Diabetic foot infection: Bacteriological causes and antimicrobial therapy

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Abstract: Background: Diabetic foot is one of the most serious complications of diabetes and is the leading cause of hospitalization in diabetic patients. This study was done to determine the common aerobic bacterial causes of diabetic foot infections and there *in vitro* antibiotic susceptibility pattern in Sohag University hospitals. **Methods:** A prospective study was performed over a period of one year in Sohag University Hospitals. The aerobic bacterial agents were isolated and their antibiotic susceptibility pattern was determined by the disc diffusion method. Members of Enterobacteriaceae were tested for extended spectrum ß-lactamase (ESBL) production by combination disc method and staphylococcal isolates were tested for susceptibility to oxacillin by screen agar method. **Results:** *Escherichia coli* (20.3%), *Klebsiella pneumoniae* (17.4%), *Staphylococcus aureus* (16.2%), and *Pseudomonas aeruginosa* (12.6%) were the most common bacterial causes of DFIs. Polymicrobial infection was observed in 39.1% of the patients. The members of Enterobacteriaceae as well as Pseudomonas spp. and Acinetobacter spp. were found to be susceptible mainly to imipenem, levofloxacin and amikacin. *Staphylococcus aureus* and Enterococcus spp. were susceptible mostly to vancomycin, levofloxacin, and ciprofloxacin with varying susceptibility to tetracycline. 80% of the isolates belonging to Enterobacteriaceae were producing ESBL and 67% of *Staphylococcus aureus* were methicillin-resistant. **Conclusion:** High prevalence of multi-drug resistant pathogens was observed. imipenem, levofloxacin and amikacin were active against gram-negative bacilli, while vancomycin, levofloxacin and ciprofloxacin were found to be active against gram-positive bacteria

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1. Introduction

Diabetic foot is one of the most serious complications of diabetes and is the leading cause of hospitalization in diabetic patients. Diabetic foot is characterized by several pathological complications such as peripheral vascular disease, neuropathy, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even limb amputation [1,2]. Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration [3]. Diabetic ulcers have 15 to 45 times higher risk of limb amputation than foot ulcers due to other causes [4]. Every year more than a million diabetic patients require limb amputation worldwide [1].

The impaired circulation in patients with diabetic foot infections limits the access of phagocytes favoring development of infection [2,5]. *Escherichia coli*, Proteus spp., Pseudomonas spp., *Staphylococcus aureus* and Enterococcus spp. are the most frequent pathogens contributing to progressive and widespread tissue destruction [2,5]. Diabetic foot infections are often polymicrobial [4,5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been commonly isolated from 30% of the diabetic wounds [6-8]. The increasing association of (MDROs) multi-drug resistant organisms with diabetic foot ulcers increases the risk of limb amputation [9]. Infection with MDROs is also responsible for the increased duration of hospitalization, cost of management, morbidity and mortality of the diabetic patients [5]. Appropriate selection of antibiotics based on the antibiogram of the isolates from the lesions is most critical for the proper management of these infections. Nevertheless, the initial empirical therapy is often decided based on the knowledge of the susceptibility profile of the prevalent microbial flora recovered from the previous cases.

Aim of the work:

To diagnose the bacteriological causes and antibiotic susceptibility patterns of the organisms isolated from diabetic foot infections in Sohag University Hospitals.

2. Patients and methods:

Samples for bacterial culture were collected from 120 patients admitted, with diabetic foot infections, to Surgery department in Sohag University Hospitals from January 2011 to December 2011. To avoid the isolation of colonizing (rather than pathogenic) flora, the investigators were instructed to first clean and debride all foot wounds and to obtain specimens by tissue biopsy, wound curettage, or aspiration rather than swab technique. The specimens

were taken immediately to the microbiology laboratory and processed without any delay. Gangerous infections and specimens suggestive of anaerobic infections were excluded from the study. The specimens were subjected to Gram staining and were simultaneously inoculated on blood agar and MacConkey agar for isolation of aerobic bacteria. After 24 hours incubation at 37°C, the bacterial isolates were identified based on standard bacteriological methods [10] and by using the biochemical reaction strips Microbact, oxoid and API, Bimourieux. Gram-negative bacilli were tested for extended spectrum β-lactamase (ESBL) production by double disc diffusion method. Staphylococcal isolates were tested for susceptibility to oxacillin by screen agar method and disc diffusion method. Anti-microbial susceptibility testing of aerobic isolates was performed by the standard disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards [11].

Imepenem, Ciprofloxacin, Levofloxacin, Cefotaxime, Ceftazidime, Ceftriaxone, Amikin, Gentamycin, Amoxy-clav, Tetracycline, Piperacillin, Sulph-trimethoprim, Cefuroxime were tested for gram-negative bacteria.

Penicillin, Amoxicillin-clavulanic acid, Erythromycin, Trimethoprim-sulfamethoxazole, Tetracycline, Ciprofloxacin, Levofloxacin, Gentamicin, Ceftriaxone, Oxacillin, Vancomycin, Cefotaxime, and Ceftazidime were tested for Staphylococcus species and Enterococcus species.

MRSA, vancomycin-resistant enterococci (VRE), Gram-negative bacilli producing ESBL, MDR *P. aeruginosa* (resistant to =3 anti-pseudomonal classes of antimicrobial agents) and MDR *Acinetobacter spp.* (resistant to =3 classes of antimicrobial agents) are defined as multi-drug resistant (MDR) pathogens [12- 14].

Combination disc method using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid was performed for detection of extended spectrum ß-lactamase (ESBL) among the members of Enterobacteriaceae [15]. Five mm or more increase in zone of inhibition for either cefotaxime-clavulanic acid or ceftazidime-clavulanic acid disc compared to the cefotaxime or ceftazidime disc respectively was taken as confirmatory evidence of ESBL production.

Staphylococcus aureus isolates were screened for methicillin resistance using oxacillin-salt screen agar containing 6µg/mL oxacillin and 4% NaCl according to CLSI guidelines [11].

3. Results:

Of the 120 patients with diabetic foot infection, 90 (75%) were males and 30 (25%) were females. The age ranged from 40 to 75 years with mean age being 54.0 ±3.5 years. A total of 167 bacterial isolates were isolated from these 120 patients. The bacteria isolated from the diabetic foot infections are summarized in Table (1). In 73 (60.9 %) patients only one pathogen was isolated, while in 47 (39.1 %) patients two pathogens were isolated. Gram-positive organisms were found as the only isolate in 29 (24.1 %) patients, while 44 (36.6 %) patients had only gram-negative organisms. The remaining 47 patients (39.1 %) had both gram-positive and gram-negative organisms. The ratio of gram-negative to gram-positive organisms isolated from diabetic foot infections was 2.2: 1.0 .Gram-negative bacteria accounted for 69.5 %, while gram-positive bacteria accounted for 30.5 %.

Table (1) Bacteria isolated from diabetic foot infections (167 isolates)

No.	Bacteria	No. of isolates (%)
1	*Escherichia coli*	35 (20.3)
2	*Klebsiella pneumoniae*	29 (17.4)
3	*Staphylococcus aureus*	27 (16.2)
4	*Pseudomonas aeruginosa*	21 (12.6)
5	Coagulase-negative staphylococci	13 (8.4)
6	*Enterococcus* spp.	11 (6.6)
7	*Proteus mirabilis*	9 (5.4)
8	*Proteus vulgaris*	8 (4.8)
9	*Citrobacter* spp.	6 (3.6)
10	*Acinetobacter* spp.	5 (3.0)
11	*Providencia* spp.	3 (1.7)

The sensitivity of the isolated gram-negative bacteria to commonly used antibiotics is summarised in Table 2. Majority of isolates of *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to imipenem, levofloxacin and amikacin, but resistant to other antibiotics tested except pipracillin for which they were showing variable susceptibility. Similarly, most of our Proteus spp. were susceptible to imepenem, ciprofloxacin, levofloxacin, ceftriaxone, amikin, and cefuroxime, with the observation that *Proteus vulgaris* is less sensitive to ciprofloxacin and levofloxacin. Citrobacter spp. and Providencia spp. were susceptible to imipenem, ceftriaxone, and cefuroxime but resistant to other antibiotics tested. Most of *Pseudomonas aeruignosa* and Acinetobacter spp. were sensitive to imepenem and amikin.

Table (2): The sensitivity pattern of Gram-negative bacteria isolated from diabetic foot infections:

Bacteria (no of isolates)	Sensitivity pattern (No- %)												
	IMP	CIP	LEV	CTX	CAZ	CRO	AK	CN	AC	TE	SXT	PIP	CUR
Enterobacteriaceae													
E. coli (35)	32-94	16-47	20-59	8-23.5	8-23.5	13-38	20-59	17-50	11-32	11-32	14-41	20-59	11-32
K. pneumoniae (29)	26-90	15-52	20-69	7-24	7-24	3- 10	22-76	11-38	9-31	9-31	11-38	15-52	10-34.5
Proteus mirabilis(9)	9-100	8-89	8-89	2- 22	2-22	7-77.7	8-89	7-77.7	1-11	2-22	5-55.5	6-66.6	8- 89
Proteus vulgaris (8)	8-100	6-75	5-62.5	1-12.5	1-12.5	5-62.5	5-62.5	4-50	0-0	2-25	2-25	4-50	7-87.5
Citrobacter sp. (6)	6-100	2-33	2-33	0- 0	0- 0	4-66.6	5-83	2-33	1-17	1-17	3-50	3-50	5- 83
Providencia sp (3)	3-100	1-33	2-66.5	0- 0	0- 0	3-100	0- 0	1-33	0-0	0-0	1-33	1-33	2-66.5
Non fermenters													
Pseudomonas aeurginosa (21)	17-81	9-43	12-57	0- 0	2-9.5	0- 0	15-71.5	12-57	0-0	0-0	0- 0	5-24	0- 0
Acinetobacter sp. (5)	4-80	1-20	1-20	1- 20	1-20	0- 0	3-60	1-20	0-0	0-0	1-20	3-60	2- 40

IMP= imepenem, CIP= Ciprofloxacin, LEV= Levofloxacin, CTX= Cefotaxime, CAZ= Ceftazidime, CRO= Ceftriaxone, AK= Amikin, CN= Gentamycin, AC= Amoxy-clav, TE= Tetracycline, PIP= piperacillin, SXT= Sulph-trimethoprim, CUR= Cefuroxime

The antibiotic susceptibility patterns of the gram-positive bacteria isolated from diabetic foot infections are shown in Table 3. *Staphylococcus aureus* were most often susceptible to Ciprofloxacin, Levofloxacin, and Vancomycin, but were relatively less susceptible to Amoxicillin-clavulanic acid, Erythromycin, Trimethoprim-sulfamethoxazole, Tetracycline, Gentamicin, Ceftriaxone, Oxacillin, Cefotaxime, and Ceftazidime. 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, Ciprofloxacin, and Levofloxacin. High-level aminoglycoside resistance was observed in about 20% of the *Enterococcus* spp.

Table (3): The sensitivity pattern of Gram-positive bacteria isolated from diabetic foot infections:

Bacteria (no of isolates)	Sensitivity pattern (No - %)												
	P	AC	E	SXT	TE	CIP	LEV	CN	CRO	OX	VA	CTX	CAZ
Staphylococcus aureus (27)	0-0	12-44.5	15-55.5	13-48	15-55.5	19-70	19-70	12-44.5	16-59	9-33.3	27-100	13-48	13-48
Coagulase-negative staphylococci (13)	3-23	4-30	9-69	6-46	7-54	10-77	11-85	8-61.5	9- 69	11-85	13-100	6- 46	7- 54
Enterococcus sp. (11)	5-45.5	2-18	5-45.5	3-27	7-64	8-73	8-73	9-82	2- 18	3-27	3-27	10-91	4- 36

P= Penicillin, A/C= Amoxy-Clav, E= Erythromycin, SXT= Sulph-trimethoprim, TE= Tetracycline, CIP= Ciprofloxacin, LEV= Levofloxacin, CN= Gentamycin, CRO= Ceftriaxone, OX= Oxacillin, VA= Vancomycin CTX= Cefotaxime, CAZ= Ceftazidime.

Eighteen of the 27 (66.6%) *Staphylococcus aureus* were resistant to oxacillin and were therefore considered as methicillin resistant *Staphylococcus aureus* (MRSA). ESBL production was detected in 71 of the 89 (80%) isolates belonging to Enterobacteriaceae. *Proteus* spp. (14 out of 17 isolates, 82%), *Klebsiella pneumoniae* (22 out of 29 isolates, 76%) and *Escherichia coli* (26 out of 3 isolates, 76%) were frequently ESBL producers. Twenty six multi-drug resistant non-fermenting gram-

negative bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp. were observed in our study.

4. Discussion:

DFIs are a major and increasing problem worldwide. In the United States about 25% of the more than 18 million diabetic patients develop foot ulcerations during their lifetimes, and over half of these become infected [16]. Diabetic patients often have chronic non-healing foot ulcers due to several underlying factors such as neuropathy, high plantar pressures and peripheral arterial disease [17]. Such chronic long-standing ulcers are more prone for infection which further delays the wound healing process. A wide range of bacteria can cause infection in these patients. To avoid selective antibiotic pressure that causes the development of resistance, most authorities give the treatment only for clinically infected wounds and use the narrowest-spectrum therapy possible [18]. On the other hand, failure to treat appropriately patients with these potentially limb-threatening infections can result in a poor outcome. Our study shows that in patients with DFIs who have not recently received antibiotic therapy, these infections are mainly caused by single organism which is usually gram-negative, so the treatment is much easier if given guided by culture and sensitivity. Also, less than half of these infections are caused by mixed gram-negative and gram-positive species in ratio of 2.2:1.

As mentioned above, in this study, gram-negative bacteria were the predominant pathogens, *E. coli* being the commonest aetiological agent, followed by *Klebsiella pneumonia, Pseudomonas aeruginosa* and then *Staphylococcus aureus* as the commonest garm-positive bacteria. Similarly, in two recent studies, gram-negative bacteria were the commonest agents [5,7]. But other earlier studies have documented gram-positive bacteria as the predominant organisms associated with diabetic foot infections [17, 19, 20]. Therefore, there seems to be a changing trend in the organisms causing diabetic foot infections, with gram-negative bacteria replacing gram-positive bacteria as commonest agents. Polymicrobial infection was observed in 39.1 % patients, which is less than other studies [2,4,7, 20]. This is because in our study we isolate only aerobic bacteria, with the fact that, polymicrobial infections in diabetic foot ulcers contain anaerobes.

Our study demonstrates the large number and variety of organisms that can be isolated from properly obtained specimens that are optimally processed. While many factors must be considered, including previous antibiotic therapy, knowledge of the usual causative organisms in these infections and their antibiotic susceptibilities will allow clinicians to make informed choices. Certainly, empirical antibiotic therapy should include coverage for oxacillin-susceptible *S. aureus* or for MRSA in a patient with risk factors for infection with this pathogen. Because specimens from many patients with diabetic foot infections have polymicrobial cultures, empirical therapy should be relatively broad spectrum, especially for patients with severe infections and those who are immunocompromised. The antimicrobial susceptibility data from our study suggest that Imepenem, Levofloxacin or Amikin would be appropriate single agents for empirical coverage (except for MRSA). In our study, most of the isolated gram-negative bacteria are sensitive to amikin, this may be due to the decrease in the use of amikin in diabetic patients because of its nephrotoxic effect. Because of the high rates of resistance among staphylococci, the use of fluoroquinolones alone might be inadequate and infections with these organisms may require vancomycin as all the isolated staphylococci are sensitive to vancomycin. *Staphylococcus aureus* isolates in our study were found to be uniformly susceptible to vancomycin, but were often resistant to most other antibiotics except levofloxacin, ciprofloxacin and ceftriaxone. Moreover 67 % of them were MRSA. This is very high compared to various other studies on diabetic foot infections which have reported only 10 – 44% MRSA [5-8]. Most of the *Enterococcus* spp. were susceptible only to vancomycin, though they showed varying susceptibility to other antibiotics. Similarly, in another study all enterococcal isolates were noted to be uniformly susceptible to vancomycin and linezolid [5, 20]. Hence, vancomycin can be considered as an important drug in the empirical regimen for treatment of diabetic foot infections especially in settings with high resistance to other antibiotics.

The main limitation of this study is the failure to detect the anaerobic bacteria. Moreover, the risk factors for the occurrence of MDR pathogens and the production of ESBL have not been well studied. Anaerobic bacteria especially in necrotizing fasciitis will be studied in another thesis.

A combination regimen consisting of amikacin, floroquinolone, or imipenem and vancomycin seems to be the most appropriate empirical treatment of diabetic foot infection. This empirical therapy can be later modified appropriately based on the antibiogram of the isolates from the individual patients.

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5. References:

1- Khanolkar MP, Bain SC, Stephens JW. The diabetic foot. QJM, 2008; 101: 685-95.
2- Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M, *et al*. Bacteriology of diabetic foot lesions. Indian J Med Microbiol., 2004; 22: 175-8.
3- Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA, 2005; 293: 217-28.
4- Alavi SM, Khosravi AD, Sarami A, Dashtebozorg A, Montazeri EA. Bacteriologic study of diabetic foot ulcer. Pak J Med Sci., 2007; 23: 681-4.
5- Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A Clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care, 2006; 29: 1727-32.
6- Tentolouris N, Jude EB, Smirnof I, Knowles EA, Boulton AJ. Methicillin-resistant *Staphylococcus aureus*: an increasing problem in a diabetic foot clinic. Diabet Med., 1999; 16:767-71.
7- Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot infections in South India. Eur J Intern Med., 2005; 16: 567-70.
8- Goldstein EJ, Citron DM, Nesbit CA. Diabetic foot infections. Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. Diabetes Care, 1996; 19: 638-41.
9- Yoga R, Khairul A, Sunita K, Suresh C. Bacteriology of diabetic foot lesion. Med J Malaysia, 2006; 61:14-6.
10- Mackie TJ, McCartney JE. Practical medical microbiology. 14th ed. New York: Churchill Livingstone; 1996.
11- National Committee for Clinical Laboratory Standards: *Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Standard*. M100-S12, vol. 22, no. 1. Villanova, PA, National Committee for Clinical Laboratory Standards, 2002.
12- Siegel JD, Rhinehart E, Jackson M, Chiarello L. Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in healthcare settings, 2006; [cited 2010 Aug 27]. Available from: http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroGuideline2006.pdf.
13- Hachem RY, Chemaly RF, Ahmar CA, Jiang Y, Boktour MR, Rjaili GA, *et al*. Colistin is effective in treatment of infections caused by multidrug-resistant Pseudomonas aeruginosa in cancer patients. Antimicrob Agents Chemother 2007; 51: 1905-11.
14- Davis KA, Moran KA, McAllister CK, Gray PJ. Multidrug-resistant Acinetobacter extremity infections in soldiers. Emerg Infect Dis., 2005; 11: 1218-24.
15- Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved standard, 9th ed. CLSI document M2-A9. Wayne, PA: CLSI; 2006.
16- Lavery, L. A., D. G. Armstrong, R. P. Wunderlich, M. J. Mohler, C. S. Wendel, and B. A. Lipsky**.** 2006. Risk factors for foot infections in individuals with diabetes. Diabetes Care 29**:**1288-1293.
17- Mantey I, Hill RL, Foster AV, Wilson S, Wade JJ, Edmonds ME. Infection of foot ulcers with Staphylococcus aureus associated with increased mortality in diabetic patients. Commun Dis Public Health, 2000; 3: 288-90.
18- Lipsky, B. A., D. G. Armstrong, D. M. Citron, A. D. Tice, D. E. Morgenstern, and M. A. Abramson**.** 2005. Ertapenem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): prospective, randomised, controlled, double-blinded, multicentre trial. Lancet 366**:**1695-1703. 61.
19- Dang CN, Prasad YD, Boulton AJ, Jude EB. Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet Med., 2003; 20: 159-
20- Sivaraman Umadevi, Shailesh Kumar, Noyal Mariya Joseph, Joshy M Easow, G Kandhakumari, Sreenivasan Srirangaraj, Sruthi Raj, Selvaraj Stephen. Microbiological Study of Diabetic Foot Infections. Indian Journal of Medical Specialities, 2010; ISSN: print - 0976-2884.