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Study for Microbiological Pattern and *In vitro* Antibiotic Susceptibility in Patients Having Diabetic Foot Infections at Tertiary Care Hospital in Abbottabad

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Abstract: Diabetic foot infections can cause substantial morbidity and frequent visits to healthcare professionals and may lead to amputation of a lower extremity. Patients with foot infections not exposed to antibiotics, are not comprehensively studied before. The aim of our study is to evaluate the microbiological pattern and to assess *in vitro* antibiotic susceptibility in patients having diabetic foot infections at a tertiary care hospital in Abbottabad. This prospective study has been conducted at Northern Institute of Medical Sciences (NIMS) Abbottabad from 1st May 2009 to 30th April 2010. Appropriate samples for pathogens culture have been collected from 85 consecutive patients admitted in medical wards with diabetic foot infections. Antibiotic susceptibility testing of aerobes was performed by Kirby Bauer's disc diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) guidelines. All anaerobes were tested for susceptibility to metronidazole and amoxicillin/clavulanic acid by micro-broth dilution test. A vancomycin screen agar (6ug/ml) has also been used to detect vancomycin intermediate isolates of staphylococcus. Clinical grading and microbiological study of 85 patients (90 specimens) having diabetic foot infections revealed monomicrobial growth in 64 (71.11%) and polymicrobial growth in 22(24.44%), while 4(4.44%) were sterile. Among the bacterial isolates, gram negative organisms comprised of 58(68.19%) patients, while 23 (27.05%) gram positive and 4(4.70%) fungi. *Pseudomonas aeruginosa* was the most common gram negative pathogen accounting for 27.05%, while *Staphylococcus aureus* was the most frequent gram positive pathogen comprising for 17.64%. Infection with anaerobes has accounted for 2(2.35%) patients. *Pseudomonas aeruginosa* and *Staphylococcus aureus* have exhibited a high frequency of resistance to tested antibiotics and rate of antibiotic resistance was 65% among the isolates. All isolates were uniformly susceptible to imipenem, fosfomycin, amikacin, vancomycin and levofloxacin. Ill-fitting shoes and habitual bare-foot walking were main causes for diabetic foot lesions in our study. Main outcome was complete healing (with or without amputation) of the foot within the maximum follow up period. In our study, pseudomonas species (especially *Pseudomonas aeruginosa*), *Staphylococcus aureus*, *Escherichia coli*, proteus species, *Streptococci* and *Staphylococcus epidermidis* were the most common pathogens involved in diabetic foot infections. All isolates were uniformly susceptible to imipenem, fosfomycin, amikacin, vancomycin and levofloxacin. Furthermore, public perception regarding foot-care needs to be explored via media for awareness in general population.

Key words: Amputation of lower extremity · Culture · Diabetic foot infections · Microbiological pattern · Glycemic control · Gram negative organisms · *In vitro* antibiotic susceptibility

INTRODUCTION

Worldwide, the prevalence of diabetes mellitus has estimated to be 2.8% in 2000 and projected to 4.4% in the year 2030, with total number of people with diabetes expected to rise from 171 million in 2000 to 366 million in 2030 [1].

Foot ulcers are much feared complication of diabetes, with recent studies suggesting that lifetime risk of developing a foot ulcer in diabetic patients may be as high

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as 25% [2]. Diabetic foot infections are one of the major cause of morbidity and mortality, especially in developing countries like Pakistan [3, 4] due to illiteracy, poor socioeconomic status, bare-foot walking and inadequate facilities for diabetes care. Infection is most often a consequence of foot ulceration, which typically follows trauma to a neuropathic foot [5].

Foot infections in patients with diabetes mellitus are a common, complex and costly problem [6-8]. They are now the most common proximate and non-traumatic cause of leg amputation [9, 10]. It has been estimated that risk of leg amputation is 15-46 times higher in diabetics than in non-diabetics. In addition, foot complications now account for the most frequent reason for hospitalization in diabetic patients [11]. The most common organisms involved in diabetic foot infections are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Proteus* [11]. A high frequency of gram negative anaerobic infection is not uncommon [12] and mixed infections are also quite frequent [13]. Obtaining a proper wound culture specimen allows the clinician to define the pathogens involved and their antibiotic susceptibility. Unfortunately, results of culture are generally not available for at least 2-3 days. Thus, most antibiotic therapy for diabetic foot infections is selected empirically [14]. But increasing incidence of antibiotic-resistant pathogens as a cause of diabetic foot infections make selecting empiric antibiotic therapy more difficult. Furthermore, emergence of resistance among organisms against commonly used antibiotics has been clearly outlined in various studies as being largely due to their indiscriminate use [15, 16].

Diabetic foot infections require careful attention and coordinated management plan, preferably by a multidisciplinary foot-care team [17-20]. Optimal management of diabetic foot infections can potentially reduce the incidence of infection-related morbidities, the need for and duration of hospitalization and the incidence of limb amputation. Unfortunately, these infections are frequently inadequately managed due to lack of understanding of current diagnostic and therapeutic approaches, insufficient resources devoted to the problem, or a lack of effective multidisciplinary collaboration.

In our setup, diabetic foot infections have been neglected by healthcare research and planning and clinical practice is mainly based more on opinions than scientific facts and figures. Hence this study is important in Abbottabad context where there is no awareness about foot-care, diabetic patients pay little attention towards disease progression and mostly concern quacks for advice and treatment. In addition, majority are illiterate, living in an unhygienic surroundings and poor socioeconomic status especially rural population who also walk bare-footed and hence inviting foot infections. Furthermore, there is inadequate management of these patients by primary care physicians due to lack of effective multidisciplinary collaboration and insufficient health budgets.

The aim of this study is to evaluate the microbiological pattern and to assess *in vitro* antibiotic susceptibility in patients having diabetic foot infections at a tertiary care hospital in Abbottabad.

MATERIALS AND METHODS

This prospective descriptive study has been conducted at Northern Institute of Medical Sciences (NIMS) Abbottabad from 1st May 2009 to 30th April 2010. The study group comprised of 85 consecutive diabetic patients with foot ulcers-being non-healing and lasting longer than three weeks. They were hospitalized for specific duration, underwent relevant investigations and their glycemic index tightly controlled until cured. The local Ethical Committees of the institutes approved the study protocol and all patients gave written and informed consent.

A pre-designed proforma has been developed to record history, examination details and investigation reports. Recorded data include demographics, co-morbidities and data on diabetic foot ulcer characteristics. A detailed medical history was inquired about age, sex, foot trauma, ill- fitting shoes or in-growing toe-nails, type of diabetes and its duration, type of treatment received including patient's compliance to drugs and status of glycemic control. Patients were also asked about ischemic symptoms like intermittent claudication or rest pain, visual (blurring or dimness of vision), neurological (sensory loss or tingling sensation in hands or feet), cardiac (chest pain, breathlessness or pedal edema) and renal (oliguria or puffiness of face). Foot ulcers in diabetic patients were categorized into six grades (from grade 0 to V) based on Meggitt-Wagner's Criteria [21]; in which grade 0- intact skin, grade I- superficial ulcer, grade II- deep ulcer to tendon, bone or joint, grade III- deep ulcer with abscess or osteomyelitis, grade IV- localized gangrene, grade V- whole foot gangrene. In addition, we also determine exact location of an ulcer (plantar and non- planter) and its duration, clinical outcome as well as duration of hospital stay for each patient. Furthermore, disabling co-morbidities were assessed; presence of retinopathy (inability to read a newspaper after correction), nephropathy (serum creatinine =150μ mol/L or presence of micro- or macro albuminuria), heart failure (New York

Heart Association [NYHA] classification III or IV) and organic neurological disorder resulting in loss of motor or sensory function (e.g. stroke). However, all patients currently taking antibiotic therapy, those with critical limb ischemia necessitating limb amputation and severely ill patients with life expectancy of less than one year, were excluded from the study group.

All patients underwent a standardized examination according to the PEDIS system. This was developed by the International Consensus on the Diabetic Foot to enable classification of patients for clinical research purposes [19, 22] and classifies foot ulcers according to five categories; extent depth, infection, perfusion and sensation. Extent (i.e. size) was determined by multiplying the longest and widest diameters and expressed in centimeters squared. Depth was described as either deep or superficial if a full-thickness lesion of the skin was or was not extending through subcutis respectively. Infection was diagnosed if two or more of the following symptoms and signs were present; frank pus, local warmth, erythema, lymphangitis, edema, creptius, pain, pyrexia and foul smell. Perfusion assessment included evaluation of the presence of pedal pulses (i.e. posterior tibial and dorsalis pedis arteries) and measurement of the ankle- brachial index (ABI) using a handheld Doppler ultrasound device; peripheral arterial disease (PAD) was considered to be present if ABI was less than 0.9 and/ or two foot pulses were absent. Evaluation of sensation (i.e. peripheral neuropathy) included pressure sensation (absence of perception with 10g Semmes-Weinstein monofilament at two of ten standardized planter sites on either foot), tactile sensation (cotton wisp on dorsum of foot), vibration sensation (128 Hz tuning fork on dorsum of the hallux) and blunt/sharp discrimination (dorsum of foot). Peripheral neuropathy (PNP) was diagnosed if the results of two or more of the aforementioned tests were abnormal.

The laboratory evaluation include complete blood count (CBC), fasting and post-prandial plasma glucose estimations, HbA_{1c}, urine examination for proteinuria, ECG, fundoscopy, serum(fasting) cholesterol level, serum creatinine, x-ray of affected foot (AP and lateral views) and/or MRI of involved foot where required.

Sample Collection: Sample collection (pus, wound exudates or tissue biopsy) had been undertaken in medical wards, after wound vigorously washed with normal saline solution. Pus and wound exudates were collected from margins and base of the ulcer in 75 and 10 patients respectively using a sterile swab stick- being transported in a clean and sterile test tube. Tissue biopsies were taken with a sterile surgical blade/ knife in wedge-shapped section involving base and margins of the ulcer along with wound swabs from same site in 5 patients and then transported in normal saline solution and sterile test tubes respectively. Thus 90 samples were collected from 85 patients and quickly sent to microbiological laboratory for further processing.

Antibiotic Susceptibility Test: Identification, isolation, culture of the micro-organisms and antibiotic susceptibility tests have been carried out according to standard microbiological procedures. Antibiotic susceptibility testing of aerobic isolates was performed by Kirby Bauer's Disc diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) guidelines [23]. All anaerobic isolates were tested for susceptibility to metronidazole and amoxicillin/clavulanic acid by micro-broth dilution test. A vancomycin screen agar (6μg/ml) has also been used to detect vancomycin intermediate isolates of staphylococci.

Treatment of Study Group: All patients were treated according to protocols based on the International Consensus on the Diabetic Foot [19], which include treatment of infection, tight glycemic control with insulin and regular wound debridement.

We gave empirical antibiotic therapy with intravenous amoxicillin/ clavulanic acid in dose of 1.2g every 8 hourly, to treat diabetic foot infections. Metronidazole (intravenous 500mg every 8 hourly) can be added to this regimen if cellulitis and or gangrene also evident. Antibiotics were then adapted according to the results of culture and sensitivity to target the most specific organisms.

Statistical Analysis: The statistical data of all patients having diabetic foot infections was analyzed by SPSS version 10.0. The quantitative variables were expressed as means of standard deviation (±SD), while qualitative variables expressed by percentages. A p-value of less than 0.05 ($p<0.05$) was considered as significant.

RESULTS

In the 85 diabetic foot patients studied, 60(70.58%) were males and 25(29.41%) females having M to F ratio of 2.4:1. The age ranged from 35 to 70 years (mean age 54.73± 3.7). Mean duration of diabetes ranged from 3 to 25 years (mean 15.6±8.7). Majority were obese, type 2 diabetics having poor glycemic control. About 50(58.82%) were compliant to drugs and majority 55(64.70%) had taken oral hypoglycemic agents. Most of 78(91.76%) foot lesions were on planter surface and right foot mainly affected

Table 1: Baseline characteristics of the study group having diabetic foot infections (n=85)

Parameters	No. of patients	Percentage (%)
Age (years)	Mean (54.73 ±3.7)	Range (35-70)
Duration of diabetes (years)	Mean (15.6 ±8.7)	Range (3-25)
Body mass Index (BMI) kg/m2	Mean (28.35 ±4.72)	-
Postprandial plasma glucose (mg/dl)	Mean (241.15 ±65.7)	-
Fasting Plasma glucose (mg/dl)	Mean (183 ±43.5)	-
Serum Cholesterol (mg/dl)	Mean (210 ±54.3)	-
Duration of Hospital stay (days)	Mean (17 days)	Range (7-61 days)
HbA1c (%)	Mean (8.1 ±1.7)	-
Sex		
Males	60	70.58
Females	25	29.41
M to F ratio	2.4:1	
Smoking		
Yes	40	47
No	45	53
Type of Diabetes		
Type II	78	91.76
Type 1	7	8.23
Type of medication used		
Oral hypoglycemic drugs	55	64.70
Insulin	12	14.11
Mixed therapy (insulin + oral Agent)	15	17.64
No medication used	3	3.52
Compliance to medication		
Compliant to Drugs	50	58.82
Non compliant to Drugs	35	41.17
Location of Lesion		
Planter	78	91.76
Non Planter	7	8.23
Lesions Involved		
Right foot	52	61.17
Left foot	27	31.76
Both feet	6	7.05
Duration of lesions (days)		
< 10 days	5	5.88
10-29 days	37	43.52
> 30 days	43	50.58
Glycemic control (%)		
HbA1c > 7	62	72.94
HbA1c < 7	23	27.05
Comorbidities		
Peripheral arterial disease (PAD)	57	67.05
Hypertension	51	60
Diabetic neuropathy	43	50.58
Diabetic retinopathy	31	36.47
Coronary artery disease	27	31.76
Diabetic nephropathy	21	24.70
Osteomyelitis	17	20

Table 2: Frequency and severity of diabetic foot lesions according to Meggit-Wagner's Criteria and pattern of bacterial flora isolated (n=85)

Grades	No. of Patients	Percentage (%)	Bacterial flora isolated		
0	0	0	Sterile	Monomicrobial	Polymicrobial
I	2	2.35	0	2	0
II	12	14.11	2	8	2
III	23	27.0	2	17	4
IV	42	49.41	0	30	12
V	6	7.05	0	2	4
Total	85		4 (4.76%)	59 (69.41%)	22 (25.88%)

Table 3: Frequency of organisms isolated from diabetic foot infections (n=85)

Organisms isolated	Number of patients	Percentage (%)
Pseudomonas aeruginosa	23	27.05
Pseudomonas pyocyaneus	2	2.35
Proteus vulgaris	8	9.41
Proteus mirabilis	3	3.52
Escherichia coli	10	11.76
Klebsiella pneumonia	7	8.23
Staphylococcus aureus	15	17.64
Streptococci	4	4.70
Staphylococcus epidermidis	4	4.70
Citrobacter	3	3.52
Enterococci (anaerobes)	2	2.35
Candida albicans	2	2.35
Candida tropicalis	2	2.35

Table 4: Showing antibiotic susceptibility/ resistance of common gram negative bacteria in diabetic foot infections (n=85)

	Pseudomonas species n=25 (29.40%)		Proteins species n=11 (12.93%)		*Escherichia coli* n=10 (11.76%)	
Antibiotic drug	Susceptible (S) (%)	Resistant (R) (%)	Susceptible (S) (%)	Resistant (R) (%)	Susceptible (S) (%)	Resistant (R) (%)
Imipenem	25 (100%)	0(0%)	11(100%)	0(0%)	10(100%)	0(0%)
Amikacin	24 (96%)	1(4%)	11(100%)	0(0%)	10(100%)	0(0%)
Fosfomycin	25(100%)	0(0%)	11(100%)	0(0%)	10(100%)	0(0%)
Levofloxacin	24(96%)	1(4%)	11(100%)	0(0%)	10(100%)	0(0%)
Ceftriaxone	14(56%)	11(44%)	11(100%)	0(0%)	5(50%)	5(50%)
Cefotaxime	12(48%)	13(52%)	11(100%)	0(0%)	6(60%)	4(40%)
Ceftazidime	18(72%)	7(28%)	4(36.36%)	7(63.63%)	5(50%)	5(50%)
Cefoperazone	11(44%)	14(56%)	7(63.63%)	4(36.36%)	5(50%)	5(50%)
Cefazolin	11(44%)	14(56%)	7(63.63%)	4(36.36%)	5(50%)	5(50%)
Cefuroxime	4(16%)	21(84%)	4(36.36%)	7(63.63%)	3(30%)	7(70%)
Gentamycin	12(48%)	13(52%)	9(81.81%)	2(18.18%)	7(70%)	3(30%)
Co-amoxiclav	11(44%)	14(56%)	3(27.2%)	8(72.72%)	4(40%)	6(60%)
Ciprofloxacin	12(48%)	13(52%)	11(100%)	0(0%)	10(100%)	0(0%)
Ofloxacin	10(40%)	15(60%)	11(100%)	0(0%)	10(100%)	0(0%)
Ampicillin	0(0%)	25(100%)	0(0%)	11(100%)	0(0%)	10(100%)

Table 5: Showing antibiotic susceptibility/ resistance of common gram positive bacteria in diabetic foot infections (n=85)

	Staphylococcus aureus n=15(17.64%)		*Streptococci* n=4 (4.70%)		*Staphylococcus epidermidis* n=4 (4.70%)	
Antibiotic drug	Susceptible (S) (%)	Resistant (R) (%)	Susceptible (S) (%)	Resistant (R) (%)	Susceptible (S) (%)	Resistant (R) (%)
Imipenem	15(100%)	0(0%)	4(100%)	0(0%)	4(100%)	0(0%)
Amikacin	15(100%)	0(0%)	4(100%)	0(0%)	4(100%)	0(0%)
Fosfomycin	15(100%)	0(0%)	4(100%)	0(0%)	4(100%)	0(0%)
Levofloxacin	15(100%)	0(0%)	4(100%)	0(0%)	4(100%)	0(0%)
Vancomycin	15(100%)	0(0%)	4(100%)	0(0%)	4(100%)	0(0%)
Ceftriaxone	10(66.6%)	5(33.3%)	4(100%)	0(0%)	2(50%)	2(50%)
Cefotaxime	9(60%)	6(40%)	4(100%)	0(0%)	2(50%)	2(50%)
Ceftazidime	7(46.66%)	8(53.33%)	4(100%)	0(0%)	4(100%)	0(0%)
Cefoperazone	10(66.66%)	5(33.33%)	4(100%)	0(0%)	4(100%)	0(0%)
Cefazolin	5(33.33%)	10(66.66%)	4(100%)	0(0%)	2(50%)	2(50%)
Cefuroxime	11(73.33%)	4(26.66%)	4(100%)	0(0%)	0(0%)	4(100%)
Gentamycin	11(73.33%)	4(26.66%)	4(100%)	0(0%)	3(75%)	1(25%)
Co-amoxiclav	7(46.66%)	8(53.33%)	4(100%)	0(0%)	4(100%)	0(0%)
Ciprofloxacin	11(73.33%)	4(26.66%)	4(100%)	0(0%)	4(100%)	0(0%)
Ofloxacin	12(80%)	3(20%)	4(100%)	0(0%)	4(100%)	0(0%)
Ampicillin	0(0%)	15(100%)	4(100%)	0(0%)	0(0%)	4(100%)

Table 6: Factors likely responsible for development of diabetic foot lesions (n=85)

Factors	No. of patients	Percentage (%)
Ill-fitting shoes/shoe related	45	52.94
Bare-foot walking	20	23.52
In-growing toe nail/corn	12	14.11
Trivial trauma	6	7.05
Unknown cause	2	2.35

52(61.17%). Majority 43(50.58%) had duration of lesions lasting more than 30 days. The duration of hospital stay in our patients ranged from 7 to 61 days (mean 17 days). Regarding co-morbidities; 57(67.05%) had peripheral arterial disease, 51(60%) were hypertensive, 43(50.58%) had neuropathy, 31(36.47%) retinopathy, 27(31.76%) coronary artery disease and 21(24.70%) had diabetic nephropathy. Osteomyelitis has been found in 17(20%) of our patients. The details of these findings were shown in table 1.

The diabetic foot lesions were graded according to Meggitt-Wagner's classification. Majority 42 (49.41%) of our patients were in grade IV, followed by 23(27%) in grade III. This shows most of patients acquire deep-seated foot infections and 21(24.70%) of our patients have suffered from gangrenous foot. The type of bacterial flora isolated from diabetic foot lesions were predominantly monomicrobial 59(69.41%). This has been shown in Table 2.

Microbiological Observations: In the 85 studied group, 115 organisms (111 bacteria and 4 fungi) were isolated from 90 specimens, which represent an average of 1.27 organisms per patient. Out of 90 specimens, 64(71.11%) were monomicrobial, 22(24.44%) polymicrobial, while 4(4.44%) were sterile.

Among the bacterial isolates, gram negative organisms comprised of 58(68.19%) patients, while 23(27.05%) gram positive and 4(4.70%) fungi. *Pseudomonas aeruginosa* was the most common gram negative isolate accounting for 27.05%, followed by proteus species, *Escherichia coli* and *Klebsiella pneumonia* comprising 12.93%, 11.76% and 8.23% respectively. Infection with anaerobic gram negative bacteria (enterococci) has been observed in 2(2.35%) patients. *Staphylococcus aureus* was the most common gram positive bacteria comprising of 17.64%, followed by *Streptococci* and *Staphylococcus epidermidis* for 4.70% each respectively. Fungal isolates accounted for 4.70% and consist of mainly candidia albicans and tropicalis mainly. When compared, duration of diabetes, HbA_{1c}, prevalence of peripheral arterial disease and neuropathy were similar in both gram negative and gram positive groups. The profile of various isolated organisms has been shown in Table 3.

Pseudomonas species (especially *Pseudomonas aeruginosa*) has shown a high frequency of resistance to tested antibiotics. It exhibited maximum resistance to ampicillin, cefuroxime, ofloxacin, co-amoxiclav, cefazolin, cefoperazone, cefotaxime and gentamycin. All isolates were uniformly susceptible to imipenem, fosfomycin, amikacin and levofloxacin. The antibiotic susceptibility results of gram negative organisms were shown in table 4. Similarly *Staphylococcus aureus* has shown a high frequency of resistance to tested antibiotics. It exhibited maximum resistance to ampicillin, cefazolin, ceftazidime, co-amoxiclav and cefotaxime. All isolates were uniformly susceptible to imipenem, amikacin, fosfomycin, vancomycin and levofloxacin. Their antibiotic susceptibilities were shown in table 5. Comparatively gram negative organisms have displayed a high degree of resistance to tested antibiotics than gram positive pathogens. The overall frequency of resistance to tested antibiotics was 65% in this study.

We also found possible factors responsible for the development of diabetic foot lesions. Ill-fitting shoes/ shoe related and habitual bare-foot walking were the most common causes for developing and worsening of foot lesions in our study. This has been shown in table 6. Furthermore, main clinical outcome of this study was complete healing (with or without amputation) of the foot within the maximum follow-up period. So, within the eleven months follow- up, 75(88.2%) of our patients have healed foot, 9(10.58%) underwent amputation and 1(1.17%) died.

DISCUSSION

Our study has demonstrated a comprehensive clinical and microbiological survey of infected diabetic foot lesions in hospitalized patients. Foot ulcers are a significant complication of diabetes mellitus and often precede lower extremity amputation. The most frequent underlying causes are neuropathy, trauma, deformity,

high plantar pressure and peripheral arterial disease [24]. Although infection is rarely implicated in the etiology of diabetic foot ulcers, yet these are susceptible to it once an ulcer formed.

Male sex preponderance was obvious from our study showing M to F ratio 2.4:1. In addition mean age of presentation in our study was 54 years which is quite similar to results of a local study [25]. Most of our patients were type 2 diabetics having poor glycemic control which is quite similar to results of another local study [26]. In our study, peripheral arterial disease (PAD) has been shown to be main contributory factor in delayed or non-healing of diabetic foot lesions, which is quite closer to results of Rizvi F [25] who found in 64% of their patients. According to Meggitt-Wagner's criteria, majority (50%) of our patients have grade IV diabetic foot lesions, which is quite closer to studies [9, 26]. This may be related to duration of diabetes, late presentation to healthcare professionals and presence of co-morbidities.

In the present study, a total of 115 organisms were isolated from 85 patients which represent an average of 1.27 organisms per patient. This is quite similar to study by Vishwanathan V [15], where cultures yielded an average of 1.21 organisms per case. Majority (71%) of our patients with diabetic foot infections have monomicrobial growth pattern on culture, which is quite similar to studies by Rizvi F and Khoharo KH [25, 26]. According to previous studies [11, 15, 27], *Staphylococcus aureus* was the main pathogen involved in diabetic foot infections, but recent studies by Goldstein EJC [12] and Khoharo KH [26] have reported a predominance of gram negative aerobes. Gram negative aerobic bacteria have also been predominantly found in our study, which is in concordance with the studies by Rizvi F [25], Khoharo KH [26] and Shankar EM [28]. In our study, *Pseudomonas aeruginosa* (27.05%)-gram negative bacteria and *Staphylococcus aureus* (17.64%)-gram positive bacteria, were commonest pathogens isolated from diabetic foot infections; while prevalence of other organisms like proteus species, *E. coli* and *Streptococci* were (13%), (12%) and (4.70%) respectively. Almost similar results have been shown for *Pseudomonas aeruginosa* by Rizvi F [25] and Bansal E [29] and for *Staphylococcus aureus* by Khoharo KH [26] and Bansal E [29]. The prevalence pattern of Proteus species, *E. coli* and *Streptococci* were also closer to studies by Bansal E [29] and Khoharo KH [26] respectively. As regard to antibiotic susceptibility pattern of gram negative bacteria, all are uniformly susceptible to imipenem, fosfomycin, amikacin and levofloxacin, which is quite similar to studies by Khoharo KH [26] and Raja NS [30]. However, we observed large number of multi-drug resistant *Pseudomonas aeruginosa* in our study which is quite similar to studies by Khoharo KH [26] and Gadepalli R [31]. Furthermore, gram positive bacteria are uniformly susceptible to imipenem, vancomycin, amikacin, fosfomycin and levofloxacin. Similar results were found by Khoharo KH [26] and Raja NS [30] in their studies. Our study proved that infection with multi-drug resistant organisms is quite common in hospitalized patients with diabetic foot lesions. Based on susceptibility testing, isolated bacteria showed 65% resistance to tested antibiotics which is quite similar to studies by Alavi MS [11] and Khoharo KH [26]. This may be due to indiscriminate usage of broad spectrum antibiotics at tertiary care settings by junior doctors which must be discouraged. In addition, inadequately controlled blood glucose (i.e. hyperglycemia) has been associated with higher mortality rates in patients with diabetic foot infections [32]. In this region (i.e. Abbottabad) ill-fitting shoes and shoe-related conditions were more common than habitual bare-foot walking. This is probably attributed to hilly terrain, poverty and improper pressure transmission on sole with conventional foot-wear.

Limitations: During this study, we lacked the facility of modern diagnostic tests such as polymerase chain reaction (PCR), which can detect most species of pathogens rapidly within hours and can be helpful for clinicians to optimize antibiotic therapy for diabetic foot infections. Although it is expensive, yet able to detect much smaller concentrations of microorganisms than standard cultures. Furthermore, it has shown promise results in various clinical situations, including identification of antibiotic-resistant gram negative organisms and would be much less likely to cause false-negative results.

Recommendations: Further research be undertaken to elucidate and validate systems for classifying diabetic foot infections, diagnosing osteomyelitis and defining optimal antibiotic regimens in various situations. Public perception regarding foot-care need to be explored via media for awareness in general population and organization of regular interactive workshops (Continued Medical Education Program) at primary healthcare centers among healthcare professionals. In addition, foot infections in diabetics require careful attention and coordinated management, preferably by a multidisciplinary foot-care team. The aims of these

objectives are to reduce infection-related morbidities (i.e. foot amputation), duration of hospitalization and hence healthcare costs in our setup.

CONCLUSION

On conclusion, males, obese, type 2 diabetics having poor glycemic control were commonly affected by diabetic foot infections which frequently involve right foot and deep-seated in nature. They predominantly have monomicrobial growth pattern on culture. In our study pseudomonas species (*Pseudomonas aeruginosa*), proteus species and *E. coli* were gram negative, while *Staphylococcus aureus*,*Streptococci* and *Staphylococcus epidermidis* were gram positive bacteria isolated from diabetic foot lesions. All these isolates were uniformly susceptible to imipenem, amikacin, fosfomycin, levofloxacin and vancomycin. In addition, public perception regarding foot-care needs to be explored via media for awareness in general population.

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REFERENCES

1. Wild, H., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-53.
2. Singh, N., D.G. Armstrong and B.A. Lipsky, 2005. Preventing foot ulcers in patients with diabetes. JAMA, 293: 217-228.
3. Faiz-ur-Rehman and S. Nadir, 2004. Diabetic foot. J. Postgard Med. Inst., 463-469.
4. Ali, S.M. and Basit A, Fawad, 2008. A. Presentation and outcome of diabetic foot at tertiary care unit. Pak J. Med. Sci., 24: 651-656.
5. Rao, N. and B.A. Lipsky, 2007. Optimising antimicrobial therapy in diabetic foot infections. Drugs, 67: 195-214.
6. Lipsky, B.A., 2004. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes Metab. Res. Rev., 20(Suppl 1): S68-77.
7. Jeffcoate, W.J. and K.G. Harding, 2003. Diabetic foot ulcers. Lancet, 361: 1545-51.
8. Tennvall, G.R., J. Apelqvist and M. Eneroth, 2000. Costs of deep foot infections in patients with diabetes mellitus. Pharmacoeconomics, 18: 225-38.
9. Sharma, V.K.I., P.B. Khadka, A. Joshi and R. Sharma, 2006. Common pathogens isolated in diabetic foot infection in Bir hospital. Kathmandu Univ. Med. J. 4(3)15: 295-301.
10. Rooh, U.M., M. Ahmed and S. Griffin, 2003. Evaluation and management according to wagner's classification. A study of 100 cases. J. Ayub. Med. Coll. Abbottabad, 15: 39-42.
11. Alavi, M.S., D.A. Khosravi, A. Sarami, A. Dashtebozorg and A.E. Montazeri, 2007. Bacteriologic Study of Diabetic Foot Ulcer. Pak J. Med. Sci. Oct-Dec (Part-1) 23(5): 681-84.
12. Goldstein, E.J.C., C.V. Merrian, B.J. Lipsky, D.M. Citron and M.A. Abramson, 2007. Bacteriology of moderate to severe diabetic foot infection and *in vitro* activity of antimicrobial agent. J. Clin. Microbio. 45(9): 2819-2828.
13. Anandi, C., D. Alaguraja, V. Natarajan, M. Ramanathan, C.S. Subramaniam and M. Thulasiram Bacteriology of diabetic foot lesions. Indian J. Med. Res., 22: 175-178.
14. Lipsky, B.A., 2007. Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? Clin Microbiol Infect 2007; 13:351-353.
15. Vishwanathan, V., J.J. Jasmine, C. Snehalatha and A. Ramachandran, 2002. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. J. Assoc. Physicians India, 50: 1013-6.
16. Chincholikar, D.A. and R.B. Pal, 2002. Study of fungal and bacteriological infections of the diabetic foot. Indian J. Pathol. Microbiol., 45: 15-22.
17. Crane, M. and B. Werber, 1999. Critical pathway approach to diabetic pedal infections in a multidisciplinary setting. J. Foot Ankle. Surg., 38: 30-3;discussion 82-3.
18. Dargis, V., O. Pantelejeva, A. Jonushaite, L. Vileikyte and A.J. Boulton, 1999. Benefits of a multidisciplinary approach in the management of recurrent diabetic foot ulceration in Lithuania: a prospective study. Diabetes Care. 22: 1428-31.
19. Schaper, N.C., J. Apelqvist and K. Bakker, 2003. The international consensus and practical guidelines on the management and prevention of the diabetic foot. Curr. Diab. Rep., 3: 475-479.
20. Ragnarson Tennvall, G. and J. Apelqvist, 2001. Prevention of diabetes-related foot ulcers and amputations: a cost-utility analysis based on Markov model simulations. Diabetologia, 44: 2077-87.

21. Wagner, F.W., 1981. The dysvascular foot: A system for diagnosis and treatment. Foot Ankle, 2: 64-72.
22. Schaper, N.C., 2004. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. Diabetes Metab. Res. Rev., 20(Suppl 1): S90-S95.
23. Performance Standards For Antimicrobial Susceptibility Testing. 12th Informational Supplement. NCCLS document M100, Pennsylvania, USA, 1: S12-22.
24. Fryberg, R.G., D.G. Armstrong, J. Giurini, A. Edwards, M. Kravette and S. Kravitz, 2000. Diabetic Foot Disorders: A clinical practice guidline. American College of Foot and Ankle Surgeons. J. Foot Surg. 39(Suppl 5): 51-60.
25. Rizvi, F., H.Z. Khawaja, N. Mehmood, S. Zeeshan, M. Afzal and A. Baig, 2009. Frequency and extent of foot lesion and the susceptibility pattern of infective organisms in diabetic foot. Ann. Punj. Med. Coll. Faisalabad Janu-Jun 3(1): 36-40.
26. Khoharo, K.H., S. Ansari and F. Qureshi, 2009. Diabetic Foot Ulcers: Common isolated pathogens and *in vitro* antimicrobial activity. Profess Med. J. Mar., 16(1): 53-60.
27. Tentolouris, N., E.B. Jude and I. Smirnof, 1999. Knowles EA, Boulton AJ. Methicillin- Resistant Staphylococcus Auerus: an increasing problem in a diabetic foot clinic. Diabet Med., 16: 767-771 [Medline].
28. Shankar, E.M., V. Mohan, G. Premalatha, R.S. Srinavasan and A.R. Usha, 2005. Bacterial etiology of diabetic foot infections in South India. Eur. J. Intern Med., 16: 567-570.
29. Bansal, E., A. Garg, S. Bhatia, A.K. Attri and J. Chander, 2008. Spectrum of microbial flora in diabetic foot ulcers. Indian J. Pathol. Microbiol., 51: 204-208.
30. Raja, N.S., 2007. Microbiology of Diabetic Foot Infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. J. Microbiol. Immunol. Infec, 40: 39-44.
31. Gadepalli, R., B. Dhawan, V. Sreenivas, A. Kapil, A.C. Ammini and R.A. Chaudhry, 2006. Clinico-microbiological Study of Diabetic Foot Ulcers in an Indian Tertiary Care Hospital. Diabetes Care, 29: 1727-32.
32. Ikem, R.T., B.A. Kolawole and I.C. Ikem, 2002. The Prevalence, presentation and outcome of diabetic foot lesions in a Nigerian teaching hospital. Trop. Doct., 32: 226-227[Medline].