AEROBIC BACTERIA ISOLATED FROM DIABETICS FOOT INFECTION
LOCALLY AND ANTIBIOTICS INFLUENCE

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ABSTRACT

Diabetic foot infection is one of the terrible complications of diabetes leading to morbidity and mortality. This study evaluated the resistance and sensitivity of isolated aerobic bacterial frequencies that were isolated from patients with diabetic foot for the commonly used antibiotics to assist in identifying empirical therapy. This study aimed to determine the antibiotic sensitivity and resistance pattern to detect aerobic bacterial pathogens present in diabetes pus against 13 commonly used standard antibiotics: erythromycin, oxacillin, chloramphenicol, ciprofloxacin, vancomycin, methycillin, amoxicillin, cephalprazin, and biphenylpropionone. Forty patients with a diabetic foot infection, including 28 males and 12 females from Al Najaf center for diabetes and endocrine in Al-Sadir medical city, were clinically sampled for 5 months. All swabs from these 40 patients showed a positive culture and a total of 98 isolates were isolated. Standard aerobic microbiological techniques were used to process clinical specimens. Also the Kirby-Bauer disc diffusion method was used to study the antimicrobial susceptibility pattern. The Infections are generally due to Gram-positive bacteria. The most isolated Gram-positive bacteria was Staphylococcus aureus bacteria and streptococcus spp. followed by Staphylococcus aureus and Bacillus spp. (49%, 34%, 13% and 3%), respectively. While Proteus spp. and Pseudomonas spp. the most isolated Gram-negative bacteria followed by Escherichia coli and Klebsiella spp. and Enterobacter spp. and Acenetobacter baumannii (32%, 27%, 19%, 11%, 5% and 5%), respectively.

Keywords: Aerobic Bacteria; Diabetics Foot; Empirical therapy; Antibiotics

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INTRODUCTION

Diabetes is one of the most important chronic diseases of our time. The World Health Organization predicts that the number of diabetics around the world will rise to nearly 366 million by 2030 [1]. It is a metabolic disorder that affects carbohydrate metabolism in particular. Diabetes leads to high blood sugar as a result of weakening the body's ability to regulate blood glucose levels [2]. The development of chronic diabetes complications is associated with a large part of the burden of the disease, as well as the cost of health care as the most common complication is diabetic foot disease that diabetics fear [3].

Diabetic foot infection develops in areas with inappropriate foot care and increases the risk of lifelong foot ulceration. This infection is considered as a global concern. Superficial wounds are the onset of infection, but weak body defense mechanisms and delay in treatment can spread to other subcutaneous tissues and to deeper structures, leading eventually to horrific complications such as amputation and gangrene.

Diabetic foot ulcers significantly affect both the patient and the health care system. These ulcers are cured slowly and require intensive care. They can be complicated by infection and gangrene, resulting in long-term hospitalization treatment and/or amputation. Foot ulcers, a complication of diabetes, which is frequently feared.

In nature, these infections are multiple in microbes. Frequent organisms isolated from cases of diabetic foot inflammation have been reported: Escherichia coli, Proteus SP. The most common pathogens in acute, untreated, foot wounds have been found in diabetic patients to be Gram-positive bacteria, especially Staphylococcus aureus and beta-hemolytic streptococci (group B), A, B, and others.

Multidrug-resistant Gram-negative bacteria are a major therapeutic challenge in hospitals, particularly in the society at large, where the doctor or surgeon faces treatment for diabetes, and ulcers without resorting to amputation are another challenge.

In recent years, the increased incidence of multidrug-resistant organisms has resulted in increased hospital stay, morbidity, cost, and higher mortality. One study has shown that the type of life of diabetic foot ulcers and that of patients with recurrent breast cancer were similar.

The challenges of the increase of multidrug resistant organisms led us to investigate to seek the most effective antimicrobial to treat the diabetic foot ulcers.

Present study aims are to:

- Examination of clinical pathogens and sensitivity of diabetic foot ulcer patients.
- Study the role of antibiotics in wounding diabetic patients.
- Evaluation of the bacteriology of diabetic foot ulcers in the al Najaf center for diabetes and endocrine in the Sadir Medical City.
- Test antibiotic susceptibility for major antibiotics against prevalent aerobic bacteria and identification of bacterial pathogens associated with diabetes wounds.

Diabetes Mellitus

Chronic disorder affects a large number of people worldwide and is a major public health problem known as diabetes. About a quarter of persons with diabetes develop lifelong ulcers, and half become infected.

Two types of diabetes, a type arising from the destruction of beta pancreas cells, resulting in a deficiency of total insulin and this is known as type 1 diabetes, while the other type is caused by the defect of gradual insulin secretion against the background of insulin resistance, which results in a relative lack of insulin and this is known as diabetes Type 2. Requires lifelong drug therapy with blood glucose-lowering agents, a modified lifestyle that includes a balanced and healthy diet and regular exercise in both cases.

Diabetes and foot ulcers

People with diabetes are more susceptible to foot ulcers than non-diabetics. Any foot injury is serious in the case of patients with diabetes, as the severity of diabetic foot infections ranges from superficial to deep involvement of the bone [18]. Types of infection include cysts, cellulitis, myositis, septic arthritis, necrotizing fasciitis, osteomyelitis, and tendonitis. The most common and serious complication of diabetes is foot infections that are associated with hospital length and an increased frequency and risk of amputation of the lower limbs [19,20].

Diabetic foot ulcers significantly affect both the patient and the healthcare system. These ulcers are cured slowly and require intensive care [21]. They can be complicated by infection and gangrene, resulting in long-term hospitalization treatment and/or amputation [22].

Foot ulcers often precede amputation and are an important complication of diabetes. Neuropathy, deformity, trauma, peripheral arterial disease, and high plantar pressures are the most common pathogens and appropriate treatment guidance requires systematic and systematic assessment and classification of foot ulcers [23,24].

**Common therapy**

The primary objective in the treatment of diabetes is to decrease the progression of macro and microvascular complications. There is evidence that the risk can be significantly reduced and prevent the development of complications in all types of diabetes if well-controlled blood glucose levels [25,26]. The management of both high blood pressure and blood lipids is beneficial both to patients, as well as physical activity, reducing stress, and a smoke-free lifestyle [27,28].

Through an active and physically distinct lifestyle, reduced intake of saturated fats, balanced and calorie intake, smoking cessation, early detection of signs of complications by screening, prescription medication, and an intensive education program for patients and enabling them to take on their own responsibility about treatment and control goals are reached [11,29].

**MATERIAL AND METHODS**

**Device**

The devices that used in this study are Autoclave model (Yx – 280 B – China), Microscope (Motic – Germany), Sensitive Balance (Sartorious – Germany), Incubator (Fisher Scientific – Germany), Sterile cotton swabs were used to collect culture specimens, the forceps.

**Culture media**

Autoclave sterile culture media were used at 121°C > 15 psi pressure for 15 minutes as described in the instructions, which are Methylened blue agar, Macconkey agar Methylene blue agar, Nutrient broth, Blood agar, UTIchrom agar, Nutrient agar, Molin Hinton agar, and Mannitol salts.

**Indicators**

Gram's stain kit and capsule stain kit which consists of crystal violet stain and Nigrosin stain.

**Study design**

Forty patients with diabetes and foot ulcers which included in this study were received at Al Najaf center for diabetes and endocrine in Al Sadir medical city in Al Najaf-Iraq, between October 2016 to February 2017. Patients underwent widespread surgical operation of diabetic foot ulcers, and a few patients that suffer from diabetes and foot ulcers weretaking antibiotics. For each patient demographic data and lesions were recorded, including age, sex and antibiotic uptake as shown in Table (2).

**Table 2: Patient Characteristics**

<table>
<thead>
<tr>
<th>Characters</th>
<th>Details</th>
<th>No. of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 40</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>51-60</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>61-70</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>71-80</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>&gt; 80</td>
<td>6</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>12</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>With</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>10</td>
</tr>
</tbody>
</table>

**Sample**

After removing the wound and rinsing the wound area with saline, Sterile cotton swabs were used to collect culture specimens. After that, the study were excluded superficial ulcers from to eliminate the possibility of isolating colonized bacteria and immediately transferred a cotton swab for culture in nutrient agar to the laboratory.

**Characterization of isolates bacterial**

On nutrient agar, the specimens were cultured and then subcultured on blood agar, MacConkey agar, mannitol salt agar, Eosin methylene blue agar and UTIChrom agar at 37 ° C for 24 hr. the plates were aerobically incubated. Based on the morphology of the colony, the presence of the capsule, the results of Gram staining and biochemical tests, isolates were identified [27,30].

Anaerobic bacteria were not included in this study due to short timing and lake of laboratory equipment that is necessary to grown it.

**Antibiotic Susceptibility Test using disc diffusion method.**
The recommendation by the Institute of Clinical and Laboratory Standard (CLSI) through disc diffusion methods was determined the sensitivity of all isolates to different antibiotics \cite{31,32}. In this study this antibiotics Amikacin, Amoxyclav, Cefotaxime, Ciprofloxacin, Oxacillin, Chloramphenicol, Erythromycin, Vancomycin, Meropenem, methicillin, ciprofazone, pipracillin and Tetracycline were used to determine an antibiotic susceptibility pattern.

Muller Hinton agar was prepared and sterilized and then poured 25 ml into the Petri dishes of sterile MHA and left hardened. We’ve made serial dilutions of the bacterial samples to get 0.5% McFarland degree standard for susceptibility testing \cite{33,34}.

At an equal distance on the plates were placed antimicrobial tablets and by the use of forceps the tablets were gently compressed after incubation of the plates for 24 hours at 37°C, and with a metal ruler in mm was measured inhibition zone and using the standard area of the inhibition diagrams were interpreted.

From the figure 3, we can shows the methods of diagnosis and antibiotic susceptibility used in this study from the first step when the specimen arrived the laboratory till the inhibition zones of the tested Petridishes.
Figure 3: The methods of diagnosis and antibiotic susceptibility
RESULTS

Demographic Characteristics

The demographic characteristics are shown in Table 4 and the results showed that forty patients included in the study were diabetic, of whom 12 (30%) were males and 28 (70%) females had an average age of patients 65.7 ± 14.75 years (mean ± SD; the range from 40 to 80 years) In addition, patients taking diabetes medications 30 (75%) and the other patients without taking diabetes medications10 (25%).

All the samples were positive cultured in the case of the 40 patients studied and 98 isolates were obtained. 62% (number = 61) of isolates were accounted for Gram-positive bacteria, while 38% (number = 37)of isolates were accounted for Gram-negative bacteria as shown in Table 4.

Table 3: Characteristic of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Range of Age</td>
<td>65.7 ± 14.75</td>
</tr>
<tr>
<td>Total No. of Female patients</td>
<td>12</td>
</tr>
<tr>
<td>Total No.of Male patients</td>
<td>28</td>
</tr>
<tr>
<td>Total No.of patients without antibiotic</td>
<td>10</td>
</tr>
<tr>
<td>Total No.of patients taking antibiotic</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 4: The characteristic of bacteria isolated from diabetic foot

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>TS</td>
<td>40</td>
</tr>
<tr>
<td>TPI</td>
<td>98</td>
</tr>
<tr>
<td>GB(-)</td>
<td>37</td>
</tr>
<tr>
<td>GB(+)</td>
<td>61</td>
</tr>
<tr>
<td>NPP</td>
<td>40</td>
</tr>
</tbody>
</table>

TS= Total number of specimens
TPI= Total number of pathogens isolated
GB(-)=Gram-negative bacteria
GB(+) =Gram-positive bacteria
NPP= number of patients with positive culture

The bacterial species most commonly isolated among the Gram-positive bacteria were Staphylococcus aureus [30 (49%) isolates], followed by Streptococcus spp., Staphylococcus epidermidis, and bacillus spp., [21 (34%) isolates] [8 (13%) isolates] [2 (3%) isolates], respectively. while, Proteus spp. and Pseudomonas spp. followed by, Escherichia coli, Klebsiella spp., Enterobacter spp. and Acinetobacterbaumannii, were among the Gram-negative bacteria [12 (32%) isolates] [10 (27%) [7 (19%) isolates] [4 (11%) isolates] [2 (5%) isolates] [2 (5%), respectively.

**Microbiology and susceptibility testing**

Tables (4) and (5) show the types of antibiotic resistance commonly used for bacteria isolated using the Kirby Bauer method. Of 61 isolates, Gram-positive organisms were isolated from them. The table 4 and 5 shows that 27(90%)cases of *Staphylococcus aureus* resistance to Erythromycin, 7(23%) resistance to Oxacillin,18(60%) resistance to Chloramphenicol; 20(67)% resistance to Methicillin,11(37%) resistance to Amikacin and 15(50%) resistance to Ciprofloxacin; 7(88%) of *Staphylococcus epidermidis* was resistance to Erythromycin, 1(13%) resistance to Oxacillin, 4(50%) resistance to Chloramphenicol;4(50%) resistance to Ciprofloxacin; and 100% sensitive to Methicillin and Amikacin while 20(95%) of *streptococcus spp.*, was resistance to Erythromycin,15(71%) resistance to Chloramphenicol, 5(24%) resistance to Ciprofloxacin,16(76)% resistance to Methicillin,100% sensitive to Amikacin, and 100% sensitive to Oxacillin; all of this strain was sensitive to Vancomycin; 1(50%) of *Bacillus spp.* was resistance to Erythromycin, 1(50%) resistance to Chloramphenicol and 100% sensitive to Oxacillin, Methicillin,Amikacin, Ciprofloxacin and Vancomycin.
Table 5: The percentage of antibiotic resistance pattern of Gram Positive Organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Tot. No. of isolates</th>
<th>Percentage of antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER</td>
<td>VAN</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

(-- sensitive indicates; ER-Erythromycin; OXA-Oxacillin; CH-Chloramphenicol; CIPR-Ciprofloxacin; VAN-Vancomycin; ME-Methicillin; AMIK-Amikacin)

Gram-negative organisms were isolated in 37 isolates. There were no resistance of *Escherichia coli* to Amoxyclav, 1(14%) resistance to Amikacin, 100% sensitive to Meropenem, Cefoperazone and Piperacillin, 5(71%) resistance to Tetracycline and 2(29%) resistance to Cefotaxime;3(75%) of *Klebsiella spp.*, was resistance to Amoxyclav, no resistance to Amikacin, 1(25%) resistance to Meropenem, 1(25%) resistance to Tetracycline and 100% sensitive to Cefotaxime, Piperacillin and Cefoperazone; 6(50%) of *Proteus spp.*, was resistance to Amoxyclav, 2(17%) resistance to Amikacin, 100% sensitive to Meropenem and Piperacillin,6(50%) resistance to Tetracycline, 3(25%) resistance to Cefotaxime and 2(17%) resistance to Cefotaxime;2(100%) of *Enterococcus spp.*, was resistance to Amoxyclav,2(100%) resistance to Tetracycline and 100% sensitive to Amikacin, Meropenem, Piperacillin, Cefoperazone and Cefotaxime; 7(70%) of *Pseudomonas spp.*, was resistance to Amoxyclav, 1(10%) resistance to Amikacin,2(20%) resistance to Cefoperazone,5(50%) resistance to Piperacillin, 3(30%) resistance to Cefotaxime, 2(100%) resistance to Tetracycline,And 100% sensitive to Cefotaxime.2(100%) of *Acinetobacter baumannii* was resistance to Amoxyclav and Tetracycline, 100% sensitive to Amikacin,Meropenem and Cefoperazone, 1(50%) resistance to Piperacillin and Cefotaxime.

Table 6: The percentage of antibiotic resistance of gram negative organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Tot. No. of isolates</th>
<th>percentage of antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMC</td>
<td>AK</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>1(14)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4</td>
<td>3(75)</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>2</td>
<td>2(100)</td>
</tr>
</tbody>
</table>

DISCUSSION

Foot infections are a common, expensive and complex problem for people with diabetes in this study; we found that the elderly (over 70 years old) account for the majority of foot inflammatory patients and this is explained by the fact that older people and people with sensory neuropathy are more likely to develop foot infection, among diabetics. In our study, we found that males were more infected than females and this finding was similar to another study [35]. In contrast, a previous study showed that males and females had the same susceptibility to foot inflammation [36].

The role of anaerobic bacteria in diabetic foot infections wasn’t clear due to differed result, there are studies that indicated that anaerobic bacteria play a simple role in diabetic foot infections [37,38], while other studies indicated that they play a large role in diabetic foot infections [39,40]. Present study resulted that the patients that have been taken antibiotics, their foot cultures bacteria were lower than patients that did not take antibiotics. This can be due to the fact that antibiotics have influenced the bacterial growth.

In this study, the dominant cultures are gram-positive bacteria. In agreement with our current study, some previous studies of diabetes when examining the isolation of Methicillin-resistant *Staphylococcus aureus* have demonstrated the prevalence of Gram-positive bacteria in diabetic foot infections [41]. In addition, in another study of diabetic foot infection, the results showed that isolated *Staphylococcus aureus* accounted for about 38% of infected cases and other organisms accounted for 72.6%. The study also indicated that about 89.5% of Gram-positive bacteria, which give them dominance, while Gram-negative bacteria of different types account for about 10.5% [40]. Conversely, some other studies have reported the prevalence of Gram-negative bacteria for diabetics in certain areas [2, 42]. As well as, in study to identify antibiotic sensitivity and microbial analysis of organisms isolated from diabetic foot, it indicated that Gram-negative bacteria aerobes were prevalent, accounting for about 51.4%, aerobes Gram-positive bacteria about 33.3% and in anaerobic 15.3% of diabetic foot infections [43]. In part of these results, it indicates differences in the severity and type of infections [44, 45]. In a previous study, in patients with previously treated infections or still suffering from chronic infections, Gram-negative bacteria are usually isolated side by side with Gram-positive cocci, mainly *Enterobacteriaceae* and sometimes *Pseudomonas aeruginosa* in aerobic Gram-negative bacteria [19, 46, 47], Which is in agreement with our results.

In this study, there are 27(90%) cases of *Staphylococcus aureus* isolated from Gram-positive organisms resistance to Erythromycin, Oxacillin, Chloramphenicol; Methicillin, Amikacin and Ciprofloxacin; 7(88%) of *Staphylococcus epidermidis* was resistance to Erythromycin, Oxacillin, Chloramphenicol;
Ciprofloxacin; and 100% sensitive to Methicillin and Amikacin while 20(95%) of *streptococcus spp.*, was resistance to Erythromycin, Chloramphenicol, Ciprofloxacin, Methicillin, 100% sensitive to Amikacin, Oxacillin; all of this strain was sensitive to Vancomycin; 1(50%) of *Bacillus spp.* was resistance to Erythromycin, Chloramphenicol and 100% sensitive to Oxacillin, Methicillin, Amikacin, Ciprofloxacin and Vancomycin. The *Staphylococcus spp.*, comprised the most prevalent isolates recovered, 51% of these were *Staphylococcus aureus*. These results are compatible with the finding of other studies [41, 48]. A possible reason for such high incidence of *Staphylococcus spp.*, might be due to the presence of these bacteria as normal flora on the skin [36].

Also, There are no resistance of *Escherichia coli* isolated from Gram-negative organisms to Amoxycyclav however, it resistance to Tetracycline, Cefotaxime and Amikacin, 100% sensitive to Meropenem, Cefoperazone and Piperacillin; 3(75%) of *Klebsiella spp.*, was resistance to Amoxycyclav, Meropenem and Tetracycline, 100% sensitive to Cefotaxime, Piperacillin and Cefoperazone, no resistance to Amikacin; 6(50%) of *Proteus spp.*, was resistance to Tetracycline, Cefoperazone, Cefotaxime, Amoxycyclav and Amikacin, 100% sensitive to Meropenem and Piperacillin; 2(100%) of *Enterococcus spp.*, was resistance to Amoxycyclav, Tetracycline and 100% sensitive to Amikacin, Meropenem, Piperacillin, Cefoperazone and Cefotaxime; 7(70%) of pseudomonas spp., was resistance to Amoxycyclav, Amikacin, Cefoperazone, Piperacillin, Cefotaxime, Tetracycline and 100% sensitive to Cefotaxime; 2(100%) of *Acephobacterbaumannii* was resistance to Piperacillin, Cefotaxime, Amoxycyclav and Tetracycline, 100% sensitive to Amikacin, Meropenem and Cefoperazone. Our findings are in accordance with the results of Roberts et al., (2012) [41, 49, 50].

CONCLUSION

The researcher concluded that *Staphylococcus aureus*, or polymicrobial infection, was the leading cause of diabetic foot infection as a gram-positive bacterium. It is obvious from the results that Diabetics are more likely to suffer from a higher risk of ulceration. It is found that the best drug of choice against both positive and negative Gram organisms are Amikacin antibiotic negative. The Vancomycin were the best choice against Gram-Positive Organisms while Tetracycline is the worst choice to terminate gram negative organisms since a high rate of resistant were found. The antibiotic Meropenem is the best choice against gram negative organisms. While Erythromycin is the worst choice to terminate gram positive organisms since a high rate of resistant was found.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.
CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING: Self-funding

REFERENCES


