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Common Microorganisms Present in Diabetic Foot Infection and It's Spectrum of Antibiotic Sensitivity

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Abstract

Introduction: *Diabetic patients have foot problems secondary to neuropathy, micro-vascular changes and impaired resistance to infection. They are at increased risk of infection and ulceration. Diabetic ulcers are frequently involved in both aerobe and anaerobe microorganisms. It is important to culture the specimen on several different media. The Gram stain can provide valuable information regarding the range of organisms under consideration. The aim of this study is to identify the sensitive antibiotics to the specific aerobic bacteria, to prevent the random misuse of broad spectrum antibiotics and make awareness about diabetic foot care.* ***Materials and Methods:*** *This study was a retrospective analysis and was carried out in general surgery and diabetic wards at North Bengal Medical College Hospital (NBMCH), Sirajganj, Bangladesh from August, 2019 to July, 2020. To identify common aerobic microorganism and spectrum of antibiotic sensitivity from 54 cases of diabetic foot ulcers, wound swabs taken and cultured using standard aerobic microbiological techniques. Antibiotic sensitivity testing to different antimicrobial agents were carried out using the disc diffusion method.* ***Result:*** *Among 54 aerobic bacterial isolates, Gram negative bacteria 41(75.9%) and Gram positive bacteria 13(24.1%). Escherichia Coli 28(51.9%), Proteus spp 09(16.7%), Pseudomonas 03(5.6%), Klebsiella spp 01(1.9%) were isolated Gram negative bacteria. Staphylococcus aureus 13(24.1%) was only Gram positive bacteria. Gram negative bacterial isolates were sensitive to Imipenem, while Vancomycin showed good activity against Gram positive bacteria.* ***Conclusion:*** *The antibiogram results of this study suggest that bacteria remain sensitive to a limited number of used agents, while found resistant to a number of widely used agents. Imipenem was most effective against Gram negative bacilli and also effective against Gram positive cocci.*

Key words: *Diabetes, Imipenem, Foot ulcer.*

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Introduction:

Diabetes is a metabolic disorder of endocrine system. The incidence of diabetes is rising. Globally it is estimated that 415 million people had diabetes in 2015, which was about 10% of the total adult population. This figure is expected to reach 642 million by 2040[1] in 2017 an estimated 54.4 million(7.6%) people of working age in India had diabetes[2]. Each year over 700000 new cases are diagnosed; 12000 to 14000 of which are children, teenagers and young adults, while this life threatening disease can be controlled. Diabetes is often accompanied by serious complications, and still today there is no cure[3].

The life time risk of a patient with diabetes developing a foot ulcer may be as high as 25%[4]. Overall diabetes mellitus accounted for 83% of all major amputations related to foot ulcers[5]. Poorly controlled diabetes is prone to skin infections because elevated blood sugar reduces the effectiveness of bacteria fighting cells. Carbuncles, boils, and other skin infections may be hazardous if not properly treated. Even a small cut may progress to a deep, open sore, called an ulcer[6]. In most cases ulceration is a consequence of the loss of protective sensation that is, the loss of awareness of trauma that can cause the breakdown of the skin.

Diabetes is a major burden on health care facilities in all countries. Globally, in 2015, Diabetes caused 5 million death in those aged 20-79 years. Health care expenditure attributed to diabetes was estimated to be at least 673 billion US dollars or 12% of total healthcare expenditure[1].

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Bangladesh has a total population of more than 160 million and is among the countries with the highest number of people with diabetes worldwide. The international diabetes federation (IDF) estimated 8.4 million people with diabetes in Bangladesh and almost an equal number with undetected diabetes[7].

Diabetic patients have foot problems secondary to neuropathy, micro-vascular changes and impaired resistance to infection. They are at increased risk of infection and ulceration. Ulceration can lead to major morbidity and amputation. Ulcers need to be treated urgently. It is a surgical emergency and needs optimal management with urgent admission, radiological and clinical assessment, followed by debridement, antibiotics if required[8]. A great variety of organisms are involved in diabetic infections. Diabetic ulcers are frequently involved in both aerobe and anaerobe microorganisms. It is important to culture the specimen on several different media under different atmospheric conditions. The Gram stain can provide valuable information regarding the range of organisms under consideration[9].

Patient with diabetes are reported to have up to 50% higher perioperative mortality than patient without diabetes. Hyper-glycaemia impair wound healing and innate immunity, leading to increased risk of infection[1].

Diabetes with ulcers commonly experience infection with Gram positive organisms such as staphylococcus aureus, Enterococcus and Gram negative organisms like Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp. Proteus spp etc, and anaerobes[10]. Among the gram positive aerobes Staphylococci are more prevalent. Gram negative organisms were most frequently isolated(80%) bacteria[11]. Many of these microorganisms are developing resistance to commonly used antibiotics largely due to their indiscriminate use[12]. The present study was undertaken to determine the microbiology of the diabetic foot ulcers and the antimicrobial sensitivity pattern of the isolates. Swabs were collected from ulcers that were macroscopically examined and classified based on Wagner's method of evaluation[13,14].

Materials and Methods:

The study was a retrospective analysis, was carried out among a total number of 54 diabetic patients admitted with foot ulceration fulfilling the inclusion criteria. The study was carried out in general surgery ward and diabetic ward of North Bengal Medical College Hospital, Sirajganj, Bangladesh during the period of August 2019 to July 2020. Inclusion criteria were clinically diagnosed as diabetic foot ulcer in both sex. Exclusion criteria were foot ulcer due to other cause, diabetic foot ulcer with no growth in culture. Swabs were collected from ulcers that were macroscopically examined and classified based on Wagner's method of evaluation. Swabbing was done on slouphy or inflammed tissue as bacteria tend to present in greater number in these areas. From each patient two swabs were collected. The sterile cottons were moistened with sterile saline before collecting the specimens. One of the swabs was used for the isolation of bacteria. The other swab was used for wet mount microscopy.

For the isolation of bacteria the media used were blood agar, and Mac Conckey's agar, which were incubated at 37^{0}C for 48 hours. The organisms isolated were subjected to antibiotic susceptibility testing on Muller-Hinton agar using Kirbey-Bauer disc diffusion method. The study and procedure was explained to the patients and written consent was obtained from the patients. Hospital authority were informed about the study and permission was obtained. Detailed information were obtained in each cases according to protocol. Collected data was classified, edited, coded and entered into the computer for statistical analysis by using MS EXCEL.

Results:

Total 54 diabetic foot patients studied, 26 were males and 28 were females, the male: female ratio being 1:1.07. Their ages ranged from 8 years to 95 years with an average of 48 years. The maximum number of patients having diabetic foot infections belonged to the age group of 40-60 years.

Of the 54 patients, 30 patients had some other complications, such as peripheral vascular disease, neuropathy, nephropathy, retinopathy, cataract, ischemic heart disease or hypertension along with diabetes mellitus. Peripheral neuropathy has a central role and is present over 80% of diabetic patients with foot lesions. From the 54 patients studied, aerobic bacteria in the pure form were isolated in all the cases in which 13 were Staphylococcus aureus(24.1%), 28 were Escherichia Coli(51.9%), 01 Klebsiella spp(1.9%), 03 Pseudomonas aeruginosa(5.6%), 09 were Proteus spp(16.7%)(Table I).

Table-I: Isolation of aerobic bacteria.

Culture isolate	Number of isolates	Percentage %
Staphylococcus aureus (S. aureus)	13	24.1
Escherichia coli (E. coli)	28	51.9
Klebsiella spp	01	1.9
Pseudomonas aeruginosa	03	5.6
Proteus spp	09	16.7

Over 71.4% strains of Escherichia Coli were sensitive to Imipenem but only 3.6% of strains were sensitive to Ceftriaxone and none of strains is sensitive to Cefuroxime but 28.5% of strains were sensitive to Ciprofloxacin, 39.2% were sensitive to Amikacin (Table II).

Table-II: Antibiotic sensitivity pattern of Escherichia Coli.

Antibiotics	Escherichia Coli (n=28) Number of Sensitive Strains (%)
Amikacin	11 (39.2)
Cloxacillin	0
Netilmycin	17 (60.7)
Chloramphenicol	12 (42.8)
Ciprofloxacin	08 (28.5)
Cotrimoxazole	01 (3.6)

Antibiotics	Escherichia Coli (n=28) Number of Sensitive Strains (%)
Gentamycin	05 (17.8)
Ceftriaxone	01(3.6)
Teracycline	03 (10.7)
Amoxicillin	01 (3.6)
Cephalexin	01(3.6)
Cefuroxime	0
Erythromycin	03 (10.7)
Fusidic acid	04 (14.2)
Imipenem	20 (71.4)
Nitrofurantoin	10 (35.7)

Escherichia coli was highly sensitive to the antibiotics tested, Pseudomonas was highly resistant to them. Amikacin and Imipenem were highly sensitive to Pseudomonas but Gentamycin and Cefuroxime were resistant to them (Table III).

Table-III: Antibiotic sensitivity pattern of aerobic Gram negative bacteria.

Antibiotics	E. Coli n=28	Klebsiella spp. n=01	Proteus n=09	Pseudomonas n=03
Ciprofloxacin	08 (28.5)	0	02 (22.2)	-
Netilmycin	17 (60.7)	0	06 (66.7)	02 (66.7)
Cefuroxime	0	0	0	0
Ceftriaxone	01 (3.6)	01 (100)	01 (11.1)	01 (33.3)
Amikacin	11 (39.2)	0	05 (55.6)	03 (100)
Imipenam	20 (71.4)	0	06 (66.7)	03 (100)
Chloramphenicol	12 (42.8)	01 (100)	03 (33.3)	02(66.7)
Gentamycin	05 (17.8)	0	03 (33.3)	0
Erythromycin	03 (10.7)	0	0	0
Antibiotics	**E. Coli n=28**	**Klebsiella spp. n=01**	**Proteus n=09**	**Pseudomonas n=03**
Co-trimoxazole	01 (3.6)	0	01 (11.1)	0
Tetracycline	03 (10.7)	0	01 (11.1)	0
Amoxicillin	01 (3.6)	0	0	0
Cephalexin	01 (3.6)	0	01 (11.1)	0
Fusidic acid	04 (14.2)	0	0	0
Nitrofurantoin	10 (35.7)	0	01 (11.1)	0

Over 69% strains of Staphylococcus aureus were sensitive to Vancomycin, Chloramphenicol and Amikacin. But none of strains were sensitive to Ceftriaxone and Cefuroxime. Only 38.5% of strains were sensitive to ciprofloxacin and 61.5% of strains were sensitive to Imipenem and only 15.4% sensitive to Cloxacillin (Table IV).

Table IV: Antibiotic sensitivity pattern of the aerobic Gram positive isolates

Antibiotics	Staphylococcus aureus (n=13 number of sensitive strains (percentage)
Amikacin	09 (69.2)
Amoxicillin	02 (15.4)
Netilmycin	08 (61.5)
Chloramphenicol	09 (69.2)
Ciprofloxacin	05 (38.5)
Cotrimoxazole	02 (15.4)
Gentamycin	05 (38.5)
Cefuroxime	0
Teracycline	05 (38.5)
Ceftriaxone	0
Erythromycin	02 (15.4)
Vancomycin	09(69.2)
Cloxacillin	02(15.4)
Fusidic acid	08 (61.5)
Imipenem	08 (61.5)
Nitrofurantoin	05 (38.5)

Discussion:

The presence of Staphylococcus aureus(S.aureus), Escherichia coli(E. coli) and other aerobic Gram negative bacilli in septic complications of infected diabetic feet have been reported in various studies. The infections are usually polymicrobial in nature, caused by aerobic Gram- positive S. aureus, and by Gram- negative bacilli like E. coli, Klebsiella species and Proteus[15]. Of the isolates; 75.9% were found to be Gram negative while 24.1% were Gram positive bacteria. This corresponds with the findings of Bansal et al, in which 76% of the microbes were Gram negative and 24% were Gram positive[16]. Pseudomonas aeruginosa was the most common isolate, accounting for 21.67%, followed by Staphylococcus aureas, E.Coli and Klebsiella comprising 18.88%, 18.18% and 16.78% respectively[16]. In the present study, E. coli (51.9%) was predominantly isolated. As regards the other aerobic Gram negative bacilli, Pseudomonas aeruginosa (5.6%), Proteus (16.7%) and Klebsiella spp (1.9%) and Gram positive Staphylococcus aureus (24.1%) were the only Gram positive cocci isolated.

Aminoglycosides are bactericidal drugs especially useful against many Gram negative rods. Amikacin and Gentamycin are most useful drugs against Gram negative rods such as E. coli, Pseudomonas[9]. Hefni AAH, et al[17], found 100% sensitivity of Pseudomonas and E. coli to both Amikacin and Gentamycin. In this study ,Sensitivity of E. coli to Amikacin was 39.2%, where Pseudomonas was 100% sensitive to Amikacin. Sensitivity of E. coli to Gentamycin was 17.8% but Pseudomonas was resistant to this drug.

Cephalosporins are B-lactam drugs. The first generation Cephalosporins are active primarily against Gram positive cocci. Second ,third and fourth generations having expanded coverage against certain Gram negative rods[9]. Journal of Diabetes and Metabolic disorder 2020 shows, most of the organisms are resistant to all forms of Cephalosporins[18]. In this study, E. Coli was not sensitive to Cefuroxime but only 3.6% were sensitive to Ceftriaxone. Pseudomonas was also resistant to Cefuroxime but only 33.3% sensitive to Ceftriaxone. Staphylococcus aureus was resistant to both Cefuroxime and Ceftriaxone.

Carbapenems are Beta lactam drugs that are structurally different from Penicillins and Cephalosporins. Imipenem, the currently used carbapenem, has the widest spectrum of activity of the beta lactam drugs and has excellent bactericidal activity against many Gram positive, Gram negative and anaerobic bacteria[9]. According to Gadepalli R et al Imipenem was found 100% sensitive to all types of organisms.19 In this study, E. coli was 71.4%, Pseudomonas was 100% sensitive to Imipenem but Staphylococcus aureus was 61.5% sensitive to Imipenem.

Vancomycin is a bactericidal agents, effective against certain Gram positive bacteria. It's most important use is in the treatment of infections by Staphylococcus aureus strains, that are resistant to the Penicillinase-resistant penicillins[8]. In a study Nageen A showed, Vancomycin is sensitive to Staphylococcus in 49.47% cases[20]. In this

study, Staphylococcus aureus was 69.2% sensitive to Vancomycin.

Among the 28 isolates of E. coli, Imipenem, Netilmycin and Amikacin showed a higher sensitivity than to commonly used Cephalosporin and Quinolone group of antibiotics. 09 isolates of Proteus were resistant to major group of antibiotics but a high sensitivity pattern was shown towards Netilmycin and Imipenem. In Pseudomonas the total isolates were resistant to the most commonly used antibiotics like ciprofloxacin, Cefuroxime, Gentamycin but all were sensitive to Amikacin and the beta lactum antibiotic Imipenem. Only one Klebsiella spp was found and sensitive to Ceftriaxone.

Among the 13 isolates of Staphylococcus aureus, Vancomycin, Chloramphenical, Amikacin, Netilmycin and Imipenem showed a higher sensitivity than to commonly used Cloxacillin, Cephalosporin and quinolone group of antibiotics.

Conclusion:

54 aerobic bacteria were isolated from diabetic foot ulcer. Escherichia coli and Staphylococcus aureus were the most common bacteria isolated. Most of the Gram negative and Gram positive isolates were sensitive to Imipenem, which was resistant to inactivation by most bacterial beta lactamases. So, it has the widest spectrum of antibacterial activity. Imipenem should therefore be used as a monotherapy against polymicrobial infections. It produces a better response comparable to that of second and third generation cephalosporins. Though, most of the bacterial isolates were sensitive to Imipenem, it is matter of great regret that, the widely used antibiotics such as Cephalosporin and Quinolone groups were resistant to most of the bacterial isolates. It shows the terrible picture of antibiotic resistance in our community which is a great danger for future. So, irrational use of antibiotics must be prohibited and make awareness among the mass people. All diabetic patients should be prescribed and educated about foot care to prevent diabetic foot infection.

Conflict of Interest: None.

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References:

1. Pearson ER, McCrimmon RJ. Diabetes mellitus. In: Ralston SH, Penman ID, Strachan MWJ, Hobson RP, editors. Davidson's Principles and practice of Medicine. 23rd ed. Edinburgh: Elsevier ltd; 2018: 719-762.

2. Zomer E, Banker KK, Liew D, Ademi Z, et al. The impact of diabetes on productivity in India. Diabetes care. 2021; 44(12): 2714-2722.
https://doi.org/10.2337/dc21-0922
PMid:34675058

3. Benjamin A, Lipsky, B.A., Roger E, et al. Outpatient management of uncomplicated lower-extremity infection in diabetic patients. Arch Intern Med. 1990; 150(4): 790-797. https://doi.org/10.1001/archinte.1990.00390160058013
PMid:2183732

4. Boulton AJM. The pathway to foot ulceration in Diabetes. Med Clin N Am. 2013; 97(5): 775-790. https://-doi.org/10.1016/j.mcna.2013.03.007
PMid:23992891

5. Hicks CW, Selvarajah S, Mathiousdakis N, at el. Burden of Infected Diabetic Foot Ulcers on Hospital Admission and Costs. Annals of Vascular Surgery. 2016; 33: 149-158. https://doi.org/10.1016/j.avsg.2015.11.025
PMid:26907372 PMCid:PMC6048950

6. Boulton AJM. The diabetic foot: a global view. Diabetes Metab Res Rev. 2000; 16: 52-55. https://-doi.org/10.1002/1520-7560(200009/10)16:1+<::AID-DM-RR132>3.0.CO;2-T

7. Whiting DR, Guariguata L, Weil C, Shaw J. Global estimates of the prevalence of diabetes for 2011 and 2030. IDF Diabetes Atlas. 2011; 94(3): 311-321.
https://doi.org/10.1016/j.diabres.2011.10.029
PMid:22079683

8. Sharp B. Foot and Ankle. In: Williams SN, O'Connell PR, McCaskie AW, editors. Bailey & Love's Short Practice of Surgery. 27th ed. Boca Raton, FL: CRC Press; 2018: 524-533.

9. Levinson W, Jawetz E. Medical Microbiology & Immunology: Examination & Board Review. 7th ed. The McGraw-Hill, United States of America; 2002: 53-72.

10. Shankar EM, Mohan V, Premalatha G, et al. Bacterial aetiology of diabetic foot infections in South India. Eur J Intern Med. 2005; 16: 567-570.
https://doi.org/10.1016/j.ejim.2005.06.016
PMid:16314237

11. Paul S, Barai L, Jahan A, Haq JA. A bacteriological study of diabetic foot infection in an urban tertiary care Hospital in Dhaka city. Ibrahim med. Coll. J. 2009; 3(2): 50-54. https://doi.org/10.3329/imcj.v3i2.4216

12. Banu A, Noorul Hassan MM, Rajkumar J, et al. Prospective study of multidrug Resistant Bacteria causing Diabetic foot ulcers in South India. Journal of Science. 2015; 5(8): 626-629.

13. Romani A, Ramani R, Shivananda PG, Kundaje GN. Bacteriology of Diabetic Foot Ulcers. Indian J Pathol Microbiol. 1991; 34(2): 81-87.

14. Robert G, Frykberg. Diabetic Foot Ulcers: Pathogenesis and Management. Am Fam Physician. 2002; 66(9): 1655-1663.

15. Benwan KA, Mulla AA, Rotimi VO. A Study of the Microbiology of Diabetic foot infections in a teaching Hospital in Kuwait. Journal of Infection and Public Health. 2012; 5(1): 1-8. https://doi.org/10.1016/j.jiph.2011.07.004
PMid:22341838

16. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol. 2008; 51(2): 204-208.

https://doi.org/10.4103/0377-4929.41685

PMid:18603682

17. Hefni AAH, Ibrahim AMR, Attia KM, et al. Bacteriological study of diabetic foot infection in Egypt. JASMR. 2013; 8: 26-32.

https://doi.org/10.4103/1687-4293.123792

18. Hamid MH, Arbab AH, Yousef BA. Bacteriological profile and antibiotic susceptibility of diabetic foot infections at Ribat University hospital; a retrospective study from Sudan. Journal of Diabetes and Metabolic Disorder. 2020; 19(2): 1397-1406.

https://doi.org/10.1007/s40200-020-00660-8

PMid:33553032 PMCid:PMC7843840

19. Gadepalli R, Kapil A, Dhawan B, et al. A clinico-microbiological study of diabetic foot ulcers in an Indian Tertiary care Hospital. Diabetes Care. 2006; 29(8): 1727-1732. https://doi.org/10.2337/dc06-0116

PMid:16873771

20. Nageen A. The most prevalent organism in diabetic foot ulcers and its Drug sensitivity and resistance to different standard antibiotics. J Coll Physicians Surg Pak. 2016; 26(4): 293-296.