Napata Collage
Program of Medical Laboratory Sciences
Department of Microbiology

Identification of Common Aerobic Bacteria Associated With Type 2 Diabetic Patients in Khartoum State

Submitted to the Napata College in Partial Fulfilment of the Requirements for the Award of Degree of BSc. in Medical Laboratory Sciences : Microbiology

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الآية

قال تعالى:

(وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا ۖ وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَىٰ كَثِيرٍ مِّنْ عِبَادِهِ الْمُؤْمِنِينَ)

صدق الله العظيم

سورة النمل الاية (15)

DEDICATION

This research is dedicated to:

The sake of Allah, our Creator and my Master, our great teacher and messenger, Mohammed (May Allah bless and grant him),

Who taught us the purpose of life.

Our great parents, who never stop giving of themselves in countless ways, our beloved brothers and sisters, our friends who encourage and support us, and all the people in our life who touch our heart, we dedicated this research

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In the name of God, the Merciful Praise be to Allah. And prayers and peace be upon Muhammad, His servant and Messenger. First of all, we thank God that we succeeded in completing this research. We would like to thank our supervisor Dr. Mona Omar Ahmed for her mighty effort. Also thank AL Moalem Hospital and Bahri Diabetes Hospital for allowing us to take samples patients, and finally thank for the volunteers for trusting us.

Also thank Dr. Reel Gamal We are so grateful for your many help to support our graduation research, because without your help we might never have been able to complete the research in it form, your generosity and help has been an inspiration to us, thank you for your encouragement and support thank you endlessly.

Abstract

Background: Diabetic foot ulcers is among the most common complications of diabetes mellitus which significantly causes hospitalization and is the most prevalent etiology of non-traumatic amputation worldwide. Knowledge of the microbial burden in the ulcers and antibiotics susceptibility pattern may improve patients' care and management. this study was aim to isolate, identify and carry out antibiotic susceptibility testing on bacterial isolates associated with foot ulcers among type 2 diabetic mellitus.

Method: A cross-sectional laboratory based study was conducted in selected Hospitals in Khartoum swabs samples were collected before dressing from patients suffering diabetic foot ulcers attended diabetic center and surgical department during the study period .Data were collected using structured questionnaire. The clinical isolates were purified by streaking on suitable selective and differential culture media. They were identified on the basis of the results of microscopically examinations, Gram reactions, cultural characters, biochemical tests and antibiotic susceptibility testing was done according to clinical and laboratory standard institutes guidelines.

Result: A total of Ninety two pathogenic bacteria were isolated from patients with a diabetic foot ulcer. The most prevalent detected bacteria were Staphylococcus .aureus(34%) , Klebsiella sp. (10.9%), Proteus.mirabilis (27.2%) ,Proteus.vulgaris (12%) ,pseudomon.aeruginosa (17.4%).Gram-positive (Staphylococcus .aureus) isolates were susceptible to Gentamycin, Vancomycin and Imepenm Gram-negative isolates were also sensitive to Imepenem and Ciprofloxacin The most resistant antibiotic was Amoxacillin

Conclusion : The pathogens causing diabetic foot ulcers show vary in sensitivity and resistant to many of the routinely used medications. However, resistance is being developed to some of the antibiotics such as Ceftrixone Therefore, the culture of the specimen to identify the causative agent and adequate knowledge of the susceptibility pattern are critical for the appropriate management of diabetic foot ulcers.

المستخلص

الخلفية :قرح القدم السكرية هي من بين المضاعفات الأكثر شيوعًا لمرض السكري والتي تسبب بشكل كبير دخول المستشفى وهي السبب الأكثر انتشارًا للبتر غير الرضحي في جميع أنحاء العالم .قد تؤدي معرفة المسبب الجرثومي في القرحة ونمط الحساسية للمضادات الحيوية إلى تحسين رعاية المرضى وإدارتهم .هدفت هذه الدراسة إلى عزل وتحديد وإجراء اختبار الحساسية للمضادات الحيوية على العزلات البكتيرية المصاحبة لتقرحات القدم بين مرضى السكري من النوع الثاني.

الطريقة :أجريت دراسة مقطعية مستعرضة في مستشفيات مختارة بالخرطوم ، تم جمع عينات من المرضى الذين يعانون من قرحة القدم السكرية الذين حضروا إلى مركز السكري وقسم الجراحة خلال فترة الدراسة ، وتم جمع البيانات باستخدام استبيان منظم .تمت تنقية العزلات السريرية عن طريق وضع خطوط على وسط زراعي انتقائي وتفاضلي مناسب .تم تحديدهم على أساس نتائج الفحوصات المجهرية ،تفاعلات الجرام ، مورفولوجيا المستعمرة ، الاختبارات البيوكيميائية واختبار الحساسية للمضادات الحيوية وفقًا لإرشادات المعاهد القياسية السريرية والمخبرية.

النتيجة :تم عزل مجموعه 92 بكتيريا ممرضة من مرضى قرحة القدم السكرية .كانت البكتيريا الأكثر انتشارا هي المكورات العنقودية الذهبية34) ٪ (أنواع الكليبسيلا10.9) ٪ (، بروتيس ميرابليس27.2)٪ (بروتيوس فولغاريس (12%) سودوموناس اريجونوسا.(17.4%)والبكتريا سالبة الجرام كانت حساسة للايميبنم و السيبروفلوكساسين. المضاد الحيوي الاكثر مقاومة هو الاموكسيسيلين.

الخلاصة: أن مسببات الأمراض المسببة لقرحة القدم السكرية تختلف في الحساسية والمقاومة للعديد من الأدوية المستخدمة بشكل روتيني .ومع ذلك ، يتم تطوير المقاومة لبعض المضادات الحيوية مثل سيفتريكسون ، لذلك ، فإن ثقافة العينة لتحديد العامل المسبب والمعرفة الكافية بنمط الحساسية أمر بالغ الأهمية للإدارة المناسبة لقرحة القدم السكرية.

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ABBREVIATIONS

Meaning	**Symbol**
Diabetes Mellitus	DM
Type 2 Diabetes Mellitus	T2DM
Diabetic Foot Ulcer	DFU
Diabetic Foot Syndrome	DFS
Glutamic Acid Decarboxylase	GAD65
Myo-Inositol Oxygenase	MIOX
Diabetic ketoacidosis	DKA
Intravenous	IV
Mannitol Salt Agar	MSA
kligler iron agar	KIA
Motility Indol urease	MIU

Chapter one

Introduction

Introduction

1.1 Background:

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. Approximately 415 million adults between the ages of 20 to 79 years had diabetes mellitus in 2015, DM is proving to be a global public health burden as this number is expected to rise to another 200 million by 2040. More than 95% of all adults with diabetes mellitus have type 2 diabetes mellitus. DM complications and co-morbidities are more frequent in old diabetics compared to their young counterparts. Diabetes is amongst the diseases with higher complications (perhaps even the highest) and these complications lower the quality of life in patients significantly [5,6]. Diabetes is a systemic disease as it affects various body systems to some extent, for instance diabetes can disrupt proper function in cardiovascular, gastrointestinal, immune and nervous systems therefore the functional impairment of peripheral nervous system can lead to diabetic foot and in worst cases to amputation and hence physical disability. In 2003, a new classification system based upon the etiological factors at work in diabetes was proposed by the WHO and this has now become the accepted system for classifying diabetes mellitus (Parveen and Michel, 2005). Type 1 diabetes it is immune mediated and idiopathic forms of b cell dysfunction, which lead to absolute insulin deficiency. It's known as insulin dependent diabetes mellitus (INDDM) (Parveen and Michel, 2005). Type 2 diabetes it is a disease of

adult onset, which may originate from insulin resistance and relative insulin deficiency or from a secretary defect. It's known as non insulin dependent diabetes mellitus (NINDDM) (Parveen and Michel, 2005). Type 3 diabetes is gestational diabetes (GM) (Parveen and Michel, 2005).

Foot ulceration is the most frequently recognized complication. The diabetic patients with foot infections especially gangrene require long-term hospitalization and carry the risk of limb amputation. Patients in whom their foot ulcer progressed to diabetic foot infections not only suffer from prolonged hospitalization but also leads to amputations of their foot which increases the rates of mortality. The presence of infection is a common finding in diabetic foot ulcers which act as an entry route for pathogens. Infections must be diagnosed and treated promptly and adequately as they may rapidly progress to a limb-threatening condition. Also, high levels of bacteria can delay or event prevent wound healing and impede surgical closure of diabetic ulcers.

The microbial etiology of diabetic foot ulcers is usually complex. Many of these infections are either mono-microbial or poly-microbial. Also, multidrug-resistant organisms have been reported very frequently, which has further complicated the treatment regimens [8, 9]. Pathogenic bacteria that cause these infections either originated from the external environment or physiological microflora of the skin (10). Furthermore, most of foot ulcerations may contain mixed flora, that consist of aerobic strains such as *S. aureus, Streptococcus pyogenes, E. coli, Proteus mirabilis, Pseudomonas aeruginosa, E. fecalis, Klebsiella spp.*, and anaerobic bacteria, for example, *Bacteroides fragilis Clostridium perfringens* and *Peptostreptococcus spp.* [11]. Diabetic patients with foot infection have several factors that might be associated with a high risk of multi-drug resistant microorganisms (MDR), such as inappropriate antibiotic treatment, chronic course of the wound, reduced antibiotic concentration in

the tissue and frequent hospital admission (Zubair). Also antibiotics use may result in antimicrobial resistance, high financial cost, and drug-related adverse effects, many published evidence discourage therapy of uninfected ulcers with antibiotics, either to enhance wound healing or as prophylaxis against infection [14].

14. Boulton AJM, Armstrong DG, Hardman MJ, Malone M, Embil JM, Attinger CE, et al. Diagnosis and Management of Diabetic Foot Infections. Arlington (VA): Am. Diabetes Assoc.2020.

Zubair M. Prevalence and interrelationships of foot ulcer, risk-factors and antibiotic resistance in foot ulcers in diabetic populations: A systematic review and meta-analysis. World J Diabetes. 2020 Mar 15;11(3):78-89. doi: 10.4239/wjd.v11.i3.78. PMID: 32180896; PMCID: PMC7061236.

1.2 Problem Identification and Justification:

Diabetic foot ulcer are most common medical complications of patients with diabetes, are responsible for more hospitalization than any other complications of diabetes. Ulcerations can have potential devastating complication as they cause up to 90% of lower extremely amputation in patients with diabetes. Diabetic foot ulceration and infection are major medical ,social ,economic problem and leading cause of morbidity and mortality especially in developing countries , Inappropriate use of antibiotics encourages increase the incidence of multidrug resistance microorganisms and represents one of the main challenges in managing and treating these infections, as it further complicated the treatment regimens and increased the hospital stay and the cost .Therefore, there is an urgent need to study the prevalence and the causative microorganisms of diabetic foot ulcer in Sudanese hospitals. Hence, this study aimed to determine the bacteriological profile and antimicrobial susceptibility pattern of organisms isolated from patients with diabetic foot ulcer.

1.3General objective

To determine the common aerobic bacteria associated with diabetic foot ulcers and their antibiotic susceptibility patterns.

1.4 Specific objectives

- To isolate and identify aerobic bacteria associated with diabetic foot ulcers.
- To assess the antimicrobial susceptibility patterns of the isolates.
- To compare between prevalence of gram negative and gram positive aerobic bacteria among diabetic foot ulcers.
- To assist Correlation between the risk factors (age-gender) and the isolated bacteria.
- To assess the prevalence of multidrug resistant bacteria among diabetic foot ulcers.

Chapter Two

Literature Review

Literature Review

2.1 Diabetic mellitus

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia and high glycated hemoglobin with or without glycosuria.Glucose metabolism disorder results from a defect in insulin secretion by the pancreas, insulin action on the target tissues (or insulin resistance), or both. Chronic hyperglycemia leads to damage and failure of various organs, especially the heart, blood vessels, eyes, kidneys, and nerves

2.2 Epidemiology of diabetic mellitus

The prevalence of diabetes is increasing worldwide, with the greatest increases occurring in low- and middle-income countries. With an 8.5% global prevalence of diabetes in 2014; various estimates suggest that the number of affected people will be risen from 422 million to 642 million in the world by 2040. Between 1990 and 2010; the rank of the disease has moved from 15 to 9, which corresponds to a 92.7% increase in the burden during the period. Over the past decade, the prevalence of diabetes has risen rapidly due to an increase in the average age of the community, hereditary background, unhealthy dietary habits, sedentary lifestyle and increased obesity in line with the growth of urbanization [16,19]. Diabetes mellitus is a disorder stemming from glucose dysregulation. Of those people with diabetes mellitus, 90% to 95% have type 2 diabetes mellitus, whereas 5% to 10% have type 1 diabetes mellitus, Within the United States >30 million Americans have diabetes mellitus and an additional 84 million Americans meet diagnostic criteria for prediabetes mellitus.

2.3 Classification of diabetes Mellitus

In 1999 WHO established a classification for diabetes based on the clinical stages and the different etiological types. Recently in 2019 WHO established new classification system for diabetes which facilitate the three primary

purposes: clinical care, aetio-pathology and epidemiology. Commonly most diabetic patients affected by one of the following types:

2.3.1 Type 1 diabetes

Type 1 diabetes results from autoimmune destruction of the pancreatic beta-cells.Markers of immune destruction of the beta-cell are present at the time of diagnosis in 90% of individuals and include antibodies to the islet cell (ICAs), to glutamic acid decarboxylase (GAD65), tyrosine phosphatases IA-2 and IA-2b, ZnT8, and insulin auto-antibodies (IAAs). Type 1 diabetes is associated with childhood-onset diabetes but most commonly diagnosed in adults.

2.3.2 Type 2 diabetes

Is characterized by insulin resistance and, at least initially, a relative deficiency of insulin secretion.In absolute terms, the plasma insulin concentration (both fasting and meal-stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis, howeverwith time there is progressive beta cell failure and worsening insulin deficiency ensues.

2.4 Complications of type 2 diabetic mellitus

2.4.1 Nephropathy,retinopathy

Diabetic nephropathy, diabetic retinopathy is caused due to prolonged high blood sugar level over timefurthermore , when the level of blood glucose is high for a long period, it can increase myo-inositol oxygenase (MIOX) enzyme activity and enhances myo-inositol catabolism, the enzymatic degradation of myo-inositol alters the activity of Na+/K+ ATPase and phosphatidylinositol synthases, the very important molecules in the secondary signaling pathway,thus high blood glucose levels due to DM results in diabetic nephropathy, retinopathy, neuropathy, and diabetic cataracts, on the other hand in uncontrolled diabetes the high blood glucose

level in the delicate vessels of the retina increases osmotic pressure, and the vessels get leaked or rupture in some instances resulting in an impaired supply of blood to the retina, therefore to compensate for the ruptured retinoid vessels, collateral blood vessels grow out of the retina and cause scar tissue to form resulting in impaired vision, also uncontrolled diabetes can affect kidneys, damaging the basement of glomerular capillaries, disrupting protein crosslinking, and allowing proteins in the urine to leak through, a process known as diabetic nephropathy.

2.4.2 Ketoacidosis

Ketoacidosis is common in diabetic patients due to the continuous production of ketone bodies, diabetic ketoacidosis (DKA) is a feature of insulin insufficiency rather than resistance, which characterizes T2DM .

2.4.3 Diabetic Neuropathy

At least half of the people with diabetes will develop clinically significant peripheral neuropathy also sensory nerve dysfunction leads to the loss or weakening of skin protection. Motor neuropathy, which increases plantar pressure that directly destroys the tissue, causes plantar capillary occlusion, local tissue ischemia, and destruction. The autonomic neuropathy of sweat glands in people with diabetes leads to reduced skin sweating, abnormal temperature regulation, and dry and chapped skin, which in turn damages the integrity of the skin, leading to a reduced barrier to infection, also it leads to perturbations in the skin blood flow and microcirculatory disorders such as loss of peripheral sympathetic nerve innervation and tension, leading to vasomotor dysfunction and abnormal arteriovenous shunting, as a result the abnormal blood flow distribution and nutritional capillary ischemia could occur

2.4.4 Impaired wound healing

Impaired wound healing is a common complication of diabetes mellitus, healing in patients with diabetes mellitus is characterized by reduced tensile

strength of wounds when compared with controls, suggesting either defective matrix production or depositionmoreover in the human mammal, diminished perfusion resulting from the presence of peripheral arterial disease as well as decreased sensory nerve function caused by peripheral neuropathy may contribute to impaired healing, also it is presumed that diabetic complications result from periods of poor glycemic control. However, aberrant growth factor expression or factors secondary to diabetes, such as advanced glycation and cross-linking of matrix protein, may also be involved.

2.4.5 Diabetic foot lesions

DM is associated with numerous complications related to microvascular, macrovascular, and metabolic etiologies, one of those is diabetic foot syndrome (DFS), which was defined as an array of foot abnormalities, resulting from peripheral neuropathy, macroangiopathy, and other consequences of metabolic disturbances. The lifetime incidence of developing diabetic foot ulcer (DFU) can reach 25%. Different factors and causes can lead to DFU, such as uncontrolled diabetes may lead to neuropathy and minor trauma can be associated with delayed healing.

2.4.6 Microbiological Considerations

Selecting appropriate antimicrobial therapy for diabetic foot infections requires knowledge of the likely etiologic agents. Various skin disorders and environmental exposures, as well as recent antibiotic therapy, can alter the colonizing flora of skin wounds . Although acute infections in previously untreated patients are usually caused by aerobic gram-positive cocci (often as monomicrobial infections), chronic wounds develop complex flora. Determining the microbial etiology of an infected wound will usually assist in subsequent management. The etiologic agents can be identified by culture only if specimens are collected and processed properly. Antibiotic-susceptibility results generally help tailor (and in many cases constrain)

antibiotic regimens. Deep tissue specimens, obtained aseptically at surgery, contain the true pathogens more often than do samples obtained from superficial lesions. A curettage, or tissue scraping with a scalpel, from the base of a debrided ulcer provides more accurate results than does a wound swab. Therapy directed against organisms isolated from culture of a swab sample is likely to be unnecessarily broad and may occasionally miss key pathogens. If multiple organisms are isolated, the clinician must decide which require specifically targeted therapy. Less virulent bacteria, such as enterococci, coagulase-negative staphylococci, or Corynebacterium, may represent pathogens but can sometimes be ignored. Organisms isolated from reliable specimens that are the sole or predominant pathogens both on the Gram-stained smear and in the culture are likely to be true pathogens. *S. aureus* is the most important pathogen in diabetic foot infections; even when it is not the only isolate, it is usually a component of a mixed infection. Gram-negative bacilli, mainly of the family Enterobacteriaceae, are found in many patients with chronic or previously treated infections. *Pseudomonas* species are often isolated from wounds that have been soaked or treated with wet dressings or hydrotherapy. *Enterococci* are commonly obtained by culture from patients who have previously received a cephalosporin. Obligate anaerobic species are most frequent in wounds with ischemic necrosis or that involve deep tissues. Anaerobes are rarely the sole pathogen; most often they constitute a mixed infection with aerobes.

2.4.7 Antibiotic Therapy

The route of administration and type of antimicrobial agent to be used are determined by the results of a microbiological culture, the severity of the clinical signs, the body structures involved, and the immunocompetence of the patient, during routine care broad-spectrum antibiotics are typically used first, before switching to a more targeted agent once the bacterial culture results are available. In severe, non-responsive, or spreading infections, or

where serious osteomyelitis is suspected, hospitalization and intravenous (IV) antibiotic therapy may be done. The most commonly used broad-spectrum agents are carbapenems β-lactam, or β-lactamase inhibitor combinations, such as piperacillin/tazobactam, ampicillin/sulbactam, and ticarcillin/clavulanic acid.Carbapenems are a mainstay in the treatment of multidrug-resistant Gram-negative bacteria; however, resistance to this group of drugs is increasingly being reported in the clinics. Additional agents and combinations used in the clinic include cefepime plus tazobactam,Imepnem, amikacin, and gentamicin .Antimicrobial therapy, along with surgical treatment or debridement, is essential for treating any chronic deep infections in the bone.

2.5 Previous studies

Ogba*et al.*, in 2019, study aerobic bacteria associated with diabetic foot ulcers and their susceptibility pattern on patients attending diabetic clinic of University of Calabar Teaching Hospital in Nigeria (19 males and 31 females) submitted swabs for culture. The most common isolated bacteria were *Staphylococcus aureus*at (32.9%) followed by *Pseudomonas.aeruginosa* (24.7%). Age group (50–59) years had the highest number of isolates (38.1%) while age group(70–79) years had the least number of isolates (15.5%). The susceptibility rate of isolates to commonly used antibiotics was 11.7 to 75.0% *Staphylococcus aureus* susceptibility profile was 48.4% for quinolones, 32.3 to 48.4% for cephalosporins, 19.4 to 61.2% for the beta-lactam antibiotics and 67.7% for erythromycin. *Pseudomonas aeruginosa* susceptibility profile was 45.8 75.0% for quinolones, 20.8 to 25.0% for cephalosporins and 25.0% for gentamycin. The gram-positive isolates showed higher susceptibility to erythromycin (67.7%), followed by amoxicillin (61.2%), The gram-negative isolates were more susceptible to ciprofloxacin (80.4–100%). Quinolones and cephalosporins are given to the patients according to the hospital

protocol for antibiotic treatment, thus there was multiple antibiotic resistance of isolates to quinolones and cephalosporins.

Hamad in 2020, conducted a study about bacteriological profile and antibiotic susceptibility of diabetic Foot infections at Ribat University hospital, among type 2 diabetic patients. Regarding culture results of 250 patients (73.2% of them were males, and 86.4% of them had type 2 diabetes mellitus), atotal of 335 bacterial isolates were identified, gram-negative were more prevalent than gram-positive organisms. The most frequently isolated organisms were *Proteus spp. Staphylococcus aureus*, and *Escherichia coli*. Furthermore, antibiotic susceptibility pattern showed that Imepnem, amikacin and vancomycin have the highest activity against isolated bacteria, and all isolates were found to be completely resistant to different cephalosporin drugs.

Mukhtar, Maowia, *et al.*, was conducted a study during December 2017 - March 2018 in a Diabetic Center, Sudan. A total of 152 diabetic patients with different grades of foot ulcers were randomly enrolled in the study (135 patients (88.8%) were male and 17 patients (11.2%) were female).The most common isolates were *P. Mirablis*, *Staphylococcus.aureus* and Coliform.The most sensitive antibiotics for Gram negative rods were Amikacin, 80.6 %, Imepnem 77.2 % and Meropenem 77%. For Gram positive the most sensitive antibiotics were Imepnem 85% and Vancomycin 69%. The most sensitive antibiotic among all isolates was Meropenem.

Chapter Three

Materials And Methods

Materials And Methods

3.1 Study design

A cross-sectional laboratory based study.

3.2 Study area and duration

Study was conducted in bahhri diabetes center , AL Moalem medical center and Zinam Hospital inKhartoum state from August- November 2022.

3.3 Study population

This study was conducted among diabetic mellitus type 2 with foot ulceration.

3.4 Inclusion criteria:

Diabetic mellitus type 2 patients suffering from foot ulcer

3.5 Exclusion criteria

Diabetic mellitus type 1 patients who were insulin dependent .

Non diabetic patients with foot ulcer.

3.6 Sample size

A total of 1oo swabs samples were collected from diabetic patients suffering from foot ulcer attended Diabetic Center and Surgical Department in hospital during the study period.

3.7 Sampling technique

Non-probability convenience sampling technique.

3.8 Ethical approval

Ethical clearance of this study was obtained from the Napata college, program of medical laboratory sciences, the specimens and information were collected from the individuals under privacy and confidentially and was not be used for any purpose rather than this study.

3.9 Data Collection :

The data was collected by using a questionnaire (Appendix I). A questionnaire was designed to include all needed information **(** gender, age, medication of patients) .

3.10 Specimens collection

Samples were collected before dressing by experienced nurse by using sterile cotton swabs and inserted into amies transport media, then transferred to microbiology lab at Napata College where optimal microbiological investigation and culture techniques were used**.**

3.11 Culture

The swabs were inoculated in both blood and macConkey agar plates and incubated overnight, aerobically in incubator at 37□C. Identification of growth was based on colonial morphology, Gram staining and appropriate biochemical tests.

3.12 Direct Gram stain

Direct Gram stain from samples were performed by rolling the swab on a slide and fixed by heat, then regular Gram stain procedures were followed.

3.13 In direct Gram stain

Firstly, smear prepared by taken one drop of normal saline into clean slide and just a touch from colony by wire loop and emulsified then spread n slide and fixed by heat, then gram stain procedure:

- cover the fixed smear with crystal violet stain for 1minute and wash by tap water
- cover the smear with iodine for 1minute and wash by tap water
- decolorized by acetone alcohol for 5_10 seconds and wash by tap water
- Cover the smear with safranin stain for 2 minutes wash by tap water, then clean the back of slide and placed on rack to air-dry finally

Examined the smear under microscope with the oil immersion objective lens x100.

- Gram positive bacteria stained blue to purple color and Gram negative bacteria stained pink to red color.

3.14 Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Method: 2ml of 3% of hydrogen peroxide solution into a test tube then take sterile wooden stick and take colonies of tested organism and immersed in solution and look for immediate active air bubble if positive catalase test and no active air bubble negative catalase test.

3.15 Coagulase test

This test is used to identify S. aureus which produces the enzyme coagulase.

Method: Place a drop of physiological saline on each end of a slide then emulsify a colony of the test organism in each of the drops to make thick suspension. Add a drop of plasma to one of the suspensions, and mix gently and look for clumping within 10 seconds if positive Coagulase test and no clumping within 10 seconds if negative Coagulase test.

3.16 Mannitol Salt Agar (MSA)

differentiation of pathogenic Staphylococci spp by inoculation in mannitol salt agar media and incubation overnight and immersed the growth if positive MSA test and no growth if negative MSA test.

3.17 Citrate utilization test:

Method: Using sterile straight wire, inoculates the test organism into 2 ml Simmon`s citrate medium Incubate the medium at 37oC for 24 hrs. Look for color change on the medium and immersed the blue color if positive citrate test and no blue color if negative citrate test.

3.18 kligler iron agar (KIA) test

using a straight wire loop to stab agar butt close to the opening and then streak the top slope (zigzag). and Incubate the medium at 37oC for 24 hrs. and result based on fermentation of glucose, lactose, Gas, and hydrogen sulfide is production. Fermentation of sugar(s) change a pH, so indicator (phenol red) will change the color of the media from red to yellow the presence of a black color indicates that H_2S was produced. H_2S reacts with the ferrous sulfate in the media to make ferrous sulfide, which is black.

3.19 Motility Indol urease test (MIU)

Test was used to identified of gram negative bacilli especially Enterobacteriaceae. Three tests in a single tube that helps to differentiate the organisms on the basis of motility, urease, and indole production. Test organisms in MIU agar after incubation show either diffused growth or turbidity extending away from the stab inoculation line in the case of motile organisms while no motile organisms appear as restricted growth along the stab line. Organisms that produced urease hydrolyses urea and release ammonia and carbon dioxide. Ammonia reacts in solution to form ammonium carbonate, which is alkaline leading to an increase in pH of the test medium. Phenol red present in the medium changes its color from yellow to pink-red acetic acid are produced from tryptophan present in casein enzyme hydrolysate by the enzyme, tryptophan's. The indole formed reacts with p-dimethyl amino benzaldehyde present in Kovac's reagent to form a quinoidal red-violet compound.

3.20 Antimicrobial susceptibility test:

Antibiotic susceptibility test was performed using the Kirby Bauer disk diffusion method according to th guidelines of the Clinical and Laboratory Standards Institute guidelines (CLSI),Three to five pure colonies were transferred to a tube containing 5ml of sterile normal saline andMixed gently until forms a homogenous suspension. A sterile cotton swab was dipped into

the suspension and lawn uniformly over the entire surface of Mueller-Hinton agar. The antimicrobial disk including Amoxicillin (AX), Ciprofloxacin(CIP), Vancomycin(VA), Imepnem(IPM), Ceftriaxone(CRO), Gentamycin(CN) were used. After overnight incubation at 37□ C, the zone of inhibition was measure to the insert millimeter using a ruler. The isolate was classified as sensitive, intermediate sensitive, or resistant based on the clinical laboratory standards institute (CLSI)criteria.

3.21 Data analysis

Data was analysed by using Statistical Package for Social sciences (SPSS) computer program version 26.

Chapter Four

Results

4.1 Results

A total of 100 samples from type 2 diabetic patients with active foot ulcers were included in this study, 24 of them from Bahhri Diabetes center, 18 from Zinam Hospital and 58 from ALMoalem

Among them (62%) were males, whereas (38%) were females (1 female: 1.6 male). Their ages ranged between (25-90) years and the mean age was 56 ± 11. (Table 1).

Regarding medications protocol that used in hospitals, the most commonly used drugs were ceftriaxone (67.4%), then ciprofloxacin (12%), Meropenem (8.7%), ceftriaxone and ciprofloxacin combination (6.5%), ceftriaxone and ciprofloxacin combination (2.2%), and finally cefuroxime (1.1%), This protocol wasnot standardized among all hospitals as, Bahri Diabetes Hospital uses ciprofloxacin and Meropenem mostly, while Zenam and al-Moalem hospitals use more ceftriaxone, and this is shown in (Table-2).

A total of 92 pathogens were isolated, (34%) were Gram positive including (30 *Staphylococcus aureus)* and (66%) were Gram negative including *Klebsiellapneumoniae* (10 (10.9%)), *Proteus mirabilis* (25 (27.2%)), *Proteus vulgaris* (11 (12%)), *Pseudomonas aeruginosa*(16 (17.4%)). The frequency of different isolated pathogens is described in (Table 3- Figure 1).

The results of antibiotic sensitivity tests revealed the most sensitive antibiotics were Imepenem (81%), Gentamycin (80%) and Ciprofloxacin(78%) .The most sensitive antibiotic among all isolates was Imepenem . The most resistant antibiotics were Amoxacilline (56%), Ceftrixone(44%) and Vancomycin(37%).The most resistant antibiotic among all isolates was Amoxacilline . Need to write the most resistant one which found also

There was no statistically significant relationship between the isolated pathogens and the gender (P = 0.77, P-value ≥ 0.05), 62 pathogens were isolated from males and 38 pathogens were isolated from females. The same

types of bacteria affected the males also affected and isolated from the females. Even though, also there was no statistically significant relationship between the isolated pathogens and the age groups (P =0. 99, $P \geq 0.05$). All the three age groups affected with same type of bacteria and in similar proportions.

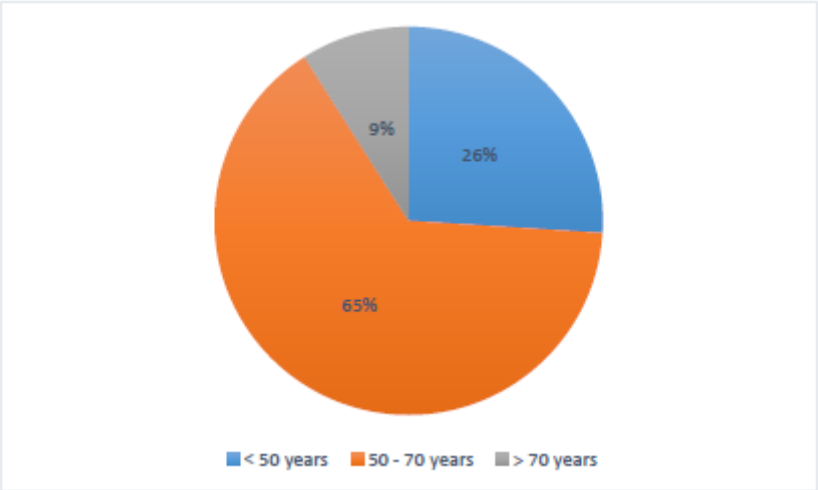

Figure4.1 Age distribution of 100 patients with type 2 diabetic foot ulcers.

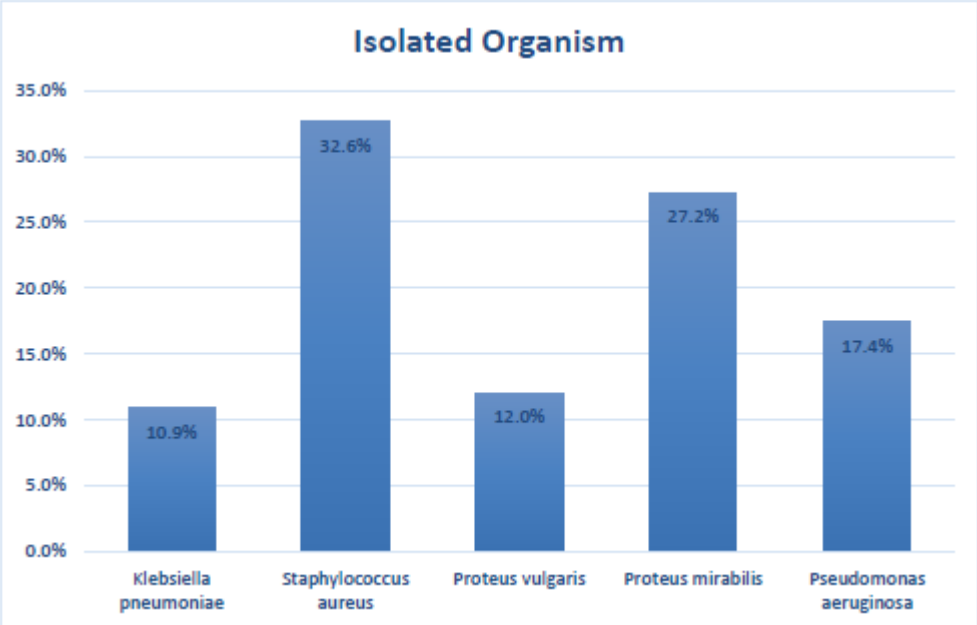

Figure4.2: Frequency of different isolated pathogens from patients with diabetic foot ulcers.

Table4.1: Frequency of medication according to hospitals:

Percent	Frequency	Medication
12.0	11	Ciprofloxacin
67.4	70	Ceftriaxone
8.7	8	Meropenem
2.2	2	Ceftriaxone, Ciprofloxacin
6.5	6	Meropenem, Ciprofloxacin
100.0	100	Total

Table4.2 Susceptibility of isolates to antibiotics:

Resistance	Intermediate	Sensitive	Antibiotics
15 (15.0%)	4 (4.0%)	81 (81.0%)	Imepnem
20 (20.0%)	2 (2.0%)	78 (78.0%)	Ciprofloxacin
44 (44.0%)	4 (4.0%)	52 (52.0%)	Ceftriaxone
15 (15.0%)	5 (5.0%)	80 (80.0%)	Gentamycin
37 (37.0%)	1 (1.0%)	62 (62.0%)	Vancomycin
56 (56.0%)	2 (2.0%)	42 (42.0%)	Amoxicillin

Table4.3 Correlations between isolated organisms and risks factors (age group, gender).

Gender		**Age (years)**			
Female	**Male**	**> 70**	**50 – 70**	**< 50**	**Isolated Organisms**
3	7	1	7	2	*Klebsiella. pneumoniae*
7.9%	11.3%	11.1%	10.8%	7.7%	
10	20	3	18	9	*Staphylococcus.aureus*
26.3%	32.3%	33.3%	27.7%	34.6%	
2	6	1	5	2	*Staphylococcus.epidermidis*
5.3%	9.7%	11.1%	7.7%	7.7%	
4	7	2	7	2	*Proteus.vulgaris*
10.5%	11.3%	22.2%	10.8%	7.7%	
12	13	2	17	6	*Proteus.mirabilis*
31.6%	21.0%	22.2%	26.2%	23.1%	
7	9	0	11	5	*Pseudomonas.aeruginosa*
18.4%	14.5%	0.0%	16.9%	19.2%	
38	62	9	65	26	**Count**
0.788		0.961			**P. Value**

Table4.4 Susceptibility of isolates to antibiotics:

Resistance	Intermediate	Sensitive	Antibiotics
15 (15.0%)	4 (4.0%)	81 (81.0%)	Imepnem
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56 (56.0%)	2 (2.0%)	42 (42.0%)	Amoxicillin

Chapter Five

Discussion, Conclusion, Recommendations

5.1 Discussion

Diabetes is believed to impact 422 million people worldwide, one in every four diabetics will develop a diabetic foot ulcer (DFU) over their lifetime [44] .

Foot ulcers cause morbidity and mortality, all over the world. Correct diagnosis of the etiological agents associated with foot ulcers, as well as proper treatment, care, and management, are the keys to lowering the incidence of diabetic foot ulcers. We attempted to identify and evaluate the susceptibility patterns of bacterial pathogens correlated with diabetic foot ulcers in this study.

Our study shows that, subjects aged 50-70 years old are more susceptible to foot ulcers. This finding is in agreement with the study of saada *et al*., (2020) in a Diabetic Center in Sudan with most of their subjects with average age of 54 years old and older than 25 years. The mean age of the subjects in this study was 54.31 with SD ± 12.1 years. Also in the current study, we found that DFUs were higher in males (62%) than in females (38%). This could be attributed to the fact that males are more exposed to hard works in the outer environment.

All DFUs in the current study showed different isolated , whereas *Staphylococcus aureus* was the only gram-positive bacterial isolate (30 (34%)) , *Proteus mirabilis* (25 (27.2%)) was the most common gram-negative isolate followed by *Pseudomonas aeruginosa(17%) Proteus vulgaris* (12%) and *Klebsiella pneumoniae* (10.9%) . These results agree with many studies done in Sudan and worldwide, one of them a study done by Mahgoub, *et al*., (2015)isolated 187 isolates, they include *Staphylococcus. aureus* (52%) and *Proteus. mirabils*(25%) as the commonest bacterial isolates. Another study done by Ogba*et al.,* (2029) in Nigeria Showed that *Staphylococcus aureus* had the highest degree of occurrence (32 (32.9%)).

None of the isolates showed 100% susceptibility to any of the antibiotics tested. Amoxicillin is the antibiotic with the highest resistance (56.0%) followed by ceftriaxone (cephalosporins) (44. 0%) .The high resistance of isolates to amoxicillin may be attributed to the fact that amoxicillin has been widely abused and frequently implicated in self-medication in Sudan. The resistance to ceftriaxone is probably because the hospital uses it routinely in her protocol to treat foot ulcers. These results are totally in agreement with a study done by Ogba*et al.,* (2019) in Nigeria which showed that the multiple antibiotic resistance of isolates is for cephalosporins and amoxicillin.

Imipenem resistance is increasing public health problem in all populations especially in the hospitalized patients and Imipenem resistance was show against Gram positive and Gram-negative bacteria in 2010 Kashan University the study proved that Imipenem had resistance against *E. coli*, *Klebsiella,* coagulase-negative Staphylococci, *Pseudomonas aeruginosa*, vancomycin-resistance was high and many (VRSA) have been isolated and in USA 2002–2006 study VRSA was isolated from ulcers and the resistance was very high (86%) . Ciprofloxacin resistance was high may be due to hospital protocol, in 2017 in Sudan the study shows that the resistance observed among most organisms because these Ciprofloxacin have been in use for a long period and must have been abused and as a result the organisms must have developed mechanisms of circumventing their mode of action. In our research Ceftriaxone resistance was a high and almost was a Gram-negative bacterium and that may due to hospital protocol and most patients that take it as treatment without culturing and there was study proved that 19% of isolated bacteria from wound discharge was a CeftriaxoneA global response. *Bull. World Health Organ.* **80**, 126–133 (2002).Amoxicillin shows the highest resistance among Gram positive and Gram negative bacteria and Ogba*et al.,* (2019) in Nigeria the study proved that there a multiple antibiotic resistance to cephalosporins and amoxicillin

MDR defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, 23% of patients was resistant to 3 to 5 of antibiotics and about 17% of them was carriers to Gram negative bacteria. A study was carried out in Addis Ababa Ethiopia (2017) showed that *Staphylococcus aureus* , *Acinetobacter* and *Pseudomonas* were multidrugs resistant.

5.2 Conclusion:

Among DFUs studied samples, gram-negative bacteria were more commonly isolated than gram-positive bacteria(*Staphylococcus.aureus*). The most frequently isolated organisms were *Proteus spp.* for the gram-negative bacteria and *S. aureus* for gram-positive bacteria. The isolated pathogens are completely resistance to Amoxicillin.The most sensitive antibiotics were Imepenm, Ciprofloxacin and Gentamycin.

5.3 Recommendations

- All Diabetic foot ulcer should be mandatary manage by using the most suitable antibacterial agents as a strategy of reducing the treatment failure and antibiotics resistance.
- The multiple antibiotic resistance of the bacterial isolates calls for the need to monitor resistance , therefore should be perform antibiotic susceptibility testing before treatment.
- The surveillance of antimicrobial resistance is necessary, and antibiotic policy should be formulated in the hospital.

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APPENDIX I

Questionnaire

Department of Microbiology

Isolation and Identification of Aerobic Bacteria Associated with Diabetic Foot Ulcer Infections in Patients Attending bahhri diabetes center , AL Moalem medical center and Zinam Hospital

1.Gender

Male

Female

2.Age

……

……

3.Antibiotic intake

Yes

No

4. Name of antibiotic Used?

……

……

5.Treatment?

……

……

6. Insulin type?

……

…….

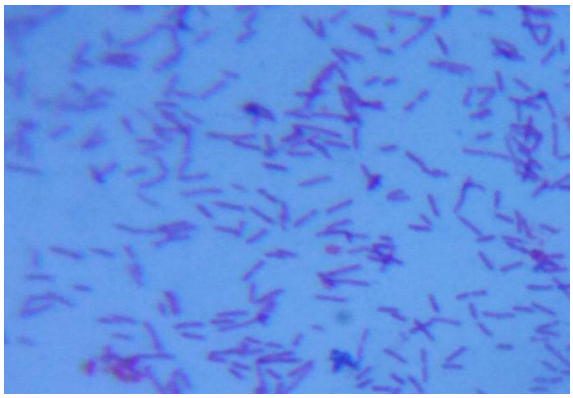
APPENDIX II

Color plates instruments

Color plate (1): Blood Agar and MacConkey

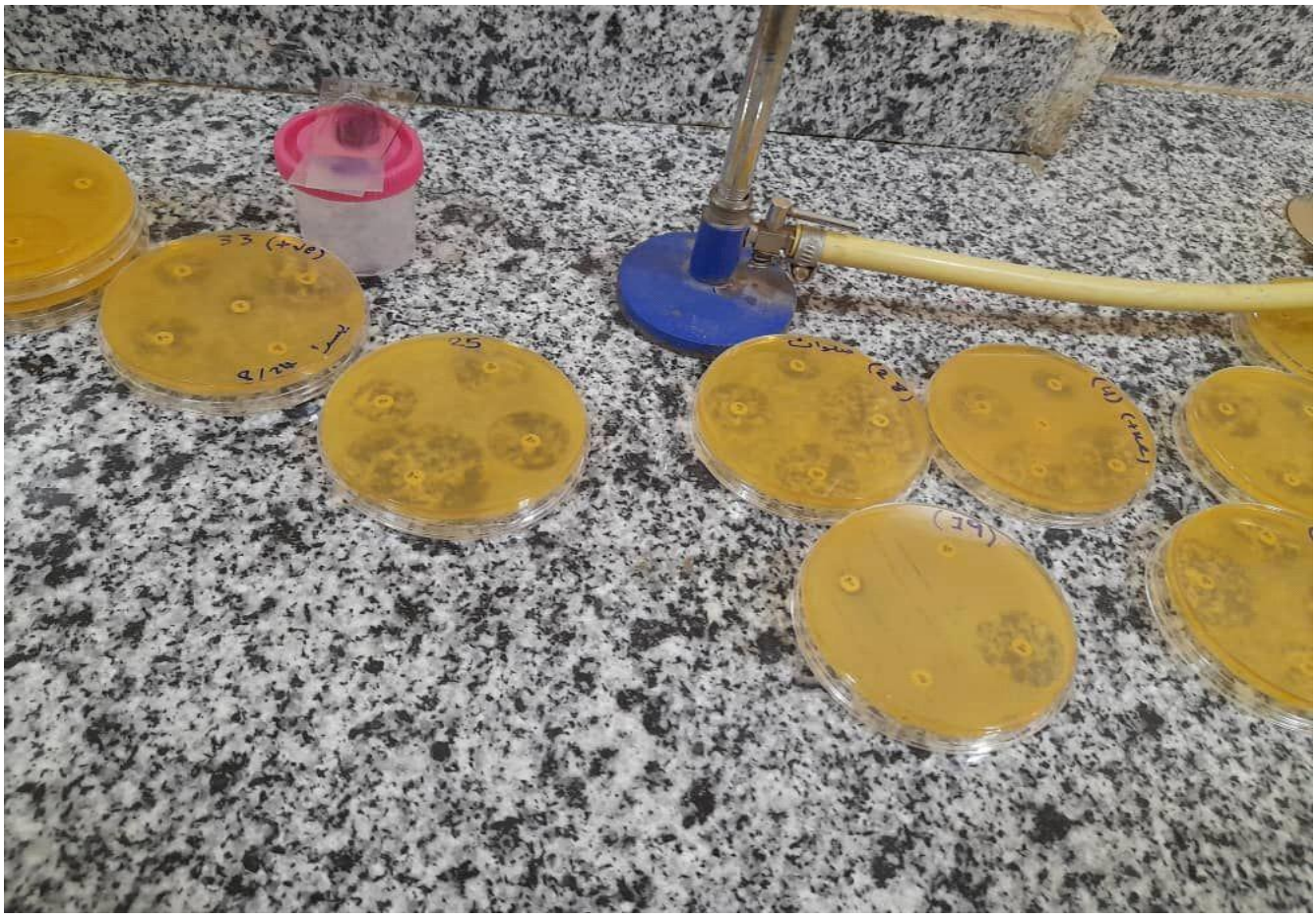
Color plate (2) :Amies transport media

Color plate (3):biochemical results *proteus vulgaris*



Color plate(4) : Gram negative bacilli short

Color plate (5):colonial morphology of Gram negative bacteria



Color plate (6): Antimicrobials susceptibility test using Kirby Bauer dise diffusion

Appendix Iii

Zone Taple

Antimicrobial drugs, abbreviations, concentration and zone size interpretation

Interpretation standards((mm))			Concentration	Abbreviation	Name
S (susceptible)	**I (intermediate)**	**R (Resistant)**			
≥ 23	20-22	≤ 19	10 µg	IPM-10	Imepnem
≥ 21	16-20	≤ 15	5 µg	CIP-5	Ciprofloxacin
≥21 ≥23	14-20 20-22	≤13 ≤19	30 µg	CRO-30	Ceftriaxone **Staphylococcusspp*. **Enterobacteriaceae*.
≥15	13-14	≤12	10 µg	CN-10	Gentamycin
≥15	……..	…..	30 µg	VA-30	Vancomycin
≥21	…….	<16	25 µg	AX-25	Amoxicillin