Sudan University of Science and Technology

College of Graduate Studies

Frequency of Antimicrobial Resistant Bacteria Isolated from Circumcised Women Suffering from Urinary Tract Infections in Different Clinics in Khartoum locality

تكرارالبكتيريا المقاومة لمضادات الميكروبات المعزولة من النساء المختونات اللآئي يعانين من عدوى المسالك البولية في عيادات مختلفة بولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Science (Microbiology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى :

قُلْ هُوَ الَّذِي أَنشَأَكُمْ وَجَعَلَ لَكُمُ السَّمْعَ وَالْأَبْصَارَ وَالْأَفْئِدَةَ ۖ قَلِيلًا مَّا تَشْكُرُونَ

صدق الله العظيم

سورة الملك – الآية (23)

Dedication

To who is always supporting me and I am gaining my strength and patience from him My father (my Allah have mercy on him).

To whom give me a beautiful prayer to Allah in every single dais ……my mother

To whom gives me the love and security… My husband .

To dears brothers and sisters

To my lovely Teachers

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First of all I thanks our **ALMIGHTY ALLAH** for success and completion of this research, and I hope this research to have a benefit for the personal and social levels. After that I would like to thank my great supervisor **Dr. Wafaa Mohammed Abdalla** who helped me when never I need a help through this work. I would like to thank all those who assisted and encouraged me to complete this research by providing me the necessary information that required to complete this study. A special thanks to my friend Duaa Alteeb for her support and help in specimens collections.

Abstract

Urinary tract infections (UTIs) are one of the diseases that are widely spreading among women, due to several factors. The most important of which is the different nature of the composition of the urinary system, which is characterized by the shortage the urinary and its proximity to the anus in addition to the change caused by circumcision that leads to the narrowness of the opening of the urinary system and vigilantes on the other.

This cross-sectional study was conducted from April 2019 to February 2021 to detect the frequency of antimicrobial resistance bacteria isolated from circumcised women attending Dan Medical Complex and Nobatia Medical Clinic. Isolation, identification and antimicrobial susceptibility testing were done by conventional methods. Eighty (n = 80) mid-stream urine specimens were collected from all eligible volunteers, in which 40 were circumcised and other 40 were not, their ages ranged from 7 to 70 years with mean of age 29.3 ± 13.0 S.D. About 16/40 (40 %) of circumcised women were married and 23/40 (60%) were single, while among non-circumcised group there were 7/40 (17.5%) married and 33/40 (82.5%) were single.

The growth rate was 34/40 (85%) among circumcised females and 6/40 (15%) among non-circumcised participants and there were significant association (*P-value*= 0.000) between circumcision and UTIs. Based on the odd ratio (O.R) the risk factor of UTIs was 32 times greater among circumcised females than non-circumcised one.

The isolated bacteria were 24/40 (60%) Gram negative rods and 10/40 (15%) were Gram positive cocci among circumcised women while there were only 6/40 (15%) Gram negative rods and no isolated Gram positive among non-circumcised one. There were meaningless association between circumcision and bacteria according to Gram's stain (*P-value*=0.125). Among the isolated bacteria; *Escherichia coli* was the most predominant isolate among circumcised and non-circumcised women (15 (37.5%)).

Concerning circumcised women; the isolated bacteria were moderately sensitive to Augmentin (22/34 (67.7%)) and Gentamycin (20/34 (58.8%)) compared to other antimicrobial agents (Ciprofloxacin 16/34 (47.1%), Cefuroxime (12/34 (35.3) and Amoxycillin (10/34 (29.4%)) and all isolated Gram negative rods bacteria were highly resistant to nalidixic acid (100.0%). In contrast to non-circumised women; all the isolated bacteria (G-ve rods) were highly sensitive to Gentamicin (6/6 (100.0%) and cefuroxime

(5/6 (83.3%), and moderately sensitive to Augmentin (4/6 (66.7%) and Ciprofloxacin (4/6(66.7%). Also all isolated bacteria were highly resistant to Nalidixic acid and Amoxycillin except *K.pnemoniae* was higly sensitive (100.0%) for both antimicrobial agents.

In conclusion, the frequency of UTIs and antimicrobial resistance were high among circumcised women.

المستخلص

عدوى المجاري البولية من الأمراض المنتشرة بكثرة بين النساء وذلك لعدة عوامل أهمها يرجع إلى إختلاف طبيعة تركيب الجهاز البولي لديها الذي يمتاز بقصر المجرى البولي وقربه من فتحة الشرج بالإضافة إلى التغير الذي يسببه الختان والذي يؤدي إلى ضيق لفتحة الجهاز البولي. تم إجراء هذه الدراسة الوصفية المستعرضة في الفترة من شهر إبريل 2019 إلى فبراير 2021 للكشف عن معدل إنتشار إلتهاب المجاري البولية بين النساء المختونات اللآتي حضرن مجمع دان الطبي ومجمع نوباتيا الطبي. تم عزل البكتريا والتعرف عليها وإختبار الحساسية لمضادات الميكروبات بالطرق التقليدية. تم جمع عدد 80 عينة منتصف تيار البول من المتطوعين المؤهلين بحيث 40 عينة من نساء مختونات و 40 عينة من نساء لم يتم ختانهم، وتراوحت أعمارهن ما بين 7 إلى 70 سنة بمتوسط أعمار 29.3 سنة وإنحراف معياري 13.8. حوالي 40 /16 (40٪) من النساء المختونات كن متزوجات و 40/23 (57٪) كن عازبات. أما في النساء الغير المختونات كان 40/7 (17.5%) متزوجات و 40/33 (82.5%) غير متزوجات.

كان معدل نمو البكتيريا وسط المختونات 40/34 (85%) بينما الغير مختونات 40/6(15%)، وتوجد علاقة معنوية (القيمة الإحتمالية= 0.000) بين الختان وإلتهاب المجاري البولية. إستناداً إلى نسبة التعرض؛ وجد أن خطر تعرض الإصابة بإلتهاب المجاري البولية 32 مرة ضعف للنساء المختونات مقارنة بغير المختونات. مثلت البكتيريا المعزولة من النساء المختونات نسبة 60.0%(24/40) من البكتريا سالبة الجرام العصوية و40/10(15.0%) من البكتريا موجبة الجرام الكروية، بينما كانت نسبتها وسط النساء الغير مختونات 40/6(15.0%) من البكتريا سالبة الجرام العصوية ولم يتم عزل أي من البكتريا موجبة الجرام الكروية (0%). ولا توجد علاقة ذات أهمية بين الختان ونوع البكتريا حسب صبغة جرام (القيمة الإحتمالية= 0.125).

ومن بين البكتيريا المعزولة كانت الإشريكية القولونية الأكثر إنتشاراً في المجموعتين (15(37.5%).

فيما يتعلق بالنساء المختونات : كانت البكتيريا المعزولة تتمتع بحساسية متوسطة ل الأوقمنتين (22/34)(67.7%) والجنتاميسين (20/34)(58.8%) مقارنة لمضادات الميكروبات الأُخرى (السيبروفلوكساسين (16/34)(47.1%) والسيفيوروكسيم (12/34)(35.3%) و الأموكسيسيلين(10/34(29.4%)) وكانت كل البكتريا المعزولة سالبة الجرام مقاومة بصورة عالية ل الناليديكسيك أسيد(100.0%).

بالمقابل لغير المختونات كل البكتيريا المعزولة كانت عالية الحساسية مع الجنتاميسين (6/6(100.0%)) والسيفيوروكسيم (5/6(83.3%)) و متوسطة الحساسية مع الأوقمنتين (4/6(66.7%)) والسيبروفلوكساسين (4/6(66.7%) وكانت كل البكتريا المعزولة ذات مقاومة عالية ل الناليديكسيك ماعدا الكلبسية الرئوية (100.0%) كانت حساسة لهذين المضادين الحيويين.

وفي خلاصة هذه الدراسة وجد أن: زيادة معدل إنتشار إلتهاب المجاري البولية ومقاومة مضادات الميكروبات أعلى وسط النساء المختونات.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
Amp	Amoxycillin
Aug	Augmentin
BC	Before Christ
Cef	Cefuroxime
CFU	Colony-forming units
Cip	Ciprofloaxacin
EAU	European Association of Urology
FGM	Female genital mutilation
Gen	Gentamicin
KIA	Kligler Iron Agar
L-UTIs	Lower urinary tract infection
MUI	Mixed urinary incontinence
Nal	Nalidixic acid
PBP	Penicillin binding protein
R	Resistant
S	Sensitive
SPP	Species
SPSS	Statistical package of the social science
SUI	Stress urinary incontinence
UI	Urinary infection
UTIs	Urinary tract infections
UUI	Urgency urinary incontinence
U-UTIs	Upper urinary tract infection

CHAPTER I

INTRODUCTION

CHAPTER I

1. INTRODUCTION

1.1. Introduction

Urinary tract infections (UTIs) are an infection in any part of urinary system and women at greater risk of developing a UTIs than are men. The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs, in which the female urethra is relatively short compared with the male urethra and also lies in close proximity to the warm, moist, per rectal region, which is teeming with microorganisms (Paticia, 2017).

UTIs typically occur when bacteria enter urinary tract through urethra and being to multiply in the bladder. Although the urinary system is designed to keep out such microscopic invaders, these defenses some time fail. When that happens, bacteria may take hold and grow into a full blown infection in the urinary tract. Women experience more than one infection during their lifetimes (Hertting, 2011).

Risk factors specific to women for UTIs include; female anatomy (a women has a shorter urethra than men), sexual activity, certain type of birth control and menopause (after menopause a decline in circulating estrogen causes change in the urinary tract that make women more vulnerable to infection) (Hertting, 2011).

Other risk factor for UTIs also can be urinary tract abnormalities (babies born with urinary tract abnormalities), blocking in the urinary tract; as kidney stone, catheterize (people who can't urinate on their own and use a tube to urinate (a suppressed immune system), urinary procedure (which can be recent urinary surgery or an exam of the urinary tract that involves medical instrument) (MAYO CLINIC, 2019).

Also female genital mutilation (FGM) also known as female genital cutting or female circumcision that don't allow the urine to leave completely or cause urine to back up in the urethra have an increased risk of UTIs and menstrual problem. The most serious type of (FGM) is type three that create covering seal and small opening is left for urine and menstrual blood to escape (https://en.wikipedia.org/wiki/Female_genital_mutilation).

All those risk factors can lead us to recurrent infection and may result in antibiotic resistant bacteria. Also geographic variation in etiologic agents of UTIs and their resistance patterns in antibiotics (Gupat, 2003; Akoackere *et al*., 2012).

1.2. Rationale

UTIs is the second most common infection presenting in community practice which associated with elevated antibiotic resistance in Sudan (Amir *et al*., 2017).

Female genital mutilation may be increasing UTIs (Elduma, 2018).

For my knowledge, there are few studies concerning the consequences of circumcision on urinary system and its risk for increasing antibiotic resistant bacteria associated with UTIs. So, the ultimate goal of this study to provide information about this problem which may highlight on circumcision and its association with antibiotic resistant bacteria causing UTIs.

1.3. Objectives

1.3.1. General objective

To determine the frequency of antibiotic resistant bacteria isolated from circumcised women suffering from Urinary Tract Infections infection in different clinics in Khartoum locality.

1.3.2. Specific objectives

1.To isolate and identify bacteria from circumcised women (study group) and non-circumcised women (control group) by routine cultivation methods.

2. To associate between types of isolated bacteria among circumcised and non-circumcised females.

3- To determine the frequency of bacteria isolated from circumcised and non-circumcised females.

4. To perform antimicrobial susceptibility testing against the isolated pathogens using Kirby-Bauer CLSI modified disc diffusion technique.

5- To compare between the results of antibiotic resistance bacteria in circumcised and non-circumcised women.

6-To find out possible association between the female circumcision and urinary tract infections.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2. LITERATURE REVIEW

2.1. Urinary tract infections (UTIs)

Urinary tract infections (UTIs) are characterized as being either upper (U-UTIs) or lower (L-UTIs) based primarily on the anatomic location of the infection. The lower urinary tract encompasses the bladder and urethra and the upper urinary tract encompasses the ureters and kidneys (Paticia, 2017).

The urethra has resident microbial that colonize its epithelium in the distal portion; these organisms are *Lactobacilli*, *Corynebacteria*, *Enterococci*, and coagulase–negative *Staphylococci*. Potential pathogens, including Gram-negative aerobic bacilli (primarily *Enterobacteriaceae*) and occasional yeasts which are present as transient colonizers (Paticia, 2017).

All areas of the urinary tract above the urethra in a healthy human are sterile, so bacteria can invade and cause UTIs via three major routes ascending, hematogenous, and lymphatic pathways. Although the ascending route is the most common course of infection in females (Paticia, 2017).

2.1.1. Types of UTIs and their clinical manifestations

There are several types and clinical presentation of UTIs which may vary from asymptomatic infection to more specific symptoms and can be distinguished by location in to lower-UTIs and hi-UTIs and the sings of both types can be considerably similar to each other. Lower UTIs involve urethra (urethritis), symptoms associated with urethritis, dysuria and frequency, cystitis (infection of the bladder) complains of dysuria, frequency, and urgency. Often, there is tenderness and pain over the area of the bladder (Mahon *et al*., 2015).

While upper UTIs (inflammation or infection within the ureters) is considered in combination with kidney infections which indicates that; organisms have begun or are in the process of ascending into the kidneys and in some time there is asymptomatic bacteriauria (Mahon*etal.*, 2015).

So if the ascending to the kidney occurs, pyelonephritis happen, which is inflammation of renal parenchyma and the typical clinical presentation includes; fever, flank (lower back) pain, chills, nausea, vomiting and lower tract symptoms (frequency, urgency, and dysuria) (Mahon *et al*., 2015).

On another hand the lower UTIs were defined by International Continence Society 2002 according to symptoms as storage symptom (i.e. frequency, nocturia, urgency, urgency urinary incontinence (UUI), stress urinary incontinence (SUI), mixed urinary incontinence (MUI), and other urinary infection (UI); voiding symptoms (i.e. intermittency, slow stream, straining, and terminal dribble); and post-micturition symptoms (i.e. sense of incomplete emptying and post-micturition dribble) (Barry *et al.*,1992).

Furthermore, the European Association of Urology (EAU) guideline severity of UTIs should be graded clinically as; (a) asymptomatic, (b) causing local symptoms such as dysuria, frequency and urgency,(c) causing general symptoms including fever, flank pain, vomiting and nausea, (d) systemic inflammatory response syndrome with fever or hypothermia , hyper leukocytosis and leucopenia, (e) circulatory or organ failure (Tonolini, 2018).

Also complicated UTIs which defined by EAU as those associated with structural or functional abnormalities urinary tract .While uncomplicated UTIs define as whether they occur in normal genitourinary tract with no prior instrumentation or comorbidities (Tonolini, 2018).

2.1.2. Causative agents

The Gram-negative rod *Escherichia coli* is the commonest cause of ascending UTIs and other members of the *Enterobacteriaceae* are also included. *Proteus mirabilis* is often associated with urinary stones (calculi), probably because this organism produces a potent urease, which acts on urea to produce ammonia, convert the urine to alkaline. *Citrobacter*, *Klebsiella, Enterobacter, Proteus* and *Pseudomonas aeruginosa*are more frequently found in hospital-acquired UTIs because their resistance to antibiotics favors their selection in hospital patients (Goering *et al*., 2012).

Among the Gram-positive species, *Staphylococcus saprophyticus* has a particular propensity for causing infections, especially in young sexually active women. *Staphylococcus epidermidis* and *Enterococcus* species are more often associated with UTIs

in hospitalized patients (especially those with AIDS). When there has been hematogenous spread to the urinary tract, other species may be found, e.g. *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (renal tuberculosis) (Goering *et al*., 2012).

2.1.3. Complications of UTIs

Normally UTIs treated accurately resolve quickly and are not associated with severe complications. The most common complications are recurrent infections and its consequences. In a minority, however, UTIs may cause severe infection and long-term disabilities. Infants younger than 3 months are at increased risk of bacteremia (Richardson and Lakhanpaul, 2007).

2.1.3.1. Recurrent UTIs

Women will have recurrent UTIs following their primary infection although they do not have obvious urinary tract anatomical abnormalities (Finer and Landau, 2004).

One of the many abnormalities is cutting and sealing creating a covering which lead to incomplete remove of all urine, however about 30% of type three female genital mutilation (FGM) suffering from recurrent UTIs while occur by 10% in type one FGM (Zambon *et al*., 2018).

The affected canal feels sever when acidic urine retention and the microbes multiply in the stagnant urine of the society by the bladder and may lead to inflammation of the bladder may extend to the ureters (Mariam, 2016).

These recurrences are often caused by the same bacterial strain, suggesting incomplete resolution of the primary infection rather than a new infection (Russo *et al*., 1995).

2.1.3.2. Renal scaring

If the host response fails, bacteria may ascend to the upper urinary tract and cause pyelonephritis. The inflammation caused by this type of infection can lead to renal scarring, especially in children. Renal scaring is a process involving a number of actors. A crucial event is the influx of neutrophils. As a consequence, granulocytic cytotoxic products including lysozyme, elastase and myeloperoxidase are released and oxidative bursts occur (Heinzelmann *et al.*, 1999).

These processes, aimed to killing and removing the pathogens may paradoxically be deleterious to the host with tissue destruction and fibrosis to follow (Jahnukainen *et al*., 2005).

2.1.4. Diagnosis

The differential diagnosis of dysuria may include pyelonephritis, cystitis and urethritis and a good history and physical examination usually gives the clinician enough information to make a correct diagnosis. The most available diagnostic test for UTIs is a urine analysis (Tenke, 2011)

2.1.4.1. Urine microscope

Is the use of a microscope to look at urine to find elevated white blood cells in patient sample (pyuria), hematuria (red blood cells in urine) and sometimes bacteria can be seen. The presence of WBCs casts indicates pyelonephritis rather than cystitis. A urine sample that has abundant squamous epithelial cells suggests that it is contaminated and the results of the culture are not reliable (Levinson, 2014).

2.1.4.2. Urine dipstick

Dipstick test results are suggestive of infection, is a rapid screening test for defecting pyuria, as it can detect the presence of leukocyte esterase. Also detect nitrate, which is signifies the presence of *Enterobacteriaceae* (Tenke, 2011).

2.1.4.3. Urine culture

Usually used mid-stream urine allows to identification of the organism causing infection. A numeric threshold of colony-forming units (CFUs) per milliliter has been established to confirm infection. In samples obtained from a midstream void, $\geq 1 \times 10^5$ CFU/uL is consistent with infection. In samples collected via catheterization, $\geq 1 \times 10^2$ CFU/uL is consistent with infection (Levinson, 2014).

2.1.5. Prevention

General measure for preventing urinary tract infection include taking enough fluid to ensure voiding at least four or five times daily, voiding immediately after sexual intercourse, and wiping from front to back after defecation to minimize fecal contamination of the vagina and urethra. Preventing recurrent infection may require taking daily a small dose of antibiotic that is concentrated in urine (Nester *et al.,* 2009).

2.1.6. Treatment

Treatment usually easily carried out with a few days of antimicrobial medication to which the causative bacterium is susceptible (Nester *et al.*, 2009).

UTIs are often treated with broad-spectrum antibiotics that affect both Gram-positive and Gram-negative bacteria. However, it might be more appropriate to use an antibiotic with a narrow spectrum activity that affects only Gram-positive or Gram-negative bacteria because of concerns about infection with resistant organisms. Moreover, the extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which has become a major problem worldwide (Kumar *et al*., 2006).

In other hand resistance is there, the etiology of UTIs and their antibiotic resistance have been changed over the past years, both in community and hospital-contracted infection (Manges *et al*., 2006; Kahan *et al*., 2006).

2.1.7. Antibiotic resistant

There are two types of bacterial resistance, first natural resistance occurs when an antimicrobial drug cannot kill a bacterium: for example, *Pseudomonas aeruginosa* is naturally resistant to penicillin-G, as its cell envelope is impenetrable for this antibiotic. Second acquired resistance develops when a bacterial strain becomes resistant to a drug to which it had been sensitive before. There are many mechanisms of resistance in bacteria, the most frequently observed, showing high prevalence in clinical isolates they was enzymatic inhibition, penicillin binding protein (PBP) modifications, porin mutations, efflux pumps, and target changes (Kon and Rai, 2016).

The etiology of UTIs and their antibiotic resistance have been changed over the past years, both in community and hospital-contracted infection (Manges *et al*., 2006; Kahan *et al*., 2006).

Unfortunately, there is not much information on etiology and resistance pattern of community acquired UTIs in Sudan is available, but helpfully, area-specific monitoring studies providing knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the right empirical treatment (Beyene and and Tsegaye, 2011).

There are many studies were done to determine the prevalence of urinary tract infections in Khartoum State and antimicrobial susceptibility pattern of isolated bacterial species. One

of these studies found that; 34.5 % of 200 urine samples had significant bacterial growth and the common isolates *were E.coli, E.faecalis* and *S.aureus*. Unfortunately *E.coli* is the most utilized antibiotic since the resistance rate were 65% (Saeed *et al.*, 2017).

Other study found that the most common urinary isolates were highly resistant when they were tested against ampicillin, amoxicillin, co-trimoxazol, tetracycline, sulfonamide, trimethoprim, streptomycin, and carbenicillin (Ahmed *et al*., 2000).

2.2. Female genital mutilation or female circumcision (FGM)

The WHO defines the female genital mutilation as procedures that intentionally alter or cause injuries to the female genital organs for non-medical reasons. No doubt, this procedure can cause many complications such as problems in urination, bleeding and infections (Elduma, 2018).

Female genital mutilation or female circumcision or cutting is common practice in Africa, Middle East and Egyptians were found practicing male and female circumcision around the middle of the fifth century B.C. But Infibulations or Pharaonic circumcision, is the most prevalent type in Africa and some studies indicated that; the Infibulation or Pharaonic circumcision title was applied by Sudanese when this practice spread from Upper Egypt to the North Sudan where was called as Sudanese circumcision (Elduma, 2018).

2.2.1. Types of FGM

There are five types of female genital mutilation in Africa namely; Mild Sunna which include the pricking of the prepuce of the clitoris with a sharp instrument. The second is called Modified Sunna in which partial or total removal of the clitoris is applied. Furthermore, the third type is called clitoridectomy excision in which the removal of all or part of the clitoris is performed plus a partial or all removal of the Labia minor. Moreover, the Infibulations or Pharaonic circumcision includes clitoridectomy, excision of Labia minor and the inner wall of the Labia major. The fifth type is introcision where enlargement of the vaginal orifice with a sharp instrument is practiced (Elduma, 2018).

2.2.2. Urological complications related to FGM

Clinicians should be aware of the short and long-term complications of FGM, in which has short-term complications are: hemorrhage, urinary retention and genital swelling, while long terms are: genital scarring, urinary tract complications, psychological squeal and genital infection and pelvic inflammatory disease. Furthermore, lower urinary tract

symptoms are more common in women with FGM, particularly those with type two or type three where there is poor urinary flow beneath the infibulation scar may result in symptoms of urinary obstruction, and stasis of urine may lead to recurrent urinary tract infection (Royal College of Obstetricians and Gynecologists, 2015).

The overall prevalence of urological complications in genital mutilated women ranges from 10 to 30% and type three circumcisions is most suffering from this complication. The most prevalent complications reported are; recurrent urinary tract infections, lower urinary tract symptoms, urinary retention, urogenital fistula, meatus stenosis, urethral stone and megaurethra (Zambon *et al.*, 2018).

2.2.2.1. Recurrent urinary tract infections

The prevalence of recurrent UTIs in women underwent type one genital mutilation was 10%, whereas in type three is up to 30%. Patients underwent type three FGM or type four FGM was more common to develop recurrent UTIs. In these cases the etiology is bladder outlet obstruction and incomplete bladder emptying (Zambon *et al.*, 2018).

2.2.2.2. Lower urinary tract symptoms and urinary retention

Lower urinary tract symptoms are more common in women with circumcision, particularly those with type two or type three circumcisions. Poor urinary flow beneath the infibulation scar may result in symptoms of urinary obstruction, and stasis of urine may lead to recurrent urinary tract infection (Royal College of Obstetricians and Gynecologists, 2015).

2.2.2.3. Urogenital fistula

The risk factors for post-procedure urogenital fistula formation are many for example lack of anatomical knowledge by local practitioners, use of non-sterilized crude instruments and urogenital infection. Women underwent infibulations are at the highest risk of post-procedure fistula formation because labia major and/or minor closure predispose these women to hematocolpos. Thus, chronically it increases the risk of infection, drainage and urogenital fistula. The most common clinical complaints in these cases are urinary incontinence, dyspareunia, chronic pelvic pain, and recurrent UTIs (Zambon *et al.*, 2018).

2.2.2.4. Meatus stenosis

There is one study in the literature describing a retraction technique for self-catheterization in women with infibulation who developed vaginal and urethral meatus stenosis. Toubia (1994) studied 162 mutilated women from 6 African countries: Chad, Egypt, Eritrea,

Ethiopia, Somalia, and Sudan. All of them had vaginal and urethral meatusstenosis and emptied their bladder doing self-catheterization. Also he found that; the most severe cases required a retraction technique to visualize and catheterize the retracted urethral meatus (Toubia, 1994).

2.2.2.5. Urethral stone

There is one case report of a 32years old Somali woman who had been infibulated earlier in her life and presented with dyspareunia, dysmenorrhea and chronic pelvic pain. A urethral stone measuring0.8 cm was accidentally found during de fibulation and labia major are construction (Ampofo *et al*., 1990).

2.2.2.6. Megaurethra

There is one a case report of a 21years old woman with type three genital mutilation who developed megaurethra due to repetitive urethra lcoitus (Mabeya, 2004).

2.3. Previous studies

In review published in Nigeria 2004, it was estimated that the prevalence of acute urinary retention accounts for 12% on all types of FGM. Agugua and Egwuatu, analyzing the consultations in a gynecological hospital in Nigeria, demonstrated that 28.8% of the patients suffered from urological problems. Straining and retention of urine associated with metal obstruction and urethral stricture occurred to the patients. Also patients of an antenatal outpatient clinic in Melbourne were found out of 51 women (27.5%) reported "urinary tract infection," but just one woman (1.9%) reported "recurrent UTIs (Vella *et al*., 2015).

Other review encompasses articles published between 1980 and November 2016 in Somalia by Joao, in which the prevalence of recurrent UTIs in women underwent type one genital mutilation was 10%, whereas in type three was up to 30%. Type three and four were more prone to develop recurrent UTIs. In these cases the etiology is bladder outlet obstruction and incomplete bladder emptying. Intermittent urinary stream and incomplete bladder emptying were reported by 20% of mutilated women, Terminal dribbling, urinary straining, and weak stream were reported by 19, 13, and 10% of women respectively (Zambon *et al*., 2018).

Seventy-three pediatric and adult female patients presented with post-circumcision complications at the University of Nigeria Teaching Hospital, Enugu, within the 9-year period 1973 to 1981. Seventy-two patients were circumcised within 21 days of birth, and one patient in the seventh month of the first pregnancy. Analysis of the complications reveals that lesions associated with urinary problems were the commonest (28.8%). Of the urinary complications, complete labial fusion contributed to 57.2%, while 23.8% of patients had meatal obstruction. Urinary infection and urethral stricture each accounted for 9.5% of patients (Agugua and Egwuatu, 1982).

A study was conducted from January, 2009 through January, 2012 by majdy at The Urogyencology clinic of Sohag University Hospital, Egypt. Released that nocturia was the most prevalent LUTIs (38.6%), intermittency (23.5%) was the most prevalent voiding LUTs, and incomplete voiding (22.7%) was the most common post-micturition LUTIs. All three type of LUTIs were reported by (11.6%) from circumcised women. However women with FGM are significantly more likely to have LUTIs (Amin *et al*., 2013).

While in Sudan, Gezira village, a study performed to adverse health effects on the child. The form of FGM was under-reported in an anatomical sense, 39% of forms being reported as "Sunna", extending to the labia major. Girls under the age of 7 there was a significant association between FGM and suspected UTIs. Symptoms from the urogenital tract in girls were heavily under-reported (Almroth *et al*., 2005).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

3. MATERIALS AND METHODS

3.1. Study design

This is cross sectional, case control, hospital based study.

3.2. Study area

This study was conducted in Dan Medical Complex and Nobatia Medical Clinic in Khartoum locality

3.3. Study duration

The study was carried out between July 2019 to March 2021.

3.4. Study population

The study was done on circumcised and non-circumcised women.

3.4.1. Inclusion criteria

Sudanese women with genital mutilation (case group) and non-genital mutilation women (serve as control group) were included, both with UTIs symptoms and with different age.

3.4.2. Exclusion criteria

The criteria of exclusion based on any women with kidney disease, birth defect on urinary system, catheter used.

3.5. Ethical considerations

Ethical approval to conduct this study was obtained from the Scientific Research Committee, Collage of Medical Laboratory Science, Sudan University of Science Technology, Dan Medical Complex and Nobatia Medical Clinic in Khartoum Locality. Verbal informed consent was obtained from participants before collection of the urine samples.

3.6. Sample size

The calculation of sample size was done according to below formula,

$N=t^2*p\ (1-p)/M^2$

N=sample seize

T=confidence level (95%)

P=prevalence of the problem (50%)

M=margin of error at (5%)

N=384

But Eighty (n=80) women (40 with FGM and 40 without FMG) were enrolled in this study due to financial cost.

3.7. Sampling technique

Non- probability, using convenience sampling technique.

3.8. Data collection

Data were collected using direct interview the patients, which provided information conceding each case examined.

3.9. Laboratory processing

3.9.1. Specimen collection and processing

Mid-stream urine specimens were collected by participants after informed with the methods of collection of specimen as possible aseptically in a sterile wide-mouth container and all specimens were processed within 2 hours of collection, or kept refrigerated at 4ºC until transported to the laboratory, and subsequently processed no more than 18 hours after initial collection.

3.9.2. Isolation

Under aseptic condition; a loop full of well mixed urine was cultured on blood and CLED agar plates and incubated aerobically at 37°C overnight.

3.9.3. Identification

3.9.3.1. Colonial morphology

The characteristics features of the colonies include shape (circular, regular), size (small, medium, large), color (yellow, green, etc.), elevation (elevated, convex, etc.), surface (smooth, rough, etc.), structure (opaque, translucent or transparent) and degree of growth (scanty, moderate, etc.) were observed.

3.9.3.2. Gram's stain

On clean, dry, labeled slide thin smear was made and allowed to air dry near the flame, the smear was fixed through passing three times over the flame. The fixed smear was covered with crystal violet stain for 1minute, washed off with clean water. Water was wiped off and applied the mordant Lugol's iodine for1 minute, then washed out and discoloration was done by using acetone alcohol mixture for a few (5-15) seconds, then washed out.

Then counter stain sufranin was added for 2 minutes, washed, the back of slide was wiped and allowed to air dry. Smear was examined microscopically with oil immersion objective to detect the bacterial Gram reaction, morphology and arrangement. Gram positive bacteria give violet color while Gram negative bacteria appear red in color (Goldman and Green, 2009).

3.9.3.3. Biochemical reactions of Grams negative rods

3.9.3.3.1. Kligler Iron Agar (KIA)

KIA can be used to determine whether an organism is capable of fermenting lactose. This media contains glucose and lactose in a peptone and casein base with a phenol red PH indicator. An agar slant is inoculated by first stabbing through the center of the medium and then streaked the surface of the slant, used an inoculum of 18- to 24-hr growth. If the organism was able to fermented glucose, acid would be produced in the agar, lowered the pH and changed the color of the medium from red to yellow. If gas were produced during fermentation, bubbles would be formed along the stab line, fractured or displaced the medium (Goldman and Green, 2009).

3.9.3.3.2. Indole test

Liquid medium with tryptone water content is used for a tube test, the medium was inoculated and incubated for 24 to 48 hours. A few drops of Kovacs reagent were added, if the organism produced tryptophanase, a pink color would be developed at the surface other with resulted as negative (Goldman and Green, 2009).

3.9.3.3.3. Citrate utilization test

Solid Simmons' citrate agar was used. Contains bromothymol blue as an indicator, original color of the medium was green. A part of colony was picked up with a straight wire loop and inoculated into media. Incubated at 37°C for 96 hours. Positive result indicated by blue color and streaked grew, negative result: original green color and no grown (Kumar, 2016).

3. 9.3.3.4.Oxidase test

A strip of filter paper was removed after soaked in oxidase reagent. Tested the colony by picked up with wooden loop and smeared over the moisturized area. Positive reaction was indicated by an intense deep-purple color, appeared within5–10 seconds. Negative reaction indicated by absented of coloration or by colorated later than 60 seconds (Kumar, 2016).

3.9.3.3.5. Motility test

Motility is demonstrated macroscopically, it was done by made a straight stab of growth into semi-solid medium, incubated for 24 to 48 hours at 35°C to 37°C, and then observed whether the organism migrated out from the stab line, caused visible turbidity in the surrounding medium (Goldman and Green, 2009).

3.9.3.3.6. Urease test

Urease test was done by inoculated heavily the tested organism in a bottle containing 3 ml sterile Christensen' modified urea broth. Incubated at 37°C overnight, looked for a pink color in the medium which indicated positive urease test and no pink color indicated negative urease test (Cheesbrough, 2006).

3.9.3.4. Biochemical reactions of Gram positive cocci

3.9.3.4.1. Litmus milk decolorization test

A 0.5 ml of sterile litmus milk media were inoculated with the tested organisms, incubated at35°C to 37°Cfor up to 4 hours(not more than 4 hours) and were examined at half hour intervals for a reduction reactions showed by a changed in color from mauve to white or pale yellow. White or pale yellow-pink color suggested of *Enterococcus*, no change or a pink color probably not *Enterococcus* (Cheesbrough, 2006).

3.9.3.4.2. Catalase test

A small amount of the culture to be tested was picked from a nutrient agar slope with a clean wooden loop and put it in a drop of 10% hydrogen peroxide on a clean glass slide. Positive test: immediate bubbled, easily observed (O_2 formed). Negative test: no bubbled (no O_2 formed) (Kumar, 2016).

3.9.3.4.3. Coagulase test (detected bound coagulase) slide method

A drop of distilled water was placed on each end of a slide or on two separate slides. A colony of the tested organism was emulsify in each of the drops to made two thick suspensions. A loop full (not more) of plasma was added to one of the suspensions, and was mixed gently. Looked for clumping of the organisms within 10 seconds. Clumping within 10 seconds is positive result while no clumping within 10 seconds (Cheesbrough, 2006).

3.9.3.4.4. DNA-ase Test

A DNA-ase plate was divided into the required number of strips by marking the underside of the plate and the tested bacteria were inoculated by a sterile loop in spots form. The plate

was incubated at 37°C overnight. The surface of the plate was covered with 1 mol/l hydrochloric acid solution. The excess acid was tipped off. Looked for clear zone around the colonies within 5 minutes of adding the acid as positive result and absent of clear zone indicated negative result (Cheesbrough, 2006).

3.9.3.4.5. Mannitol salt agar

A useful selective medium for *S.aureus* which fermented mannitol and is able to grow on agar containing 70–100g/l sodium chloride. Mannitol salt agar containing 75 g/l sodium chloride (Cheesbrough, 2006).

3.9.4. Susceptibility Testing

3.9.4. 1. Modified Kirby-Bauer susceptibility testing technique

By using a sterile wire loop, 3–5 of well-isolated colonies of fresh purified of the tested organism were touched and emulsified in 3–4 ml of sterile physiological saline. The turbidity of the suspension was matched to the standard turbidity (0.5%McFarland standard) on good light. Sterile swab was used to inoculate a plate of Mueller Hinton agar. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. Streaked the swab evenly over the surface of the medium in three directions, rotated the plate approximately 60̊ to ensure even distribution. With the petri dish lid in place, allowed about 3–5minutes (no longer than 15 minutes) for the surface of the agar to dry. By used a sterile forceps, placed the appropriate antimicrobial discs, evenly distributed on the inoculated plate (Cheesbrough, 2006). After overnight incubation, examined the plates ensure the growth is confluent, by a ruler measured the diameter of each zone of inhibition in mm. Interpreted zone sizes of each antimicrobial don by interpretative chart, according to CLSI guidelines (Cheesbrough, 2006).

3.10. Statistical analysis

Data obtained from this study was analyzed using statistical package of the social science (SPSS) version 20.0. Frequencies were expressed in form of table and Chi-Square test used to determine the significant differences at *p-value* ≤ 0.05.

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

A total of 80 urine specimens had been collected from Dan Medical Complex and Nobatia Medical Clinic in Khartoum locality. In which 40 were circumcised women and other 40 were not, and their ages ranged from 7 to 70 years old with a mean age of 28.8± 13.8 S.D. About 16/40 (40 %) of circumcised women were married and 23/40 (57%) were single, while among non-circumcised group there were 7/40 (17%) married and 33/40 (82%) single as in table (4.1).

The growth rate was 34/40 (85%) among circumcised patients and 6/40 (15%) among non-circumcised participants and there were significant association (*P-value*= 0.000) between circumcision and UTIs. Based on the odd ratio (O.R) the risk factor of UTIs was 32 times greater among circumcised females than non-circumcised one as showed in table (4.2).

The isolated bacteria were 24/40 (60%) Gram negative rods and 10/40 (15%) were Gram positive cocci among circumcised women while there were only 6/40 (15%) Gram negative rods and no isolated Gram positive among non-circumcised one. There were meaningless association between circumcision and bacteria according to Gram's stain (*P-value*= 0.125) as displayed in table (4.3).

Among the isolated bacteria; *Escherichia coli* was the most predominant isolate among circumcised and non-circumcised women (15(37.5%)).

Among the isolated bacteria; *Escherichia coli* was the most predominant isolate among circumcised and non-circumcised women (12 (35.2%) and 3 (50.0%) respectively) as illustrated in table (4.4).

Concerning circumcised women; the isolated bacteria were moderately sensitive to Augmentin (22/34 (67.7%)) and Gentamycin (20/34 (58.8%)) compared to other antimicrobial agents (Ciprofloxacin 16/34 (47.1%), Cefuroxime (12/34 (35.3) and Amoxycillin (10/34 (29.4%)) and all isolated Gram negative rods bacteria were highly resistant to nalidixic acid (100.0%) as shown in table (4.5).

In contrast to non-circumcised women; all the isolated bacteria (G-ve rods) were highly sensitive to Gentamicin (6/6 (100.0%) and cefuroxime (5/6 (83.3%), and moderately sensitive to Augmentin (4/6 (66.7%) and Ciprofloxacin (4/6(66.7%). Also all isolated

bacteria were highly resistant to Nalidixic acid and Amoxycillin except *K.pneumoniae* were highly sensitive (100.0%) for both antimicrobial agents (table (4.6)).

Table 4.1: Distribution of circumcised and non-circumcised women according marital status

Marital status	**Study groups**	
	Circumcised women	Non-circumcised women
Single	23 (57.5%)	33 (82.5%)
Married	16 (40%)	7 (17.5%)
Total	40 (100%)	40 (100%)

Table 4.2: Association between circumcision and UTIs

Growth rate	**Study groups**		***P-value***	**OR**
	Circumcised women	Non-circumcised women		
Growth	34(85%)	6(15%)	0.000	32.11
No growth	6 (15%)	34 (85%)		
Total	40 (100%)	40(100%)		

Table 4.3: Association between isolates according to Gram's reaction and the study groups

Isolates	**Study groups**		***P-value***
	Circumcised women	Non-circumcised women	
G-ve rods	24 (70.6%)	6 (100%)	2.35
G+ve cocci	10 (29.4%)	0 (0%)	
Total	34 (100%)	6 (100%)	

Table 4.4: Frequency of isolated bacteria among circumcised and non- circumcised women

Isolated bacteria	Study groups	
	Circumcised women	**Non- circumcised women**
E.coli	12 (35.3%)	3 (50.0%)
K.pneumoniae	7 (20.6%)	1 (16.7%)
Pr.mirabilis	2 (5.9%)	1 (16.7%)
Ps.aeruginosa	3 (8.9%)	1 (16.6%)
S.aureus	1 (2.9%)	0 (0.0%)
S.saprophyticus	1 (2.9%)	0 (0.0%)
E.faecalis	8 (23.5%)	0 (0.0%)
Total	34 (100%)	6 (100%)

Table 4.5: Antimicrobial susceptibility of isolated bacteria among circumcised women

Isolates No. (%) of S+R	**Antimicrobial agents**					
	Amp	Cip	Gen	Nal	Aug	Cef
E.coli No. (%) of S	5/12 (41.7%)	4/12 (33.3%)	7/12 (58.3%)	0/12 (0.0%)	6/12 (50.0%)	3/12 (25.0%)
E.coli No. (%) of *R*	7/12 (58.3%)	8/12 (66.7%)	5/12 (41.7%)	12/12 (100.0%)	6/12 (50.0%)	9/12 (75.0%)
K.pneumoniae No. (%) of S	2/7 (28.6%)	4/7 (57.1)	3/7 (42.9%)	0/7 (0.0%)	6/7 (85.7%)	3/7 (42.9%)
K.pneumoniae No. (%) of *R*	5/7 (71.4%)	3/7 (42.9%)	4/7 (57.1%)	7/7 (100.0%)	1/7 (14.2%)	4/7 (57.1%)
Pr.mirabilis No. (%) of S	0/2 (0.0%)	1/2 (50.0%)	2/2 (100.0%)	0/2 (0.0%)	2/2 (100.0%)	1/2 (50.0%)
Pr.mirabilis No. (%) of R	2/2 (100.0%)	1/2 (50.0%)	0/2 (0.0%)	2/2 (100.0%)	0/2 (0.0%)	1/2 (50.0%)
Ps.aeruginosa No. (%) of S	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	0/3 (0.0%)	1/3 (33.3%)	0/3 (0.0%)
Ps.aeruginosa No. (%) of R	3/3 (100.0%)	1/3 (33.3%)	2/3 (66.7%)	3/3 (100.0%)	2/3 (66.7%)	3/3 (100.0%)
E. faecalis No. (%) of S	2/8 (25.0%)	3/8 (37.5%)	5/8 (62.5%)	-	5/8 (62.5%)	3/8 (37.5%)
E. faecalis No. (%) of R	6/8 (75.0%)	5/8 (62.5%)	3/8 (37.5%)	-	3/8 (37.5%)	5/8 (62.5%)
S. aureus No. (%) of S	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	-	1/1 (100.0%)	1/1 (100.0%)
S. aureus No. (%) of R	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	-	0/1 (0.0%)	0/1 (0.0%)
S.saprophyticus No. (%) of S	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)	-	1/1 (100.0%)	1/1 (100.0%)
S.saprophyticus No. (%) of R	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	-	0/1 (0.0%)	0/1 (0.0%)
Total No. (%) of S	10/34 (29.4%)	16/34 (47.1%)	20/34 (58.8%)	0/24 (0.0%)	22/34 (64.7%)	12/34 (35.3%)
Total No. (%) of R	24/34 (70.6%)	18/34 (52.9%)	14/34 (41.2%)	24/24 (100.0%)	12/34 (35.3%)	22/34 (64.7%)

Key words:

Amp=Amoxycillin Cip= Ciprofloaxacin Gen= Gentamicin

Nal= Nalidixic acid Aug=Augmentin Cef= Cefuroxime

S= Sensitive R= Resistant

Table 4.6: Antimicrobial susceptibility of isolated bacteria among non-circumcised women

Isolates No. (%) of S+R	**Antimicrobial agents**					
	Amp	Cip	Gen	Nal	Aug	Cef
E.coli No. (%) of S	0 /3 (0.0%)	1/3 (33.3%)	3/3 (100%)	0 /3 (0.0%)	3/3 (100.0%)	2/3 (66.7%)
E.coli No. (%) of *R*	3/3 (100.0%)	2/33 (66.7%)	0 /3 (0.0%)	3/3 (100.0%)	0 /3 (0.0%)	1 /3 (33.3%)
K.pneumoniae No. (%) of S	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)
K.pneumoniae No. (%) of *R*	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
Pr.mirabilis No. (%) of S	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
Pr.mirabilis No. (%) of R	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	1/1 (100.0%)
Ps.aeruginosa No. (%) of S	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (0.0%)
Ps.aeruginosa No. (%) of R	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	0/1 (0.0%)
Total No. (%) of S	1/6 (16.7%)	4/6 (66.7%)	6/6 (100.0%)	1/6 (16.7%)	4/6 (66.7%)	5/6 (83.3%)
Total No. (%) of R	5/6 (83.3%)	2/6 (33.3%)	0/6 (0.0%)	5/6 (83.3%)	2/6 (33.3%)	1/6 (16.7%)

Key words:

Amp=Amoxycillin

Cip= Ciprofloaxacin

Gen= Gentamicin

Nal= Nalidixic acid

Aug=Augmentin

Cef= Cefuroxime

S= Sensitive

R= Resistant

CHAPTER V

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

CHAPTER V

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

In this study; the growth rate was 34 (85%) among circumcised women compared to non-circumcised (6 (15%)) that means circumcised women are more likely to be infected and with UTIs more than non-circumcised group. This finding was similar to a study done in Sudan Elduma (2018) and Amin *et al*. (2013) in Egypt which reported highly significant different type of UTIs by 86.6% among 251 the circumcised women participate in the study).

This study reported that; the growth of Gram negative rods were 24/34 (70.6%) and G+ve cocci were 10/34 (29.4%) from circumcised women when compared to non-circumcised women, the G-ve rods were the predominant growth by 6/40 (15%) and no isolated G+ve cocci. This was harmonized to that reported in Sudan by Saeed *et al*. (2017) who found Gram negative bacteria were 45(65%) more than Gram positive bacteria (24(35%)) from 69 of significant growth urine samples.

The present study found the commonest isolate was *E. coli* 12(35.3%), followed by *E. faecalis* 8(23.5%) and *K.pneumoniae* 7(20.6%) among circumcised females. It was matched to a study carried out by Theodore (2006) in Nigeria who found 141 out of 181 growth and *E.coli* was 47 (33.3%) followed by *Klebsiella species* 28 (19.9%) and 5(3.5%) *E. faecalis*. Also among non-circumcised females *E.coli* was most microorganism (Theodore, 2006),

In the present study it was found that; the isolated bacteria from circumcised women were highly resistant to a number of the antimicrobial agents used, including: Nalidixic acid (100%), Amoxycillin (70.6%) and cefuroxime (64.7%) that was in agreement with a study performed by Ahmed and his colleagues (2000) in Sudan in which *E.coli* showed high resistant when to amoxicillin, but in comparison, low resistant rates were found against nalidixic acid. Considering non-circumcised women, 83% were resistant to nalidixic acid and Amoxycillin and 16.7% resistant to cefuroxime.

In general, circumcised women were more resistant to antimicrobial agents than non-circumcised women (Ahmed *et al* 2000).

5.2. Conclusion

The frequency of UTIs and antimicrobial resistance were higher among circumcised women than non-circumcised one.

5.3. Recommendations

Increase awareness of population about the risk of FMG which is a critical health problem.

Interesting studies in wider field including different areas in Sudan and using more advance methods such as Polymerase chain reaction (PCR) to confirm our findings.

Prevention or decrease FGM which is a traditional habit in Sudan.

REFERENCES

REFERENCES

Agugua, N.E. and Egwuatu, V.E. (1982). Female circumcision: management of urinary complication. *Journal of tropical pediatrics*, **28**:248-252.

Ahmed, A.A., Osman, H., Mansour, A.M., Musa, H.A., Ahmed, A.B., Karrar, Z. (2000). Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infections in the Dudan. *The American journal of tropical medicine and hygiene*, **5**:259–63.

Akoachere, K., Yvonne, S., Akum, N.H. and Seraphina E.N. (2012). Etiologic profile and antimicrobial susceptibility of community acquired urinary tract infection in two Cameroonian towns. *Biomedical Central*, **5**:219.

Almroth, L., Bedri, H., Musharaf, S., Satti, A., Idris, T., Hashim, M., Suliman, G and Bergstrom, L. (2005). Urogenital complications among girls with genital mutilation: a hospital-based study in Khartoum. *African Journal of Reproductive Health*, **9** (2): 118-124.

Amin, M.M., Rashid, S. and Saliem, E. (2013).Lower urinary tract symptom following female genital mutilation. *International Journal of Gynecology and Obstetrics,* **123:**21-23.

Ampofo, K., Out, T and Uchebo, G. (1990). Epidemiology of vesico-vaginal fistulae vaginal fistulae in northern Nigeria. *West African journal of medicine*, **9**: 98-102.

Barry, M.J., Fowler, F.J., O'Leary, M.P., Bruskewitz, R., Holtgrewe, H., and Mebust, W. (1992). The American Urological Association symptom index for benign prostatic hyperplasia. *The Measurement Committee of the American Urological Association*, (5):1549–64.

Beyene, G and Tsegaye, W. (2011). Bacterial uro pathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University specialized hospital, south west Ethiopia. *Ethiopian Journal of Health Science*, **21**:141−6.

Cheesbrough, M. (2006). District laboratory practices in tropical countries. 2nd edition. Part 2. United States of America: Cambridge University Press, New York: 35-70.

Elduma, A.H. (2018). Female genital mutilation in Sudan .*Open Access Macedonian Journal of medical Sciences.* **1**: 099. https://www.idpress.eu/mjms/article/view/oamjms. (Accessed on 3/4/2019, 11:45 p.m).

Finer, G and landau, D. (2004). Pathogenesis of urinary tract infections with normal female anatomy. *The Lancet infectious disease*, **4** (10):631-635.

Goering, V.R., Dockrell, H.M., Roitt, I.M and Chiodini, P.L. (2012). Mimss Medical Microbiology. 5th edition. Berlin, Elsevier, p.p. 237-241

Goldman, E and Green, L.H. (2009). Practical Handbook of Microbiology. 2nd edition. United States of America, Taylor & Francis Group, p.p.38-39.

Gupta, K. (2003). Emerging antibiotic resistance in urinary tract pathogens. *Infected Diseases Clinic of North American*, **17**: 3–321.

Heinzelmann, M., Mercer-Jones, M. and Passmore, J.C. (1999). Neutrophils and renal failure. *American journal of kidney diseases*, **34**(2):384-399.

Hertting, O. (2011). Urinary Tract Infection Pathogenesis and Complications. Sweden, Shannon Wright, p.p.1-16.

Jahnukainen, T., Chen, M. and Glesi, S. (2005). Mechanisms of renal damage owing to infection. *Pediatric nephrology*, **20**(8):1043-1053.

Kahan, N.R., Chinitz, D.P., Waitman, D., Dushnitzky, D., Kahan, E and Shapiro, M. (2006). Empiric treatment of uncomplicated urinary tract infection with fluoroquinolones in older women in Israel another lost treatment option. *Journal of clinical pharmacy and* therapeutics, **12**:7-223.

Kon, K and Rai, M. (2016). Antibiotic Resistance. London, Sara Tenney, p.p.19-113.

Kumar, M.S., Lakshmi, V. and Rajagopalan, R. (2006). Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp, isolated at a tertiary care institute. *Indian Journal of Medical Microbiology*, **3**:208–11.

Kumar, S. (2016). Essentials of Microbiology. New Delhi: Jaypee Brothers Medical, p.p. 48-55.

Levinson, W. (2014). Medical microbiology and immunology. 5th edition. New York: McGraw-Hill Education, p.p. 649-652.

Mabeya, H.M. (2004). Characteristics of women admitted with obstetric fistula in rural hospitals. The Geneva Foundation for Medical Education and Research 2004. February 23 –April 2; Geneva, Switzerland, Geneva University.

Mahon, C.R., Lehman,D.C. and Manuselis, G. (2015). Diagnostic Microbiology. 5th edition. Maryland: Saunders, p.p. 884-900.

Manges, A.R., Natarajan, P., Solberg, O.D., Dietrich, P.S. and Riley, L.W. (2006). The changing prevalence of drug-resistant *Escherichia coli* clonal groups in a community.

Evidence for community outbreaks of urinary tract infections. *Emerging infectious diseases*, **2**:425–31

MAYO CLINIC (2019). Urinary tract infection. Available at: https://www.mayoclinic.org/diseases-conditions/urinary-tract-infection/symptoms-causes/syc-20353447. (Accessed on 14/ 09/2019, 9.00 p.m.).

Nester, E.W., Anderson, D.G., Rober, C.E., and Nester, M.T. (2009). Microbiology A Human Perspective. 6th edition. New York: McGraw-Hill Education, p.p. 621-622.

Richardson, M and Lakhanpaul, M. (2007). Assessment and initial management of feverish illness in children younger than 5 years summary of NICE guidance. *British medical journal*, **334** (7604): 1163-1164.

Royal College of Obstetricians and Gynecologists (2015). Female Genital Mutation and Management. 2th edition. London: Nice, p.p.1-26.

Russo, T.A., Stapleton, A., Wenderoth, S., Hooton, T.M., Stamm, W.E. (1995). Chromosomal restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women, *the journal of infectious disease*, **172**(2): 440-445**.**

Saeed, A., Shadia, A, Bayoumi M., Shanan, S., Alouffi, S., Samir, A., Fawaz, D., and Alshammari, H. (2017). Elevated Antibiotic Resistance of Sudanese Urinary Tract Infection Bacteria. *Experimental and clinical sciences*, **3**:1073-1074.

Tenke, P. (2011). Urinary Tract Infection. Croatia: In Tech, p.p. 3-35.

Paticia, M. (2017). Bailey and Scott's diagnostic microbiology. 14th edition, Saint Louis. Elsevier, p.p. 987-992.

Paticia, M. (2014). Bailey and Scott's diagnostic microbiology. 13th edition, Saint Louis. Elsevier, p.p. 919-925.

Tonolini, M. (2018)**.** Imaging and Intervention in Urinary Tract Infection and Urosepsis. Milan, Springer Nature, p.p. 3-17.

Toubia, N. (1994). Female circumcision as public health issue. *The New England journal of medicine*, **331:** 712-716.

Theodore, M. (2006). Prevalence and antibiogram of urinary tract infections among prison inmates in Nigeria. *The Internet Journal of Microbiology*, **2**:1–5.

Vella, M., Argo, A., Pavone, C., Tarantino, L., Milone, L., Costanzo, A. (2015). Female genital mutilation: genito urinary complications and ethical legal aspects. *Urologia*, **82** (3):151-159. Available at: https://en.wikipedia.org/wiki/Female_genital_mutilation (accessed on 18/9/2019, 8.25 p.m).

Zambon, J.P., Mihai, B., Ivan, C.A., Magalhaes, R.S., Karakeci, A. and Zhao, S. (2018). Urological Complications in Women With Genital Mutilation. *Clinical and Medical Report*, **2**(1):2-4

World Health Organization (WHO) (2017). Female Genital mutilation. Available at: http://www.who.int/mediacentre/factsheets/fs241/en/. (Accessed on 25/3/2020, 9:40 a.m.).

Appendix

Appendix -I

Culture Media and Reagents

(1): Peptone water (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Peptic digest of animal tissue ……..10.0g

Sodium chloride ……..……..5.0g

Final PH (at 25° C) 7.4 + 0.2.

Preparation

Suspend 15.0 grams in 100 ml D.W. Mix well and dispense into tubes and sterilize by autoclaving at 15Ibs pressure (121°C) for 15 minutes.

(2): Mannitol salt agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Proteose peptone ……10.00g

Beef extract …….1.00g

Sodium chloride……. 75.00g

D-Mannitol……..… 10.00g

Phenol red……..……. 0.025g

Agar…….…….……... 15.00g

Final PH (at 25°C) 7.4 ±0.2

Preparation

Suspend 111.02 grams in 1000 ml D.W .heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121°C) for 15 minutes. Mix well.

(3): Hydrogen peroxide 3%

Concentrated hydrogen peroxide …….. 3ml

Distilled water …… 100ml

(4): DNase agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Casein enzymic hydrolysate…….15.0g

Soya peptone……..…… 5.00g

Deoxyribonucleic acid (DNA) ……....2.00g

Sodium chloride ……..5.00g

Agar……. 15.00g

Final PH (at 25°C) 7.3 ± 0.2.

Preparation

Suspend 42 g in 1000ml D.W. heat with agitation to dissolve the medium completely. Sterilize by autoclaving at Δ118°-121°C for 15 min. cool to 45-50°C and into sterile Petri plates.

(5): Kligler Iron Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Peptic digest of animal tissue ……..15.00g

Beef extract ……..... 3.00g

Yeast extract ……... 3.00g

Protease peptone ……. 5.00g

Lactose……10.00g

Dextrose …….. 1.00g

Ferrous sulphate…….. 0.20g

Sodium chloride……5.00g

Sodium trisulphate……...0.30g

Phenol red ……..0.024g

Agar ……...15.00g

Final PH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 57.52 grams in 1000 ml D.W. Heat until boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15Ibs pressure (121°C) for 15 minutes.

(6): Kovac's Reagent (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

P-dimethyl aminobenzaldehyde…… 10 g

Isoamyl alcohol ……...150 ml

Concentrated hydrochloric acid...…….. 50 ml

Preparation

Kovac's reagent is prepared by dissolving 10 gm of p-dimethyl aminobenzaldehyde in 150 ml of isoamyl alcohol and then slowly adding 50 ml of concentrated hydrochloric acid.

(7): Simmons Citrate Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Magnesium sulphate……. 0.20g

Ammonium dihydrogen phosphate ……...1.00g

Dipotassiumphpsphate……..1.00g

Sodium citrate …….. 2.00g

Sodium chloride ……..5.00g

Bromothymol blue ...……...0.08g

Agar …… 15.00g

Final PH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 24.28 grams in 1000 ml D.W. Heat until boiling to dissolve the medium completely. Dispense as desired in tubes or flasks. Sterilize by autoclaving at 15Ibs pressure (121°C) for 15 minutes.

(8): Urea Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Peptic digest of animal tissue……..1.00g

Dextrose …….....1.00g

Sodium chloride…….. 5.00g

Dipotassiumphpsphate....…….. 1.20g

Monopotasiumphosphate……0.80g

Phenol red……..0.012g

Agar ……. 15.00g

Final PH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 24 grams in 950 ml D.W. Heat until boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚C) for 15 minutes. Cool to 50˚C and aseptically add 50 ml of sterile 40% urea solution and mix well. Dispense into sterile tubes and allow setting on slanting position. Don't over heat or reheat the medium as urea decomposes very easily.

(9): Semisolid medium (motility test medium) (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Treptose……10g

Sodium chloride…… 5g

Agar……5g

Final PH (at 25°C) 7.2±0.2

Preparation

Suspend 20.0 grams in 1000 ml distilled water. Heat until boiling to dissolve the medium completely. Dispense as desired in tubes and sterilize by autoclaving at 15Ibs pressure (121°C) for 15 minutes. Allow the tubed medium to cool in an upright position.

(10): Mueller Hinton Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients g/L

Casein acid hydrolysate…… 17g

Meat infusion solids……..…2g

Starch, soluble ……..1.5g

Agar …….. 17g

Final PH (at 25°C) 7.3±0.1

Preparation

Suspend 38.0 grams in 1000 ml D.W. Heat until boiling to dissolve the medium completely and sterilize by autoclaving at 15Ibs pressure (121°C) for 15 minutes. Cool to 45-50°C, mix well and pour into sterile Petri plates.

(11): 0.5% McFarland standard

Prepare a 1% v/v solution of sulphuric acid by adding1 ml of concentrated sulphuric acid to 99 ml of water. Mix well, prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of D.W. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula. When stored in a well-sealed container in the dark at room temperature (20–28°C), the standard can be kept for up to 6 months (Cheesbrough, 2006).

(12) Blood agar and chocolate agar (Cheesbrough, 2006)

BLOOD AGAR

To make about 35 blood agar plates:

Nutritious agar . . . . . 500 ml

Sterile defibrinated blood . . . . . 25 ml

Preparation

Prepare the agar medium as instructed by the manufacturer. Sterilize by autoclaving at 121 C for 15 minutes. When the agar has cooled to 50 C, add the sterile blood and mix gently but well. Avoid forming air bubbles. Dispense in sterile petri dishes.

(13) Cystine lactose electrolyte deficient

(CLED medium)

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media. *Contents:* Peptone, *Lab-Lemco* powder, tryptone, lactose, L-cystine, bromothymol blue, agar.

Preparation

Sterilize by autoclaving at 121°C for 15minutes.Mix well before pouring, Cool to 45-50°C, mix well and pour into sterile Petri plates.

(14) Litmus milk medium

Skimmed milk powder …….. 2 g

Distilled water …….. 20 ml

Preparation

Dissolve the milk in the water, and add the litmus. Sterilize by autoclaving at 110 °C for 10 minutes.

Dispense in 0.5 ml amounts in sterile 13 -100 mm tubes.