

The prevalence of antibiotic resistance in aerobic and anaerobic bacteria isolated from patients with diabetic foot ulcers

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الخلاصة:

ان الهدف من هذه الدراسة هو لتحديد انواع الاحياء الجهرية المعزولة من تقرحات القدم السكري وأنماط الحساسية تجاه المضادات الحيوية, تم جمع نماذج من النسيج التالف والقيح من 46 مريض مصاب بتقرحات القدم السكري ومن ثم اجريت الفحوصات المناسبة عليها . شخّصت البكتريا المعزولة من خلال استخدام الفحوصات المجهرية , الصفات الزرعية والأختبارات البايوكيميائية . أستخدمت طريقة الانتشار بالأقراص لـ Kirby-Bauer لأجراء فحوصات الحساسية . من بين 46 مريض كان الذكور (73.9%) اكثر تعرضا وتأثيرا لأصابات القدم من الأناث (26.1%) , بينما كان مرض السكري من النوع الثاني (65.2%) اكثر تحسسا للأصابة من مرض السكري من النوع الاول (34.8%) . ان الاحياء الهوائية السالبة لصبغة كرام تكون غالبا اكثر الأنواع المعزولة (42%) , يتبعها الاحياء الهوائية الموجبة لصبغة كرام والاحياء اللاهوائية (30.4% و 27.6%) على التوالي . ان البكتريا الهوائية واللاهوائية السائدة هي *Staphylococcus aureus* (17.1%) و *Peptostreptococcus spp* (8.6%) على التوالي . جميع الاحياء المجهرية المعزولة أظهرت مقاومة عالية للمضادات الحيوية المستخدمة . ان حساسية البكتريا الهوائية واللاهوائية تجاه المضاد الحيوي Cefprozil كانت بنسبة (100%) لذلك نقترح هذا الدواء لمعالجة تقرحات القدم السكري.

Abstract:

The aim of the present study was to determine the microbiological profile and antibiotic susceptibility patterns of organisms isolated from diabetic foot ulcers.

Pus and debrided tissue samples from 46 patients with diabetic foot ulcers collected and processed.

Bacterial isolates were identified by using different microscopical examination, cultural characteristics and biochemical tests. Sensitivity tests were performed by Kirby-Bauer disk diffusion method. Out of 46 patients , male (73.9%) were found to be affected and develop foot infection more than female

(26.1%) while type two diabetes mellitus (65.2%) more susceptibility for infection than type one (34.8%).

Gram- negative bacteria were most frequently isolated (42%), followed by gram-positive aerobes and anaerobes (30.4% and 27.6% respectively). Predominant aerobic and anaerobic bacteria were *Staphylococcus aureus* (17.1%) and *Peptostreptococcus spp* (8.6%), respectively. All the microorganisms isolated showed high resistance to used antibiotics; susceptibility of both aerobic and anaerobic bacteria to ceftizoxime was (100%), so we suggest this drug for treatment of diabetic foot ulcers.

Introduction:

Foot ulceration is a leading cause of hospital admission for patients with diabetes, and an extremely expensive complication of diabetes^[1]. There are three main factors that lead to ulceration and necrosis in diabetic foot, namely neuropathy, infection, and ischemia^[2].

The feet are prone to of peripheral neuropathy leading chiefly to sensory deficits and autonomic dysfunction. Infection is rarely a sole factor, but often complicates neuropathy and ischemia^[3].

Ischemia results from atherosclerosis of the leg vessels, which in the diabetic patient is often bilateral, multisegmental, and distal, involving vessels below the knee^[4,5].

Treatment of serious foot infection remains difficult, requiring long hospital stays in many cases because these patients have impaired microvascular circulation, which limits the access of phagocytic cells to the infected area and result in a poor concentration of antibiotics in the infected tissues^[6,7].

Infection with multidrug resistant strain of bacteria are becoming a major problem in the whole world^[8] and individuals with diabetes are known to be at greater risk of foot infection than individuals without diabetes^[9].

These multidrug resistant organisms (MDROs) are frequently resistant to many classes of antibiotic so it is necessary for the clinicians to be completely aware of the prevalence rate of multidrug resistant organism and their management strategies^[10]. Since improper management MDRO might lead to devastating complications, which include systemic toxicity, gangrene formation and amputation of lower extremity^[11]. Therefore patients with wounds infected with MDRO require an early diagnosis and careful follow up ensure that appropriate and effective medical and surgical regimen is readily available to the patients^[12].

In addition to proper cleaning, debridement, local wound care, a diabetic foot infection requires carefully selected broad-spectrum antibiotics therapy based on culture and antimicrobial susceptibility results^[13]. Studies related to multidrug

resistant organism and infections in diabetic foot ulcers may be helpful as they provide basis for empirical antimicrobial therapy.

The main aim of the present study was to determine the microbiological profile and antibiotic susceptibility patterns of organism isolated from patients with foot ulcers.

Materials and Methods:

A total of 46 patients with diabetic foot ulcers from Kadhimiya Teaching Hospital and Baghdad Teaching Hospital were screened between November 2008 and March 2009.

These patients were clinically assessed and the foot ulcers were graded depending on the severity of ulcers with 3 to 5 as grade 3 deep ulcer, abscess formation and bone involvement, grade 4- localized gangrene and grade 5- gangrene of whole foot^[14].

Pus aspirates from the abscesses and debrided necrotic materials were collected for aerobic and anaerobic culture using punch biopsy. The collected specimen was processed by performing Gram stain from the direct smear, inoculating the specimen on to culture media like blood agar, MacConkey agar, chocolate agar and bile-esculin agar for aerobic and anaerobic culture. The bacterial isolates grown on the media were identified by using different microscopical examination, cultural characteristics and biochemical tests^[15,16].

Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method according to National Committee for Clinical Laboratory Standard (NCCLS) guide lines^[17]. Antibiotic discs which used manufactured by Oxoid (England). Types and concentrations of these antibiotic discs were as following:

Ceftizoxime (30mg/disc), Imipenem (10mg/disc), Augmentin (30mg/disc), Ciprofloxacin (5mg/disc), Amikacin (30mg/disc), Piperacillin-tazobactam (100/10mg/disc), Cotrimoxazole (25mg/disc), Erythromycin (15mg/disc), Ampiclox (10mg/disc), Gentamycin (10mg/disc), Colistin (10mg/disc), Rifampicin (30mg/disc), Chloramphenicol (30mg/disc), Clindamycin (2mg/disc).

Results:

Among 46 patients with diabetic foot ulcers, male patients 34 (73.9%) were found to be affected and develop foot infection more than female 12 (26.1%), while type II diabetes mellitus (65.2%) showed more susceptibility for infection than type I (34.8%) (Table-1). The age ranged from 14-65 years with mean age being 39.5 years. All patients had ulcers graded 3-5 in the Wagner classification.

Bacteriological study of patients revealed polymicrobial etiology in 37 (80.4%) and single etiology in 9 (19.6%). The profile of the organisms isolated detailed in Table 2. Of the total 105 isolates, 76 (72.4%) were aerobic and facultative bacteria, 32 (30.4%) and 44 (42%) were aerobic Gram- positive and Gram negative bacteria, respectively. There were a total equal number of Gram-positive (15.2%) and negative bacteria (12.4%). Predominant aerobic and anaerobic bacteria were *Staphylococcus aureus* (17.1%) and *Peptostreptococcus spp* (8.6%), respectively. Antibiogram test was done on aerobic-anaerobic facultative bacteria. Susceptibility of these bacteria was as following:

Ceftizoxime 100%, Imipenem 94%, Augmentin 83%, Ciprofloxacin 77%, Amikacin 56%, and their resistance to Gentamycin was 99%, Ampiclox 96%, Chloramphenicol 89%, Clindamycin 81%, Cotrimixazole 78%, Erythromycin 74%, and Piperacillin-tazobactam 64% (figure-1).

In anaerobic bacteria, susceptibility to Ceftizoxime 100%, Imipenem 91%, Augmentin 80%, Ciprofloxacin 73%, Amikacin 51%, and their resistance to Ampiclox 98%, Clindamycin 93%, Chloramphenicol 87%, Erythromycin 81%, Cotrimixazole 74%, Colistin 72% and Rifampicin 66%.

Types of DM	Male No. of cases (%)	Female No. of cases (%)	Total
IDDM (Type I)	12 (26.1)	4 (8.7)	16 (34.8)
NIDDM (Type II)	22 (47.8)	8 (17.4)	30 (65.2)
Total	34 (73.9)	12 (26.1)	46

Table-1: Numbers of diabetic patients according to sex and type of diabetic mellitus (DM).

- IDDM: Insulin-dependent DM
- NIDDM: Non- Insulin-dependent DM

Bacteria category	Frequency N (%)
Aerobic and facultative isolates	76 (72.4)
Gram-positive	32 (30.4)
<i>Staphylococcus aureus</i>	18 (17.1)
Non-coagulase staphylococci	3 (2.9)
Group D- <i>Enterococci</i>	6 (5.7)
<i>Micrococcus spp</i>	1 (0.9)
Group A – <i>streptococci</i>	4 (3.8)
Gram- negative	44 (42)
<i>Escherichia coli</i>	13 (12.4)
<i>Pseudomonas aeruginosa</i>	11 (10.5)
<i>Enterobacter spp</i>	3 (2.9)
<i>Klebsiella spp</i>	7 (6.7)
<i>Acinetobacter spp</i>	4 (3.8)
<i>Proteus spp</i>	6 (5.7)
Anaerobic isolates	29 (27.6)
Gram –positive	16 (15.2)
<i>Propionebacterium acnes</i>	2 (1.9)
<i>Peptostreptococcus spp</i>	9 (8.6)
<i>Clostridium spp</i>	5 (4.8)
Gram- negative	13 (12.4)
<i>Fusobacterium spp</i>	4 (3.8)
<i>Prevotell spp</i>	2 (1.9)
<i>Bacteriodes spp</i>	7 (6.7)

Table-2: Profile of bacteria isolated from infected foot ulcers in daibetic patients' specimens (46).
N=Number of isolates (105)

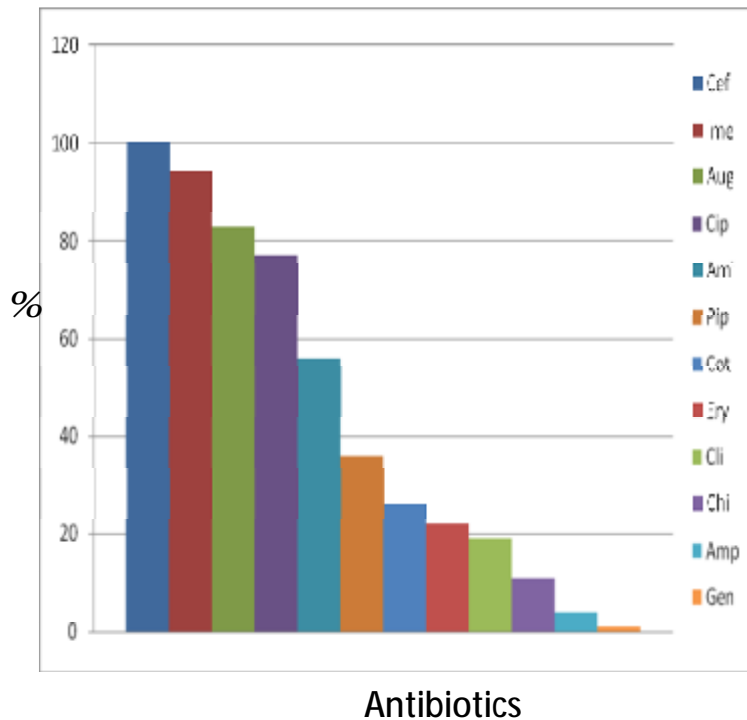


Figure-1: The prevalence of antibiotic susceptibility aerobic bacteria isolated from patients with diabetic foot ulcers.

Ceftizoxime: Cef (30mg/disc), Imipenem: Imi (10mg/disc), Augmentin: Aug (30mg/disc), Ciprofloxacin: Cip (5mg/disc), Amikacin: Ami (30mg/disc), Piperacillin-tazobactam: Pip-taz (100/10mg/disc), Cotrimixazole: Cot (25mg/disc), Erythromycin: Ery (15mg/disc), Clindamycin: Cli (2mg/disc), Chloramphenicol: Chl (30mg/disc), Ampiclox: Amp (10mg/disc), Gentamycin: Gen(10mg/disc).

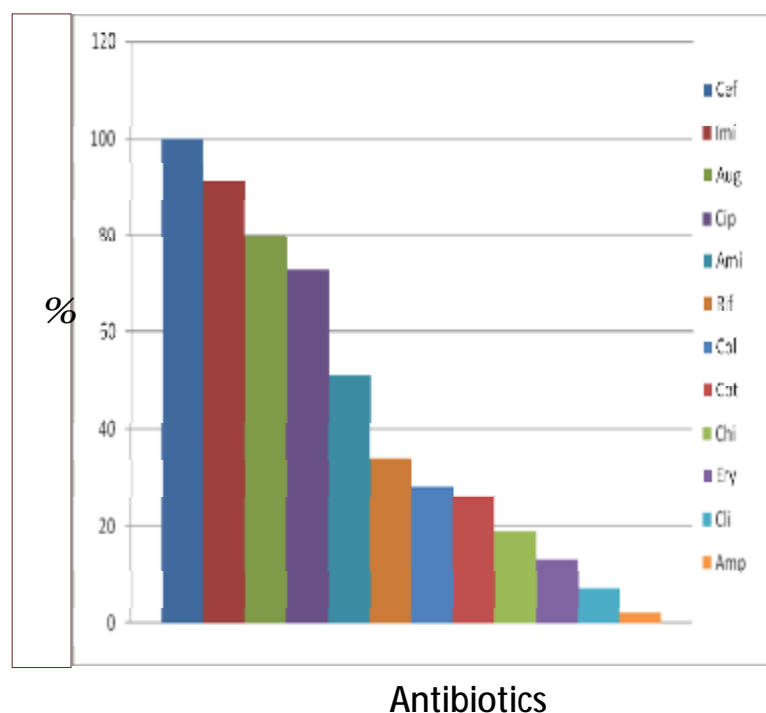


Figure-2: The prevalence of antibiotic susceptibility anaerobic bacteria isolated from patients with diabetic foot ulcers.

Ceftizoxime: Cef (30mg/disc), Imipenem: Imi (10mg/disc), Augmentin: Aug (30mg/disc), Ciprofloxacin: Cip (5mg/disc), Amikacin: (30mg/disc), Rifampicin: Rif (30mg/disc), Colistin: Col (10mg/disc), Cotrimixazole: Cot (25mg/disc), Chloramphenicol: Chl (30mg/disc), Erythromycin: Ery (15mg/disc), Clindamycin: Cli (2mg/disc), Ampiclox: Amp(10mg/disc).

Discussion:

Foot ulcers are a significant complication of diabetes and often precede lower extremity amputation. The most frequent underlying etiologies are neuropathy, trauma, deformity, high plantar pressures, and peripheral arterial disease [18]. Although infection is rarely implicated in the etiology of diabetic foot ulcers, the ulcer and susceptible to infection once the wound is present.

An understanding of the bacteriological of diabetic foot ulcers is important in guiding antibiotic selection and correlate culture result with appropriate definitive therapy [19].

Male diabetic patients is suffering more than female patient from diabetic foot problem and infection, possibly because he is subjected more to trauma, associated

with smoking and alcohol drinking. In our study we found (73.9%) male and (26.1%) female affected. It goes with study of Lavery et. al., 2006^[20].

Patients with type two show more diabetic foot problem and infection than type one. In our study 65.2% of patients with foot infection were type two while only 34.8% were type one. This result goes with study of Viswanathan et. al. 2002^[21].

Single type bacterial infection was found in 19.6% of patients .while polymicrobial was found in 80.4% of patients , this goes with most research Hunt,1992 ^[22], and Sapico et. al., 1984 ^[23] that many and different type of bacteria could be found.

All the ulcers in the patients were 3-5 grading in Wagner's grading of foot ulcers. The difference in the grades of the wound did not have any significant impact on the nature or type of the organism isolated from the wound.

Though previous studies showed Gram-positive aerobes as predominant in diabetic foot infections^[24,25], we found Gram-negative aerobic bacteria were the most frequently isolated. Thus, the major infective organisms in diabetic foot ulcers in our patients appear to be different. These variations might be due to the difference in the study setting, age, sex, composition, ulcer grades etc. between our study subjects and those of previous studies. Our study we found *Staphylococcus aureus* was the most frequent pathogen (17.1%), and *Peptostreptococcus spp*(8.6%) typically are the most common isolated anaerobic bacteria . The majority of studies also noted a high frequency of these microorganisms in foot infection of diabetic patients ^[26,27].

The unique feature about the study was that all the isolates showed resistance to more than 2-3 antibiotics. When organisms are resistant to more than 2 or 3 antibiotics they can be called as multidrug resistant organisms (MDROs). The high rates of antibiotic resistance observed in the present study may be due to the widespread usage of broad-spectrum antibiotic leading to selective survival advantage of pathogens^[28].

Susceptibility of both aerobic and anaerobic bacteria to Ceftizoxime was (100%).This approximately the same result as Martinez et. al., 2009^[29].

MDRO infection in patients with diabetic foot ulcers has become remarkably common. This finding presented by our study in agreement with the report of Hartemann et. al., 2004 ^[30].

Conclusions:

In this prospective study we found that male diabetic patient is more prone to have diabetic foot infection than female and type two diabetes showed more susceptibility for foot infection.

Polymicrobial infection is more than single type bacteria. Anaerobic gram-negative bacteria were the most frequently isolated.

Staphylococcus aureus and *Peptostreptococcus spp* were the most common causes of diabetic foot ulcers in present study.

Susceptibility of both aerobic and anaerobic bacteria to Cftizoxime was 100%, so we suggest this drug for treatment of diabetic foot ulcers.

This study reveals that there is a high frequency of occurrence of multidrug resistant organisms (MDROs) infection in diabetic foot ulcers.

There is a need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy and reduce the risk of complications.

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