World Journal of Medical Sciences 10 (4): 494-502, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.10.4.94135

Bacteriology of Diabetic Foot Ulcer among an Egyptian Population: A Retrospective Study

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Abstract: Diabetic foot lesions are a major medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes worldwide. Bacterial study from 28 cases of diabetic foot ulcers attending Abou Seifein Diabetes Center in Cairo, Egypt was carried out to determine the etiological agents and their antibiogram. Out of these patients 31 isolates were recovered, from these isolates, 21 (67.7%) were pure single bacterial and 10 (32.3%) were mixed bacterial infections. *S. aureus* (41.9%) was the predominant isolate followed by *Coagulase-negative staphylococci* (CONS), *Escherichia coli* and *Klebsiella* (9.7%) for each. *S. aureus* was sensitive to Cephalexin, Dicloxacillin, Amoxicillin- Clavulanate and Trimethoprim-Sulfamethoxazole.

Key words: Diabetic foot · *S. aureus* · *Coagulase-negative staphylococci (CONS)* · *E. coli* · *Klebsiella*

INTRODUCTION

A diabetic foot is one of the most feared complications of diabetes and it is the leading cause of the hospitalization among diabetic patients As a globally widespread disease with an increasing incidence, diabetes mellitus has afflicted 150,000,000 people across the world according to the World Health Organization (WHO); and this will be doubled by 2025 [1]. In the Middle East and North Africa Region, 1 in 10 adults have diabetes; the Region has the highest prevalence of diabetes, at 10.9%. In Egypt, 42% of people with diabetes experience early-stage eye disease and 5% of people diabetes are classified as legally blind. International Diabetes Federation (IDF) estimates that there are 34.6 million people with diabetes in the Middle-East and North Africa, a number that will almost double to 67.9million by 2035 if concerted action is not taken to tackle the risk factors fuelling the epidemic of diabetes throughout the Region [2].

Infections and ulcers accompanied by neuropathy and arteriovenous abnormalities in the foot of patients with diabetes, referred as diabetic foot, are among the most common complications which lead to the development of gangrene and which even necessitates limb amputation. Diabetic foot infection, also considered as the most important cause of hospitalization in patients with diabetes accounted for 20% of inpatient admissions [3].

The impaired micro-vascular circulation in patients with a diabetic foot limits the access of phagocytes, thus favoring the development of an infection. The local injuries and the improper foot wear further compromise the blood supply in the lower extremities [4]. While the foot infections in persons with diabetes are initially treated empirically, a therapy which is directed at the known causative organisms may improve the outcome [5]. 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 occurred over time, geographical variations, or the type and the severity of the infection, as were reported in the studies. 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 [3, 6].

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Different microorganisms are isolated from diabetic foot infections, based on severity and depth of ulcers. For instance, Gram-positive cocci are the most common germs in superficial ulcers, while anaerobic bacteria are mostly found in deeper lesions. The presence of different microorganisms along with increasing resistance to antibiotic therapy has compromised the empiric therapy in diabetic foot infection now, medical and research communities are beginning to realize that the diversity of the bacterial populations in chronic wounds may be an important contributor to the chronicity of the wounds, such as diabetic foot ulcers [7].

The current study aimed to determine the most common microorganisms responsible for diabetic foot infections in order to minimize the failure of antibiotic therapy and the risk of developing complications (including amputation) in a group of Egyptians.

MATERIALS AND METHODS

Patients: In our Diabetes clinic when we deal with diabetic patients presenting with Diabetic Foot ulcer (DFU) - apart from controlling the hyperglycemic burden - we usually prescribe a fixed antibiotic cocktail we call it ”the Triad". This triad is a wide spectrum antimicrobial chemotherapy including a 3rd generation Cephalosporin Ceftriaxone, a 2nd generation fluoroquinolone Ciprofloxacin and Lincosamide class, Clindamycin. This triad was given together for a period of two weeks.

Unfortunately, our "Triad" did not achieve required results in all cases and we felt confused rather guilty for not taking an earlier decision of doing a culture and sensitivity test for all our patients presenting with DFU before starting the empirical antibiotic Triad.

To solve this dilemma and settle an algorithm, we recruited for our study 28 adult Diabetic patients (18 males and 10 females) age ranges from 42-65. All patients were diagnosed of having diabetes (23 patients type 2 DM & 5 patients type 1 DM) [8] for a period of 20 years and all were on insulin therapy (Multiple Daily Insulin regimen) for a duration of at least 5 years.

All patients were suffering from chronic DFU for a period of at least 3 months despite a trial with the Triad and a relatively acceptable glycemic control (HBA1c level range 6.9 – 7.5%).

Our Inclusion Criteria Included:

- A positive diagnosis of infected diabetic ulcer without osteomyelitis
- The ability to attend the clinic visits during the follow-up period (3 months)
- Lab tests confirming active infection (CBC with high TLC, ESR and CRP)
- Acceptance for a written consent.

The exclusion Criteria were:

- Patients with severe infections according to the Infectious Diseases Society of American Classification [9] causing remarkable disability.
- Presence of Osteomyelitis.
- Patients with moderate to severe renal impairment (estimated Glomerular Filtration Rate (eGFR) < 50) [10].
- Patients with moderate to severe Peripheral Arterial Disease (PAD) [11] which was clinically diagnosed by absence of both distal pulse (dorsalis pedis & medial ankle pulses) and confirmed by Duplex study.

Methodology

Sample Collection: All patients were instructed to stop any antibiotic therapy for a period of 7 – 10 days prior to the study. Proper debridement of the ulcer wound was necessary before performing our culture sample. This step was important to decrease the risk of acquired infection and to reduce any peri-wound pressure. (Debridement was performed by a surgeon specialized in foot surgery).

The specimens were checked for aerobic and anaerobic microorganisms. Samples were collected from the deeper portion of the ulcers by using 2 sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and the other was used for culture. Standard methods for isolation and identification of aerobic and anaerobic bacteria were used [12, 13]. A direct Gram stained smear of the specimen was examined. The specimens were inoculated onto blood agar, chocolate agar, MacConkey’s agar and Thioglycollate medium. One inoculated Blood agar and MacConkey’s agar specimen were incubated in air at 37°C for 24 h, which was extended to 48 h if there was no growth. The Chocolate agar was incubated in CO 2 incubator for 24h;Thioglycollate medium plates were incubated in anaerobic jars under atmospheric conditions in the presence of CO 2 10% and H 2 90% for 48 h, which was extended to a total of 5 days if there was no growth after 48 h. 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.

Antibiotic Susceptibility Testing: All bacterial isolates were tested for antibiotic susceptibility by disc diffusion method against selected members of the following groups: Amikacin, Gentamycin, Clindamycin, Amoxicillin / Clavulanate, Azithromycin, Ceftazidime, Cefotaxime, Cephalexin, levofloxacin, Ciprofloxacin Ofloxacin, Piperacillin/Tazobactam, Dicloxacillin, Ipipenem, Ampicillin/sulbactam, Chloramphenicol and Penicillin. Sensitivity was estimated by measuring the diameters of inhibition zones in millimeters according to Kirby-Bauer technique [14].

Follow-up Schedule: The follow up protocol for each patient enrolled was as follows: on receiving the culture and sensitivity report including the prescribed antibiotic therapy, each patient was instructed to start taking their assigned antibiotic on the same day according to the prescribed regimen for a period of at least 2 weeks. This was documented as visit 1. All patients returned after 4 weeks which is visit 2, for clinical examination, measurement of the ulcer size and performing laboratory tests: CBC, ESR & CRP. On visit 3, which is 8 weeks after visit 2, patients had the same clinical examination, ulcer measurement and laboratory tests repeated in addition to analysis of the glycosylated hemoglobin (HBA1c test) for glycemic evaluation.

RESULTS

The bacterial growth pattern of the culture positive cases and their antibiotic susceptibility results are shown in Table (1, 2 & 3).

Staphylococcus aureus and coagulase-negative *Staphylococci* (CoNS) were the predominant organisms isolated (8 cases & 3 cases respectively), they were sensitive to Cephalexin, Dicloxacillin, Amoxicillin-Clavulanate and Trimethoprim-Sulfamethoxazole.

Pseudomonas aeruginosa was identified on 2 cases and was resistant to most tested antibiotics, it showed sensitivity to Carbenecillin and Levofloxacin but resistant to Cefotaxime.

Methicillin –Resistant *Staphylococcus aureus* (*MRSA*) was identified in 1 case and was sensitive to Clindamycin together with Trimethoprim-Sulfamethoxazole.

A number of other aerobic species was identified including *Escherichia coli* (3 cases) and *Streptococcus species* (2 cases).

Anaerobic organisms isolated included also anaerobic *Streptococci* (2cases).

Klebsiella & Proteus species were isolated as a mixed infection (3, 2 cases respectively) with *Staphylococcus aureus*.

Table 1: Growth patterns in culture of foot ulcer samples of 28 patients

Culture Results	No. of cases	Percentage (%)
Positive culture	26	92.85
Pure bacterial growth	21	75
Mixed microbial growth	5	17.85
No growth	2	7.15
Total	28	100

Table 2: Profile of 31 bacterial isolates from infected foot ulcers in diabetic patient's specimens

Name of Bacterial Isolates	pure isolate	%	Mixed with other bacteria	%	Total	%
Gram positive						
S. aureus	8	25.8	5	16.1	13	41.9
CONS	3	9.7	0	0	3	9.7
MRSA	1	3.2	0	0	1	3.2
Streptococcus species	2	6.5	0	0	2	6.5
Gram negative						
Pseudomonas	2	6.5	0	0	2	6.5
E.coli	3	9.6	0	0	3	9.6
Klebsiella	0	0	3	9.6	3	9.6
Proteus	0	0	2	6.5	2	6.5
					-	-
Anaerobic Streptococci	2	6.5	0	0	0	0
Total	21	67.7	10	32.3	31	100

Table 3: Susceptibility of the bacterial isolates to different antibiotics

Organism	Susceptible Antibiotics
S. aureus	Cephalexin, Dicloxacillin, Amoxicillin- Clavulanate, Trimethoprim-Sulfamethoxazole.
CONS	Cephalexin, Dicloxacillin, Amoxicillin- Clavulanate and Trimethoprim-Sulfamethoxazole.
MRSA	Clindamycin together with Trimethoprim-Sulfamethoxazole
Streptococcus species	Azithromycin, Amoxicillin- Clavulanate, Penicillin+Clindamycin
Pseudomonas	Carbenecillin, Levofloxacin
E. coli	Amikacin, Ipipenem, Piperacillin, Ceftazidim
Klebsiella	Ampicillin/sulbactam, Ciprofloxacin, Cefotaxime, Piperacillin/tazobactam
Proteus	Ofloxacin, Ciprofloxacin, Gentamycin
Anaerobic Streptococci	Ampicillin/sulbactam, Clindamycin, Chloramphenicol

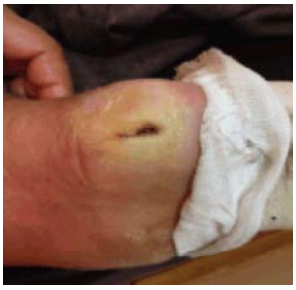

Fig. 1: Marked Improvement in Ulcer Size and Inflammatory Signs

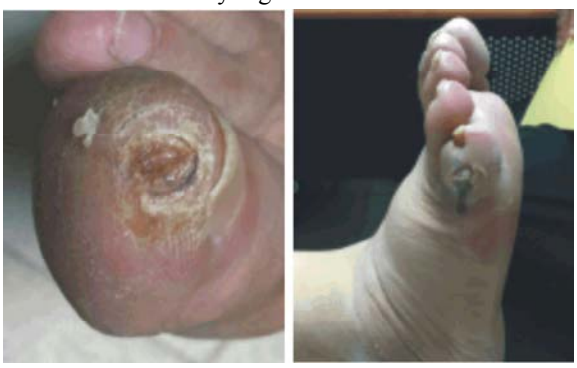

Fig. 2: Moderate Improvement in Ulcer Size and Inflammatory Signs

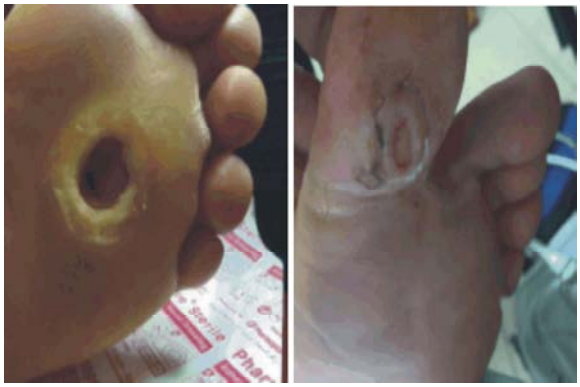

Fig. 3: Minimal Improvement in Ulcer Size and Inflammatory Signs

Two plates showed no growth raised the possibility of viral or fungal infection (excluding *Candida albicans)*.

A combination of the most sensitive antibiotics in each plate was selected for every patient and an antifungal agent was added to the two cases that showed no growth on their plates.

All patients were instructed to use this regimen for a period of two weeks and an appointment was settled to evaluate the results.

After 2 weeks of using the selected antimicrobial regimen 20(71.4%) patients out of 28 showed marked improvement in both their ulcer size and local inflammatory signs and their laboratory tests (Figure 1), 5(17.9%) patients showed mild improvement in ulcer size but with remarkable improvement in both local inflammatory signs and lab tests (Figure 2), while only 3 (10.7%) patients failed to show any improvement neither the ulcer size nor the local inflammatory signs but they did show some improvement in the lab tests. These 2 cases had Pseudomonas aeruginosa and anaerobic Streptococci on their plates, they were not following the medical instructions concerning bed rest and pressure avoidance (Figure 3).

Figure 4 showed diabetic foot ulcer before and after treatment with marked improvement in ulcer Size and inflammatory signs.

Statistical Analysis: 20 patients showed marked improvement in ulcer size and inflammatory signs and near normal lab tests (71.4%) ($P<0.005$, highly significant).

5 patients showed mild improvement in ulcer size but great improvement in both local inflammatory signs and lab tests (17.9%).

3 patients failed to show any improvement in ulcer size and local inflammation with mild improvement in their lab tests (10.7%).

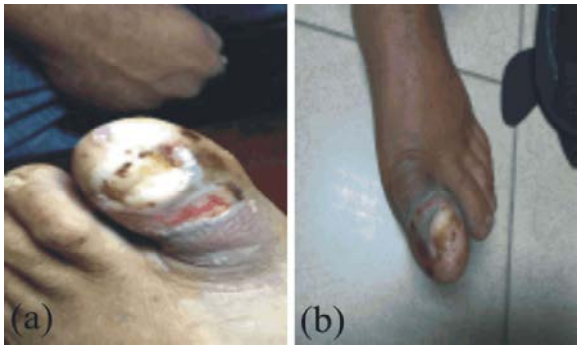

Fig. 4: (a) Before treatment & (b) After treatment; Marked Improvement in Ulcer Size and Inflammatory Signs

These results are statistically highly significant and in favor of our therapeutic approach. From our view these outcomes raise the necessity for performing a routine culture and sensitivity test for all patients presenting with chronic DFU.

DISCUSSION

Diabetic foot ulcers (DFU) are more liable to bacterial infections that spreads rapidly and ultimately leads to irreversible tissue damage. Patterns of microbial infection are not consistent in patients with diabetic foot infections and therefore repeated evaluation of microbial characteristics and their antibiotic sensitivity is imperative for the proper choice of antibiotic administration.

The extent of the severity of infection is mainly caused by under estimation of the proper procedures or the inappropriateness of the antimicrobials used [15].

Although the prevalence of diabetes type 2 is reported to be variable in several countries (Up to 117% in Iran) [7] the majority of our studied population had type 2 diabetes (82.1%).

Our study's objective was to properly identify the bacterial pathogens associated with DFU and find out its antibiotic susceptibility pattern in a limited number of patients visiting our clinic. In addition to trace the rate of improvement of the ulcer (through various criteria) within a 3 month period, follow-up.

In the present study, all 28 patients with DFU belonged to Wagner grade 1 and 2 [16]. Out of 28 patients, 26 (92.85%) yielded growth of organisms, making a total of 31 isolates. Out of these 31 isolates, 21 (75%) were single bacterial isolates and 5(17.85%) were mixed bacterial growth yielded 10 isolates Zubair *et al*. [6], Arandi *et al*.[17], Rama Kant *et al*.[18], Pappu K *et al*. (19), Citron *et al*,(5) and Shanmugam *et al*. [3] reported 56.5, 19, 23, 92, 16.2 and 50% monomicrobial infections and 33, 67,66,7.7, 83 and 50% polymicrobial infections respectively. Our results correlate with the group of Pappu study [19] regarding the monomicrobial results and slightly similar to those of Zubair's study regarding polymicrobial infection [6]. DFU is known for polymicrobial infections [20] but we observed a preponderance of monomicrobial infection and this is in accordance with a study by Dhanasekaran *et al,* [21]. Gram positive cocci were more prevalent (67.75%) than Gram negative bacilli (32.25%). The commonest isolate was *Staphylococcus aureus* (41.9%) followed by coagulase negative *staphylococcus* (CONS), *E. coli* and *Klebsiella spp*. (9.7%) for each and *Pseudomonas aeurginosa* (6.4%). These were predominant among the monobacterial isolates. *Klebsiella spp.* and *Proteus spp*. were predominant among the mixed growths.

In the work performed by Mehta *et al*.[22], they isolated Gram negative bacilli as the most common bacteria with *Pseudomonas aeruginosa* as the predominant organism in 27% of the total isolates obtained, followed by *Klebsiella spp.* and *E. coli*, in 22 and 19% respectively. The Gram positive cocci, *S. aureus* and CONS were isolated in only 7 and 1% respectively. Similarly, Shanmugan *et al*. [3] and Pappu *et al*. [19] reported that Gram negative bacilli were isolated predominantly in 65 and 76% respectively with *Pseudomonas spp*. also being the leading pathogen 16% and 23% in their studies respectively. *E. coli* was the most frequent organism isolated in the works by Hadadi *et al*. [7] and Tiwar *et al*.[15] who reported that following *E. coli, S. aureus* was the second commonly isolated organism. Our results obtained do not agree with all the aforementioned authors, neither they do with the study by Al Benwan *et al*. [23] done in Kuwait which also stated that Gram negative bacilli (51.2%) were isolated higher than Gram positive pathogens (32.3%). In contrast, Citron *et al*. [5], Zubair *et al*. [6] and Alavi *et al*. [24] reported *S. aureus* as the predominant pathogen obtained in 57.2, 28 and 26.2% of their isolates respectively. Our results are in close agreement with those obtained by the latter authors. Also, Fejfarova *et al*. [25] and Dang *et al*. [26] reported similar results. In addition, Hena *et al*. [27] isolated *S.aureus* as the common organism (43.2%) in a polymicrobial infection, followed by *Pseudomonas aeruginosa* in 24.3% of the total isolated organisms.

The difference in the age –sex composition, ulcer grades, geographical study setting, etc. between our study population and those of previously mentioned works, whose results are not in agreement with ours,

might be the reason for these discrepancies in agreement. Compared with an earlier report by Viswanathan *et al*. [28] we isolated fewer anaerobic spp.in 2(6.4%) but our result is consistent with that of Gadepalli *et al*. [29] who isolated anaerobic bacteria in 1 patient only (1.2%). They attributed this low rate of recovery that most of their patients did not have chronic draining wounds and only 9% had gangrene associated with their infection.

We find that this explanation closely agrees with our patients' situation, where all of them presented with DFU grade 1 and 2 by Wagner's classification [16]. This might be an important indication of fewer anaerobic spp. among non-threatening lower extremity infections [30].

The antibiotic sensitivity profiles of the bacteria revealed that *Pseudomonas aeruginosa* exhibited a multi-drug resistant (MDR) pattern, a finding that correlated with the work of Shanker *et al*. [31] who stated a 44% of *Pseudomonas* isolates were MDR; Vimalin and Growther [27] and Gadepalli *et al*. [29] Who observed a high recovery of MDR *Pseudomonas aeruginosa* and defining it as an aggressive Gram negative bacillus. They concluded that almost two thirds of their patients were infected with MDR organisms together with a high prevalence of MRSA isolated. This was not the case in our findings attributable to the location in which the study took place. While our study was conducted in a private clinic, the study of Gadepalli and co-workers [29], was on patients in a tertiary care hospital with wide spread usage of antibiotics leading to selection for resistant strains. *Pseudomonas* was resistant to most of the antibiotics used, with high resistance to the 3rd generation cephalosporins. It was sensitive to carbencillin and levofloxacin. Sivanmaliappan and Sevanan [32] reported that *Pseudomonas aeruginosa* strains showed high resistance to the fluroquinolone norfloxacin and were susceptible to cefotaxime. These findings were contrary to our findings where there was a high resistance to the 3rd generation cephalosporin cefotaxime. On the other hand, our results agree with those obtained by Shanugama *et al*. [3] who identified that *Pseudomonas* showing 61% resistant to 3rd generation cephalosporins and 100% sensitivity to carbencillin.

Gatepalli *et al*. [29] documented that *E. coli* was the second Extended-Spectrum Beta-lactamase (ESBL) producer in their study and was resistant to 3rd generation cephalosporins. This was also noted in the work by Sivaraman *et al* [4]. Our findings are not consistent with those latter two workers regarding susceptibility pattern which showed resistance to ciprofloxacin *E. coli* and was sensitive to 3rd generation cephalosporins. On the other hand Hena *et al*. [27] agree with our results and concluded that *E. coli* isolates showed a higher sensitivity to 3rd generation cephalosporin group than to the commonly used chloramphenicol.

Among the 5 isolates of *Klebsiella spp*. and *Proteus spp*. isolated as mixed infection culture, *Proteus spp*. showed high resistance to ciprofloxacin and sensitive to cefoperazone. This finding is in agreement with that by Shanugama *et al*. [3] who revealed *Proteus spp*. showing 80% resistance to quinolones and sensitive to cefoperazone. They also added that 80% of the *Proteus spp*. were ESBL producers. Likewise, *Klebsiella spp*. isolates were ESBL producers 40%. This latter finding regarding *Klebsiella spp*. is contrary to our findings in which all 5 *Klebsiells spp.* isolates were sensitive to ceftriaxone, cefotaxime and cefoperazone.

All the strains of *S. aureus* which were isolated in our study were sensitive to amoxicillin/clavulanate, dicloxacillin, contrary to the findings by Hena *et al*. [27] who reported a resistance to penicillin and amoxicillin/clavulanate. In addition *S. aureus* was resistant to ciprofloxacin and clindamycin, while Alvali *et al*. [24] reported that it was sensitive (91%) to ciprofloxacin, a finding that was quite different from ours. Hena *et al*. [27] stated that 63.8% of S. *aureus* strains were sensitive to gatifloxacin, while only 25.5% were sensitive to ciprofloxacin.

In our study we stated that the 1 isolate of MRSA was sensitive to clindamycin. Girish *et al*. [33] reported that 15% of MRSA strains were resistant to ampicillin and cephalosporins, but sensitive to vancomycin and linezolid. Mehta *et al.* [22] showed that out of 15 isolates of *S. aureus*, 9 (60%) were MRSA. Their Gram negative isolates were found to be sensitive to amoxicillin/clavulanate which is in agreement with our results. On the contrary to our findings, most of their Gram negative isolates were resistant to levofloxacin and gatifloxacin, the 3rd and 4th generation fluoroquinolones, amoxicillin/clavulanate respectively. Our studied isolates showed resistance to only the 2nd generation ciprofloxacin. Thus when testing the susceptibility to fluoroquinolones, individual drugs must be included, as sensitivity to one drug cannot be measured as evidence of susceptibility to other fluoroquinolones [27].

As a final objective in our study, we followed up our studied population after their intake of the selected treatment by the most sensitive antimicrobials. The follow up period began after antibiotic intake and extended up to 3 months during which the patient came for two visits

(2 and 3), after 1 month and two months following initiation of antibiotic intake (visit 1). These follow up procedures were aiming to reach an evaluation of each patient's condition through the validation of various criteria including measurement of inflammatory markers (TLC, ESR and CRP), measurement of ulcer size and observation of local inflammatory signs. We observed that 91.3% of the studied patients experienced a marked to moderate improvement, while only 8.7% showed no improvement after 3 months follow up, as seen in the figures.

In a study conducted by Tiwari *et al*. [15], where they evaluated the clinical characteristic of DFU, besides the microbial evaluation. Their results indicated a high TLC and low hemoglobin in polymicrobial compared to monomicrobial infections. TLC was higher in Gram negative compared to Gram positive infections. These findings suggest that infections with multiple organisms contributed to deterioration of the wound infection as evidenced by the clinical characteristics of TLC and hemoglobin level. Our results are in accordance with their findings. The predominant microbial pattern of the 31 isolates of our study, depicted a monomicrobial nature (80.9%), thus increasing the chance of ulcer improvement, evidenced by the high (91.3%) improvement rate of the patients' wounds. The validity of the improvement criteria was confirmed by the absence of inflammatory signs and markers (TLC, ESR and CRP) observed in the 26 (92.85%) patients. On the other hand, the remaining 2 (7.15%) patients showed no improvement of inflammatory signs, but only a slight improvement of inflammatory markers.

The culture results showed the isolation of *Pseudomonas* in 1 case and anaerobic *streptococci* in the other. Hadadi *et al*. [7] studied the risk of developing complications in a total of 113 patients with deep DFU without osteomyelitis according to Wagner's grading [16]. They reported that the isolated pathogen showed no significant correlation with severity and type of the lesion; yet the response rate to treatment was high (31.6%) in monomicrobial pathogen than in polymicrobial infection (10%). We found that their results are in agreement with our results. In addition, their results failed to report any relationship between the patient's outcome and the duration of diabetes, neutrophil count and the anatomic site of foot lesion. While in another study, the complication of treatment of DFU depended on depth of the ulcer, presence of ischemia and the severity of glycemic control [34]. Our findings are in agreement with the latter authors. All studied population group had superficial or non-osteomyelitis extended ulcer. There was no evidence of ischemia indicated by a felt distal pulse and confirmed by a clear duplex scan; in addition to a controlled glycosylated hemoglobin level range. All these selected criteria from our point of view and in accordance to the findings of Oyibo *et al*. [34] have played a role in the outcome or complication of treatment, thus selecting for a favorable outcome and improvement of ulcer condition.

The present study confirmed the association of ulcer size with MDR organisms which were also reported by Gadepalli *et al*. [29]. The 2 patients (7.15%) who showed no improvement in their DFU, exhibited a non-decrease in ulcer size or inflammatory signs. Culture of the organisms from the two ulcers yielded isolation of MDR *Pseudomonas aeruginosa* from one and anaerobic *streptococci* from the other indicating the severe depth and size of the ulcer associated with the adequate environment for anaerobic organisms. Our findings are supported by the results of Gadepalli *et al*. [29] as previously mentioned, they reported an ulcer size of greater than 4cm2 to be associated with MDR organisms and patients with MDR organisms had a tendency to develop complications with increase in hospital stay.

Consequently our findings indicate that patients with non-MDR organisms, as compared with MDR organism, ulcer had a higher tendency towards a reduction in ulcer size and hence indication of improvement. These findings were statistically significant, where 56.5% of the studied patients whose outcome was evaluated as marked improvement had a range of more than 50 and 90% reduction in ulcer size. Patients who exhibited moderate improvement (34.8%) had less than 50% reduction in ulcer size.

In conclusion, these findings are encouraging for implementation of a fixed and organized regimen for standard clinical and diagnostic procedures to assess the appropriate empirical antibiotic therapy in DFU patients.

In addition, we should take into account that proper management is a must to decrease the incidence of MDR infections in diabetic patients. Knowledge of antibiotic susceptibility pattern of the isolates from diabetic foot infections is imperative for the planning of the appropriate treatment of these cases.

REFERENCES

1. WHO (World Health Organization), 2012. Diabetes Mellitus. Fact Sheet N 138, 2012. Available from: http://www.who.int/mediacentre/factsheets/fs138/en. Make references like this style.

2. IDF (International Diabetes Federation), 2013.IDF Diabetes Atlas, 6th ed. Brussels. Make references like this style.
3. Shanmugam, P., M. Jeya and S.L. Susan, 2013. The Bacteriology of Diabetic Foot Ulcers, with a Special Reference to Multidrug Resistant Strains. J. Clin. Diagn. Res., 7(3): 441–445. Make references like this style.
4. Sivaraman, U., S. Kumar, N.M. Joseph, J.M. Easow and G. Kandhakumari, 2011. Microbiological study of diabetic foot infections. Indian J Med Specialties; 2(1): 12-17.
5. Citron, D.M., E.J.C. Goldstein, V.C. Merriam and B.A. Lipsky, 2007. Bacteriology of moderate to severe diabetic foot infections and *in vitro* activity of antimicrobial agents. J. Clin Microbial., 45(9): 2819-28.
6. Zubair, M., A. Malik and J. Ahmad, 2010. Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India. Biol Med., 2(4): 22-34.
7. Hadadi, A., H.O. Ghiasi, M. Hajiabdolbaghi, M. Zandekarimi and Reza Hamidian, 2014. Diabetic Foot: Infections and Outcomes in Iranian Admitted Patients Jundishapur. J. Microbiol., 7(7): e11680.
8. David M. Nathan, 2009 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus Diabetes Care. Jul; 32(7): 1327-1334.
9. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system, Lavery LA1, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA 2009.
10. National Kidney Foundation ESRD, 2002. Classification, Advisory Board. Clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kidney dis 2002; 39(2Suppl 2): S1-246. Adj 2012.
11. Shammas, N.W., 2007. Epidemiology and Classification of peripheral arterial disease (PAD) Vasc Health Risk Manag., 3(2): 229-34.Adj 2014.
12. Sutter, V.L., D.M. Citron, M.A.C. Edelstein and S.M. Finegold, 1985. Wadsworth Anaerobic Bacteriology Manual. 4th ed. Belmont, CA, Star Publishing.
13. The Clinical and Laboratory Standards Institute, 2011. Performance Standards for Antimicrobial Susceptibility testing, twenty first informational supplement, M100-S21, Clinical and Laboratory Standards Institute.
14. Tiwari, S.1., D.D. Pratyush, A. Dwivedi, S.K. Gupta, M. Rai and S.K. Singh, 2012. Microbiological and clinical characteristics of diabetic foot infections in northern India. J Infect Dev Ctries. 13; 6(4): 329-32.
15. O'Neal, L.W. and F.W. Wagner, 1983. The Diabetic Foot. St Louis, MO: Mosby New Book, 274: 18.
16. Anandi, C.L., D. Alaguraja, V. Natarajan, M. Ramanathan, C.S. Subramaniam, M. Thulasiram and S. Sumithra, 2004. Bacteriology of diabetic foot lesions. Indian J. Med. Microbiol. Jul-Sep; 22(3): 175-8.
17. Ramakant, P., A.K. Verma, R. Misra, K.N. Prasad, G. Chand, A. Mishra, G. Agarwal, A. Agarwal and S.K. Mishra, 2011. Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose? Diabetologia. 54(1):58-64. doi: 10.1007/s00125-010-1893-7. Epub 2010 Sep 11.
18. Pappu, A.K., A. Sinha and A. Johnson, 2011. Microbiological profile of diabetic foot ulcer. Calicut Med. Journal, 9(3)e: 1-4. 20.
19. Singh, S.K., K. Gupta, S. Tiwari, S.K. Shahi, S. Kumar, A. Kumar and S.K. Gupta, 2009. Detecting aerobic bacterial diversity in patients with diabetic foot wounds using ERIC-PCR: a preliminary communication. Int. J. Low Extreme Wounds., 8(4): 203-8.
20. Dhanasekaran, G., N.G. Sastry and V. Mohan, 2003. Microbial pattern of soft-tissue infections in diabetic patients in South India. Asian Journal of Diabetology, 5(5-6): 8-10.
21. Vaidehi J. Mehta, Kunjan M. Kikani and Sanjay J. Mehta, 2014. Microbiological profile of diabetic foot ulcers and its antibiotic susceptibility pattern in a teaching hospital, Gujarat, Int. J. Basic Clin Pharmacol., 3(1): 92-95.
22. Al Benwan, K., A. Al Mulla and V.O. Rotimi, 2012. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J. Infect Public Health., 5(1): 1-8.
23. Alavi, S.M., A.D. Khosravi, A. Sarami, A. Dashtebozorg and E.A. Montazeri, 2007. Bacteriologic study of diabetic foot ulcer. Pak J. Med. Sci., 23: 681-4.
24. Fejfarová, V., A. Jirkovská, J. Skibová and V. Petkov, 2002. Pathogen resistance and other risk factors in the frequency of lower limb amputations in patients with the diabetic foot syndrome Vnitr Lek., 48(4): 302-6.

25. Dang, C.N., Y.D. Prasad, A.J. Boulton and E.B. Jude, 2003. Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet Med., 20(2): 159-61.
26. Vimalin Hena, J. and L. Growther, 2010. Studies on Bacterial Infections of Diabetic Foot Ulcer. Afr J. Cln. Exper Microbiol., 11(3): 146-9.
27. Viswanathan, V., J.J. Jasmine, C. Snehalatha and A. Ramachandran, 2002. Prevalence of pathogen in diabetic foot infection in South Indian type 2 diabetic patients. J Assoc Physicians India, 50: 1013-16.
28. Gadepalli, R., B. Dhawan, V. Sreenivas, A. Kapil, A.C. Ammini and R. Chaudhry, 2006. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care, 29(8): 1727-32.
29. Lipsky, B.A. and A.R. Berendt, 2000. Principles and practice of antibiotic therapy of diabetic foot infections. Diabetes Metab Res Rev. Sep-Oct;16
30. Shankar, E.M., V. Mohan, G. Premalatha, R.S. Srinivasan and A.R. Usha, 2005. Bacterial etiology of diabetic foot infections in South India. Eur J. Intern Med., 16(8): 567-70.
31. Tamil Selvi Sivanmaliappan and Murugan Sevanan, 2011. Antimicrobial Susceptibility Patterns of Pseudomonas aeruginosa from Diabetes Patients with Foot Ulcers. International Journal of Microbiology, Volume 2011 Article ID 605195, 4 pages
32. Girish, M.B., T.N. Kumar and R. Srinivas, 2010. Pattern of antimicrobials used to treat infected diabetic foot in a tertiary care hospital in Kolar Int J Pharm Biomed Res, 1(2): 48-52.
33. Oyibo, S.O., E.B. Jude, Tarawneh, H.C. Nguyen, D.G. Armstrong, L.B. Harkless and A.J. Boulton, 2001. The effects of ulcer size and site, patient's age, sex and type and duration of diabetes on the outcome of diabetic foot ulcers. Diabetes Med., 18(2): 133-8.