ORIGINAL ARTICLE

Microbiological Study of Diabetic Foot Ulcer

*Mohammuddunnobi[1], T Jahan[2], AA Amin[3]

ABSTRACT

Background: Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients. Limb-threatening diabetic infections are usually polymicrobial involving multiple aerobic and anaerobic organisms.

Methodology: The present study was a cross sectional study, conducted in the department of surgery and microbiology at BIRDEM General Hospital, Dhaka, over a period of 9 months during January 2017-September' 2017. The study included a total of 77 adult patients of clinically diagnosed diabetic foot patients presenting to outpatient department and emergency ward. The standard case definition of diabetic foot is 'any pathology occurring in the foot of a patient suffering from diabetes mellitus or as a result of long term complication of diabetes mellitus'.

Results: Majority 17(22.1%) patients had *Klebsiella pneumonia,* 14(18.2%) had *Pseudomonas aeruginosa,* 11(14.3%) had *Staphylococcus aureus,* 10(13.0%) had *Escherichia coli,* 6(7.8%) had *Coagulase-negative staphylococci* and 8(10.4%) had *Providencia spp*. In *Escherichia coli* 100% sensitivity to imipenem, 70% to amoxicillin-clavulanic acid, amikacin, piperacillin-tazobactam. In Coagulase-*negative Staphylococci* 83.3% sensitivity to tetracycline, 66.7% to ceftriaxone. *In Proteus mirabilis* 100% sensitivity to tetracycline, amikacin, ceftriaxone, imipenem, piperacillin-tazobactam. In *Enterococcus spp*.75.0% sensitivity to tetracycline. In *Citrobacter spp*. 100% sensitivity to imipenem.

Conclusion: Common organism found in diabetic foot ulcer patients were *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Coagulase-negative staphylococci* and *Providencia spp*. In tetracycline, amikacin, ceftriaxone, imipenem, piperacillin-tazobactam was 100% sensitive in *Proteus mirabilis* and only imipenem found in *Escherichia coli* and *Citrobacter spp*.

Key Words: Diabetic foot, ulcer, organism.

Introduction

Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients.[1] Diabetic foot syndrome (DFS) is a complex and heterogeneous disorder that affects 15% of patients with diabetes during their lifetime.[2] Wounds of diabetic foot very often get infected due to several factors including high blood sugar level, suppressed immunity, inadequate blood supply and neuropathy.[3] Polymicrobial infections involving both aerobic and anaerobic bacteria are very common in diabetic foot ulcers, in many centres of developing countries, anaerobes are rarely isolated due to technical difficulties.[4] Approximately 85% of all diabetes-related lower-extremity amputations are preceded by foot ulcers. Diabetic foot ulcers are at high risk of infection secondary to high glucose levels and poor tissue perfusion.[5] Limb-threatening diabetic

[1]*Dr. Mohammuddunnobi, Resident Surgeon, Surgical Emergency, BIRDEM*
e-mail: drskfiroz2005@yahoo.com
[2]*Dr. Tasnuva Jahan, Medical officer, DMCH*
[3]*Prof. Abdullah -Al-Amin, Prof. & Head Dept. of Surgery BIHS General Hospital, Mirpur, Dhaka*
**Corresponding Author*
Date of submission: 15.08.2018 Date of acceptance: 21.11.2018

infections are usually polymicrobial involving multiple aerobic and anaerobic organisms. *Staphylococcus aureus, Streptococcus spp.*, *Enterobacteriaceae spp.*, *Bacteroides fragilis*, *Peptococcus* spp. And *Peptostreptococcus* spp. are the common organisms cultured from diabetic ulcers.[6] The aim of the study was undertaken to identify the aerobic, anaerobic, and fungal pathogens involved in the different grades of diabetic foot ulcers and to find out the antimicrobial sensitivity pattern of the bacterial isolates.

Materials and Methods

The present study was a cross sectional study, conducted in the department of surgery and microbiology at BIRDEM General Hospital, Dhaka, over a period of 9 months during January 2017-September' 2017. The study included a total of 77 adult patients of clinically diagnosed diabetic foot patients presenting to outpatient department and emergency ward. The standard case definition of diabetic foot is 'any pathology occurring in the foot of a patient suffering from diabetes mellitus or as a result of long term complication of diabetes mellitus'. Samples were taken from enrolled subjects using the following criteria: patient with diabetic foot pathology. All patients signed an informed consent. Samples were inoculated immediately at the bedside, on pre-reduced *Brucella* blood agar (Hi-Media) plates enriched with 5 µg/ml hemin and 1 µg/ml menadione. Each plate was immediately put inside the modified candle jar, and before closing the jar lid, anaerobiosis was initiated by lighting a small white wax candle and putting 5 g of acidified copper-coated steel wool on an open plate kept inside. This simple inhouse developed method was standardized earlier and was found suitable for the initiation of anaerobiosis at bedside. Simultaneously, a separate inoculated plate was placed in a jar with GasPak system (Anaerogas Pack- Hi-Media) and another inoculated plate for aerobic incubation. After 48 h of incubation at 37^0C, the anaerobic plates were examined for growth and used for aero-tolerance study by aerobic incubation on blood agar plate after subculture. Colony morphology was noted and bacterial morphology was observed from Gram-stained smears. Aerobic bacteria were identified based on the results of standard biochemical tests. The sensitivity tests were performed by modified Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guideline. Suspected anaerobic isolates, verified by aero-tolerance study, were put into a fresh set of modified candle jars to perform biochemical tests. The biochemical tests included fermentation, indole, nitrate disk reduction, catalase and urease tests. Special-potency disk test (vancomycin, 5 µg; kanamycin, 1000 µg; and colistin, 10 µg), sodium polyanethol sulphonate disk test, bile esculin hydrolysis test, lipase and lecithinase test, pigment production test and colony observation of fluorescence study were also included for presumptive identification of anaerobes up to the genus level[8]. Isolated anaerobes were tested for antibiotic susceptibility by the *E*-test (BioMérieux, France) in the same modified candle jar system. Antibiotics tested were metronidazole, clindamycin, cefoxitin, imipenem and penicillin.

Results

Majority 27(35.1%) patients belonged to age 51-60 years with mean age was found 51.57 ± 12.13 years. Males were predominant (70.1%), male: female ratio was 2.3:1. Almost two third (64.9%) patients had trauma, 23(29.9%) were smoker and 11(14.3%) had family history of diabetes (Table 1). More than two third (68.6%) patients had diabetes during period of 10-19 years and the mean duration of diabetes was found 12.7 ± 3.81 years (Table 2). Majority 17(22.1%) patients had *Klebsiella pneumonia,* 14(18.2%) had *Pseudomonas aeruginosa,* 11(14.3%) had *Staphylococcus aureus,* 10(13.0%) had *Escherichia coli,* 6(7.8%) had *Coagulase-negative staphylococci* and 8(10.4%) had *Providencia spp.* (Table 3). In *Klebsiella pneumonia* organism 94.1% sensitivity to imipenem, 70.6% to amikacin, 70.6% to piperacillin-tazobactam, 64.7% to meropenem. In *Pseudomonas aeruginosa* organism 92.9% sensitivity to imipenem, 74.6% to piperacillin-tazobactam, 64.3% to amikacin, 57.1% to ciprofloxacin. In *Staphylococcus aureus* organism

63.6% sensitivity to tetracycline, 54.5% to eftriaxone. In *Escherichia coli* 100% sensitivity to imipenem, 70% to amoxicillin-clavulanic acid, amikacin, piperacillin-tazobactam. In *Coagulase-negative Staphylococci* 83.3% sensitivity to tetracycline, 66.7% to ceftriaxone. In *Proteus mirabilis* 100% sensitivity to tetracycline, amikacin, ceftriaxone, imipenem, piperacillin-tazobactam. In *Enterococcus spp*. 75.0% sensitivity to tetracycline. In *Citrobacter spp*. 100% sensitivity to imipenem (Table 4).

Table 1: Demographic Profile, risk factors and clinical presentation of diabetic foot patients (n=77)

Age in years	**Number**	**Percentage**
21-30 yrs	7	9.1
31-40 yrs	8	10.4
41-50 yrs	18	23.4
51-60 yrs	27	35.1
61-70yrs	14	18.2
>70 yrs	3	3.9
Mean (±SD)	51.57(±12.13)	Range 21-78 years
Sex		
Male	54	70.1
Female	23	29.9
Risk factors		
Trauma	50	64.9
Smoking	23	29.9
Family history of diabetes	11	14.3

Table 2: Duration of diabetes mellitus (n=77)

	Number	**Percentage**
Duration of diabetes (years) (Mean SD)	12.7(±3.81)	Range 5-27 years
<10 yrs	18	23.4
10-19 yrs	53	68.8
>20 yrs	6	7.8

Table 3: Bacteria isolated from diabetic foot ulcers

	Number	**Percentage**
Klebsiella pneumoniae	17	22.1
Pseudomonas aeruginosa	14	18.2
Staphylococcus aureus	11	14.3
Escherichia coli	10	3.0
Coagulase-negative staphylococci	6	7.8
Proteus mirabilis	4	5.2
Enterococcus spp.	4	5.2
Citrobacter spp.	3	3.9
Proteus vulgaris	1	1.3
Acinetobacter spp.	1	1.3
Pseudomonas spp.	1	1.3
Providencia spp.	8	10.4

Table 4: Antimicrobial susceptibility pattern of aerobic bacterial isolates from infected foot in diabetic patients

Bacterial isolates	**Sensitivity pattern (%)**														
	AC	**TE**	**CI**	**GM**	**AK**	**NC**	**CFX**	**CTR**	**CAZ**	**IP**	**PT**	**COL**	**FA**	**LIN**	**MER**
Klebsiella pneumonia (n=17)	52.9	58.8	47.1	35.3	70.6	-	17.6	23.5	-	94.1	70.6	41.2	23.5	58.8	64.7
Pseudomonas aeruginosa (n=14)	-	-	57.1	50.0	64.3	42.9	-	-	28.6	92.9	74.6	50.0	21.4	42.9	28.6
Staphylococcus aureus (n=11)	27.3	63.6	36.6	45.5	-	-	-	54.5	-	18.2	27.3	-	-	-	-
Escherichia coli (n=-10)	70.0	30.0	30.0	40.0	70.0	-	20.0	30.0	-	100	70.0	30.0	40.0	20.0	50.0
Coagulase-negative Staphylococci (n=6)	33.3	83.3	50.0	50.0	-	-	-	66.7	-	16.7	-	-	-	16.7	33.3
Proteus mirabilis (n=4)	25.0	100	75.0	75.0	100	-	50.0	100	-	100	100	25.0	-	50.0	75.0
Enterococcus spp. (n=4)	-	75.0	50.0	50.0	-	-	-	-	-	-	-	-	-	-	-
Citrobacter spp. (n=3)	33.3	33.3	33.3	33.3	66.7	-	33.3	66.7	-	100	100	33.3	-	33.3	33.3
Proteus vulgaris (n=1)	100	100	100	-	-	-	-	100	-	100	-	-	-	-	-
Acinetobacter spp. (n=1)	-	100	-	-	-	-	-	-	-	100	100	-	-	-	-
Pseudomonas spp. (n=1)	-	-	100	-	-	100	-	-	-	100	100	-	-	-	-
Providencia spp. (n=8)	-	-	-	-	-	-		100	-	-	100	-	-	-	-

Discussion

In this study majority 27(35.1%) patients belonged to age 51-60 years with mean age was found 51.57±12.13 years. Males were predominant (70.1%), male: female ratio was 2.3:1. Almost two third (64.9%) patients had trauma, 23(29.9%) were smoker and 11(14.3%) had family history of diabetes. Reghu *et al.*[7] study observed that the age ranged from 25 to 93 years with mean age being 63.6 years. Males were predominant (73.3%) in the study subjects. Umadevi *et al.*[1] the age ranged from 32 to 73 years with mean age being 47±11 years. Of the 105 patients with diabetic foot, 84 (80%) were male and 21 (20%) were female. Anand *et al.*[2] study reported that the mean age of the patients was 52.42 years. The highest number of patients was in the older age group of 51-60 years old of 34% (17/50). The male: female ratio is 3.5:1. Sixty-eight percent (34/50) of these patients had been diagnosed with diabetes for less than 10 years while the rest have been diagnosed for more than 10 years. This is in similar to the study conducted by Zaine *et al.*[8] conducted in Sydney and Gardner *et al.*[9] from Iowa where mean age of the study participants was 67 years and 64.2 years. Increasing age may be a contributory factor to chronic wounds as the skin can easily damage. Older cells do not proliferate as fast and may not have an adequate response to stress in terms of gene up regulation of stress related proteins.[8-10] This is comparable to the study conducted by Perrin *et al.*[11] from Victoria, where they reported male predominance at 61.3%. The reason for this male predominance is unknown, although in Indian subcontinent, habit of bare foot walking and predominantly rural background may contribute to trauma leading to ulcers. Indians also sit with legs crossed for long hours of work or worship leading to repetitive, prolonged pressure over lateral malleolar areas, leading to bursae and dark hypertrophied skin, which can ulcerate and cause infection.[11] Gadepalli *et al.*[12] males were predominant (85.0%) in the study subjects. The majority of subjects had type 2 diabetes (88.8%). The mean age of the subjects was 53.9±12.1 years. The mean duration of diabetes was 11.8±5.7 years.

In current study more than two third (68.6%) patients had diabetes during period of 10-19 years and the mean duration of diabetes was found 12.7±3.81 years. Gadepalli *et al.*[12] the mean duration of diabetes was 11.8±5.7 years. Reghu *et al.*[7] study observed that the duration of diabetes ranged from 1 year to 40 years with a mean duration of 16.2 years. Most of the patients (41.3%) had a diabetic history of 11-20 years. Anand *et al.*[2] reported that sixty-eight percent of these patients had been diagnosed with Diabetes for less than 10 years while the rest have been diagnosed for more than 10 years.

In current study showed majority 17(22.1%) patients had *Klebsiella pneumonia,* 14(18.2%) had *Pseudomonas aeruginosa,* 11(14.3%) had *Staphylococcus aureus,* 10(13.0%) had *Escherichia coli*, 6(7.8%) had *Coagulase-negative staphylococci* and 8(10.4%) had *Providencia spp*. In the Reghu *et al.*[7] study, most of the isolated pathogens belonged to the genus *Staphylococcus* (20.1%), *Enterococcus* (14.3%) and *Pseudomonas* (13.6%). The organisms isolated from infected diabetic foot ulcers. Among the *Staphylococcus* species, *Staphylococcus aureus* and *Coagulase negative staphylococci* constituted 9.2% and 7.0% of the isolates, respectively. Among *Enterococcus* and *Pseudomonas* species, *Enterococcus faecalis* and *Pseudomonas aeruginosa* constituted 12.1% and 11.4% of the isolates, respectively. Other commonly isolated organisms were *Escherichia coli* (12.1%) and *Klebsiella pneumonia* (10.2%). The *Candida* species isolated included *Candida albicans* (2.6%), *Candida parapsilosis* and *Candida tropicalis* (0.7% each); and *Candida famata* and *Candida haemulonii* (0.4% each). Other organisms isolated included *Proteus mirabilis* (3.3%), *Acinetobacter baumannii* (1.8%), *beta haemolytic streptococci* (1.1%) and *Proteus vulgaris* (0.7%). Umadevi *et al.*[1] study observed that *Klebsiella pneumonia* was found 20.5%, *Pseudomonas aeruginosa* 17.0%, *Staphylococcus aureus* 17.0%, *Escherichia coli* 14.6%, Coagulase-negative staphylococci 7.0%, *Proteus mirabilis* 5.8%, *Enterococcus spp*. 5.3%, Citrobacter spp. 4.1, *Proteus vulgaris* 3.5%, *Acinetobacter spp*. 3.5%, *Pseudomonas spp*. 1.2%, *Providencia spp*. 0.6%. In study of Anand *et al.*[2] observed that on comparison of the bacterial isolate with the incident of amputation it was observed that 80% (4/5) of the patients in with Proteus mirabilis was isolated

underwent amputation, 57.12% (4/7) with Streptococcus pyogenes, 50% (1/2) with Pseudomonas aeruginosa, 33.3% (2/6) with Klebsiella pneumonia, 12% (2/16) with Staphylococcus aureus underwent amputation.

In this study observed that Klebsiella pneumonia organism 94.1% sensitivity to imipenem, 70.6% to amikacin, 70.6% to piperacillin-tazobactam, 64.7% to meropenem. In *Pseudomonas aeruginosa* organism 92.9% sensitivity to imipenem, 74.6% to piperacillin-tazobactam, 64.3% to amikacin, 57.1% to ciprofloxacin. In *Staphylococcus aureus* organism 63.6% sensitivity to tetracycline, 54.5% to eftriaxone. In *Escherichia coli* 100% sensitivity to imipenem, 70% to amoxicillin-clavulanic acid, amikacin, piperacillin-tazobactam. In *Coagulase-negative Staphylococci* 83.3% sensitivity to tetracycline, 66.7% to ceftriaxone. In *Proteus mirabilis* 100% sensitivity to tetracycline, amikacin, ceftriaxone, imipenem, piperacillin-tazobactam. In *Enterococcus spp*. 75.0% sensitivity to tetracycline. In *Citrobacter spp*. 100% sensitivity to imipenem. Reghu *et al*.[7] study reported that the antibiotic susceptibility pattern of the isolates is also essential for proper management of diabetic foot infections. Against gram positive organisms linezolid, teicoplanin, tigecycline and vancomycin showed >90% susceptibility. In their study, all Staphylococcus species isolated were susceptible to vancomycin, tigecycline, teicoplanin and linezolid and all of *Enterococcus* species susceptible to vancomycin. These antibiotics are highly effective against gram positive organisms isolated from this study and these antibiotics seem to be appropriate for empirical treatment of diabetic foot infections. *Coagulase negative staphylococcus* also showed 100% susceptibility to levofloxacin. Most of the gram positive organisms showed low susceptibility to erythromycin and Penicillin G. In the Reghu *et al*.[7] study, against *Pseudomonas* species, colistin and amikacin showed good susceptibility. However, against Klebsiella species, amikacin showed only 58% susceptibility. *Klebsiella*, *Escherichia coli* and *Acinetobacter* isolates were susceptible to colistin. Majority of the *Klebsiella* and *Acinetobacter* isolates were resistant to cefoperazone sulbactam, co-trimoxazole, and piperacillin tazobactam. Against *Escherichia coli*, meropenem and amikacin showed >80% susceptibility. *Proteus* species showed 100% susceptibility to amikacin and levofloxacin. *Acinetobacter* species showed complete resistance to levofloxacin. Management of gram negative infections is extremely challenging. Future studies should aim at identifying the risk factors for the development of these infections, so that appropriate treatment can be implemented early and can hence prevent fatal outcomes. The antibiotic susceptibility pattern of the gram negative bacteria isolated from diabetic ulcers. Against Candida species, amphotericin and fluconazole showed 83.3% and 90.9% susceptibility, respectively. Umadevi *et al.*[1] majority of isolates of *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to amikacin, piperacillin-tazobactam and imipenem, but resistant to other antibiotics tested except amoxicillin-clavulanic acid for which they were showing variable susceptibility. Similarly, most of our *Proteus* spp. were susceptible to tetracycline, ciprofloxacin, amikacin, ceftriaxone, piperacillin-tazobactam and imipenem, while being less susceptible to amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole and cefuroxime. However, *Proteus mirabilis* was relatively more susceptible than *Proteus vulgaris* to most antibiotics. *Citrobacter* spp. were susceptible to piperacillin-tazobactam, amikacin, ceftriaxone and imipenem, but resistant to other antibiotics tested.

Most of the *Pseudomonas aeruginosa* were susceptible to piperacillin-tazobactam and imipenem, while they were showing varying susceptibility to ciprofloxacin, gentamicin, amikacin and netilmicin. Similarly, majority of *Acinetobacter* spp. were susceptible to piperacillintazobactam, imipenem and trimethoprimsulfamethoxazole, while being less susceptible to gentamicin, amikacin, ciprofloxacin, tetracycline, ceftiaxone and ceftazidime. The antibiotic susceptibility patterns of the grampositive bacteria isolated from diabetic ulcers. *Staphylococcus* aureus were most often susceptible to erythromycin, tetracycline and vancomycin, but were relatively less susceptible to amoxicillin-clavulanic acid, trimethoprimsulfamethoxazole, ciprofloxacin, gentamicin and ceftriaxone. None of the *Staphylococcus aureus* were susceptible to

penicillin. Most of the *Enterococcus* spp. were susceptible only to vancomycin. However they showed varying susceptibility to tetracycline, penicillin, and ciprofloxacin. High-level aminoglycoside resistance was observed in 33% of the *Enterococcus* spp.[1] Yerat and Rangasamy[5] study observed that regarding the antimicrobial sensitivity pattern, we found that 57.14% of *S. aureus* were beta lactamase producer and 5 of the 21 isolates were methicillin-resistant *S. aureus* (MRSA) (23.80%). The GPC isolates were 100% sensitive to vancomycin while all the Gram-negative bacterial isolates were 100% sensitive to imipenem.

Conclusion

Common organism found in diabetic foot ulcer patients were *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Coagulase-negative staphylococci* and *Providencia* spp. In tetracycline, amikacin, ceftriaxone, imipenem, piperacillin-tazobactam were 100% sensitive in *Proteus mirabilis* and only imipenem found in *Escherichia coli* and *Citrobacter spp*.

Conflict of interest: none.

Reference

1. Umadevi S, Kumar S, Joseph NM, *et al*. Microbiological study of diabetic foot infections. Indian Journal of Medical Specialities 2011; **2(1):** 12-17.
2. Anand A, Biswal I, Soni RK, *et al*. A clinico-microbiological study of diabetic foot ulcer patients to identify risk factors and their correlation with prognosis in tertiary care hospital in India. Int Surg J. 2016; **3(2):** 669-673.
3. Aherrao N, Shahi SK, Dwivedi A, *et al*. Detection of anaerobic infection in diabetic foot ulcer using PCR technique and the status of metronidazole therapy on treatment outcome. *Wounds* 2012; **24:** 283-8.
4. Haldar J, Mukherjee P, Mukhopadhyay S, *et al*. Isolation of bacteria from diabetic foot ulcers with special reference to anaerobe isolation by simple two-step combustion technique in candle jar. *Indian J Med Res* 2017; **145:** 97-101.
5. Yerat RC, Rangasamy VR. A clinicomicrobial study of diabetic foot ulcer infections in South India. *Int J Med Public Health* 2015; **5:** 236-41.
6. Lipsky BA, Berendt AR, Cornia PB, *et al*. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2012; **54:** e132-73.
7. Reghu R, Padma UD, Sasankan V, *et al*. A Microbiological Study of Diabetic Foot Ulcer in a South Indian Tertiary Care Hospital. *Int. J. Pharm. Sci. Rev. Res.,* 2016; **37(1):** 167-170.
8. Zaine NH, Burns J, Vicaretti M, *et al*. Characteristics of diabetic foot ulcers in Western Sydney, Australia. *Journal of Foot and Ankle Research*. 2014; **7:** 39.
9. Gardner SE, Hillis SL, Heilmann K, *et al*. The neuropathic diabetic foot ulcer micro biome is associated with clinical factors. *Diabetes*. 2013; **62(3):** 923-30.
10. Posnett J, Franks PJ. The burden of chronic wounds in the UK. Nurs Times. 2008; 104: 44-5.
11. Perrin B. A retrospective audit of a diabetic foot clinic. *Australas J Podiatr Med*. 2006; **40:** 23-5.
12. Gadepalli R, Dhawan B, Sreenivas V, *et al*. A Clinico-microbiological Study of Diabetic Foot Ulcers in an Indian Tertiary Care Hospital. *Diabetes Care* 2006; **29:** 1727-32.