Efficiency of Different Solutions on *Escherichia coli* Isolated from Diabetic Septic Foot, Abuaglla Center for Diabetic Patients, Gezira State, Sudan (2018)

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B.Sc. in Microbiology, Shendi University (2013)

A dissertation

Submitted to the Collage of Graduates, University of Gezira in Partial Fulfillments of Requirements for the M.Sc. degree

In

Medical Microbiology

Department of Medical Microbiology

Faculty of Medical Laboratory Sciences

April, 2018
Efficiency of Different Solutions on *Escherichia coli* Isolated From Diabetic Septic Foot, Abuaglla Center for Diabetic Patients, Gezira State, Sudan (2018)

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**Supervisor Committee:**

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<td>Prof. Omer Hassan Mohammed Hassan</td>
<td>main supervisor</td>
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<tr>
<td>Prof. Mohammed Ahmed Taha</td>
<td>co supervisor</td>
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Date: 1/5/2018
Efficiency of Different Solutions on *Escherichia coli* Isolated From Diabetic Septic Foot, Abuaglla Center for Diabetic Patients, Gezira State, Sudan (2018)

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Examination Committee:

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<td>Dr. Attalla Mohammed Attalla Elhassan</td>
<td>External Examiner</td>
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<tr>
<td>Dr. Abdelrahman Eldaw Mohammed</td>
<td>Internal examiner</td>
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Date: 1/5 / 2018.
DECLARATION

This dissertation ethics for Efficiency of Different Disinfectant Solutions on *Escherichia coli* Isolated From Diabetic Foot Wounds Infections, Abuaglla Center for Diabetic Patients, Gezira State, Sudan (2018), a presentation of my original research work, wherever contributions of others are involved, and every effort is made to indicate this clearly, with due to reference to the literature, and acknowledgment of collaborative research and discussion.

The work was done under the guidance of Prof. Omer Hassan Mohammed Hassan and prof. Mohammed Ahmed Taha.

Name: Mohammed Ali Mohammed Altayeb

Place: Wad Medani

Date: 1/5 / 2018
DEDICATION

Happily, I would like to dedicate this simple attempt

To

The one who have taught me how to be a valuable member of the community

‘’MY FATHER’’

To essence of life and meaning of humanity

‘’MY MOTHER’’

To who share with me all moments of happiness and sadness and made me happy at time of sadness

‘’ My lovely family’’

To who gave me sense of everlasting warmth and beauty

‘’My best friends’’

My thanks and appreciation to the technicians staff of Abuagllla clinic especially:

Dr. Madani Ahmed Albathier

For collaboration by directing their patient to join in my study
ACKNOWLEDGMENT

All praises to Almighty Allah, the merciful and kind enough for giving me the opportunity and courage to carry out and complete this work.

A special sincere respect and thanks to the main supervisor: Prof. Omer Hassan Mohamed Hassan I shall remain ever grateful to him for his continuous and untiring guidance, active cooperation, valuable comment, constructive criticize and constant inspiration to carry out the work successfully. I would like to extend my grateful thanks and appreciation to the co-supervisor: Prof. Mohammed Ahmed Taha for his help.

I have a great pleasure to express my sincere regards and gratefulness to lab staff Department Microbiology, Faculty of Laboratory Science, University of Gezira, for his help and cooperation with me to do this research, also, I would like to express my deepest gratitude and appreciation to Dr. Hajar Mohammed who supported me help me while collecting sample and editing this thesis. Especial thanks to all my colleagues who respond for the questionnaire and blood sample without hesitate. I wish to express my appreciation to all those who contributed to data collection by enabling me to fill the questionnaire and to collect the blood samples.

Hereby, I would like to acknowledge the generous support provided by my all family for their encouragement during the study period.
Abstract

The diabetic foot ulcer is an open sore or wound affecting about 15% of diabetic patients and it commonly located in the bottom of the foot. Approximately 6% of patients with diabetic foot ulcer are hospitalized. Diabetic foot infection is a potentially hazardous complication that can progress to irreversible septic gangrene necessitating foot amputation. The objective of this study is to detect the relative frequency of bacterial isolates obtained from diabetic foot ulcer and assess their comparative in vitro susceptibility to the most used disinfectants. Total of 30 swab samples were collected from the patient with diabetic foot ulcer of different ages during the period (April-October, 2017) in Abuaglla Centre for Diabetic Patients at Wad Medani, Gezira State, Sudan. Samples were inoculated into blood agar and MacConkey agar, and then stained using gram’s stain and standard biochemical tests were accomplished to identify the microorganism. Disinfectants sensitivity was done to isolated microorganism regarding Escherichia coli include absolute methanol, Absolute ethanol, 70% methanol, 70% ethanol, iodine, hydrogen peroxide and honey with fusiderm. The results showed that the isolated gram’s positive bacteria were (56.7%) Staphylococcus aureus while gram’s negative microorganisms exhibited that (16.7%) Escherichia coli, (10%) Pseudomonas aeruginosa and (10%) Proteus spp. 6.7% of collected samples showed no growth. The sensitivity of Escherichia coli was reported as 96.67% in iodine, 80% methanol absolute, 73.33% absolute ethanol, 26.67% methanol 70%, 23.33% ethanol 70%, 16.3% in combination of Honey and fusiderm, hydrogen peroxide and 13.33%. Study recommended that disinfectant susceptibility should be encouraged for the proper choice of the disinfectant, regular dressing is important to avoid the contamination of the ulcer and the patients awareness for the antibiotic should be promoted.
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<td>Escherichia coli</td>
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<tr>
<td>TLC</td>
<td>Total lymphocyte count</td>
</tr>
<tr>
<td>ESBLs</td>
<td>Extended spectrum beta lactamase</td>
</tr>
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<td>WHO</td>
<td>World health organization</td>
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<td>DFU</td>
<td>Diabetic foot ulcer</td>
</tr>
<tr>
<td>EIEC</td>
<td>Enter invasive Escherichia coli</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>CLED</td>
<td>Cystine lysine electrolyte deficiency</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<td>ETEC</td>
<td>Entro toxigenic Escherichia coli</td>
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<td>VTEC</td>
<td>Verotoxigenic Escherichia coli</td>
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<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental protection agency</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
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CHAPTER ONE

Introduction

1.1 Introduction

Foot ulcers are the most common medical complications of patients with diabetes, with an estimated prevalence of 12-15% among all individuals with diabetes. Diabetic foot ulcers are responsible for more hospitalizations than any other complication of diabetes. Ulcerations can have potential devastating complications as they cause up to 90% of lower extremity amputations in patients with diabetes.

Several factors are involved in the decreased healing potential of a diabetic foot, all of which stem from the metabolic disorders associated with diabetes. The most important of these factors are:

- Level of uncontrolled hyperglycemia.
- Reduced circulation and arterial blood flow.
- Nutrition status.
- Inability to offload the affected region of the foot.
- Presence of infection (Boulton et al., 2004).

Failure of the wound healing process, in particular repair of lost extracellular matrix that forms the largest component of the dermal skin layer, is one of the primary culprits in diabetic foot ulcers. The most predictive marker for wound healing is serum albumin, followed by total lymphocyte count (TLC). Commonly accepted levels of these markers for wound healing are a serum albumin of 3.0 g/dL and TLC >1,500/mm3. Additional useful laboratory values to assess wound ulcers include the nutrition marker pre-albumin; the inflammatory markers serum white blood cell count, C-reactive protein, and erythrocyte sedimentation rate; and the glycemic markers serum glucose and hemoglobin A1C.

Post-operative wound infections have been a problem in the field of surgery since time immemorial. These infections may occur shortly after surgery or several days postoperatively. Studies have shown that such wound infections are universal and that the bacteria types present vary with geographical locations1. Although Staphylococcus aureus are the primary cause of such infections, in recent years, there has been a growing number of post-operative wound infections due to Gram-negative organisms mainly Escherichia coli and Pseudomonas aeruginosa (Wanger et al., 1979).

Enterobacteria are opportunistic pathogens and are responsible for a wide range of infections. They are common precipitants of sepsis by virtue of the inflammatory response activated by end toxins present in the Gram-negative cell wall. Resistance to individual antimicrobials is
rapidly increasing in Gram-negative bacilli. Of even greater concern is the propensity for Gram-negative bacilli to become resistant to multiple antimicrobials which is mainly mediated by extended spectrum beta lactamases (ESBLs) (Brody et al., 1999).

1.2 Problem identification and justification:

Wound infections after surgery and diabetic wounds are usually caused by several types of organism. Disinfectants such as Alcohols, Iodine and Hydrogen peroxide are used for the cleaning of wounds, so this study will be carried out to achieve the perfect concentration of disinfectant solution and the best type to be used for cleaning the infected wounds.

The most of diabetic patients have ulcer suffering from long time of dressing and painful disinfectant solution using in cleaning and dressing and most of the bacteria are resistant to this disinfectant solution and finally lead to amputation.

This study will do to reduce the time of dressing and to choice the best and effective concentration of disinfectant solutions.
1.3 Objectives:

1.3.1 General objectives:

To determine the efficiency of different disinfectant solutions on *E. coli* isolates from diabetic septic foot.

1.3.2 Specific objectives:

1. To isolate *Escherichia coli* from diabetic foot wound infections
2. To study the effect of methanol, ethanol, hydrogen peroxide, and iodine on *Escherichia coli* species.
3. To compare between the methanol, ethanol, hydrogen peroxide, and iodine disinfectants in dressing of diabetic foot ulcer.
4. To evaluate the effectiveness of methanol, ethanol, hydrogen peroxide, and iodine as disinfectants and their effect in diabetic patients with different wounds.
CHAPTER TWO

Literature Review

2.1 Diabetes Mellitus (D.M):

Diabetes mellitus is actually of metabolic disease characterized by hyperglycemia resulting from defect in insulin secretion insulin action or both (Michael et al., 2009). Diabetes mellitus is caused by an absolute or relative insulin deficiency. It has been defined by the world health organization (WHO) on basis on the laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140 mg/dl). Or greater than 11.1 mmol/l (200mg/dl) 2hour after carbohydrate meal or 2hour after the oral ingestion even if the fasting concentration normal (Joan et al., 1979). The term diabetes, without qualification, usually refers to diabetes mellitus, which roughly translates to excessive sweet urine (known as "glycosuria). Several rare conditions are also named diabetes. The most common of diabetes in sipidus in which large amounts of urine are produced hoes is (polyuria, which is not sweet (insipid us meaning "without taste" in Latin) (Wendy et al., 2007).

2.2 Diabetic foot ulcer (DFU):

Foot disorders such as ulceration infection and gangrene are the leading causes of hospitalization in patients with diabetes mellitus (Boulton et al., 2000). Approximately 15 to 20 percent of the estimated 16 million persons in the United States with diabetes mellitus will be hospitalized with a foot complication at some time during the course of their disease. Unfortunately; many of these patients will require amputation within the foot or above the ankle as a consequence of severe infection or peripheral ischemia. Neuropathy is often a predisposing facto to ulceration and amputation. The diabetic foot and its sequelae account for billions of dollars in direct medical expenditures, as well as lengthy hospital stays and periods of disability (Reiber et al., 1995). The most characteristic lesion of the diabetic foot is a mal performance ulceration which consequently is one of the major risk factors for amputation. Approximately 85 percent of all diabetes related lower-extremity amputations are preceded by foot ulcers (Pecoraro et al., 1990).

2.2.1 Etiology of Foot Ulceration:

The etiology of diabetic foot ulcers usually has many components. (Frykberg et al., 2000) A recent multicenter study attributed 63 percent of diabetic foot ulcers to the critical triad of peripheral sensory neuropathy, trauma, and a deformity. Other factors in ulceration are ischemia, callus formation, and edema. Although infection is rarely implicated in the etiology
of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present. Many of the risk factors for foot ulcer are also predisposing factors for amputation, because ulcers are primary causes leading to amputation. (Boyko et al., 1999).

2.2.2 Ulcer Evaluations:

A thorough evaluation of any ulcer is critical and should direct management. (Frykberg et al., 2000) An adequate description of ulcer characteristics, such as size, depth, appearance, and location, also provides for the mapping of progress during treatment. (American et al., 1999) The evaluation should determine the etiology of the ulcer and ascertain whether the lesion is neuropathic, ischemic, or neuro-ischemic. Failure to perceive the pressure of a 10-g monofilament is a proven indicator of peripheral sensory neuropathy and loss of protective sensation. (Armstrong et al., 1998) Other common modalities that can detect insensitivity are a standard tuning fork (128 cycles per second) and a neurologic reflex hammer. After describing the dimensions and appearance of the ulcer, the physician should examine the ulcer with a blunt sterile probe. Gentle probing can detect sinus tract formation, undermining of ulcer margins, and dissection of the ulcer into tendon sheaths, bone, or joints. A positive probe-to-bone finding has a high predictive value for osteomyelitis. (Grayson et al., 1995). Failure to diagnose underlying osteomyelitis often results in failure of wound healing. The existence of odor and exudates, and the presence and extent of cellulites must be noted. (Frykberg et al., 1991) Generally, limb-threatening infections can be defined by cellulites extending beyond 2 cm from the ulcer perimeter, as well as deep abscess, osteomyelitis, or critical ischemia. (Caputo et al., 1994). Aerobic and anaerobic cultures should be taken when signs of infection, such as purulence or inflammation, are present. Cultures are best taken from purulent drainage or curetted material from the ulcer base because all ulcers are contaminated, culture of non-infected wounds is generally not recommended. (Lipsky et al., 1990) Polymicrobial infections predominate in severe diabetic foot infections and include a variety of aerobic gram-positive cocci, gram-negative rods, and anaerobes. (Caballero et al., 1998) Radiographs should be obtained in most patients with deep or longstanding ulcers to rule out osteomyelitis; however, radiographs are not a very sensitive indicator of acute bone infection (Lipsky et al., 1997). When clinical suspicion indicates osteomyelitis but radiographs are negative, additional bone or leukocyte scanning is helpful in ascertaining bone involvement. However, in the neuropathic patient, bone scans are often falsely positive because of hyperemia or Charcot’s arthropathy. Leukocyte scanning or magnetic resonance imaging offers better specificity in this situation (Lipsky et al., 1997). Ultimately, bone biopsy is necessary to firmly establish the diagnosis of osteomyelitis. Vascular status must always be assessed because ischemia portends a poor prognosis for healing without vascular
intervention. The simple palpation of both pedal pulses and popliteal pulses is the most reliable indication of arterial perfusion to the foot.

Pedal pulses in the presence of a palpable popliteal pulse are a classic finding in diabetic arterial disease because of the selective involvement of the tibial arteries below the knee. (Caputo et al., 1994). Noninvasive Doppler studies should be used to augment the clinical examination as needed, although even with these tests, the severity of arterial insufficiency can be underestimated. (Caputo et al., 1994). Vascular surgical consultation is warranted when there is significant suspicion of ischemia. Classification of ulcerations can facilitate a logical approach to treatment and aid in the prediction of outcome. (Frykberg et al., 1998).

Several wound classification systems have been created, based on parameters such as extent of infection, neuropathy, ischemia, depth or extent of tissue loss, and location. The most widely accepted classification system for diabetic foot ulcers and lesions is the Wagner ulcer classification system, which is based on the depth of penetration, the presence of osteomyelitis or gangrene, and the extent of tissue necrosis (Wanger et al., 1987). The drawback of the Wagner classification system is that it does not specifically address two critically important parameters: ischemia and infection.

**2.2.3 Classification of Diabetic Foot Ulcers:**

The results of the foot evaluation should aid in developing an appropriate management plan (Frykberg et al., 2000) If an ulcer is discovered, the description should include characteristics of the ulcer, including size, depth, appearance, and location (Americans et al., 1999). There are many classification systems used to depict ulcers that can aid in developing a standardized method of description. These classification systems are based on a variety of physical findings. One of the most popular systems of classification is the Wagner Ulcer Classification System, which is based on wound depth and the extent of tissue necrosis (Table 1) (Wanger et al., 1987). Several authors have noted a disadvantage of this system in that it only account for wound depth and appearance and does not consider the presence of ischemia or infection. The University of Texas system is another classification system that addresses ulcer depth and includes the presence of infection and ischemia (Table 2) (oyibo et al., 2001). Wounds of increasing grade and stage are less likely to heal without vascular repair or amputation (Frykberg et al., 2000).
### Table 2.1 Wagner ulcer classification system:

**Table 1. Wagner ulcer classification system.**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Superficial diabetic ulcer</td>
</tr>
<tr>
<td>2</td>
<td>Ulcer extension involving ligament, tendon, joint capsule, or fascia with no abscess or osteomyelitis</td>
</tr>
<tr>
<td>3</td>
<td>Deep ulcer with abscess or osteomyelitis</td>
</tr>
<tr>
<td>4</td>
<td>Gangrene to portion of forefoot</td>
</tr>
<tr>
<td>5</td>
<td>Extensive gangrene of foot</td>
</tr>
</tbody>
</table>

### Table 2.2 University of Texas wound classification system:

**Table 2. University of Texas wound classification system.**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage A</td>
<td>No infection or ischemia</td>
</tr>
<tr>
<td>Stage B</td>
<td>Infection present</td>
</tr>
<tr>
<td>Stage C</td>
<td>Ischemia present</td>
</tr>
<tr>
<td>Stage D</td>
<td>Infection and ischemia present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Epithelialized wound</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Superficial wound</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Wound penetrates to tendon or capsule</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Wound penetrates to bone or joint</td>
</tr>
</tbody>
</table>
2.2.4 Prevention of ulcer formation:

“49-85% of all diabetic foot related problems are preventable. (Spraul et al., 2000). “This can be achieved through a combination of good foot care, provided by an interprofessional diabetes care team, and appropriate education for people with diabetes. (Modified from Bakker et al., 2005). Education of patients, careers, and healthcare providers is an essential component of an effective, interprofessional team approach, but effective systems and structures for screening, provision of chiropody (podiatry), and footwear, and prompt treatment when required must be in place. (Modified from Spraul et al., 2000). The most important aspects, for example, danger signs which require prompt action by the patient, should be summarized and repeated. (Spraul et al., 2000). Successful diagnosis and treatment of patients with chronic wounds involve holistic care and a team approach. The integration of the work of an interprofessional care team that includes doctors, nurses and allied health professionals with the patient, family and caregivers offers an optimal formula for achieving wound resolution. (Sibbald et al., 2001).

2.3 Escherichia coli:

Since its first description by Dr. Theodore Escherichia in 1885, Escherichia coli are the most extensively studied bacterial species. Much of our knowledge of bacteria at a molecular and cellular level has been obtained through studies with E. coli, particularly using the strain K12, isolated in 1922 from a patient with diphtheria. The complete genome sequence of a strain of E. coli K12 has been published revealing the degree of genome plasticity, as indicated by the presence of phage remnants and insertion elements (Blattner et al., 1997).

2.3.1 Description of the organism:

The genus Escherichia includes motile and non-motile bacteria which belong to the family Enterobacteriaceae (Edward et al., 1972). They are Gram-negative, oxidase negative, non-spore-forming, rod-shaped bacteria, facultative anaerobes, which are often motile by peritrichously arranged flagella. They are capable of fermenting a wide variety of carbohydrates with production of both acid and gas, although anaerogenic biotypes exist. Rapid fermentation of lactose is a characteristic feature of many strains, particularly those of E. coli, whilst other Escherichia species, including strains of enter invasive E. coli (EIEC), as well as some E. coli (metabolically inactive) strains ferment it slowly or fail to utilize this substrate at all. Other genera within the family Enterobacteriaceae (Klebsiella, Enterobacter, Serratia and Citrobacter), which share this ability to ferment lactose rapidly (typically within 24 h), are collectively termed coliform bacteria. Besides E. coli, other species belonging to the genus are Escherichia blattae, Escherichia fergusonii, Escherichia hermannii and
Escherichia vulneris. The species Escherichia adecarboxylata has since been assigned to the genus Lecercia, and a new species, Escherichia albertii, has been described (Abbott et al., 2003). The primary habitat of E. coli and the other species is the gastrointestinal tract of humans and other warm-blooded animals where they generally exist as harmless commensal organisms. They can also occur in water, food, and soil, but this is invariably the result of faecal contamination. Although most E. coli strains are harmless, there are others that cause disease in humans and animals that have evolved to become important pathogens in their own right.

2.3.2 Laboratory methods for isolation and detection of pathogenic E. coli:

Whilst most strains of E. coli grow well on a range of microbiological culture media, the growth and isolation of some pathogenic strains requires specific methodology. Strains of pathogenic E. coli can be phenol typically identical to communal E. coli strains, whereas others may give rise to atypical reactions with particular biochemical tests which aid the identification of E. coli. Rapid lactose fermentation remains a key diagnostic feature of media used for the initial isolation or subsequent confirmation of E. coli. MacConkey agar and E. coli broth are widely used for the initial isolation and confirmation of suspect E. coli, respectively. Strains of E. coli are commonly distinguished from other faecal coliforms by their ability to grow and produce gas from lactose at 44 °C and indole production from tryptophan. However, these two tests are not always exclusive to E. coli as other bacteria, e.g. Klebsiella, can give rise to false-positive results. Furthermore, strains of EIEC often ferment lactose slowly or not at all, which together with the absence of indole production and synthesis of lysine decarboxylase can mean that they are not recognised as E. coli. The presumptive identification of E. coli has been improved by the introduction of chromogenic media that provide better diagnostic characteristics mediated by specific enzyme activity which yields colonies of a distinct color. Most chromogenic substrates used in E. coli specific media rely upon the activity of GUD which is prevalent in approximately 95% of E. coli strains. A notable exception, however, is E. coli O157:H7, which is largely GUD negative. Another common enzyme exploited in chromogenic media is β-D-galactosidase which is responsible for lactose fermentation and common in coliform bacteria, including E. coli. Some media contain individual chromogenic substrates to enable specific identification of the target organism, whereas others contain more than one substrate, which enables a differential count or presumptive identification to be made. Fluorogenic substrates that follow the same principle are also available, although they are now less popular because of the requirement to observe plates under long-wave UV light and the problems caused by diffusion of fluorescence through the medium. Lactose fermentation remains a useful diagnostic feature of
media for the isolation of urinary pathogens, including *E. coli*. A good example of such a medium is cysteine lactose electrolyte-deficient (CLED) agar which is used for routine diagnostic urinary bacteriology. This medium is recommended because it is reported that 1.5% of *E. coli* isolated from urine require cysteine and that subsequent broths used for their identification will require cysteine supplementation (McIver *et al.*, 1990). The non-selective medium CLED supports the growth of a wide range of urinary pathogens whilst preventing swarming of *Proteus spp*. Lactose-fermenting organisms, including *E. coli*, lower the pH of the medium, which turns from green to yellow. The development of chromogenic media combining the basal CLED medium with various chromogenic substrates has the potential to improve presumptive identification of urinary isolates (Fallon *et al.*, 2003). The correlation between specific O and H antigens with different path types of *E. coli* has led to serotyping being used for identification purposes. Whilst this remains useful for certain pathotypes associated with infection, especially those that comprise limited serotypes and strains belonging to distinct colonial lineages, this becomes less reliable when the pathogenicity-associated genes are located on mobile genetic elements. Consequently, there is no longer a clear distinction between certain pathotypes of *E. coli* based on serotyping. Detection of specific antigens or toxins associated with a particular *E. coli* pathotype using appropriate immunological methods such as ELISA can improve detection and identification of these strains. This approach has been used to confirm ETEC colonies using a GM1 ganglioside ELISA to detect LT and ST, which compared favorably against a gene probe (Sommerfelt *et al.*, 1988). Numerous commercial assays are available for the detection of the somatic O antigen of *E. coli* O157 and also for the detection of VT from culture supernatants or directly from sample enrichments, thus enabling detection of all VTEC in clinical samples and foods (Bettelheim *et al.*, 2003).

**2.3.3. Antibiotic resistant:**

Most *E. coli* are sensitive to antimicrobial agents active against Gram-negative bacteria, although resistance among enteric bacteria, including *E. coli*, has increased markedly over the past 50 years since of the widespread use of antibiotics (Houndt *et al.*, 2000). Multiple antibiotic resistances can be acquired via plasmids or drug efflux systems. The chromosomal multiple antibiotic resistance locus in *E. coli*, designated marA, influences the expression of the acrAB efflux pump and other chromosomal genes, resulting in resistance to a range of antibiotics including tetracycline and many unrelated antibiotics including chloramphenicol, β-lactams and nalidixic acid (George *et al.*, 1983). Susceptibility of *E. coli* strains to amoxicillin has decreased over recent years owing to the presence of TEM-1 and TEM-2 β-lactamase. The effectiveness of cotrimoxazole and trimethoprimhas been reduced by frequent
carriage on plasmids and integrons of resistance genes (Yu et al., 2003). Because resistance is high, a fluoroquinolone or nitrofurantoin should be considered for empirical treatment. One study of E. coli urinary isolates from females in the United States from 1995 to 2001 revealed resistance rates to be constant for ampicillin (36–37%) and co-trimoxazole (15–17%), with increasing resistance to ciprofloxacin (0.7–2.5%) and low resistance to nitrofurantoin (0.4–0.8%) (Karlowsky et al., 2002), which to gather with fosfomycin-trometanol remains highly active against urinary Enterobacteriaceae with over 90% of E. coli reported to be susceptible (Chomara et al., 2000). One prospective study has identified prior UTI is a common risk factor for resistance to different antibiotics use to treat UTI (Sotto et al., 2001).

2.4 Alcohol (Ethanol and Methanol):

2.4.1. Ethanol:

Also commonly called ethyl alcohol, drinking alcohol, or simply alcohol is the principal type of alcohol found in alcoholic beverages, produced by the fermentation of sugars by yeasts. It is a neurotoxic psychoactive drug and one of the oldest recreational drugs used by humans. It can cause alcohol intoxication when consumed in sufficient quantity (Brust et al., 2010). Ethanol is a volatile, flammable, colorless liquid with a slight chemical odor. It is used as an antiseptic, a solvent, a fuel, and, due to its low freezing point, the active fluid in post-mercury thermometers. Its structural formula, CH3CH2OH, is often abbreviated as C2H5OH, C2H6O or Et OH (Brust et al., 2010).

2.4.1.1 Chemical formula of Ethanol:

Ethanol is a 2-carbon alcohol. Its molecular formula is CH3CH2OH. An alternative notation is CH3–CH2–OH, which indicates that the carbon of a methyl group (CH3–) is attached to the carbon of a methylene group (–CH2–), which is attached to the oxygen of a hydroxyl group (–OH). It is a constitutional isomer of dimethyl ether.
2.4.1.2 Medical uses of Ethanol:

Ethanol is used as an antiseptic in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% v/v as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria and fungi, and many viruses, but is ineffective against bacterial spores (McDonnell et al., 1999). Ethanol is also widely used, clinically and over the counter, as an antitussive agent and may be administered as an antidote to methanol poisoning (Calesnick et al., 1971).

2.4.1.3 Production of Ethanol:

Ethanol is produced both as a petrochemical, through the hydration of ethylene and, via biological processes, by fermenting sugars with yeast. Which process is more economical depends on prevailing prices of petroleum and grain feed stocks (Mills et al., 1987).

2.4.2 Methanol:

Methanol, also known as methyl alcohol, wood alcohol, wood naphtha or wood spirits, is a chemical with the formula CH3OH. Methanol acquired the name “wood alcohol” because it was once produced chiefly as a byproduct of the destructive distillation of wood. Modern-day methanol production occurs in a catalytic industrial process directly from carbon monoxide,
carbon dioxide, and hydrogen. Methanol is the simplest alcohol, and is a light, volatile, colorless, flammable liquid with a distinctive odor very similar to that of ethanol (drinking alcohol) (National Institute for Occupational Safety and Health, 2008). However, unlike ethanol, methanol is highly toxic and unfit for consumption. At room temperature, it is a polar liquid, and is used as an antifreeze, solvent, fuel, and as a denaturant for ethanol. Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria, and is commonly present in small amounts in the environment. As a result, there is a small fraction of methanol vapor in the atmosphere. Over the course of several days, atmospheric methanol is oxidized with the help of sunlight to carbon dioxide and water (Barceloux et al., 2002). Methanol burns in oxygen, including open air, forming carbon dioxide and water:

\[
2 \text{CH}_3\text{OH} + 3 \text{O}_2 \rightarrow 2 \text{CO}_2 + 4 \text{H}_2\text{O}
\]

**Figure 2.2 Chemical Formula of Methanol**

Methanol, which is a common laboratory solvent, is especially useful for HPLC, UV/VIS spectroscopy, and LCMS due to its low UV cutoff.

### 2.5 Disinfection:

Many disinfectants are used alone or in combinations (e.g., hydrogen peroxide and per acetic acid) in the health-care setting. These include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, *ortho*-phthalaldehyde, hydrogen peroxide, iodophors, per acetic acid, phenolics, and quaternary ammonium compounds. Commercial formulations based on these chemicals are considered unique products and must be registered with EPA or cleared by FDA. In most instances, a given product is designed for a specific purpose and is to be used in a certain manner. Therefore, users should read labels carefully to ensure the correct product is selected for the intended use and applied efficiently (CDC et al., 2008). Disinfectants are not interchangeable, and incorrect concentrations and inappropriate disinfectants can result in excessive costs. Because occupational diseases among cleaning personnel have been associated with use of several disinfectants (e.g., formaldehyde, glutaraldehyde, and chlorine), precautions (e.g., gloves and proper ventilation) should be used to minimize exposure (CDC et al., 2008).
2.5.1 Alcohol as a disinfectant:

Methyl alcohol (methanol) has the weakest bactericidal action of the alcohols and thus seldom is used in healthcare. The bactericidal activity of various concentrations of ethyl alcohol (ethanol) was examined against a variety of microorganisms in exposure periods ranging from 10 seconds to 1 hour. *Pseudomonas aeruginosa* was killed in 10 seconds by all concentrations of ethanol from 30% to 100% (v/v) and *Serratia marcescens, E.coli* and *Salmonella typhus* were killed in 10 seconds by all concentrations of ethanol from 40% to 100%. The gram-positive organisms *Staphylococcus aureus* and *Streptococcus pyogenes* were slightly more resistant, being killed in 10 seconds by ethyl alcohol concentrations of 60%–95%. Isopropyl alcohol (isopropanol) was slightly more bactericidal than ethyl alcohol for *E. coli* and *S. aureus* (CDC et al., 2008).

In tests of the effect of ethyl alcohol against *M. tuberculosis*, 95% ethanol killed the tubercle bacilli in sputum or water suspension within 15 seconds. In 1964, Spaulding stated that alcohols were the germicide of choice for tuberculocidal activity, and they should be the standard by which all other tuberculocides are compared. For example, he compared the tuberculocidal activity of iodophor (450 ppm), a substituted phenol (3%), and isopropanol (70%/volume) using the mucin-loop test (10^6 *M. tuberculosis* per loop) and determined the contact times needed for complete destruction were 120–180 minutes, 45–60 minutes, and 5 minutes, respectively. The mucin-loop test is a severe test developed to produce long survival times. Thus, these figures should not be extrapolated to the exposure times needed when these germicides are used on medical or surgical material. Ethyl alcohol (70%) was the most effective concentration for killing the tissue phase of *Cryptococcus neoformans,* *Blastomyces dermatitidis, Coccidioides immitis,* and *Histoplasma capsulatum* and the culture phases of the latter three organisms aerosolized onto various surfaces. The culture phase was more resistant to the action of ethyl alcohol and required about 20 minutes to disinfect the contaminated surface, compared with <1 minute for the tissue phase (CDC et al., 2008). The documented shortcomings of alcohols on equipment are that they damage the shellac mountings of linseed instruments, tend to swell and harden rubber and certain plastic tubing after prolonged and repeated use, bleach rubber and plastic tiles and damage tonometer tips (by deterioration of the glue) after the equivalent of 1 working year of routine use. Tonometer biprisms soaked in alcohol for 4 days developed rough front surfaces that potentially could cause corneal damage; this appeared to be caused by weakening of the cementing substances used to fabricate the biprisms. Corneal opacification has been reported when tonometer tips were swabbed with alcohol immediately before measurement of intraocular pressure. Alcohols are
flammable and consequently must be stored in a cool, well-ventilated area. They also evaporate rapidly, making extended exposure time difficult to achieve unless the items are immersed (CDC et al., 2008).
CHAPTER THREE

Materials and Methods

3.1 Materials

3.1.1 Study design:

Cross sectional laboratory based study.

3.1.2 Study area:

The Study was conducted at Wad-Medani, Abuaglla center for diabetic patients, Gezira state, Sudan.

3.1.3 Study population:

Diabetic foot ulcer people attending Abuaglla center for diabetic patients.

3.1.4 Study period:

The study was done from April to October 2017.

3.1.5 Inclusion criteria:

All diabetic foot ulcers that attending Abuaglla center for diabetic patients.

3.1.6 Exclusion criteria:

- All diabetic patients not ulcerated were excluded.
- All diabetic patients with foot ulcer who refused to participate in the study.

3.1.7 Sampling and sample size:

30 patients were included according to inclusion and exclusion criteria.

3.1.8 Materials and reagents:

- swabs
- Gram stain sets
- Nutrient broth
- MaCconkey agar
- Blood agar
- Chocolate agar
- Simmons's citrate agar
- Peptone water
- Urea agar
- Kilgler iron agar
- Nutrient agar
- Oxidase disc
- Hydrogen peroxide
- Methanol
- Ethanol
- Autoclave
- Incubator
- Oven
- Water bath
3.2 Methods

3.2.1 Data collection:
Structured questionnaire was used to collect data from study population.

3.2.2 Samples collection:
Samples were collected using sterile swab from diabetic ulcer.

3.2.3 Samples processing:
Samples were processed and transported to laboratory and wound swaps were cultivated on Blood agar, Chocolate blood agar and MaCconkey agar. After differentiation, one ml of subculture peptone water was added to 9 ml Alcohol, Iodine, Hydrogen peroxide with different concentrations starting from 100% to 70% and a lope full of the mix was streaked at 5 minutes. The media will be incubated at 37 ºC for 24 hours aerobically and then results were examined.

3.2.4 Statistical analysis:
- This study was analyzed by using statistical package for social sciences (SPSS) software. Descriptive analysis, correlation analysis, sensitivity, specificity and predictive value of every diagnostic test were done. Also, ROC or AUC analysis was done.
- The results was considered significant when p < 0.05.

3.2.5 Ethical consideration:
- The permission to conduct this study was obtained from State Ministry of health Gezira state.
- Consent of every participant was obtained.
- Information was collected from the subjects under privacy and was used for research study only.
CHAPTER FOUR

Results and Discussion

4.1 Results:

4.1.1 Gender:
The study population included 30 patients of both gender, (77 %) were males and (23 %) were females, as shown in (table 4.1).

Table (4.1): Distribution of DFU patients according to gender.

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>23</td>
<td>76.7</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.1.2 Age group:
The age of participants was grouped as: 20 – 40 years (3.3 %), 41 – 60 years (56.7 %) and more than 60 years (40 %) as shown in (Table 4.2).

Table 4.2 Distribution of DFU patients according to age group

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 40</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>41 – 60</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>more than 60</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.1.3 Period of infection:
Within 30% of DFU patients (83%) of patient suffering from infection for less than one year, (17%) suffering from 2 – 4 years, that is shown in (table 4.3).

Table (4.3): Distribution of DFU patients according to period of infection

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than one year</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td>2 – 4</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.1.4 Duration of disease:
Within 30% of DFU patients (6.7%) suffering from diabetic disease for less than 1 year, (23.3%) suffering for 3 – 6 years, (16.7%) suffering for 6 – 9 years, (53.3%) suffering for more than 9 years. That is shown in (table 4.4).

Table (4.4): Distribution of DFU patients according to duration diabetic disease.

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 1 year</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>3 - 6 years</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>6 - 9 years</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>more than 9 years</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.1.5 Times of dressing:
Within 30 patient of DFU (53.3%) were dressing the ulcer 3 times per week, (33.3%) dressing the ulcer 2 times per week, (13.4%) were dressing the ulcer daily. That is shown in (table 4.5).

Table (4.5): Distribution of DFU patients according to times of dressing

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>2 time per week</td>
<td>4</td>
<td>13.4</td>
</tr>
<tr>
<td>3 time per week</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.1.6 Grade:
Within 30 patient of DFU patients (26.7%) in grade 1, (40%) in grade 2, (33.3%) in grade 3. That is shown in (Table 4.6).

Table (4.6): Classification of DFU patients according to severity of infection (grade).

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>grade 1</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>grade 2</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>grade 3</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.1.7 Absolute methanol:

Within 30 subculture of *E. coli* (80.0%) was sensitive to absolute methanol, (20.0%) was resistant (figure 4.1).

![Figure (4.1): Frequency of absolute methanol solution according to resistant and sensitivity to *E. coli*](image-url)
4.1.8 Methanol 70%:
Within 30 subculture of *E. coli* (26.7%) was sensitive to absolute methanol (73.3%) was resistant (figure 4.2).

Figure (4.2): Frequency of methanol 70% solution according to resistant and sensitivity to *E. coli*. 
4.1.9 Absolute ethanol:
Within 30 subculture of *E. coli* (73.3%) is sensitive to absolute methanol, (26.7%) was resistant (figure 4.3).

![Figure 4.3](image)

Figure (4.3) Frequency of absolute ethanol solution according to resistant and sensitivity to *E. coli*. 


4.1.10 Ethanol 70%:
Within 30 subculture of *E. coli* (23.3%) is sensitive to absolute methanol, (76.7%) was resistant (figure 4.4).

![Pie chart showing the distribution of sensitivity to ethanol 70% solution among subcultures of *E. coli*. 76.67% are resistant, 23.33% are sensitive.]

Figure (4.4): Frequency of ethanol 70% solution according to resistant and sensitivity to *E. coli*. 
4.1.11 Iodine:

Within 30 subculture of *E. coli* (96.7%) is sensitive to absolute methanol, (3.3%) was resistant (figure 4.5).

Figure (4.5): Frequency of iodine solution according to resistant and sensitivity to *E. coli*. 
4.1.12 Hydrogen peroxide:
Within 30 subculture of *E. coli* (13.3%) is sensitive to absolute methanol, (86.7%) was resistant (figure 4.6).

![Pie chart]

Figure (4.6): Frequency of hydrogen peroxide solution according to resistant and sensitivity to *E. coli*. 
4.1.13 Fusiderm with honey:
Within 30 subculture of *E. coli* (16.3%) is sensitive to absolute methanol, (86.7%) was resistant (figure 4.7).

Figure (4.7): Frequency of fusiderm with honey solution according to resistant and sensitivity to *E.coli*. 
4.2 Discussion:

Diabetes mellitus is the major cause of non-traumatic limb amputation, lower extremities infection are serious cause of morbidity in patients with DM. in the present study all the samples yielded mono microbial isolates. This is significantly different from most studies in which results DFU is poly microbial infection.

The mono microbial nature of infection is associated with the duration of the ulcer and antimicrobial treatment. Ulcers that are shallower and that have a lesser degree of necrosis tend to be mono microbial.

In this study 30 samples were involved male were represented (76.7%) of the total sample and the female were (23.3%) (Table 4.1) and patients age above 41 – 60 year displayed (56.7%) (Table 4.2).

In this study the most frequently isolated microorganisms were gram negative *Escherichia coli* (16.7%).

In fact (6.7%) of the total isolates showed no growth. this may be due to effectiveness of antimicrobial agent used as the samples collected immediately after the wound cleaned.

The treatment of diabetic foot infection require bactericidal agent given in sufficient large dose to provide adequate tissue level.

In certain circumstances the anti-microbial treatment may have to be initiated empirically to prevent systematic invasion by pathogenic microorganism in debilitated patient while waiting microbiological result.

The sensitivity of *Escherichia coli* was reported as 96.67% in iodine (Figure 4.5), 80% absolute methanol (Figure 4.1), and 73.33% absolute ethanol (Figure 4.2), 26.67% methanol 70% (Figure 4.3) 23.3% ethanol 70% (Figure 4.4), 16.3% in combination of Honey and fusiderm (Figure 4.7) and 13.3% hydrogen peroxide (Figure 4.6) respectively. The highest effective disinfectant against *E.coli* was iodine (96.67%).
CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.1 Conclusion:

The present study demonstrated that all the samples were mono-microbial. The predominant isolate from DFU was *S. aureus*, followed by *E. coli*, *Pseudomonas aeruginosa* and *proteus Spp.*

Disinfectant susceptibility test against *E. coli* showed that the iodine is the most effective disinfectants (96.67%).

5.2 Recommendation:

- Determination of the causative organism of infection in diabetic foot patient and their antimicrobial susceptibility pattern is essential for the appropriate choice of antimicrobial therapy.
- Treatment should be initialized according to the severity of infection, costs, availability of the antimicrobial agent.
- Regular dressing is important to avoid the contamination of the ulcer.
- Blood glucose level should be monitored.
- The health care provider should advise the diabetic patient to avoid tight shoes and regularly check their feet for injuries.
- Patient's awareness for the antibiotic should be promoted.
References


American Diabetes Association Consensus development conference on diabetic foot wound care: 7–8 April (1999), Boston, Massachusetts.


Joan F Zilva (1979) PR pannallmayne Philip Clinical chemistry in diagnosis and treatment - page 206.


Appendices

Appendix (1)

بسم الله الرحمن الرحيم

University of Gezira

Efficiency of different disinfecting solutions on diabetic foot wounds infections.

Name: ................................. Number: ............

Gender: .....................................................

Address: ....................................................

Age: .........................................................

Duration of diabetic disease: .........................

Period of wound: ....................................

How many time of dressing: .........................

Type of disinfecting solution used: .................

Type of antibiotic used: ............................

Other chronic disease: ..............................

Researcher data:

Isolated microorganism: ..............................

Sensitive solution: .....................................

Resistant solution: .....................................

Sensitive antibiotic: ...................................

Resistant antibiotic: ..................................
Appendix (2)

- Swabs
- petridishes
- gloves
- cotton
- mask
- loops
- benzene burner
- Test tube
- Bijou bottle
- Racks
- Slide
- Gram stain sets
- Flask
- Cylinder
- Filter paper
- Nutrient broth
- MacConkey agar
- Blood agar
- Chocolate agar
- Simmons's citrate agar
- Peptone water
- Urea agar
- Kilgler iron agar
- Nutrient agar
- Oxidase disc
- Wooden stick
- Hydrogen peroxide
- Oil
- Pipettes
- Methanol
- Ethanol
- Gauze
- Distell water
- Microscope
- Sensitive balance
- Autoclave
- Incubator
- Oven
- Water bath