ORIGINAL ARTICLE

FREQUENCY OF COMMON BACTERIA AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN DIABETICS PRESENTING WITH FOOT ULCER

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Background: Foot ulcers are one of the most important complications of diabetes mellitus and often lead to lower limb amputation. Diabetic foot ulcers are susceptible to infection. The objective of this study was to determine the frequency of common bacteria infecting these ulcers and their antibiotic sensitivity pattern. **Methods:** This descriptive cross-sectional study was performed in the Departments of Medicine and Surgery, Khyber Teaching Hospital, Peshawar from April, 2011 to February, 2012. Specimens collected from ulcers of 131 patients were inoculated on Blood Agar and MacConkey Agar, and antibiotic sensitivity was tested using standard disc diffusion method. **Results:** Out of 131, specimens from 120 patients yielded 176 bacteria. Sixty-six patients had monomicrobial infection while polymicrobial growth was obtained in 54 patients. Overall, *Staphylococcus aureus* (38.6%) was the most common isolate followed by *Pseudomonas aeruginosa* (27.3%). *Staphylococcus aureus* was most often sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezolid while it showed varying sensitivity to Penicillins and Cephalosporins. 47.1% isolates of *Staphylococcus aureus* were resistant to Methicillin. Most of the gram negative rods were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate. Majority of gram negative bacteria were found resistant to Cephalosporins and Moxifloxacin except *Pseudomonas* which showed variable sensitivity to Ceftriaxone, Ceftazidime and Moxifloxacin. **Conclusions:** Majority of isolates were found resistant to the commonly used antibiotics. Most commonly isolated bacterium, *Staphylococcus aureus* was most often sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezolid, while majority isolated gram negative rods were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate.

Keywords: Diabetes mellitus; foot ulcers; infection; antibiotic sensitivity

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INTRODUCTION

Diabetes Mellitus (DM) is one of the leading causes of morbidity and mortality around the world and is responsible for 3.8 million deaths per year.[1] It has shown an exponential rise worldwide in the last two decades from 30 million cases in 1985 to 177 million in 2000.[2] The estimated number of diabetics worldwide were 285 million in 2010 which is estimated to rise to 439 million by 2030.[3] The International Diabetes Federation ranks Pakistan 7th in the list of prevalence of DM.[1]

Diabetes mellitus is associated with 10–30% decrease in life expectancy.[4] The morbidity and mortality associated with DM are mainly due to its complications.[2] Foot ulceration and infection is one of the major complications of DM.[2] Diabetic foot ulcers remain a common cause of admission to hospital and death among diabetic patients.[2] About 15% diabetic patients develop foot ulcers in their lifetime.[2] It is the leading cause of non-traumatic lower limb amputations in United States.[5] Poor glycaemic control, peripheral neuropathy and peripheral vascular disease all predispose to the development of foot ulcers. Such ulcers are often complicated by infection and the presence of infection increases the risk of amputation.[6] Diabetic foot infections are usually polymicrobial and pathogens are multidrug resistant. Different studies have shown that samples from these ulcers have grown a variety of bacteria. In a study conducted in Malaysia, Staphylococcus aureus (*S. aureus*) was isolated in 44% cases, *Proteus* in 28%, *Pseudomonas aeruginosa* (*P. aeruginosa*) in 25% and *Klebsiella* in 15% cases.[7] Another study conducted in Iran has shown *Escherichia coli* (*E. coli*) in 23.8% cases and *Staphylococcus epidermidis* (*S. epidermidis*) in 14.3% cases.[8] Studies conducted in Iran and Mirpurkhas have shown a high rate of antibiotic resistance among the isolated pathogens, 65% and 66% respectively.[8,9] Infection with drug resistant organisms is associated increased requirement of surgical intervention, poorer outcomes and higher healthcare costs.[10,11] Appropriate antibiotic therapy is an important but challenging part of the management. Studies have shown that empirical

antibiotic therapy for soft tissue infections presenting as ulcers is effective only in 50% cases.[12]

MATERIAL AND METHODS

This cross sectional study was conducted in the Departments of Medicine and Surgery at Khyber Teaching Hospital, Peshawar from April, 2011 to February, 2012. Both male and female patients above 35 years of age with duration of DM more than one year from diagnosis and duration of foot ulcer more than one week were included in this study while patients who had received local or systemic antibiotic therapy in the last 72 hours before presentation, patients with wounds resulting from direct trauma and patients with history of instrumentation in their ulcers were excluded. Patients with the above features were not included because these factors could have affected the growth of bacteria on culture media and could have introduced bias in the study results by acting as confounders. Informed written consent was taken from those who agreed to participate in the study. Demographic characteristic were recorded.

A swab was applied lightly to the ulcer bed and/or the exudate and pus from the ulcer base under aseptic conditions. The swab was transferred to its container after it was saturated with the culture specimen and was sent to pathology laboratory on the same day. The ulcer was dressed. The culture material was inoculated on Blood Agar and MacConkey Agar simultaneously. The culture was placed under aerobic conditions at 37 degree centigrade and monitored for the growth of colonies of *S. aureus*, *S. epidermidis*, *E. coli*, Proteus, *P. aeruginosa* and *Klebsiella* which are the common bacteria infecting foot ulcers. The isolated bacteria were recognized based on their characteristic growth pattern on culture media and biochemical reactions.

The colonies were then transferred to Nutrient Agar for sensitivity testing by Kirby-Baeur Disk Diffusion method. Ampicillin-Cloxacillin, Amoxicillin-Clavulanic Acid, Cephradine, Cefuroxime, Ceftriaxone, Ceftazidime, Cefixime, Cefipime, Moxifloxacin, Imipenem/Meropenem (against both gram positive and gram negative bacteria); Flucloxacillin, Methicillin, Vancomycin, Fusidic Acid, Linezolid (against staphylococcus aureus and staphylococcus epidermidis only); and Piperacillin/Tazobactam, Ticarcillin/Clavunalic Acid & Amikacin (against gram negative bacteria only) were tested. All patients admitted during the study period were included and results were recorded on a structured *pro forma.* The data collected was entered into SPSS for statistical analysis. Means and standard deviation for age of patient, duration of DM and duration of ulcer, and the frequencies and percentages for gender, the grade of ulcers and the common bacteria isolated were calculated. The frequencies and percentages of sensitivity of the isolated bacteria to antibiotics under study were calculated. Results were presented as tables.

RESULTS

A total of 131 patients with diabetic foot ulcer were included in the study. Among them, 48 (36.6%) were male and 83 (63.4%) were female. Male to female ratio was 1:1.73. Their age ranged between 36 and 90 years while the mean age was 56.46±9.85 years. The mean duration of DM was 11.02±4 years. The mean duration of ulcer was 3.38±1.4 weeks and 71% patients had the ulcer for more than 2 weeks before presentation at the hospital. Fifty-three (40.5%) patients had superficial ulcers (Wagner's grade 1 and 2 ulcers) while 78 (59.5%) patients had deep ulcers (Wagner's grade 3, 4 and 5 ulcers) (Table-1).

Out of 131, specimens from 120 patients were culture positive for the bacteria included in the study. Specimen from 11 patients either did not grow any bacteria or yielded bacteria other than those included in the study. A total of 176 bacteria included in the study were isolated from specimens from 120 patients with positive culture. 66 patients had a single bacterium infecting their ulcers (monomicrobial). Fifty-two patients had two bacteria isolated from their wounds while 2 patients yielded three bacteria from their ulcers (polymicrobial) (Table-1).

Cross tabulation for number of bacteria infecting each ulcer (monomicrobial versus polymicrobbial) and grade of ulcer (superficial versus deep) is shown in table-2.

Ninety-eight Gram negative bacteria and 78 Gram positive bacteria were isolated. Overall *S. aureus* (n=68) (38.6%) was the most common bacterium grown from diabetic foot ulcers, followed by *P. aeruginosa* (n=48) (27.3%). Thirty patients (22.9%) had only gram positive organisms infecting their ulcers while specimen from 36 patients (27.5%) grew only gram negative organisms (Table-3)

S. aureus was most often sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezolid. *S. aureus* showed varying sensitivity to Penicillins and Cephalosporins. 47.1% isolates of *S. aureus* were found resistant to Methicillin and hence considered as Methicillin Resistant Staphylococcus aureus (MRSA). Similarly, majority of the isolates of *S. epidermidis* were found resistant to Penicillins and Cephalosporins except Cefepime and Cefuroxime. However, most of *S. epidermidis* isolates were

sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezoild. (Table-4)

None of the gram negative bacteria were sensitive to Ampicillin-Cloxacillin and Cephradine. Majority of gram negative bacteria were found resistant to Cephalosporins and Moxifloxacin except *P. aeruginosa* which showed variable sensitivity to Ceftriaxone, Ceftazidime and Moxifloxacin. Most of the isolates of gram negative rods included in the study were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate. *P. aeruginosa* was sensitive to Amikacin in most of the cases while *E. colii*, *Proteus* and *Klebsiella* showed variable sensitivity to Amikacin. (Table-5)

Table-1: clinical features of the patients

Parameter	Number of patients (n=131)
Age	56.46±9.846 (min36 max90)
36–45 years	21
4655 years	33
56–65 years	61
More than 65 years	16
Gender	
Male	48 (36.6%)
Female	83 (63.4 %)
Duration of Diabetes in years	11.02±4.004 (min3 max21)
Less than or equal to 5 years	8
6–10 years	59
11–15 years	45
16–20 years	17
More than 20 years	2
Duration of Ulcer in weeks	3.38±1.400 (min1 max8)
Less than or equal to 2 weeks	38
More than 2 weeks and less than or equal to 4 weeks	71
More than 4 weeks	22
Grade of Ulcer	
Superficial	53
Deep	78
Number of bacteria per case	
No growth	11
Monomicrobial	66
Polymicrobial	54

Table-2: Grade of ulcer and no. of bacteria per case

No. of bacteria per case (n=120)	**Grade of ulcer**		**Total**
	Superficial	Deep	
Monomicrobial	32	34	66
Polymicrobial	13	41	54
Total	45	75	120

p= 0.006

Table-3: distribution of bacteria isolated from diabetic foot ulcers (n=176)

Bacteria (n=176)		**Frequency**	**%**
Gram Positive Bacteria (n=78)	*Staphylococcus aureus*	68	38.6
	Staphylococcus epidermidis	10	5.7
Gram Negative Bacteria (n=98)	*Pseudomonas aeruginosa*	48	27.3
	Escherichia coli	37	21
	Proteus	8	4.6
	Klebsiella	5	2.8

Table-4: antibiotic sensitivity pattern of gram positive bacteria

	Staph. aureus (n=68)	**Staph. epidermidis (n=10)**
Ampicillin-Cloxacillin	26 (38.2%)	6 (60%)
Amoxicillin-Clavulanate	40 (58.8%)	5 (50%)
Cephradine	31 (45.6%)	4 (40%)
Cefuroxime	41 (60.3%)	7 (70%)
Ceftriaxone	37 (54.4%)	5 (50%)
Ceftazidime	34 (50%)	6 (60%)
Cefixime	23 (33.8%)	5 (50%)
Cefipime	44 (64.7%)	8 (80%)
Moxifloxacin	47 (69.1%)	7 (70%)
Imepenem/ Meropenem	55 (80.9%)	8 (80%)
Flucloxacillin	33 (48.5%)	4 (40%)
Methicillin	35 (51.5%)	7 (70%)
Vancomycin	50 (73.5%)	8 (80%)
Fusidic acid	39 (57.4%)	6 (60%)
Linezolid	47 (69.1%)	8 (80%)

Table-5: antibiotic sensitivity pattern of gram negative bacteria

	Pseudomonas aeruginosa (n=48)	***E. coli* (n=37)**	**Proteus (n=8)**	**Klebsiella (n=5)**
Ampicillin-Cloxacillin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Amoxicillin-Clavulanate	3 (6.25%)	11 (29.7%)	2 (25%)	1 (20%)
Cephradine	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cefuroxime	8 (16.7%)	6 (16.2%)	1 (12.5%)	1 (20%)
Ceftriaxone	27 (56.3%)	6 (16.2%)	1 (12.5%)	1 (20%)
Ceftazidime	24 (50%)	12 (32.4%)	2 (25%)	0 (0%)
Cefixime	2 (4.2%)	2 (5.4%)	0 (0%)	1 (20%)
Cefipime	18 (37.5%)	8 (21.6%)	3 (37.5%)	2 (40%)
Moxifloxacin	27 (56.3%)	17 (46%)	4 (50%)	1 (20%)
Imepenem/ Meropenem	40 (88.3%)	31 (83.8%)	6 (75%)	4 (80%)
Piperacillin-Tazobactam	35 (72.9%)	31 (83.8%)	6 (75%)	4 (80%)
Ticarcillin-Clavulanate	41 (85.4%)	29 (78.4%)	5 (62.5%)	4 (80%)
Amikacin	36 (75%)	23 (62.2%)	3 (37.5%)	2 (40%)

DISCUSSION

Foot ulcers are one of the most important complications of DM and often lead to lower limb amputation. The most important contributory factors include diabetic neuropathy, peripheral artery disease, foot deformities, high plantar pressures and unrecognized trauma.[13] Although infection is rarely implicated in the aetiology of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present. Infected diabetic foot ulcers do not get proper antibiotic treatment due to poor understanding of the common organisms infecting these ulcers and of their sensitivity patterns.[14]

This study presents a detailed microbiological overview of infected diabetic foot ulcers in hospitalized patients. We took samples from the base

of the ulcers under aseptic conditions in all the cases. Bone biopsy may be a better specimen in cases of osteomyelitis.[15] But it requires surgical expertise, can be traumatic to the patient and is not routinely performed. Moreover, samples taken from the base of the ulcer are adequate for identifying the infecting organisms.[16]

Specimens from 120 out of 131 patients (91.6%) were found positive for bacterial growth. One hundred and seventy-six bacteria were isolated from these ulcers at a rate of 1.47 isolates per patient which is similar to that reported by Raja NS *et al* in 2005.[7] However, Carvalho CB *et al* and others have reported higher rates of isolation of bacteria from diabetic foot ulcers.[17–19] This difference may be due to the fact that most of the ulcers sampled in these studies were long-standing with the mean duration of ulcer in one study as long as 8.5 months.[19] Moreover, this study did not include isolating anaerobic bacteria which might also have contributed to the lower rate of isolation of bacteria.

A total of 45% of culture positive diabetic foot ulcers had polymicrobial infection which is consistent with the findings of studies conducted by Alavi SM *et al* and others who have reported polymicrobial growth in such ulcers in the range of 42–52.4%.[7,8,20] However, Anandi C *et al* and others have reported a much higher rate of polymicrobial infection.[21,22] This difference can be explained by the reasons already mentioned above for the lower isolate per case in this study, i.e., the duration of ulcer and the non-inclusion of anaerobic bacteria in the study. The association between polymicrobial infection and deep ulcers was statistically significant (p=0.006).

Gram negative aerobes were the most common (55.7%) isolates among all the bacteria cultured. This finding is consistent with studies conducted by Umadevi S *et al* and others.[7–9,17,18,20] Studies carried out by Mantey I *et al* and others have documented gram positive bacteria as the predominant organisms associated with diabetic foot infections.[23,24] Therefore, there seems to be a changing trend in the organisms causing diabetic foot infections with gram-negative bacteria replacing gram-positive bacteria as the commonest agents. Overall, *S. aureus* was the most common bacterium isolated (38.6%). Alavi SM *et al* and others have shown *S. aureus* to be the most common organism in diabetic foot ulcers in studies conducted both regionally and internationally.[8,22,23]

Among the gram negative bacteria, *P. aeruginosa* was the most frequent bacterium isolated (27.3%), a finding which has been consistently observed by Anandi et al and others.[21,22,25–29] However, Umadevi S *et al* and others have found gram negative bacteria like *E. coli* and Proteus more frequently than *P. aeruginosa*.[7,19,20]

Majority of *S. aureus* isolates were found sensitive to Vancomycin (73.5%), Moxifloxacin (69%) and Linezolid (69%). Vancomycin has been reported by Khoharo HK *et al* and others as the most effective antibiotic against *S. aureus* cultured from diabetic foot ulcers in many local and international studies.[9,18,20,26–28] Similarly Carvalho CB *et al* and others have also found Carbapenems and Linezoild to be the most effective antibiotics against *S. aureus*.[17,18]

S. aureus showed variable sensitivity to Penicillins and Cephalosporins, and was found resistant to most members of Penicillin and Cephalosporin group of antibiotics used in the study. This finding is similar to the observations of Yoga R *et al* where all *S. aureus* isolates were resistant to the Penicillin group.[30]

A total of 47% *S. aureus* isolates were found resistant to Methicillin, and these were considered MRSA. The MRSA rate in previous studies by El-Tahawy AT *et al* and others has shown great variability and has been reported between 30–65.5%.[18,20,28] This wide range of MRSA rates in these ulcers may be due to several reasons including the differences in the use of empirical antibiotics for these ulcers before presenting to the centre of study, the duration and grades of ulcers and the degree of contamination of wounds by the hands of hospital personnel.[31]

Most of the gram negative bacteria were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate. Imipenem has been shown to be the most effective antibiotic against gram negative bacteria by Raja NS *et al* and others.[7,26,28] Similarly the sensitivity pattern of *P. aeruginosa*, the most common gram negative bacteria isolated, to Imipenem/Meropenem, Piperacillin-Tazobactam, Ticarcillin-Clavulanate and Amikacin in this study is consistent with the findings of Carvalho CB *et al* and others. [17,30]

Majority of gram negative bacteria were found resistant to the commonly used penicillins and cephalosporins. Similar sensitivity pattern was observed by Khoharo HK *et al* and others.[9,20] This also includes *E. coli* which was isolated with a higher frequency (21%), a finding consistent with a study conducted by Alavi SM *et al* in Iran.[8] *E. coli* has never been isolated this frequently in other studies on the subject. The high frequency of resistant *E. coli* among the gram negative isolates cannot be explained. But the alarming level of antibiotic resistance seen among common organisms like *S. aureus*, *P. aeruginosa* and *E. coli* to the commonly used penicillin and cephalosporin

antibiotics can be the result of the inappropriate and casual use of these drugs without clear indication for their use. This has possibly led to the emergence of multidrug resistant strains of these common pathogens. Moreover, repeated admissions and treatment for the same ulcer could be responsible for the higher frequency of resistant organisms seen in this study, both due to inappropriate use of antibiotics and contamination of wounds by healthcare personnel before the patients are referred to specialist centres and tertiary care hospitals.

Although the study provides a general overview of microbiology of diabetic foot ulcers, there were certain limitations. We could not study the anaerobic bacteria infecting these ulcers due to the lack of proper facilities for the transport and growth of specimen for anaerobes. The production of Beta-lactamase by the isolates was not studied for the same reason. Moreover, no record of previous admissions and treatment for the same ulcer was sought which otherwise could have helped us explain the higher frequency of multidrug resistant organisms in these ulcers.

CONCLUSIONS

This study has demonstrated that a large proportion of diabetic foot ulcers are infected with a variety of gram positive and gram negative bacteria. About half of these ulcers are infected with more than one bacterium at a time. With few exceptions, majority of these bacteria are resistant to the antibiotics most commonly used in the management of these ulcers. Moreover, no single antibiotic used empirically offers adequate coverage for all the potential bacteria found in these ulcers.

Therefore, all those managing diabetic foot ulcers should have adequate evidence-based knowledge regarding the microbiology of these ulcers. Proper specimen should be collected for culture and sensitivity before initiating empirical antibiotic therapy. Treatment should be started using antibiotics with adequate coverage for the common bacteria infecting these ulcers, usually a combination of two antibiotics e.g., Vancomycin plus Imipenem/Meropenem or a Cephalosporin. The regimen should be modified according to the result of culture and sensitivity if there is no in-vivo response to the antibiotics being used.

AUTHOR'S CONTRIBUTION

FR: Concept and synopsis, data analysis/results, discussion. FU: Data Collection, Literature review. MI: Data Analysis/results. AKA: Data collection. SUR: Literature review, discussion. HR: Data collection

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