

Isolation and Identification of bacterial causes from diabetic foot ulcers

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Abstract

This study was employed to isolate and identify aerobic bacterial species of diabetic foot ulcers in diabetes mellitus patients. The isolated bacteria were also examined for their susceptibility to commonly used antibiotics. Polymicrobial infections were observed in 15(50%) of patients, single etiology in 14(46.6) of patients and 1 case was culture negative of the isolated bacteria. *Staphylococcus aureus* was the commonest 21(70%) followed by *Escherichia coli* 11(36.6) isolates.

Meropenem, ciprofloxacin, gentamicin were the most effective antibiotics against tested isolates.

Key words: Diabetes, foot, bacterial infections, antibiotics susceptibility.

Introduction

Diabetic foot ulceration and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries [1, 2]. Fifteen percent of all diabetic develop a foot ulcer at some point in their lives which is highly susceptible to infections and that spread rapidly, leading to tissue destruction and subsequent amputation [3]. The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection [4].

Patients with diabetes are susceptible to foot infection because of neuropathy, vascular insufficiency, and diminished neutrophil function [5]. Peripheral neuropathy has a central role in the development of a foot infection and it occurs in about 30 to 50 percent of patients with diabetes. Patients with diabetes lose the protective sensations for temperature and pain, impairing awareness of trauma such as abrasions, blistering, or penetrating foreign body [5]. Motor neuropathy can result in foot deformities that contribute to local pressure from footwear, making skin ulceration even more likely. Once the skin is broken (typically on the plantar surface), the underlying tissues are exposed to colonization by pathogenic organisms. The resulting wound infection may begin superficially, but with delay in treatment and impaired body defense mechanisms caused by neutrophil dysfunction and vascular insufficiency, it can spread to the contiguous subcutaneous tissues and to even deeper structures [6]. So the present study is designed to isolate and identify bacterial causes in diabetic patients and assess their susceptibility to antibiotics.

Patients and Methods

A total of 30 hospitalized diabetic untreated patients (that not taken antibiotics previously) with foot infections of both sexes from Al-Mawani General Hospital, Basrah, Iraq, were screened between September 2011 and April 2012. Samples were included Pus or discharges from the ulcers base and debrided necrotic tissues were collected by deep swab.

Isolation and identification

The specimens were inoculated to blood agar and MacConkey agar for isolation of aerobic bacteria.

After 24 hours incubation at 37°C the bacterial isolates were subjected to Gram's staining and identified by conventional biochemical tests [7, 8].

Susceptibility testing

Antibiotics susceptibility testing was performed by Kirby and Bauer's disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines [9]. The used antibiotics were: Cefotaxime, Cephalexin, Ceftazidime, Ciprofloxacin, Cloxacillin, Carbencillin, Meropenem, piperacillin, Gentamicin, Augmentin, Kanamycin, Lincomycin, Metronidazole.

Results and Discussion

A total of 30 hospitalized untreated patients with diabetic foot infections of both sexes were examined from September 2011 till April 2012.

From the 30 diabetic foot patients, 14(46.6%) were males and 16(53.3%) were females. The age ranged from 27 to 70 years, body mass index (BMI) 18.4 - 33kg / m², duration of diabetes mellitus ranged between 3 to 16 years. A total of 43 bacteria were isolated from these 30 patients. The bacterial species isolated from the diabetic foot ulcers were summarized in (Table 1).

The study revealed polymicrobial infections in 14(46.6%) patients (13 were infected with two types of bacteria, 1 had three types of bacteria), while single etiology appeared in 15(50%) patients and 1(3.3%) case was culture negative (Table 2). According to culture gram positive bacteria were found alone in 9 (30%) patients, and *Staph. aureus* was the most frequent microorganism. Gram negative rods were recorded in 7(23.3%) patients, and *E. coli* was the most predominant microorganism. The remaining 13(43.3) patients had both gram-positive and gram-negative microorganisms.

Table (1): Bacterial species isolated from diabetic foot ulcers

The isolated bacteria	No. of isolates	(%)
1 <i>Staphylococcus aureus</i>	21	70
2 <i>Streptococcus pyogenes</i>	1	3.3
3 <i>Escherichia coli</i>	11	36.6
4 <i>Klebsiella pneumoniae</i>	7	23.3
5 <i>Proteus vulgaris</i>	3	10

Table (2): Polymicrobial isolation from diabetic foot infections

Isolation type		No. of patients	(%)
1	Single bacterial isolate	15	50
2	Two bacterial isolates	13	43.3
3	Three bacterial isolates	1	3.3
4	No bacterial isolate	1	3.3

Diabetic patients often have chronic non healing foot ulcers due to several underlying such as neuropathy, high plantar pressures and peripheral arterial disease, the risk by abnormally high levels of blood sugar in the diabetic patients which damage blood vessels, causing them to thicken and leak, this make vessels less able to supply the body, especially the skin with blood to remain health, the result of poor circulation leads to ulcers, especially those located in the feet, such chronic long- standing ulcers are most prone for infections which further delays the wound healing process. So a wide range of bacteria can cause infection in those patients [10].

In this study, *Staph. aureus* was the predominant and commonest pathogen, followed by *E. coli* and *Klebsiella pneumoniae*. The earlier studies have documented that gram-positive bacteria as predominant microorganisms associated with acute, previously untreated, superficial infected foot wounds

in patients with diabetes [11,12] *Staph. aureus* and beta-haemolytic Streptococci (group A, B, and others) are the commonest pathogen in the diabetic foot infections [13,14]. Methicillin-resistant *Staph. aureus* (MRSA) is more common pathogen in patients who have been previously hospitalized or who have recently received antibiotic therapy. MRSA infection can also occur in the absence of risk factors because of the increasing prevalence of MRSA in the community [15], beside aerobic gram-negative, e.g. *E. coli*, *Proteus* species, *Klebsiella* species and anaerobic organisms e.g. *Bacteroides* and *Clostridium* species who are a part of mixed infections in patients with foot ischemia or gangrene [16].

The microbiology of the diabetic foot is unique infection can be caused by bacteria singly or in combination. As there may be a poor immune response of the diabetic patients, even bacteria normally regarded as skin commensalisms may cause severe tissue damage [17].

The results showed that most of isolated bacteria were susceptible mainly to meropenem, ciprofloxacin, gentamicin, followed by kanamycin and lincomycin, then piperacillin, While, all isolates were highly resistant to the other types of tested antibiotics (Table 3).

Table (3): Antibiotics susceptibility test applied against isolated bacteria

Antibiotic	Symbol	Concentration	Diameter of inhibition zone (mm)	
1	Meropenem	MEM	10 mg	18 - 40
2	Ciprofloxacin	CIP	5 mg	22 - 40
3	Gentamicin	CN	30 mg	17 - 35
4	Kanamycin	K	30 mg	12 - 31
5	Lincomycin	L	2 mg	20 - 26
6	Piperacillin	PRL	30 mg	10 - 21
7	Cefotaxime	CTX	30 mg	R
8	Cephalexin	CL	30 mg	R
9	Ceftazidime	CAZ	30 mg	R
10	Cloxacillin	CX	1 mg	R
11	Carbencillin	PY	100 mg	R
12	Augmentin	AMC	30 mg	R
13	Metronidazole	MET	5 mg	R

The development of infection constitutes a foot care emergency which requires referral to specialized foot-care team within 24 hours. The underlying principles are to diagnose infection, culture the bacteria, treat aggressively with antibiotic therapy and consider the need for debridement and surgery. Usually therapy is commenced with wide spectrum therapy which is then focused according to the microbiology culture results [18,19, 20].

Also important to assess the arterial supply to the foot and consider revascularization either by angioplasty

or bypass if the foot is ischemic. It is also important to achieve metabolic control. Thus infection in the diabetic foot needs full multidisciplinary treatment [21, 22, 23, 24]. So the study deduce that the proper management of bacterial infections requires appropriate antibiotics selection based on culture of the isolates from the lesions and antimicrobial susceptibility testing. Prevention, prompt diagnosis and treatment are necessary to prevent morbidity, especially amputation.

References

1. Ako-Nai, A. K.; Ikem, I. C.; Akinloye, O. O.; Aboderin, A. O.; Ikem, R. T. and Kassim, O. O. (2006). Characterization of bacterial isolates from diabetic foot infections in Illefe, Southwestern Nigeria. *The foot. J.*, 16(3): 158-164.
2. Gadepalli, R.; Dhawan, B.; Sreenivas, V.; Kapil, A.; Ammini, A. C. and Chaudhry, R. A. (2006). Clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diab. car. J.*, 29:1727-1732.
3. Khanolkar, M. P.; Bain, S. C. and Stephens, J. W. (2008). The diabetic foot. *Quarter. J. Med.*, 101:685-695.
4. Anandi, C.; Alaguraja, D.; Natarajan, V.; Ramanthan, M.; Subramaniam, C. S. and Thulasiram, M. (2004). Bacteriology of diabetic foot lesions. *Indian J. Med Microbiol.*, 22:175-178.
5. Lipsky, B. A.; Berendt, A. and Deery, H.G. (2004). Diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis.* 39(7):885-910.
6. Abdulrazak, A.; Bitar, Z. I.; Al-Shamali, A. A. and Mobasher, L. A. (2005). Bacteriological study of diabetic foot infections. *J. Diab. Compl.* 19(3):139-141.
7. Sivaraman, U.; Shailesh, K.; Noyal, MJ.; Joshy, ME.; G. K.; Sreenivasan, S.; Sruthi, R. and Selvaraj, S. (2011). Microbiological study of diabetic foot infections. *Indi. J. Med. Speci.*, 2(1):12-17.
8. Macki, T.J. And McCartney, JE. (1996). *Practical Medical Microbiology*. 14th ed. New York, Churchill Livingstone.
9. Performance standards for antimicrobial susceptibility testing. 12th informational supplement. (2002). NCCLS document M100-S12, 22, No. 1, Pennsylvania, USA.
10. Altavilla, D. ; Feiterety, M. K. and Ferneberg, U. Y. (2001). Inhibition of lipid per oxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. *Diabetes*, 50:667-674.
11. Alavi, S. M.; Khosravi, A. D.; Sarami, A.; Dashtebozorg, A. and Montazeri, E. A. (2007). Bacteriologic study of diabetic foot ulcer. *Pak J. Med Sci.*, 23(5):681-684.
12. Mantey, I., Hill, R. L.; Foster, A. V.; Wilson, S.; Wade, J. J. and Edmonds, M. E. (2000). Infection of foot ulcers with *Staphylococcus aureus* associated with increased mortality in diabetic patients. *Commun Dis public health.*, 3:288-290.
13. Bader, M. S. (2008). Diabetic foot infection. *Amer. Fam. Phys.* 78(1): 81.
14. Dang, C. N.; Prasad, Y. D.; Boulton, A. J. and Jude, E. B. (2003). Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: a worsening problem. *Diabet. Med.*, 20:159-161.
15. Tentolouris, N. ; Petrikos, G. ; Vallianou, N. (2006). Prevalence of methicillin- resistant *Staphylococcus aureus* in infected and uninfected diabetic foot ulcers. *Clin. Microbiol. Infect.*, 12(2):186-189.
16. King, M. D. ; Humphery, B. J. ; Wang, Y. F. ; Korbatova, E. V. ; Ray, S. M. and Blumberg, H. M. (2006). Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft tissue infections. *Ann. Intern. Med.*, 144(5):309-317.
17. Edmonds, M. (2009). The treatment of diabetic foot infections: focus on ertapenem. *Vascular Health and risk Management*, 5:949-963.
18. Zubair, M. ; Malik, A. and Ahmed, J. (2010). Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in north India. *Biol. Med.* 2(4):22-34.
19. Nelson, E. A.; Omeara, S.; Golder, S.; Dalton, J.; Craig, D. and Iglesias, C.(2006). For the DASIDU steering group. Systematic review of antimicrobial treatments for diabetic foot ulcers. *Diabet. Med.* 23(4):348-359.
20. Armstrong, D. G. and Lavery, L. A. (2005). Diabetic foot study consortium. Negative pressure wound therapy after partial diabetic foot amputation: a multi centre, randomised controlled trail. *Lanc.* 366(9498):1704-1710.
21. Lipsky, B. A. and Stoutenburgh, U. (2005). Daptomycin for treating infected diabetic foot ulcers: evidence from a randomized , controlled trail comparing daptomycin with vancomycin or semi-synthetic penicillins for complicated skin and skin-structure infections. *J. Antimicrob. Chemother.*, 55(2):240-245.
22. Spencer, S. (2000). Pressure relieving interventions for preventing and treating diabetic foot ulcers. *Cochrane Database Syst. Rev.*, (3):CD002302.
23. Wolcott, R. D. and Rhoads, D. D. (2008). A study of biofilm-based wound management in subjects with critical limb ischemia. *J. Woun. car.*, 17:145-154.
24. Dowd, S. E.; Wolcott, R. D.; Sun, Y.; McKeehan, T.; Smith, E. and Rhoads, D. (2008). Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX Amplicon pyrosequencing (bTEFAP). *Plos One.*, 3(10):e3326.

عزل وتشخيص المسببات الجرثومية من قرح القدم السكري

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الملخص

أجريت هذه الدراسة لعزل وتشخيص الجراثيم الهوائية الممرضة المرافقة للقرح في اقدام مرضى السكري. كذلك تم اختبار حساسية الجراثيم المعزولة للمضادات الحيوية. لوحظت الأصابات الجرثومية المتعددة في 15 (50%) من المرضى والأصابات الجرثومية المفردة في 14 (46.6%) من المرضى. ولم تعطي حالة واحدة أي نمو جرثومي. كانت جرثومة المكورات العنقودية الذهبية والأشريشيا القولونية هي الجراثيم الأكثر تردداً في الأصابات الجرثومية لمعظم المرضى وبواقع 21 (70%) و 11 (36.6%) لكلا العزلتين الجرثوميتين على التوالي. المضادات ميروبنيم ، سايبروفلوكساسين وجنتاميسين كانت الأكثر فعالية تجاه الأنواع الجرثومية المعزولة.