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Identifying the Resistant Bacterial Pattern in Patients with Diabetic Foot Ulcer

Sura Qusay Ali[1] and Yasir Majid Kamil[2]

[1]College of Education, Al-Iraqia University, Baghdad, IRAQ.

[2]College of Pharmacy, Al-Farabi University, Baghdad, IRAQ.

[1]Corresponding Author: sorra.q.ali@aliraqia.edu.iq

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ABSTRACT

Background: Diabetes is a term used to describe a group of metabolic disorders that are hyperglycemic due to deficiencies in insulin secretion, insulin action, or both. Diabetes complications are common in both type 1 and type 2 diabetes patients, and they are responsible for significant morbidity and mortality. One of the persistent diabetes complications is a foot ulcer associated with neuropathy. These ulcers eventually lead to infections in the diabetic foot. Diabetic foot diseases such as ulceration, gangrene, Charcot joint, and fracture are common causes of amputation.

Methodology: The study was conducted of 23 samples from foot ulcers diabetic disease. Relevant clinical, biochemical, and microbiological sensitivity evaluations were carried out on the subjects.

Results: This study has shown that the highest ratio of isolated bacteria from diabetic foot ulcer patients were *Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes* respectively. The isolated Gram-positive bacteria were more than isolated gram-negative bacteria. Both *Streptococcus pyogenes and Staphylococcus aureus* showed a high resistance to Benzylpenicillin and Oxacillin, Whereas some isolates of *Pseudomonas aeruginosa* showed resistance to Imipenem, meropenem and Piperacillin/Tazobactam. *Escherichia coli* were resistant to Ticarcillin, Aztreonam, Cefepime and Ceftazidime. *Klebsiella pneumoniae* show high resistant to all of antibiotics. *Proteus mirabilis* resist to Aztreonam, Cefepime, Ceftazidime, Gentamicin, meropenem, Piperacillin/ Tazobactam, Trimethoprim/ Sulfamethoxazole and Tobramycin.

Conclusion: The outcome of current study has shown that the isolated Gram-positive bacteria were more than isolated gram-negative bacteria in foot ulcer patients, with different pattern of resistance to the studied antibiotics.

***Keywords*-** Diabetes, foot ulcer, resistant bacteria.

I. INTRODUCTION

Diabetes is a group of metabolic disorders considered to be hyperglycemic due to defects in insulin secretion, the action of insulin, or both. Hyperglycemia with chronic diabetes is characterized by long injury, dysfunction, and loss of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels[(1)]. Type 1, type 2, gestational and other rare genetic and syndromic variants, such as monogenic adolescent diabetes (MODY), are known as diabetes mellitus types [(2)]. Current defines by the ADA and WHO with the following criteria: Fasting hyperglycemia (plasma glucose ≥ 126 mg/dL (7.0 mmol/L) after at least 8 hours of fasting), hemoglobin A1c (HbA1c) ≥6.5% (48 mmol/mol), or a random plasma glucose >200 mg/dL (11.1 mmol/L) with associated symptoms of hyperglycemia, or a plasma glucose >200 mg/dL after a 75 g glucose load on an OGTT [(3)]. Nowadays, diabetes is considered one of the modern world's most daunting public health issues, with an impacted population of approximately 463 million adults with ages of (20 – 79) in 2019, this is expected to rise to 700 million by 2045. Although diabetes affects people of all ages, the most affected age group is between 60 and 69 years. Around half of the estimated diabetic population, which is about 232 million, is undiagnosed and leads to around 4.2 million deaths worldwide due to diabetes. Around 10 % of adults' total health spending, around $760

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billion, is globally attributed to diabetes. In low and middle-income countries, the number of deaths and expenses per person due to diabetes is much higher, as can be seen from the fact that approximately 87 % of diabetes-related deaths occurred in low and middle-income countries, although only 35 % of diabetes-related health expenditures existed there [(4)].

Complications with diabetes are common in patients with type 1 or type 2 diabetes but are responsible for substantial morbidity and mortality at the same time. Chronic diabetes complications are commonly categorized into microvascular and macrovascular complications, with the former showing a significantly greater prevalence than the latter [(5)]. Neuropathy, nephropathy, and retinopathy are microvascular complications, while cardiovascular disease, stroke, and coronary artery disease are macrovascular complications. The occurrence of foot ulcer associated with neuropathy, and infection has been identified as diabetic foot syndrome, and it is a major cause of lower limb amputation [(6)].

This type of nerve injury is caused by about 50 % of those with type 2 diabetes and 20 % of those with type 1 diabetes [(7)].

Diabetic Foot Ulcer (DFU) is characterized by a timely and orderly failure to self-repair [(8)] and arises as a function of the involvement of many contributory variables. These contributory causes can be classified by scheme into intrinsic (neuropathy, peripheral vascular disease and severity of diabetes) and extrinsic (infection of the wound, development of calluses and undue site pressure) variables [(9)]. A triad of causes ultimately lead to ulceration: the involvement of peripheral neuropathy, foot deformities, and repeated acute (or chronic) trauma. Peripheral sensory neuropathy in the diabetic foot is blamed for pain insensitivity, while autonomic neuropathy induces decreased activity of the sweat gland, resulting in dry, atrophic skin. In the metatarsal plantar area with minimal fat padding, motor neuropathy usually induces inherent muscle wasting with characteristic foot with joint contractures and conspicuous bones [(10)]. Both the three elements together assess lack of feeling, changes in the anatomy of the foot with consequent deformity, and changes in the skin. Consequently, with weak defenses, the diabetic foot is more vulnerable to damage. Of note, it has been shown that loss of peripheral sensory and autonomic nerves and reduced development of neuropeptides precede clinical neuropathy symptoms [(11)]. In addition, skin biopsies of T2DM patients with active foot ulcers, with and without peripheral sensory neuropathy, demonstrated extreme denervation, independent of clinically observable sensory neuropathy [(12)]. The next main aspect is typically internal or external traumas, and they are usually related to the development of abnormally elevated foot pressures while walking. Usually, internal traumas originate from repeated pressures from high-pressure regions; external traumas, such as an item in the shoe, are derived from the environment instead [(13)]. The foot ulceration pathway. The first factor is sensory neuropathy, synonymous with insensitivity to pain. The next main part is mental or external traumas. The third factor contributing to the production of chronic ulceration is poor wound healing.

Complications of the foot ulcer are a significant public health concern and put a heavy load on health care [(14)]. For the bulk of diabetes-associated hospital admissions, foot infections are responsible. Around 15 % of all diabetics are estimated to develop foot ulcers and ultimately progress to osteomyelitis [(15)]. Approximately 20 % of diabetic patients experience diabetic foot ulcer because of owing peripheral neuropathy, muscle atrophy, foot deformity and neuropathic fractures. Eventually, these ulcers result in infections of the diabetic foot. Ulceration, gangrene, Charcot joint, or fracture can be diabetic foot diseases which are a significant cause of amputation [(16)]. Approximately half of the diabetic patients with serious diabetic foot infections in the previous study needed amputation at some stage of their lives before recovery or death [(17)]. The diabetic polymicrobial foot nature is because of the aerobics (*Staphylococcus species*, *Streptococcus spp*. and *Enterobacteriaceae*), anaerobic flora (*Bacteroides species*, *Clostridium spp*. and *Peptostreptococcus spp*.) and fungi [(18,19)].

Diabetic foot ulcer (DFUs) is considered a leading cause of clinical hospitalization, infections, chronic illness, and death. Currently, 20 million persons globally are projected to have a DFU, and at the risk of developing a DFU of around 130 million because of DPN. The most common route to developing a DFU is via intense mechanical tension on senseless neuro-pathic plantar tissue. It results in subdermal trauma, inflammation and finally a DFU will develop if mechanical stress remains excessive. Neuropathy, unfortunately, not only results in the individual's failure to perceive extreme levels of mechanical tension but can also induce gait irregularities and foot deformities that increase mechanical stress or tension levels higher [(20)].

II. MATERIALS AND METHODS

This study includes a 23 samples of diabetic foot ulcer patients. This study was carried out in the city of Baghdad from 1st of March 2021 to 5th of May 2021. The samples were collected from a total of 23 diabetic foot ulcer patients who had been visiting the Medical Nursing clinics in Baghdad city, from both sexes, with age range (43-71) years, a swab from wound samples were obtained after the debridement of exudates, disposable sterile swabs were used, the specimens were obtained by scraping the ulcer base or the deep portion of the wound edge with a sterile curette. Specimens were obtained. then transferred immediately to the lab and inoculated on Blood, MacConkey agar and mannitol salt agar, incubated at 37ºC o for 24-48 hours, then an antibiotic susceptibility test was tested.

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Sterilization

Most of the culture media used in the study were sterilized using the Autoclave device, according to the manufacturer's instructions, at a temperature of 121 ° C (1.5 lb / inch2 pressure) for a period of 15 minutes. Then the media was left to cool until it reached a temperature of 45 ° C, then poured into the Petri dishes and then left to harden, and then it was poured into the Petri dishes and then left to harden and then put in the incubator at a temperature of 37 ° C for a 24-hour duration to ensure that no contamination occurred and to eliminate moisture, after which it was stored in the refrigerator at a temperature of 4° C until used. As for glassware, they were sterilized using an electrical oven at a temperature of (180-160 ° C) for two hours. As for the solutions that deteriorate with high temperature, they were sterilized using millipore filters with a diameter of 0.22 m [(21)].

Preparations of culture media

The prepared implant media were prepared according to the instructions fixed by the company on its package, where it was sterilized with the oxidizer at a temperature of 121 ° C for a period of 15 minutes, after which the media was left to cool until its temperature reached 45 ° C, then poured into tubes or dishes according to the state of the medium (liquid - solid). Then it was placed in the incubator at a temperature of 37 ° C for a period of 24 hours to get rid of moisture and to make sure that no pollution occurred, and then it was kept at a temperature of 4 ° C until its use. All agricultural communities were prepared according to [(22)].

Culture characteristics

The bacterial isolates were diagnosed by studying the general agricultural characteristics of colonies growing on the culture media, where the visible colony shapes were studied and determined on the basis of texture, color, shape, and size, as well as observing other general characteristics such as lactose fermentation or lack thereof and their decomposition of blood [(23)].

1. Blood agar:

Where bacterial isolates were diagnosed by the type of hemolysis on this medium.

2. Mannitol salt agar:

Diagnostic and selection for Selective Media to isolate Staphylococcus aureus, which is characterized by its ability to grow, at a concentration of (10 - 7.5%) NaCl. Where it was incubated at 37 ° C for a period of (48-24) hours to distinguish the fermented aureus from the non-fermented mannitol sugar, where the color of the medium changed from red to yellow due to the presence of Methyl red reagent [(24).]

Antibiotic susceptibility test

Antibiotic susceptibility testing was carried out according to [(25)] for many pathogenic bacteria that were isolated from diarrhea and wounds, for different groups of antibiotics, by the disc diffusion method according to [(26)] and as follows: colonies of the growing bacterial isolates were transferred to the nutrient hemocyte medium at the age of 18-24 hours using a standard culture conveyor to a test tube containing 5 ml of physiological salt solution. The density of the bacterial suspension was adjusted with a tube containing a McFarland turbidity scale 0.5 giving a count of about 1.5-1 x 108 cells / ml. Then a cotton swab was dipped in the bacterial suspension and pressed against the inner wall of the tube to remove the excess inoculum from it, then inoculated with the Muller-Hinton Agar (MHA) plate by wiping the swab on the surface of the middle in several directions in order to obtain homogeneous growth. Then the dishes were left for about (10-15) minutes at laboratory temperature to dry. The antibiotic tablets were transferred using sterile forceps to the surface of the inoculated (MHA) and pressed gently in order to fix them well on the surface of the dens, with a distance of not less than 25 mm between the center of each tablet and the other. The plates were incubated airily and inverted at a temperature of 37 ° C for 24 hours, and the results were recorded by measuring the diameter of the inhibition zone in mm, formed around each disc, and then compared with the standard rates for the diameter of the inhibition zone for those antibiotics mentioned in [(26)], and on the basis of which the bacteria are known to be resistant or sensitive to those antibiotics.

Statistical analysis

Using SPSS (Statistical Package for Social Sciences) version 16.0.0. SPSS Inc., Chicago, statistical analysis was assisted. Regression analysis and analysis of variance for one dependent variable by one or more factors and/or variables is given by the Univariate method. Variables of the factor divided the population into classes. The null hypotheses about the effects of other variables on the means of different groupings of a single dependent variable using this General Linear Model method. We have used the method of Bivariate Correlations to compute the correlation coefficient of Pearson with their significance levels. Correlations test the interaction of variables or rank orders [(27)].

III. RESULTS

Age and Sex characteristics

In this study, the total number of DFU group were of 23 patients, 4 of them were females and 19 were males. The age ranged from 43-71, with total mean age: 55.57±6.37 years. while the total number of control group was 23 of 3 females and 20 males, the age ranged from 21-49 with total means age of 32.35±7.72, Table 1.

Table 1: Means and Standard deviations of Age in the three studied groups.

CASE	SEX	N	AGE Mean. (Years)	Std. Deviation
DFU	Female	4	53.00±	6.33
	Male	19	56.11±	7.57
	Total	23	55.57±	6.37

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Microbiological pattern isolated from diabetic foot ulcer patients.

The organisms isolated from different specimens are summarized in Table 2. The most isolated organisms were *Staphylococcus aureus* (30.40%), *Pseudomonas aeruginosa* (21.7%), *Streptococcus pyogenes* (17.4%), *Escherichia coli* (13%), *Klebsiella pneumoniae* (8.7%), *Proteus mirabilis* (4.3%), *Enterococcus faecalis* (4.3%). (Fig 1)

Table 2: Isolated bacteria with its frequency and percentage.

Gram stain	Bacteria Names	Frequency *N*	Percent (%)
G-ve	***Pseudomonas aeruginosa***	**5**	**21.7**
	Escherichia coli	**3**	**13**
	Klebsiella pneumoniae	**2**	**8.7**
	Proteus mirabilis	**1**	**4.3**
G+ve	***Staphylococcus aureus***	**7**	**30.40**
	Streptococcus pyogenes	**4**	**17.4**
	Enterococcus faecalis	**1**	**4.3**
	Total	**23**	**100**

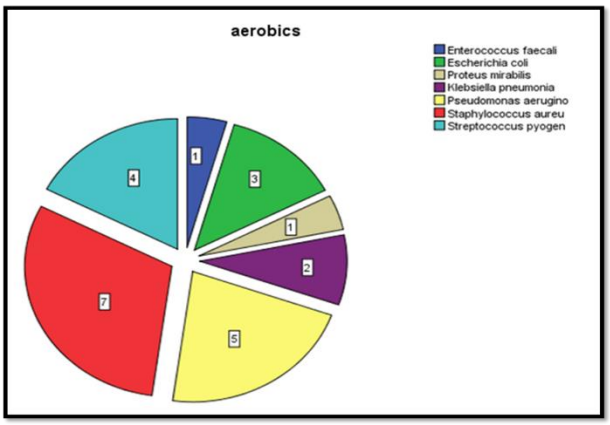

Figure 1: Pie chart of isolated bacteria from diabetic foot ulcer patients.

The most frequently isolated Gram-negative pathogens were *Pseudomonas aeruginosa* (50%), *Escherichia coli* (30%) and *Klebsiella pneumoniae* (20%) of the total Gram-negative isolates. The most frequently isolated Gram-positive pathogens were *Staphylococcus aureus* (58.33%), *Streptococcus pyogenes* (33.33%) and *Enterococcus faecalis* (8.33%) of the total Gram-positive isolates.

Antimicrobial susceptibility test

The antibiotic susceptibility test Multiple antibiotic resistance forms in the big picture and medically dangerous, through the use of this model in the random use of antibiotics, and this is a different use in use depending on the reliance on sensitivity testing, [28]. Antibiotic sensitivity using the hard disk diffusion method on Muller-Hinton Agar medium (MHA). The sensitivity of 14 antibiotics from different antibiotics to Gram-negative bacteria and 17 antibiotics against Gram-positive bacteria were tested [29] and the results were interpreted as stated in [26]. As shown in the tables (3,4).

Table 3: G-negative resistant bacterial isolates isolated from diabetic foot ulcer.

Bacterial isolates / Antibiotics	*Pseudomonas aeruginosa*		*Escherichia coli*		*Klebsiella pneumoniae*		*Proteus mirabilis*	
	№	%	№	%	№	%	№	%
Amikacin	0	0	0	0	2	100	0	0
Aztreonam	0	0	2	66.6	2	100	1	100
Cefepime	0	0	2	66.6	2	100	1	100
Ceftazidime	0	0	2	66.6	2	100	1	100
Ciprofloxacine	0	0	0	0	2	100	0	0
Gentamicin	0	0	0	0	2	100	1	100
Imipenem	1	20	0	0	2	100	0	0
Meropenem	1	20	0	0	2	100	1	100
Minocycline	0	0	1	33.3	2	100	0	0
Piperacillin	0	0	1	33.3	2	100	0	0
Piperacillin/ Tazobactam	1	20	0	0	2	100	1	100

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Ticarcillin	0	0	3	100	2	100	0	0
Tobramycin	0	0	0	0	2	100	1	100
Trimethoprime/ Sulfamethoxazole	0	0	1	33.3	2	100	1	100
Total Tested Samples	5		3		2		1	

Table 4: G-positive resistant bacterial isolates isolated from diabetic foot ulcer.

Antibiotics	***Staphylococcus aureus* NO.**	**(%)**	***Enterococcus faecalis* NO.**	**(%)**	***Streptococcus pyogenes* NO.**	**(%)**
Benzylpenicillin	**6**	**85.7**	**0**	**0**	**2**	**50**
Oxzacillin	**6**	**85.7**	**0**	**0**	**2**	**50**
Gentamicin	**1**	**14.2**	**0**	**0**	**1**	**25**
Tobramycin	**1**	**14.2**	**0**	**0**	**0**	**0**
Levofloxacin	**1**	**14.2**	**0**	**0**	**0**	**0**
Moxifloxacin	**1**	**14.2**	**0**	**0**	**0**	**0**
Erythromycin	**3**	**42.8**	**0**	**0**	**0**	**0**
Clindamycin	**3**	**42.8**	**0**	**0**	**0**	
Rifampicin	**4**	**57.1**	**0**	**0**	**1**	**25**
Teicoplanin	**0**	**0**	**0**	**0**	**0**	**0**
Fucidic Acid	**5**	**71.4**	**0**	**0**	**1**	**25**
Vancomycin	**0**	**0**	**0**	**0**	**0**	**0**
Nitrofuranation	**0**	**0**	**0**	**0**	**0**	**0**
Tetracycline	**3**	**42.8**	**0**	**0**	**0**	**0**
Tigecycline	**0**	**0**	**0**	**0**	**0**	**0**
Linezole	**0**	**0**	**0**	**0**	**0**	**0**
Trimethoprime/Sulfa methoxazole	**0**	**0**	**0**	**0**	**0**	**0**
Total samples Tested:	**7**		**1**		**4**	

IV. DISCUSSION

Diabetic foot ulcer is the result of complex amalgamation of multiple risks like peripheral neuropathy, peripheral vascular disease, foot deformity, trauma, arterial inadequacy and poor infection resistance [30]. These permanent, long-lasting ulcers are often more susceptible to infection, hence delaying the recovery process of wound. In these patients, a large variety of pathogens can lead to infection. Gram-positive bacteria were the predominant pathogens in this research, similar to [31,32], although several other studies have reported Gram-negative predominant infections [33,34,35], As a result, there appears to be a shifting pattern species that triggers diabetic foot infection, with gram-negative bacteria replacing gram-positive bacteria as the most prevalent organism. This study shows the predominance of Gram-positive cocci, *Staphylococcus aureus* was the most commonly isolated cocci, and several studies have found the same outcome [36,37,38]. The other Gram-positive cocci of the most frequently isolated was *Streptococci*. This can refer to the primary phases of superficial infection [39,40]. Together with *staph. aureus*, the presence of *Enterococcus faicalis* applies to patients who have undergone long or inadequate or broad-spectrum antibiotics or who have had a long hospitalization, persistent wound or surgical procedure [35]. The Gram-negative *P. aeruginosa*, *E. coli* and *Proteus spp*. were other typical isolates, with findings similar to several recent studies indicating the predominance of Gram-negative bacteria, as stated [30]. This study was similar to [41] study with the isolate percentages, for *P. aeruginosa* (16.9%) followed by *E. coli* (16.1%) and *Proteus spp*. (8.8%). While many studies showed more *Klebsiella pneumoniae* isolates, like in [35,42, 43], this study was similar to [44] for both isolates' percentage of *Klebsiella* species (8.2%) and *Proteus* spp. (4.3%). Various bacterial profiles have been recorded with various degrees of wounds, in which exacerbated wounds and infections have been associated with an increase Gram-negative species [45]. *Staphylococcus aureus* as it showed a high resistance to Benzylpenicillin and Oxacillin, at a rate of

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85.7%, these antagonists inhibit the construction of the cell wall of the bacterial cell, as they affect the process of manufacturing peptidoclycan, the main component in the cell wall.

This antagonist works to inhibit the bacterial growth as it prevents the protein synthesis process in the bacterial cell, the reason for its resistance to these antagonists is due to the possibility of developing strains resistant to these antagonists due to their production of-Lactamase enzymes whose genes are either on the plasmid or chromosome, or as a result of their production of broad-spectrum-lactamase enzymes. These enzymes neutralize the-Lactam antagonists by breaking the beta-lactam ring in the bacterial cell's synthesis [(46)]. Or, the bacterial cells may produce these penicillin-related proteins present in the bacterial cell wall and work to change the target site of these antagonists and bacterial resistance to them occurs [(47)], so the antibiotic is modified by modifying enzymes such as Adenylating, Phosphorylating and Acetylating, or the permeability of the antagonist to the bacterial cell may be reduced due to a chromosomal mutation in the gene that encodes the core Target routine for the 30S ribosome unit, [(48)]. As for the antagonist Fucidic acid, which works on the elongation factor G (EF-G), which is important in the translation process for protein building, as this antagonist binds to the ribosome and the elongation factor, preventing protein synthesis. Whereas some isolates of *Pseudomonas aeruginosa* showed resistance to Imipenem, meropenem and Piperacillin/Tazobactam 20%. The reason for its high sensitivity to anti-Carbapenems is due to the mechanism of action of these antagonists, as they work to inhibit the construction of the cell wall of the bacterial cell and thus its death. As for the cause of the bacteria's resistance to β-lactam antagonists, the ability of the bacteria to produce-lactamase enzymes, as well as by reducing the permeability of the outer membrane, which prevents the entry of the antibiotic into the bacterial cell [(49)]. *Streptococcus pyogenes* showed resistance to to Benzylpenicillin and Oxacillin 50% and Gentamicin, Rifampicin and Fucidic Acid 25% ,no resistance of other antibiotics have been shown, *Escherichia coli* resist to Ticarcillin 100%, 66.6% to Aztreonam Cefepime Ceftazidime, The reason for its high resistance to these antibiotics is through the production of enzymes that break down the beta-lactam ring in the antibiotic molecule, which leads to a loss of its effectiveness and becomes resistant to it. These enzymes are called β-lactamase enzymes [(50)], *Klebsiella pneumoniae* show high resistant to all of antibiotics This is due to the emergence of isolates of Klebsiella that produce the broad-spectrum beta-lactamase enzymes (ESBLs), which led to an increase in their resistance to various antibiotics, as these resistances include pencillins, cephalosporin, aminoglycosides, and fluoroqunones. As reported in [(51)], where their resistance to both Cefixime and Piperacillin was 100%. *Proteus mirabilis* was resist to Aztreonam, Cefepime ,Ceftazidime, Gentamicin, meropenem, Piperacillin/Tazobactam, Trimethoprime /Sulfamethoxazole, Tobramycin 100%.

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