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Polymicrobial Chronic Wound Infections and Antibiotic Sensitivity Profiles in Diabetic Patients Attending to Dongola Diabetic Center, Northern State, Sudan 2023-2025

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ABSTRACT

Background: Chronic wounds are frequently infected with multiple bacterial or fungal species, which can both promote or inhibit each other. Network analyses are helpful to understand the interplay of these species in polymicrobial infections. This study aimed to determine bacterial isolates and antibiotic sensitivity in diabetic foot wounds.

Methods: Swabs (n = 160) from chronic wound infections (Dongola diabetic centers, Sudan, 2023–2025) were screened for bacterial isolates using non-selective agars and selective media. Species identification was done with cultural methods and biochemical reactions, antimicrobial susceptibility tests. Network analysis was performed to investigate co- occurrence of different species within one patient. All species with ≥ 105 isolates were taken into account.

Results: Of the 160 specimens from 160 diabetic patients, 88 (55%) were males, 152 (95%) specimens revealed positive bacterial pathogen(s) isolation. According to the gender 86 (97.7%) and 66 (91.6%) females showed positive bacterial pathogen(s) isolation. Positive bacterial pathogen(s) isolation rate in relation to the age groups was 100% in; 19-29, 40-49, 60- 69years and 70-more than years. The isolated bacterial patterns were 34 (22.5%) with a single pathogen, 44 (28.9%) with two pathogens, 52 (34.2%) with three pathogens, 14 (9.9%) with four pathogens, 8 (5.5%) more than four pathogens. The common isolated pathogen was Staphylococcus. Aureus Antimicrobial sensitivity testing of bacterial isolates showed that Imipenem was effective in all bacteria.

Conclusion: The culture of chronic wounds in Dongola diabetic center patients is high and characterized by the co-occurrence of multiple pathogens, more than two pathogens. The broad-spectrum antibacterial agent Imipenem is the most suitable antibiotic for treatment of diabetic wound infections. Further studies including anaerobic bacteria is needed.

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Introduction

Diabetes Mellitus

It is expected that the number of chronic wounds will increase worldwide due to the increase of lifestyle diseases, such as Diabetes Mellitus (DM), obesity, and cardiovascular diseases. It is estimated that 246 million people had diabetes worldwide in 2007 and this is expected to increase to 380 million people by the year 2025 (Diabetes Atlas).

DM is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance, or both. Diabetes is usually irreversible, and although patient can have a reasonably normal lifestyle, its late complication result in reduced life expectancy and major health cost.

The long-term complications include macro vascular disease, leading to coronary artery disease, peripheral vascular disease and stroke, and microvascular damage causing diabetic retinopathy and nephropathy and neuropathy.

Diabetic foot disease is a serious public health problem worldwide, this problem was redoubled with rising prevalence of DM inevitable rise in foot ulcers. Contributory factors include peripheral neuropathy, vascular disease, foot deformities, local trauma and pressure.

Infections remain a serious hazard for the diabetic patient. Patients with uncontrolled diabetes (hyperglycemia) are considered immunosuppressed due to the negative effects of elevated blood sugars on the immune system. some microorganisms become more virulent in a high glucose environment. Diabetic patients are particularly prone to infections with rare organisms such candida [1].

Diabetes mellitus represent a group of metabolic disorders characterized by hyperglycemia. The chronic hyperglycemia diabetes appears from defect in insulin secretion, insulin action or both.

Diabetes mellitus is classified into type 1 diabetes which include type 1A or immune _ mediated diabetes and type 1B or idiopathic diabetes ; type 2 diabetes in which individuals have insulin resistance and relative insulin deficiency, Gestational Diabetes Mellitus (GDM) and it is hyperglycemia that is first recognized during pregnancy, pre-diabetes which include Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG) , other specific type of diabetes which include ; genetic defect of the B-cell, disease of exocrine pancreas , endocrinopathies and drug or chemical- include diabetes [2].

The interaction of multiple species within the wound is complex as they affect each other by the secretion of molecules. These molecules can alter the tolerance to antibiotics, virulence or biofilm formation as shown for Pseudomonas Aeruginosa and Staphylococcus aureus [3]. The S. aureus protein toxin Panton-Valentine Leukocidin (PVL) is of particular interest in resource limited settings as it is associated with Severe Skin and Soft Tissue Infections (SSTI) and much more common among S. aureus from sub-Saharan Africa (up to 74%) compared to Europe (1.4%) [4-6]. One in vitro study suggests that PVL could be a competitive advantage for S. aureusin the interplay with P. aeruginosa, but this finding has not been yet confirmed in vivo [7].

Signs and Symptoms of Diabetes

Diabetes often goes undetected because symptoms can be attributed to many other causes and some patients experience no symptoms or fail to heed warning signs. Possible indicators of diabetes include:

Excessive thirst (polydipsia), Excessive urination (polyuria) and dehydration, Excessive hunger or appetite (polyphagia), unexplained weight loss, Blurred vision, nearsightedness or other vision problems, Frequent infections, including skin infections, thrush, gingivitis, urinary tract infections and yeast infections, Slow healing of sores – Skin problems, such as itchiness or acanthosis Nigerians, Fatigue, lethargy or drowsiness, Shakiness or trembling, Mood swings or irritability, Dizziness or fainting numbness, tingling or pain in the feet, legs or hands.

Diabetes is associated with long-term organ damage particularly in the eyes, kidneys, nerves, heart, and blood vessel. Patients with type 2 diabetes have a higher prevalence of obesity (particularly abdominals obesity), hypertension, and lipid disorders, as an increased risk of macro-vascular disease in coronary, peripheral. and cerebral arterial circulations, than people without diabetes. Micro-vascular complications of diabetes include retinopathy, which can lead to loss of vision, nephropathy (leading to renal failure), neuropathy (with an increased risk of foot ulcers, and foot deformations), and autonomic neuropathy, causing cardiovascular, gastrointestinal, and genitourinary dysfunction [8].

Development of risk factors for diabetes mellitus is determined by genetic (30% to 40%) and environmental factors (60% to 70%) These include: metabolic risk factors (Obesity, dyslipidemia, elevated blood pressure, dysglcemia), genetic risk factors (individuals with a first-degree relative with DM are at an increased risk of developing DM), and lifestyle factors (a transition to a ‘‘westernized’’ lifestyle has been blamed for changes in dietary patterns, exercise habits, and smoking leading to increased prevalence of DM) WHO (2006) [9].

Infectious diseases are more prevalent in individuals with DM. If pathogen is able to invade the host without the assistance of the innate immune system, an increased risk of infection is expected. Hyperglycemia causes undesirable changes in the function of the immune system such as decreased leukocyte adherence and bactericidal activity [10]. Furthermore, DM has been associated with response of T cells, neutrophil function, and disorders of humoral immunity [11].

Furthermore, some microorganisms become more virulent in a high glucose environment. Another mechanism, which can lead to the increased prevalence of infections diabetic patients, is an increased adherence of microorganisms to diabetic compared to nondiabetic cells.

Bacteria’s ability to thrive in the presence of elevated blood sugars activates the immune response to combat such infections. In addition, a hyperglycemic state negatively affects the body’s ability to respond to antimicrobial therapy.

Common bacterial infections include gram-negative organisms such as Pseudomonas aeruginosa, Klebsiella pneumonia, and E. coli. Gram-positive organisms, such as Staphylococcus & Streptococcus are common, also. Anaerobic organisms may be present as well due to decreased blood and oxygen perfusion throughout the blood vessels for the synthesis of leukocytes. Glycosuria is found in poorly regulated diabetics patients. Glycosuria enhances bacterial growth of different Escherichia coli strains, which probably plays a role in the increased incidence of urinary tract infections in diabetic patients [12].

Wound Infection

Wound infection follow surgery or trauma that disrupts the skin or mucosal surface. These lesions are universally colonized with various potentially pathogenic bacteria.

Infection occurs when the presence of multiplying bacteria in body tissues results in spreading cellular Injury. Normal function of skin: prevent colonization and invasion of underlying tissue by potential microbial pathogens Loss of skin integrity (wound) provides moist and nutritious environment for microbial proliferation. Presence of foreign material and necrotic tissue facilitates microbial proliferation ("dirty" wound). Acute wounds: external damage to intact skin (surgical wounds, burns). Chronic wounds: endogenous mechanisms compromising epidermal and dermal tissue (diabetes mellitus, poor nutrition, immunosuppression).

Predisposing Factors for Wound Infection

Poor blood perfusion with hypoxia (pO2 < 20 mm Hg) inhibits granulation tissue response and wound repair. Cell death and tissue necrosis due to hypoxia creates ideal growth conditions for wound microflora, Hypoxia compromises oxygen radical dependent killing of bacteria by polymorph nuclear neutrophils. Density of microorganisms the critical factor determining whether or not a wound heals Presence of specific microbial pathogens of primary significance in delayed wound healing. Most likely, both factors important in delayed wound healing due to infection.

Polymicrobial Wound Infection

Mechanisms

Oxygen consumption by aerobic bacteria induces tissue hypoxia and favorable growth conditions for anaerobic bacteria. Nutrients produced by one organism supports growth of other fastidious and potentially pathogenic organisms.

Vitamin K production by Staphylococcus aureus supports growth of vitamin K- dependent Prevotella melaninogenic. Succinate produced by Klebsiella pneumoniae a critical growth factor for Prevotella melaninogenica.

Clinical Signs of Wound Infection

- Purulent Discharge
- Painful Spreading Erythema
- Failure to Heal

Wound Specimens

Tissue, Wound fluid (purulent exudate), and Superficial swabs

Polymicrobial Wound in DM

Infections in diabetic foot are usually polymicrobial due to aerobic bacteria and fungi. Severe infections usually yield polymicrobial isolates, whereas mild infections are frequently monomicrobial. In cases of a severe diabetic foot infection, three to five organisms may be cultured [13].

Most DFIs are polymicrobial, with aerobic gram-positive cocci (GPC), and especially staphylococci, the most common causative organisms. Aerobic gram- negative bacilli are frequently pathogens in infections that are chronic or follow antibiotic treatment, and obligate anaerobes may be pathogens in ischemic or necrotic wounds) IDSA Guideline for Diabetic Foot Infections 2012).

As in most wound types, S. aureus is a prevalent isolate in diabetic foot ulcers, together with other aerobes including S. epidermidis, Streptococcus spp., P. aeruginosa, Enterococcus spp., and coliform bacteria. With good microbiological techniques, anaerobes have been isolated from up to 95% of diabetic wounds, the predominant isolates being Peptostreptococcus, Bacteroides, and Prevotella spp.

Polymicrobial infection was identified in (75%) of the patients. the predominate organism isolated were members of Enterobacter family (28.5%). Pseudomonas aeruginosa (17%), Staphylococcus aureus (11.8%), methicillin-resistance S-aureus (7.7%), anaerobic Gram-negative organism (10.8%).

Vancomycin was the most effective treatment for Gram-positive bacteria, whereas Imipenem, Pipercacillin-Tazobactam and Amikacin were most effective to Gram- negative [14].

Bacteriology of Superficial and Deep Tissue Infection

Taking Good Samples

Open lesions (such as varicose, ischemic and diabetic ulcers, burns and pressure sores). Such lesion are nearly always sampled by swab taken from the surface of the lesions. These swabs yield mixed cultures of bacteria, which are often surface colonizers of uncertain pathogenicity.

Transport

Swabs may be transported either to the laboratory dry or in transport medium. The latter is preferable unless there is minimal delay in culture.

Many types of transport medium are available commercially: they can be obtained in bijoux bottle into which separate swab may be broken off or pre-packed in a plastic tube accompanied by a swab in sterile pack.

General Methods

Wound swabs are by far the commonest type of specimen received from soft tissue infection, and most laboratories receive large numbers every day. Because of this, and also because they are repeatable specimen and they rarely come from life-threatening infection, culture method are shorter and identification often less detailed than for samples of pus, fluid or tissue.

Microscopic Examination

Microscopy takes second place to culture unless more than one swab is received from the same site. Only a Gram stain is performed.

Rationale

This research started from the observation on that hospital's bacteriologists do not make well isolation and identification of causative agent, so missing of isolation of some pathogens mainly from mixed infected wound may lead to none specific choice of antibiotics for treatment of the causative agent resulting in lack of proper drug choice and leading to a delayed wound healing. This study was aimed to reducing the problem of wound infections by increasing the health care awareness about diabetic wound infections.

Objectives

General Objective

To determine the prevalence of polymicrobial wound infections among diabetic patients in Dongola diabetic centers.

Specific Objective

- To determine the prevalence of different bacterial types associated with wound infections within the medicine wards at the Dongola Hospitals, and different diabetic centers.
- To identify the factors contributing to wound infections at the Dongola Hospitals, and different diabetic centers.
- To estimate different types of bacteria and fungi, which cause wound infections and fully identify the species.
- To determine the susceptibility pattern of most common isolates against a wide spectrum of antibiotics in target hospitals.

Literature Review

The study was undertaken to evaluate the bacteriology and antibiogram of isolates from diabetic patients with chronic foot ulcers in Nigeria. A total of 150 pus samples were collected and processed according to standard aerobic and anaerobic microbiological methods. Antibiogram was done using Kirby-Bauer method. Biofilm tests, ESBL & AmpC production was conducted using Congo red agar, Double disc synergy test and Cefoxitin disc test respectively. Total number of isolates obtained was 210. The Plasmid profiles of some of the Multi-Drug Resistance (MDR) isolates were carried out using the alkaline lysis method for plasmid extraction and electrophoresis on agarose gel with standard markers. The most frequently isolated aerobic organism in the study was Escherichia coli (32.1%) while the least occurring was Enterobacter spp (1.57%). For the anaerobes, Peptostreptococcus spp (40%) was the highest isolated bacterium. Percentage of Extended Spectrum -lactamase (ESBL) producers among E. coli isolates was 44%. Percentages of biofilm formation potential among the isolates were: E. coli (36.8%), S. aureus (23.1%) and Proteus vulgaris (4.2%). Escherichia coli and S. aureus showed considerable levels of resistance to some common antibiotics. No methicilin resistant S. aureus was encountered. AmpC producers encountered were Klebsiella pneumonia (10%) and E. coli (8.1%). Post-curring antibiogram tests revealed that nine isolates carried plasmids, suggesting that the mode of resistance may be plasmid mediated [15].

A total of 101 patient records were analyzed. The mean age of patients was 57.1 ± 9.1 years. There were more males (64.4%), mostly with type 2 diabetes (99%), with a median duration of 9 years (IQR: 4 - 14 years). Their median blood sugar on admission was 246 mg/dL. Five percent of patients died and 23% had a major amputation. Two hundred and twenty-five (225) germs were isolated, with an average of 2.25 germs per patient. Gram-negative bacteria were more frequent (75.2%). These were mainly Morganella morganii (13.8%), Klebsiella pneumonia (12%), Escherichia coli (11.6%), Proteus spp. (10.7%), and Pseudomonas aeruginosa (8.9%). Gram-positive bacteria (24.8%) were mainly Staphylococcus aureus (9.3%), Streptococcus spp. (7.6%), and Enterococcus spp. (7.1%). Gram-negative bacteria showed a high resistance to amoxicillin-clavulanic acid (78%), fluoroquinolones (55%), and gentamycin (50%). They were susceptible to imipenem (95%), amikacin (88%), and show moderate susceptibility to third generation cephalosporins (62%). Gram-positive bacteria were susceptible to vancomycine (94%), and moderately susceptible to pristinamycine (82%) and fusidic acid (67%) (Mesmin Dehayem Yefou, etal,. 2022).

In 2015, Mousumi in Bangladesh studied the association of bacteria and their antimicrobial profile in diabetic foot wounds infection and reported that, the common pathogen isolated was Pseudomonas spp (29%), Bacillus spp (12%), Enterobacter spp (22%), Staphylococcus spp (13.3%), Acinetobacter spp (10%), Enterococcus spp (9%), Klebsiella spp (8%), and Citrobacter (29%). The most isolate showed resistant to antibiotic used: Cephalosporin, Cefatzidime, Ceftriazone, and Cefurozime [16].

A recent study done in Sudan included collection, purification, identifications of 187 clinical strains isolated from patients with diabetic septic wounds. The samples were collected entirely from Jabir abo Alezz diabetic center in Khartoum State. The clinical strains identified as the following: Staphylococcus aureus (46%), Enterococcus faecalis (8%), coagulase-negative staphylococci (5.9%) Streptococcus pyogenes (2.1%), Pseudomonas aeruginosa (13.4%), Proteus spp (9.1%), Escherichia coli (4.8%), Klebsiella pneumonia (3.7%), Povidencia spp (1.1%), Citrobacter freundii (1.6%) and serratia spp (4.3%) [17].

One handered and fifty (150) wound swabs were collected from diabetic patients attending clinic in Irrua specialist Teaching Hospital, Irrua, Nigeria, The occurrence of bacterial isolates in the wounds investigated was in the following decreasing order of frequency; Staphylococcus aureus (38%), Escherichia coli (24%), Proteus spp. (20%) Klebsiella spp. (10%) and pseudomonas aeruginosa (8%). All the isolates were resistant to sensitivity of bacterial isolates, to antibiotics tested were in the decreasing order of Perfloxacin (13%), Augmentin (10%), Rocephih/Zinnacef (8%), Cioproflaxacin (6%), Gentamicin (3%), Streptomycin (2%) and Amoxicillin (1%) [18].

Out of 105 samples collected from wounds of diabetic patients in a private hospital and government hospital in Pondicherry, India, 95 showed bacterial infection. Staphylococcus spp. was the primary, pathogen in most of wound infection in the study. The frequent reported bacterial pathogens of wound infections such as Klebsielaa spp. and pseudomonas spp. were no so common. Almost all isolates showed resistance against most of the broad spectrum and narrow spectrum antibiotics tested (Penicillin, Amoxycillin, Ciprofloxacin, Vancomycin, cotrimoxazole, and Cephalothin) [19].

A study done in Coimbatore, India, the common pathogen isolate from the diabetic pus included Gram-positive cocci like (Staphylococcus aureus and Streptococcus pyogenes) and Gram-negative bacilli like (Pseudomonas spp, Escherichia coli, Klebsiella spp, and Proteus spp). Gram- positive bacteria were present in greater number than Gram-negative bacteria in the pus sample. In this study bacterial pathogens showed resistance to most antibiotic used: Amikacin, Ampicillin, Cefotoxime, Ceftazidime, Cefazolin, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Ofloxacin, Penicillin-G, Piperacillin, Sulphamethazole, Trimethoprim and Vancomycin [20].

The bacteriological isolation and antimicrobial sensitivity tests of the isolates were done by standard microbiological methods. Gram-negative bacilli were tested for extended spectrum β lactamase (ESBL) production by double disc diffusion method. Culture was positive in 92% of the cases which yielded 135 pathogens. Of the positive culture, 75.3% had multiple organisms. Polymicrobial infection was more in higher grade of foot ulcers. Gram-negative organisms were most frequently isolated (80%) bacteria. Pseudomonas (48%) and Proteus sp. (33%) was the most common Gram negative organisms isolated. Staphylococcus aureus was the most commonly isolated gram-positive organism (21.3%). ESBL production was noted in 31.5% Gram-negative bacilli and methicillin resistance was noted in 43.8% of Staphylococcus aureus. Most of the Gram-negative bacilli were resistant to various classes of antibiotics. Imepenem was the most effective agent against Gram negative organisms, while vancomycin was for staphylococcus. The present study has shown that infection with multidrug resistant Gram-negative bacilli is the most common cause of DFI in BIRDEM hospital.) [21].

A one-year retrospective study was carried out analyses the bacterial isolates of all patient admitted with diabetic foot infection –Bir Hospital Kathmandu, Nepal. Gram – positive bacteria were isolate more often than Gram- negative ones in the patient's screened. The most frequent bacterial isolate were Staphylococcus aureus (38.4%), Pseudomonas aeruginosa (17.5%), and Proteus (14%). Imipenem was the most effective agent against Gram-

negative organism's. Vancomycin was found to be most effective against Gram-positive organisms [22].

Materials and Methods

Study Design
Cross-sectional observational institution-based study.

Study Area
The study was performed in the Microbiology Laboratory, Faculty of Medicine Laboratory- University of Dongola, Sudan.

Study Duration
The study was conducted during the period: March 2023 to October 2025.

Study Population
Diabetic patients with wound infections who were attending to hospitals or centers of Diabetics at Dongola city, Northern State, Sudan were enrolled in the study.

Sample Size and Sampling Technique
Convenient sampling technique was used and 160 samples were received from diabetic patients with wound infections during the study period.

Data Collection
An interviewed questionnaire used to collect data from diabetic patients including: age, gender and history of disease.

Data Analysis
Data were analyzed by SPSS (23). Frequencies and percentage were used for categorical variables. A p-value of <0.05 was considered statistically significant.

Ethical Clearance
The study was approved by the ethical committee of Ministry of health of Northern state. Permission was granted from the diabetic center and hospitals. Confidentiality of the data was secured.

Specimens' Collection
The following recommendations can be used as a guide and should be used in conjunction with local protocols

- When a swab is indicated, the patient should be given a concise explanation of the need for microbiological investigation and what the procedure involves, for example, that swabs are mainly used to recover species from the surface layers rather than from the deep tissues of a wound.
- Before a representative sample is collected, any contaminating materials such as slough, necrotic tissue, dried exudate and dressing residue should be removed by cleansing the wound with tap water, sterile saline or debridement.
- Sterile swabs with cotton or rayon tips are usually used. If the wound is moist a swab can be used straight from the packaging – if the wound is dry, then the swab tip should be moistened with sterile saline to increase the chances of recovering organisms from the site Swabs with a transport medium that incorporates charcoal enhance the survival of fastidious organisms (BA Lipsky, et al 2006).

Specimens Processing
Wound swabs were transported to Bacteriology Laboratory dry within one hour. When there was dry a delay in culture, swabs were transported in Amie's transport medium within 24 hours at room temperature.

Laboratory Testing
Inoculation
Every wound swab was inoculated on sterile Blood and MacConkey agar media. First, a well rea (area A) was seeded then by using a sterile wire loop a serial of streaks were made on three areas (B, C, and D) and finally a zigzag was made (area E).

Cultural Conditions
Inoculated plates were incubated aerobically at 37oC for 18-24 hours.

Colonial Morphology
After incubation, culture plates were observed for colonial morphology (shape, margin, elevation, size, texture, appearance, and optical property) colour change on MacConkey agar medium and type of hemolysis on Blood agar medium.

Indirect Gram Stain
Smears were prepared from either Blood agar or MacConkey agar plate and stained with Gram stain to identify the microscopic appearance of the organism, including the Gram reaction, cell shape, and arrangement, the following staining procedure was preformed.

- Primary staining with 2% crystal violet, 1 minute.
- Mordant 1% iodine solution, 1 minute.
- Decolorization with absolute alcohol, 30 seconds.
- Counterstaining with 0.5% safranin, 2 minutes.

Identification
According to the result of the Gram-stained smears bacterial pathogens that showed Gram - positive reaction with spherical – shaped cell were subjected to Catalase, Coagulase, litmus milk decolorization and esculin Hydrolysis tests.

Bacterial pathogens that showed Gram – negative reaction with rod – shaped cell were subjected to Motility, Citrate Utilization, Utilization, Urease, Indole, Oxidase and Kligler Iron Agar (KIA) reaction tests.

Catalase Test (Slide Method)
Requirements
- Hydrogen peroxide, 3% H2O2
- Wooden stick
- Glass slide

Procedure
1-2 drop of Hydrogen peroxide solution placed on a glass slide then several colonies of the test organism removed by using a wooden stick and several emulsified in the hydrogen peroxide solution and looked from immediate bubbling.

Interpretations
- Active bubbling: Positive catalase test
- No bubbles: Negative catalase test

Coagulase Test (Slide Method)
Requirements
Undiluted human plasma Glass slide

Procedure
- A drop of normal saline placed on each end of the slide.
- A colony of the test organism emulsified in each of the drops to make two thick suspension.
- A loopful of plasma added to one of the suspensions and mixed gently.

- Looked for clumping of the organism within 10 seconds.

Interpretations
- Clumping within 10 seconds: Staphylococcus aureus
- No clumping within 10 seconds: No bound coagulase

Litmus Milk Decolorization Test
Requirements
Sterile Litmus Milk Medium

Procedure
- 0.5-1.0 ml of sterile litmus milk medium inoculated with the test organism by emulsification. A heavy inoculum of the test organism used.
- Inoculated medium incubated aerobically at 35-37 ℃ for 18-2 hours.

Interpretations
- White or pale yellow-pink colour: Suggestive of Enterococcus
- No change or a pink colour: Probably not Enterococcus

Aesculin Hydrolysis Test
Requirements
Sterile Bile Aesculin Agar Slope

Procedure
- By using a sterile straight wire, a small portion of test organism's colony removed and incubated by streaking the slope in a zigzag pattern.
- Inoculated medium incubated aerobically at 35-37 ℃ for 18-24 hours.

Interpretations
- Positive Black colour (Blackening)
- Negative: No change

Motility Test
Requirements
Sterile Semi-Solid Nutrient agar Medium

Procedure
By using a sterile straight wire, a small portion of test organism's colony removed and incubated by making a single stab down the center of the tube to about half the depth of the medium.

Inoculated medium incubated aerobically at 35-37 ℃ for 18-24 hours.

Interpretations
- Positive (Motile): Diffuse growth around the stab line
- Negative (Non-Motile): No diffuse growth around the stab line.

Citrate Utilization Test
Requirements
Sterile Citrate Agar Medium (Slope)

Procedure
By using a sterile straight wire, a small portion of test organism's colony removed and incubated by streaking the slope in a zigzag pattern.

Inoculated medium incubated aerobically at 35-37 ℃ for 18-24 hours.

Interpretations
- Positive: Blue colour
- Negative: No change in colour of medium

Urease Test
Requirements
Sterile Urea Agar Medium (Slope)

Procedure
By using a sterile straight wire, a small portion of test organism's colony removed and incubated by streaking the slope in a zigzag pattern.

Inoculated medium incubated aerobically at 35-37 ℃ for 18-24 hours.

Interpretations
- Positive: Red/pink colour
- Negative: No change in colour of medium

Indole Test
Requirements
Sterile Peptone water Medium

Procedure
By using a sterile wire loop, a small portion of test organism's colony removed and incubated by emulsification in the Peptone water Medium.

Inoculated medium incubated aerobically at 35-37 ℃ for 18-24 hours.

After 0.5 ml Kovac's reagent by layering.

Interpretations
- Positive: Red ring developed in surface after adding the Kovac's reagent
- Negative: No red ring developed in surface after adding the Kovac's reagent

Oxidase Test
Requirements
Oxidase disc

Procedure
By using a wooden stick, well – isolated test organism's colony removed and smeared on the oxidase disc.

Interpretations
- Positive: Blue-purple colour (within 10 seconds)
- Negative: No blue-purple colour (within 10 seconds)

Kligler Iron Agar (KIA) Reactions
Requirements
Sterile Kligler Iron Agar Medium

Procedure
By using a sterile straight wire, a small portion of test organism's colony removed and incubated by stabbing the butt and returning in the same direction to make a zigzag pattern in the slope

Inoculated medium incubated aerobically at 35-37oC for 18-24 hours.

Interpretations

- A red/pink slope and a yellow butt: No lactose – fermenter
- A yellow slope and a yellow butt: lactose - fermenter
- A red/pink slope and a red/pink butt: non - fermenter
- Gas product ion indicated by air bubbles, cracks or both.
- Hydrogen Sulphide production indicated blackening along the stab line or throughout the medium.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out on Mueller-Hinton agar using the standard disk diffusion technique, and the following antibiotics These antibiotics is wide spectrum: Imipenem (10mcg), Amikiacin (30mcg), Ciprofloxacin (5mcg), Ticarcillin ( ), and Ceftazidime (30mcg).

Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.

In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use).

Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60 to ensure even distribution. With the Petri dish lid in place, allow 3-5 minutes (no longer than 15 minutes) for the surface of the agar to dry.

Using sterile forceps, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate. Should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35-37 oC for 16–18 h, After overnight incubation, examine the control and test plates to ensure the growth. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Results

In the present study, a total of 160 wound swab specimens were collected from different diabetic patients. Which collected from the Dongola diabetic centers and clinics, in the period from March 2023 to October 2025. The study involved 88 (55%) males and 72 (45%) females.

Of the 160 specimens, 152 (95%) specimens revealed positive bacterial pathogen(s) isolation. According to the gender 86 (97.7%) males and 66 (91.6%) females showed positive bacterial pathogen(s) isolation. The gender distribution is shown in Table 1.

Table 1: Distribution of Positive Bacterial pathogen(s) Isolation in Relation to Gender Among Diabetic Patients with Wound Infection at Dongola, Northern State, Sudan, 2023-2025 (n=160).

Gender	Positive Growth *No (%)*	Negative Growth *No (%)*	Total
Male	86 (97.7)	2 (2.3)	88 (55)
Female	66 (91.7)	6 (6.3)	72 (45)
Total	**152 (95)**	**8 (5)**	**160 (100)**

The distribution of positive bacterial pathogen(s) isolation from diabetic wound infections in relation to the age range, as demonstrated in Table 2, was 100% among the age groups 19-29, 40-49, 60-69 and 70 or more years.

Table 2: Age Distribution of Positive Bacterial Pathogen(S) Isolated From wound swabs of diabetic patients from Dongola, Northern State, Sudan in 2023-2025 (n=160).

Age groups (years)	Total	Number with Positive Growth	Percent
19-29	10	10	100%
30-39	8	6	75%
40-49	36	36	100%
50-59	56	50	89.2%
60-69	40	40	100%
70 or more	10	10	100%
Total	160	152	95%

The number of isolated bacteria from diabetic patients is indicated in table 3. There were 34 (22.5%) with a single pathogen (Figure-1) whereas 118 (77.6%) with multiple pathogens (Figure 2 & 3).

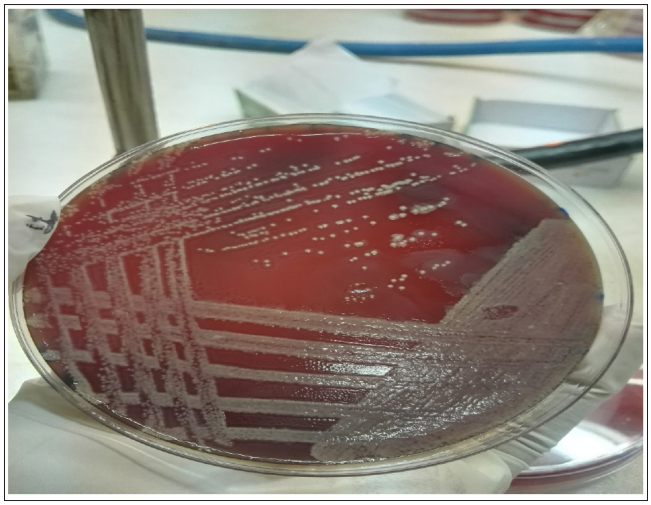

Figure 1: Culture on Blood Agar Medium showing Proteus mirabilis and Staphylococcus aureus

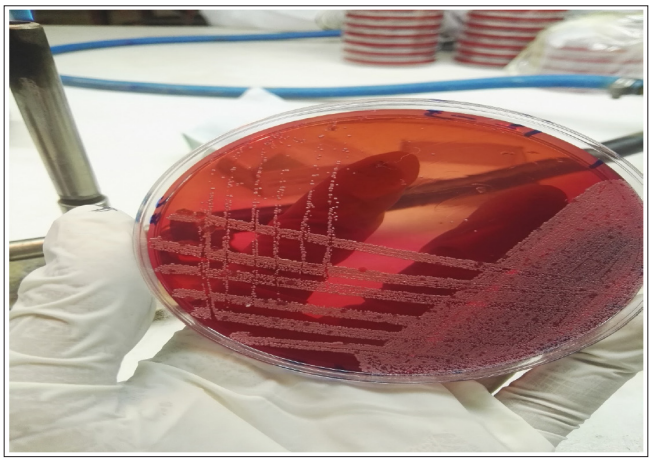

Figure 2: Culture on MacConkey Agar Medium showing three organisms (Klebsiella pneumonia and E.coli and Staphylococcus aureus)

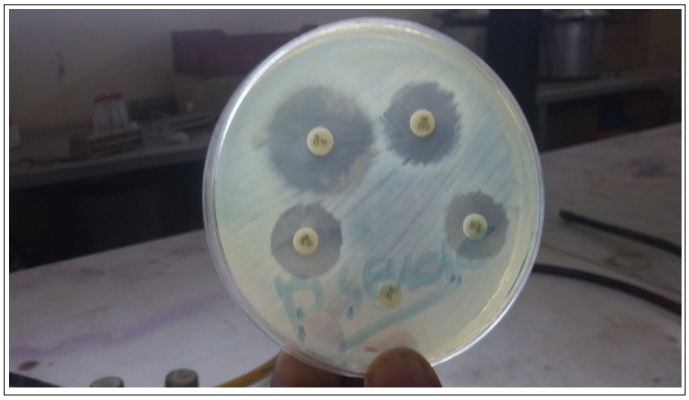

Figure 3: Antimicrobial sensitivity testing on Muller Hinton Agar Medium for Pseudomonas aeruginosa

Table 3: Number of Bacterial Species Isolated from Diabetic Wound Infections in Dongola City, Northern State, Sudan, 2023-2025 (N=152)

Number of Isolated bacteria	No	(%)
Single pathogen	34	22.5
Two pathogens	44	28.9
Three pathogens	52	34.2
Four pathogens	14	9.2
More than four pathogens	8	5.5
Total	**152**	

The commonest bacterium isolated from the diabetic wound swabs was Staphylococcus. aureus which was detected from a third of patients (33%), followed by Proteus mirabilis (20.3%) and Pseudomonas aeruginosa (18%) as demonstrated in Table-4.

Table 4: Bacterial Species Isolated from Wound Swabs of Diabetic Patients from Dongola, Northern State, Sudan In 2023-2025

The sensitivity testing of bacterial isolates to antimicrobial agents showed that Imipenem was effective in all bacteria isolated from diabetic wound swabs (Table 5)

Bacterial isolates	Frequency	Percent
Gram-positive aerobes		
Staphylococcus aureus	62	33.1%
Enterococcus faecalis	18	9.6%
Gram-negative aerobes		
Escherichia coli	12	6.4%
Klebsiella pneumonia	23	12.2%
Pseudomonas aeruginosa	34	18.1%
Proteus mirabilis	38	20.3%

Table 5: Sensitivity Test Results the Isolated Organisms from Wound Swabs of Diabetic Patients from Dongola, Northern State Sudan, 2023-2025

Organisms	IMP	AK	CIP	TIC	CAZ
Gram -positive bacteria					
Staphylococcus aureus	S	S	S	S	S
Enterococcus faecalis	S	R	S	S	R
Gram -negative bacteria					
Escherichia coli	S	S	S	S	S
Klebsiella pneumonia	S	S	R	S	R
Pseudomonas aeruginosa	S	R	S	R	S
Proteus mirabilis,	S	S	R	S	S

Key: IPM = Imipenem, AK= Amikiacin, CIP= Ciprofloxacin, TIC= Ticarcillin, CAZ= Ceftazidime. S = Sensitive, R=Resistant

Discussion

In the present study, a total of 160 wound swab specimens were collected from diabetic patients, 152(95%) specimens revealed positive bacterial pathogen(s) isolation. This is similar to the isolation rate of a study in India (94%) but lower than that of another study from India (100%) [23,24].

According to the gender 43(97.7%) males and 33(91.6%) females showed positive bacterial pathogen (s) isolation similar to the study of [25].

The mean age at presentation was 19-more than 70 years and the most common age group affected was 50-59 years, it is in this age group that the type 2 diabetes is usually diagnosed. It is also the age group in which patients are working and liable to trauma. As the age increases, they have nutritional deficiencies and decreased immunity.

The frequency of aerobic bacterial infection among diabetic patients, the most predominant bacterial type and the most common isolates and sensitivity pattern were carried out in this study. The gram staining of the organisms showed that the gram-negative bacteria were more frequent than gram positive ones, This is also in accordance with studies [15,23].

It has been found that the most frequently isolated organism is Staphylococcus aureus which agrees with the studies of [15,17-19,20,22].

In one specimen three different strains of Staphylococcus aureus with different colonial morphology and antimicrobial sensitivity were isolated.

Imipenem was the most effective agent against all the isolated bacteria. Which agrees with [15,22].

Although this study is the first to determine bacterial isolations and antibiotic sensitivity in diabetic foot infections, there are some limitations. The study was carried out on a relatively small

scale and need to be carried out on a larger scale involving other geographical areas to see any difference in the bacteriology of diabetic food ulcer. The cross-sectional type of the study and being limited to Dongola, the capital of the Northern State are also among the limitations. The study did not include isolation of anaerobic bacteria.

Conclusion

Bacterial isolation rate from diabetic foot infections was 95%. More than two pathogens were isolated from 59 (77.6%) diabetic wound infections. Gram-negative aerobes were more commonly isolated but the common isolate was Staphylococcus aureus. The broad-spectrum antibacterial agent Imipenem is the most suitable antibiotic for treatment of diabetic wound infection. Gram-negative aerobes were more commonly isolated but the common isolate was Staphylococcus aureus.

Knowledge of the local isolates from diabetic wound and their subsequently, has ten wound healing.

Recommendations

- In further studies, wound swab specimen must be inoculated on two Blood agar media and incubated anaerobically and aerobically to recover the anaerobic bacterial causes of wound infection.
- Proper wound care is recommended and the antibiotic should be selected based on the knowledge of the causative organism and antibiotic sensitivity.
- Another wound swab should be taken from the diabetic patient after the completion of the treatment especially in mixed wound infections to evaluate the state of the wound.
- Further large-scale studies are recommended to help understand the magnitude of the problem and promote, prevent and properly treat diabetic wound infections.

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