ceftazidime , was 27%, while resistance to imipenem was seen in 3 (9%) of isolates. In Proteus species, resistance to third generation cephalosporins was 90% while to imipenem resistance was 60%. Resistance to commonly prescribed quinolones was more than 70% among all the bacterial isolates.

Conclusions: Common Gram positive and Gram negative organisms responsible for infection in DFU were Staphylococcus aureus, Pseudomonas species and Proteus species. The isolates were multi-drug resistant (MDR). Resistance to antibiotics used as empiric therapy was high.

Keywords: Diabetic foot ulcer, antibiotic resistance, empiric therapy

Introduction

Diabetes mellitus is a growing health care concern in Pakistan. The prevalence of the disease was 26.3% in the National Diabetes Survey of Pakistan (NDSP) in 2016-17, an increase of 17.6% from the previous survey carried out in 1994-98.[1] It is the fourth leading cause of death in developed countries, while Pakistan ranks at seventh position currently. This significant increase in the prevalence of diabetes is attributed to an aging population, unhealthy dietary practices, sedentary life style, obesity and smoking.[2]

Diabetic foot ulcer (DFU) is a common and serious complication in diabetic patients. Most hospitalizations in diabetic patients are due to diabetic foot ulcers.[3] Approximately 15% of diabetics develop foot ulcer at some point in their lives which can lead to infection, tissue destruction and may result into amputation if inadequately treated. The rate of amputation in diabetic foot ulcers accounts for 50% of all

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Submission Date: 01-09-2020
1st Revision Date: 13-10-2020
Acceptance Date: 18-11-2020

non-traumatic amputations carried out. The rate of recurrence of diabetic foot ulcers is similarly high, reoccurring in 50% of patients within three years.[4] Risk factors for the disease include peripheral arterial disease, peripheral neuropathy and foot deformaties.[5] Different classifications are used to describe the lesion on the foot, but the most commonly used classification is of Meggitt-Wagner. In this classification the ulcer is graded from 0 to 5, first four grades describe physical depth of the ulcer while last two describe the extent of gangrene.

Diabetic foot infection (DFI) management involves empirical antibiotic therapy and supportive care initially followed by definitive antibiotic regimen based on culture and antibiotic sensitivity reports.[3,6] DFI are mostly polymicrobial including both Gram positive and Gram negative organisms.[6,7] Patients with diabetic foot ulcers are exposed to several antibiotics that is the leading cause of development of resistance to antibiotics.[8] Early diagnosis and appropriate antibiotic therapy in DFI can minimize the complications.[6,7]

Microorganisms isolated and their sensitivity pattern in DFI vary in different regions and different institutions[9,10], therefore it is essential that empirical antibiotic therapy is based on local guideline which takes into account the prevalence of different microorganisms and their sensitivity patterns to antibiotics. This will ensure that empirical antibiotic therapy provides appropriate coverage. It will also reduce the use of multiple antibiotics and resistance to antibiotics. The purpose of present study is to contribute to this field of research by identifying the bacteriological profile and antibiotic sensitivity patterns in patients with DFI.

Methods

This prospective observational study was done in collaboration with Diabetes Management Centre (DMC) of Endocrinology Department and Microbiology Pathology Department of Services Institute of Medical Sciences & Services Hospital Lahore. Prior approval was obtained from institutional review board. Patients with diabetes mellitus type 2 presenting to DMC with infected foot ulcer were included after informed consent from patients. Fifty specimens of discharge from ulcer were received in Amies transport media. A filled performa containing information regarding patient identification, ulcer grade according to Meggitt-Wagner's classification of diabetic foot ulcer based on depth of wound[12], antibiotics prescribed and HbA1c results were received from 24th June 2019 to 27th December 2019.

All specimens were inoculated on blood agar and MacConkey agar plates. Incubation was done aerobically at 35°C for 24 hours. Identification was based on colony morphology, Gram stain reaction and biochemical tests. For Gram positive organisms catalase test and DNAse were done. For Gram negative organisms oxidase test was done and for oxidase negative colonies urease, citrate utilization, motility and triple sugar iron tests were performed. If results were ambiguous API20E was set up.[13]

Antibiogram was performed on Mueller Hinton agar (Oxoid,UK) by Kirby Bauer disc diffusion method. OxoidTM antibiotic discs were used.[14]

For Gram positive organisms antibiotics applied were penicillin (P10µg), Cefoxitin (FOX30µg) Vancomycin (VA30µg), Gentamicin (CN10µg), Amikacin (AK30µg), Erythromycin (E15µg), Doxycycline DO(30µg), Ciprofloxacin (CIP5µg), Clindamycin (DA2µg), Trimethoprim-Sulphamethoxazole (SXT1. 25/23.75µg), Linezolid (LZD30µg). Cefoxitin was used as surrogate for methicillin sensitivity. Sensi-tivity of Gram negative organisms were tested against Ampicillin (AMP10 µg), Amoxacillin – clavulanate (AMC 20/10 µg), Piperacillin-tazobactam (TZP100/ 10 µg), Cefuroxime (CXM30 µg), Cefotaxime (CTX 30 µg), Ceftriaxone (CRO 30µg), Ceftazidime (CAZ 30µg), Imipenem (IPM 10 µg), Meropenem (MEM10 µg), Gentamicin (CN10 µg), Amikacin AK (30µg), Doxycycline (DO30µg), Ciprofloxacin (CIP5µg), Trimethoprim-Sulphamethoxazole SXT(1.25/ 23.75µg).[14]

Statistical Analysis was done on Microsoft excel.

Data was presented as Mean±SD for continuous variables and frequency with percentage for categorical variables.

Results:

Of the fifty DFI samples 36 were obtained from males and 14 from females. The age range of patients was between 35 - 80 years. Mean age was 53 ± 9.5years.

HbA1c results were above normal limits >7% going upto 14.2% in all except one patient with normal HbA1c 5.1%.

Most patients had grade 2 or grade3 ulcer as shown in Figure 1. Twenty four (48%) patients had grade 2 ulcer while 20 (40%) had grade 3 ulcer.

On culture monomicrobial growth was obtained in 62% while seven specimens yielded no growth Figure 2. Most common isolates were Gram negative organisms Figure 3. The different organisms isolated are shown in Figure 4.

Methicillin resistance in Staphylococcus aureus was 43% (MRSA). Resistance to ciprofloxacin was >93% in Staphylococcus aureus. All MRSA were sensitive to vancomycin and linezolid Table 1.The second most common isolate was Pseudomonas species. Twenty-seven percent Pseudomonas sp. showed resistance to third generation cephalosporins, ceftazidime, while only 1 (09%)showed resistance to imipenem. The Enterbacteriaceae and Acinetobacter species isolated and their sensitivity pattern is shown in Table 2. Resistance to ciprofloxacin was very high 73-100 % in Gram-negative organisms. Antibiotics taken by patients before arriving at DMC are shown in Table 3.

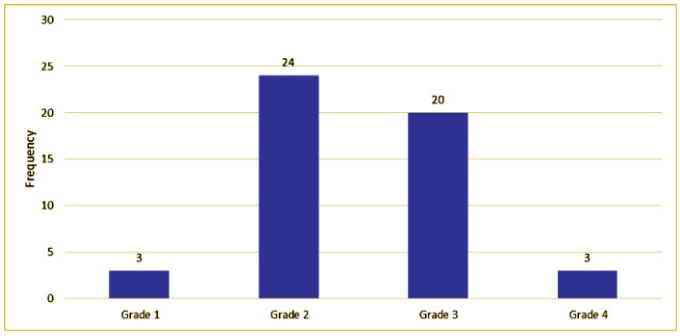


Figure-1: *Grading of Diabetic Foot Ulcer Patients According to Magitt Wegener's Classification n=50*

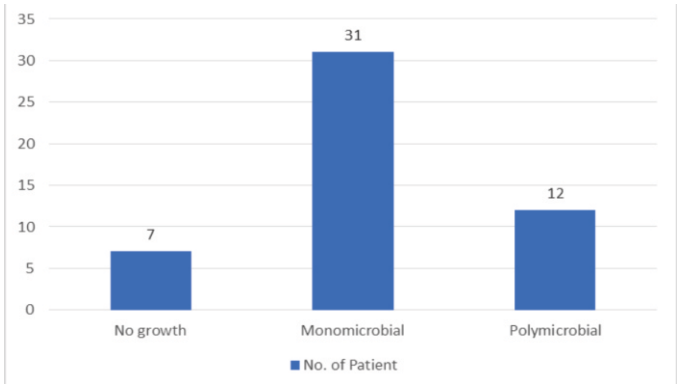


Figure-2: *Frequency of Monomicrobial and Polymicrobial Organisms in Diabetic Foot Ulcer Samples. n=50*

Table 1: *Resistance Pattern of Gram Positive Organisms*

Organism Identified	P	FOX	VA	CN	AK	E	DO	CIP	DA	SXT	LZD
Staph aureus (n=14)	14 (100%)	6 (43%)	0 (0%)	1 (7%)	2 (14%)	7 (50%)	13 (93%)	13 (93%)	7 (50%)	11 (79%)	0 (0%)
Streptococcus spp (n=1)	1 (100%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)

n =15

Table 2: *Resistance Pattern of Gram Negative Organisms*

Enterobac-teriaceae	AMP	AMC	TZP	CTX	CRO	CAZ	IPM	MEM	CN	AK	DO	CIP	SXT	CT
E.coli (n=6)	4 (67%)	6 (100%)	6 (100%)	2 (33%)	1 (17%)	-	2 (33%)	1 (17%)	2 (33%)	0 (0%)	6 (100%)	6 (100%)	6 (100%)	-
Citrobacter spp (n=1)	1 (100%)	1(100%)	0(0%)	0(0%)	0 (0%)	-	0(0%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	-
Klebsiella (n=5)	IR*	0 (0%)	3 (60%)	4 (80%)	4 (80%)	-	1 (20%)	1 (20%)	2 (40%)	2 (40%)	5 (100%)	5 (100%)	5 (100%)	-
Proteus (n=10)	IR*	IR*	1 (10%)	9 (90%)	9 (90%)	-	6 (60%)	5 (50%)	4 (40%)	1 (10%)	IR*	8 (80%)	9 (90%)	-
Non Fermentors														
Pseudomonas (n=11)	IR*	IR*	1 (9%)	IR*	IR*	3 (27%)	1 (9%)	0 (0%)	6 (55%)	5 (45%)	IR*	8 (73%)	IR*	-
Acinetobacter (n=4)	IR*	IR*	4 (100%)	4 (100%	4 (100%)	-	3 (75%)	3 (75%)	2 (50%)	1 (25%)	IR*	4 (100%)	4 (100%)	0 (0%)

n=37
*Intrinsic resistance

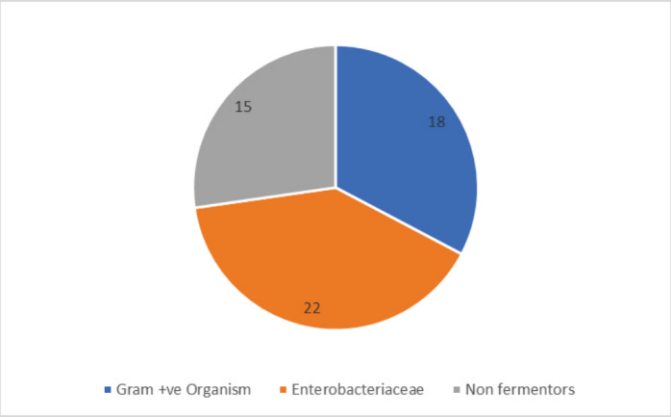
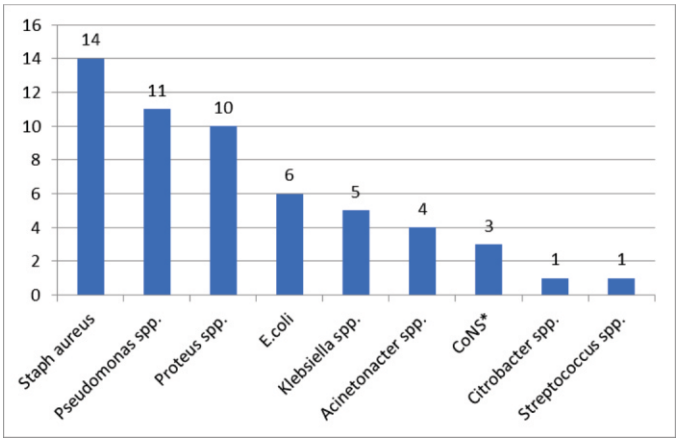


Figure-3: *Most Common Isolates in Diabetic Foot Ulcers*



**Coagulase negative Staphylococcus*

Figure-4: *Frequency of Various Organisms Isolated from Diabetic Foot Infection Patients*

Table 3: *Empiric Therapy Prescribed to Patients*

Antibiotics Received	No. of patients
Augmentin& Moxifloxacin	28
Moxifloxacin	9
Augmentin	7
Linezolid	3
Augmentin , Moxifloxacin & Amikacin	2
Augmentin & Amikacin	1
Total	**50**

Discussion:

Diabetes mellitus and its complications like DFU are increasing and have become leading cause of morbidity and mortality in Pakistan and worldwide.[1,15,16,17] Patients of DFU are predisposed to infections which if not prevented and treated early with appropriate drugs lead to gangrene & amputation. Moreover, due to prolonged use of multiple antibiotics, it often results in development of antibiotic resistance.[7] Present study was conducted to study the organisms causing infections in DFU and their sensitivity to various antibiotics, which will help in formulating appropriate empiric antibiotic therapy.

The majority of patients with diabetic foot infection (DFI) in the present study were males. This is because diabetes mellitus and diabetic foot ulcer are more common in males as compared to females.[4,18,19] Another reason may be due to males involvement in outdoor activities in hot humid environment , lack of appropriate foot care and absence of formal education.[8,20] Other studies carried out on DFU also show preponderance of male patients in Pakistan and the subcontinent.[7,9,10,21]

The age group most affected was 53±9.5. Past studies carried out in Islamabad, Karachi and Peshawar also show a similar age group affected with diabetic foot ulcer and infection.[4,7,10]

The glycemic control of patients was poor as evidenced by HbA1c results; only one patient out of the 50 affected had normal value. This was as expected: poor glycemic control leads to peripheral neuropathy and to peripheral arterial disease which leads to formation of ulcers and infections.[4,8,22] Infected diabetic foot ulcer takes longer to heal in the presence of high HbA1c level; hence, prolonged antibiotic therapy is required with appropriate antibiotics as per culture sensitivity report.[23,24]

Most of the patients had grade 2 or grade 3 ulcer (Figure1) which might be why monomicrobial growth was more common than polymicrobial growth in the present study (Figure 2). It has been observed that as the infection starts to involve deeper layers, multiple organisms are more likely to be isolated, making it polymicrobial.[3] Studies in which patients presented early to hospital were more likely to have monomicrobial growth.[24,8]

Seventy four percent isolates were Gram negative in present study Figure 3. This finding is similar to study carried out by Miyan et al. In this study on 473 samples 76.2% of isolates were Gram negative.[7]

Staphylococcus aureus was the most common Gram positive organism isolated. Of these, 43% were MRSA. All MRSA remained sensitive to vancomycin and linezolid. Resistance to quinolones, ciprofloxacin was 93% (Table 1). Other studies in Pakistan and

the subcontinent also reveal Staphylococcus aureus to be the most common Gram positive causative agent responsible for infections in DFU.[7,3] Different studies carried out in Pakistan showed resistance to ciprofloxacin between 53.68% to 73%.[6,7,10]

Pseudomonas species Figure 4, was the second most common isolate. This is similar to the study carried out in Islamabad by Chadury et al on 50 patients.[25] In present study resistance in Pseudomonas species to carbapenem was 27% and to ciprfloxacin was 73%. In another study in which the number of pseudomonas species isolates was seven, resistance to quinolones was 71.4% and to carbapenem was 28.6% very similar to the present study. In a study carried out in Karachi by Miyan et al ninty three Pseudomonas species were isolated from DFI in which 39.5% were resistant to quinolones and 6.17% resistant to carbapenem.[7,10] The marked variation in the sensitivity pattern in these studies is due to difference in the number of Pseudomonas species isolates.

Proteus species were the most common isolates among the Enterobateriaceae family Table 2. Most other studies reported E.coli to be the most common isolate in Enterobateriaceae family.[6,9,10]

The standard treatment patients were prescribed included Moxifloxacin with Augmentin or as monotherapy (Table 3). As the results of sensitivity of both Gram positive and Gram negative organisms reveals high degree of resistance to quinolones, ciprofloxacin, and augmentin. Thus there is an urgent need to develop new empiric therapy.

Conclusion

Staphylococcus aureus, Pseudomonas species and Proteus species are the most common pathogens responsible for DFI. Most of the Gram positive and Gram negative organisms isolated were resistant to multiple antibiotics. Moreover resistance to quinolones and augmentin, commonly prescribed antibiotics was very high.

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