

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

**Isolation and Antimicrobial Susceptibility Patterns of
Escherichia coli in Patients with Urinary Tract Infections
in AL-Ribat University Hospital - Khartoum**

العزل وأنماط حساسية مضادات الجراثيم للإشريكية القولونية

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**A Dissertation Submitted in Partial Fulfillment of the
Requirement of M.Sc. in Medical Laboratory
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الآية

قال تعالى: {رَبَّنَا آمَنَّا بِمَا أَنْزَلْتَ وَاتَّبَعْنَا الرَّسُولَ فَاكْتُبْنَا مَعَ
الشَّاهِدِينَ}

صدق الله العظيم

سورة آل عمران: الآية (53)



Dedication

I dedicate this work to my parents for their kind auspice and to my Wife for her continues helping.

And also I dedicate this work to all my friends who offer me great help to accomplish this work.



Acknowledgement

First of all a great thanks to ALMIGHTY AllAH for helping me to finish this study.

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Abstract

Urinary tract infections are among the most common bacterial infections both in the community and hospital setting. In the majority of cases, antibiotics are given empirically before the final susceptibility testing results are available. Therefore, the studies that detect the bacteria species that cause UTIs and their antimicrobial susceptibility is mandatory for helping the selection of an effective treatment. This study aimed to isolate *E.coli* from patients with UTIs and to determine antimicrobials susceptibility patterns.

During the period from January to May 2016, 336 patients with urinary tract infection symptoms who attending Al-Ribat University Hospital - khartoum, were enrolled in this study.

Urine specimens were collected and investigated by standard conventional microbiological methods.

Kirby Bauer's method was used to determine the antimicrobials susceptibility patterns.

Most of study population were females (239 (71%)) and their age ranged between 1 – 92 years with mean 31 and 50% of them were belong to 21 – 40 age range.

The over all results showed that out of the total, uropathogens were detected significantly in 134 (39.8%) of specimens, of which 100 (74.6%) were *E. coli*.

This study revealed that the rate of resistance in uropathogenic *E. coli* is highest for Trimethoprim Sulfamethoxazole (54%), followed by Cephalexin (37%), and Ciprofloxacin (29%), and lowest for Gentamycin (12%) and Nitrofurantoin (9%).

This study concluded that *E. coli* was the major isolated pathogen, and the rate of resistance in uropathogenic *E. coli* was highest for trimethoprim sulfamethoxazole and lowest for gentamycin and nitrofurantoin.

Further studies recommended for different study area using additional culture media with CLED and incubate in different incubations to cover other fastidious pathogens.

الخلاصة

إلتهابات المسالك البولية من اهم عدوى البكتيريا في المجتمعات والمستشفيات، وفي معظم الحالات يتم العلاج مباشرة قبل توفر النتيجة النهائية لإختبارات الحساسية، وعليه الدراسات التي تحدد انواع البكتيريا التي تسبب إلتهابات المسالك البولية وإختبارات الحساسية لها مهمة لإختيار العلاج الفعال.

هذه الدراسة تهدف لعزل الإشريكية القولونية من مرضى عدوى إلتهابات المسالك البولية ولتحديد أنماط وإختبارات الحساسية لمضادات الجراثيم لها.

خلال الفترة من يناير حتى مايو 2016 ، 336 مريض يعانون من أعراض إلتهابات المسالك البولية من مستشفى الشرطة – الخرطوم .تم إحتوائهم في هذه الدراسة.

تم جمع عينات البول وفحصت بالطريقة القياسية وتم إستخدام طريقة كير باي باور لإختبارات الحساسية للمضادات الحيوية.

معظم مجموعة الدراسة كن نساء 239 (71%) وأعمارهن في المدى بين 1- 92 سنة مع وسط حسابي 31 سنة و 50% منهن في الفئة العمرية 21- 40 سنة.

من المعدل الكلي تم عزل 134 (39.8%) بكتيريا ممرضة في الجهاز البولي، منها 100 (74.6%) إشريكية قولونية.

هذه الدراسة لخصت ان معدل مقاومة الإشريكية القولونية الممرضة بالجهاز البولي اعلى في التراي ميثوبرايم سلفاميثازول (54%) ، سفالكسين (37%) والسبروفلوكساسين (29%) وأقل في الجنتاميسين (12%) والنايتروفيранتوين (9%).

هذه الدراسة لخصت أن معدل مقاومة الإشريكية القولونية المعزولة من عينات البول كان أعلي ضد التراي ميثوبرايم سلفاميثازول، وأقل ضد الجنتاميسينوالنايتروفيранتوين .

الدراسات القادمة ينصح أن تكون في عدد من المستشفيات وفي ولايات مختلفة وباستخدام أوساط زراعية إضافية وأن تحضن في بيئات مختلفة وذلك لعزل الميكروبات الحساسة الأخرى.

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List of Abbreviations

Abbreviation	Complete word
<i>E. coli</i>	<i>Escherichia coli</i>
UTIs	Urinary Tract Infections
UPEC	Uropathogenic <i>Escherichia coli</i>
ESBLs	Extended-spectrum β -lactamases
TMP-SMX	Trimethoprim sulfamethoxazole
PCR	Polymerase chain reaction

CHAPTER ONE

Introduction

1 Introduction

1.1 Introduction:

The term UTI encompasses a range of infections from simple cystitis involving the bladder to full-blown infection of the entire urinary tract, including the renal pelvis and kidney (pyelonephritis). The primary feature of cystitis is frequent urination, which has a painful burning quality. In pyelonephritis, symptoms include fever, general malaise, and flank pain in addition to the frequent urination. Cystitis is usually self-limiting, but infection of the upper urinary tract carries a risk of spread to the blood stream. It is the leading cause of Gram-negative sepsis and septic shock (Kenneth *et al.*, 2014).

Such an infection manifests either solely in the lower urinary tract (urethritis, cystitis, urethrocystitis) or affects the renalpelvis and kidneys (cystopyelitis, pyelonephritis). Urinary tract infections result from ascension of the pathogen from the ostium urethrae. Development of such an infection is also furthered by obstructive anomalies, a neurogenic bladder or a vesicoureteral reflux. Urinary tract infections that occur in the absence of any physical anomalies are often caused by the pathovar UPEC (uropathogenic *E. coli*). UPEC strains can attach specifically to receptors of the renal pelvis mucosa with pyelonephritis-associated pili or nonfimbrial adhesins. They produce the hemolysin HlyA (Fritz *et al.*, 2005).

Some patients have what are called “complicated urinary tract infections.” This includes individuals who have congenital or acquired anatomic abnormalities of the urinary tract. Obstruction of the urinary tract as a result of either a stone or a malfunctioning bladder secondary to a neural injury also predisposes to infection and makes infections more difficult to treat (Richard *et al.*, 2008).

E. coli accounts for more than 90% of the more than 7 million cases of cystitis and 250,000 of pyelonephritis estimated to occur in otherwise healthy individuals every year in the United States. UTIs are more common in women, 40% of whom have an episode in their lifetime, usually when they are sexually active. The reservoir for these infections is the patient's own intestinal *E. coli* flora, which contaminate the perineal and urethral area. In individuals with urinary tract obstruction or instrumentation, environment sources assume some importance (Kenneth *et al* ., 2014).

Urinary infections are the commonest type of bacterial infection that causes women to seek medical care. In a given year, about 1 in 20 women have acute cystitis. Acute pyelonephritis is one of the most common infections that require hospital admission for intravenous antibacterial therapy. Urinary infections are recurrent in about 5 percent of women, and the recurring cystitis and pyelonephritis cause substantial morbidity. Recurrent urinary infection in women can be due to a number of underlying causes. Sexual intercourse, the syndrome referred to as “honeymoon cystitis”, is responsible for about one-half of urinary infections in sexually active adult women. These infections are not acquired from the sexual partner but rather are due to the mechanical irritation associated with intercourse. Unfortunately, women who acquire frequent infections that are associated with intercourse often have difficulties developing healthy, normal sexual relations and this may require special attention (Richard *et al* ., 2008).

The most pressing problem facing medicine is the growing number of microorganisms that are now resistant to a wide range of antibiotics. The term antibiotic resistance describes the condition where bacteria have developed or acquired means to overcome the inhibitory effect of one or more antibiotics and the mechanisms by which bacteria develops resistance to antibiotics are either due to mutation or acquisition of resistance genes from other organisms (Simon , 2002).

Antibiotic resistance is an emerging and serious public health problem resulting in increased morbidity and mortality. In urinary tract infections (UTI), resistance rates against commonly prescribed antimicrobial agents are constantly rising. Now a days, in many countries more than 20% of responsible uropathogens are resistant to trimethoprim /sulfamethoxazole and to cephalosporins. This increasing resistance is also being observed for fluoroquinolones with resistance rates, risen up to 10% (Schito *et al.*, 2009).

A detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance. A nationwide knowledge base is also important for optimal patient management, control of nosocomial infection and for the conservation of antibiotics (Tawfiq , 2006).

1.2 Rationale

Antibiotic resistance is a worldwide problem. New forms of antibiotic resistance can cross international boundaries and spread between continents with ease. Many forms of resistance spread with remarkable speed. World health leaders have described antibiotic resistant microorganisms as “nightmare bacteria” that “pose a catastrophic threat” to people in every country in the world (Neal , 2009).

Urinary tract infections (UTIs) are among the most common bacterial infections both in the community and hospital setting. In the majority of cases, antibiotics are given empirically before the final bacteriology results are available. Therefore, area-specific monitoring studies to document the microorganisms causing UTIs and their antimicrobial susceptibility is mandatory for helping the selection of an effective empirical treatment (Smith and Coast , 2002).

Urinary tract infections (UTI) are the most common bacterial infections during pregnancy and untreated UTI can be associated with serious obstetric complications (Masinde *et al.*, 2009).

Hence, it is important to focus new studies on resistant strains of *Escherichia coli* isolates which is the commonest species of the bacteria that cause this infection.

1.3 Objectives

1.3.1 General Objective

To Isolate and assess Antimicrobial Susceptibility pattern of *Escherichia coli* from patients suffering of Urinary Tract Infections.

1.3.2 Specific Objectives

1. To isolate and identify *Escherichia coli* from urine specimens using standard microbiological method.
2. To assess antimicrobial susceptibility pattern for isolated *E. coli* using standard Kirby Baur's method.
3. To determine the distribution of UTIs according to gender, age and different clinical remarks of patients.

CHAPTER TWO

Literature review

2. Literature review

2.1 Urinary Tract Infections

2.1.1 Definition

Urinary tract infection (UTI) is an infection involving any part of the urinary system, including urethra, bladder, ureters, and kidney (Gould *et al.*, 2009).

2.1.2 Clinical Manifestations

Acute cystitis is a superficial inflammation of the bladder and urethra which leads to urinary frequency, painful urination, a feeling of fullness following voiding, and suprapubic discomfort. Acute pyelonephritis is due to bacterial invasion of the renal tissue with inflammation and swelling, leading to fever, back pain, and sometimes renal dysfunction. Acute cystitis occurs together with acute pyelonephritis in about one-third of patients. Acute prostatitis occurs when bacteria invade the prostate, causing perineal pain and fever. Infection can spread within the urinary tract and patients often have recurrences of cystitis, sometimes interspersed with episodes of pyelonephritis. Symptoms that persist and recur are often referred to as chronic cystitis, chronic pyelonephritis or chronic prostatitis. However, these chronic conditions are much more difficult to define (Richard

et al., 2008).

Such infections may cause high blood pressure, kidney damage, uremia, or death. In some instances the infections are inapparent and may go unnoticed for some time (Benson, 2001).

Recurring kidney infection in childhood sometimes leads to renal damage and ultimate kidney failure. Hypertension is also an occasional outcome of chronic renal infection. Asymptomatic infections of the urinary tract—*asymptomatic bacteriuria*—are common. In childhood, about 1 percent of girls have

asymptomatic bacteriuria. The prevalence increases to 3 to 5 percent among adult women and 10 to 50 percent in elderly men and women. Occasionally, individuals do have symptoms such as incontinence or ongoing malaise that is not recognized as due to bacteriuria until it is diagnosed and treated (Richard *et al.*, 2008).

2.1.3 Epidemiology:

The UTIs are very common, and each year result in over 7 million physician office visits and about 1 million hospitalizations. Most urinary tract infections result from contamination of the urethra with organisms found in the colon (Neal , 2009).

2.1.4 Acquisition

Bacterial infection is usually acquired by the ascending route from the urethra to the bladder. The infection may then proceed to the kidney and occasionally bacteria infecting the urinary tract invade the blood stream to cause septicemia, less commonly, infection may result from hematogenous spread of an organism to the kidney, with the renal tissue being the first part of the tract to be infected. From an epidemiological viewpoint, UTIs occur in tow general settings: Community acquired and hospital (nosocomial) acquired, the latter most often being associated with catheterization. Hospital acquired UTIs, while less common than community acquired , contribute significantly (ca. 40%) to overall nosocomial infection rates (Richard *et al.*, 2008).

2.1.5 Pathogenesis

Relatively minor trauma or the mechanical effect of sexual intercourse have been shown to allow bacteria access to the bladder. In most instances, these bacteria are purged by the flushing action of voiding. Factors that violate bladder integrity (urinary catheters) or that obstruct urine outflow (enlarged prostate) are also

associated with infection, however, this cannot be the whole story; fewer than 10 *E. coli* serotypes account for the majority of UTI cases, and these UTI serotypes are not the dominant ones in the fecal flora. The ability of uropathic *E. coli* (UPEC) to produce UTI is related to general virulence factors such as hemolysins, together with pili-mediated adherence to uroepithelial cells (Kenneth *et al.*, 2014).

2.2 *Escherichia coli*

E. coli is the commonest urinary pathogen causing 60–90% of infections. Some strains are more invasive, e.g. capsulated strains are able to resist phagocytosis, other strains are more adhesive (Cheesbrough , 2006).

Escherichia coli is the most common cause of urethritis, cystitis, prostatitis, and pyelonephritis (Neal , 2009).

Escherichia coli is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation. It is the leading pathogen causing urinary tract infections. And is among the most common pathogens causing blood stream infections , wounds, otitis media and other complications in humans (Kibret and Abera , 2011).

2.2.1 General characteristics of *E. coli*: (Cheesbrough , 2006)

Morphology:

- On microscopic examination:

The morphology of *E. coli* is a Gram negative usually motile rod. Inactive strains (formerly described as *Alkascens-Dispar*) are non-motile. A minority of strains are capsulated.

E. coli is an aerobe and facultative anaerobe. Optimum temperature for growth is 36–37 °C with most strains growing over the range 18–44 °C.

- On culture media:

Blood agar: *E. coli* produces 1–4 mm diameter colonies after overnight incubation. The colonies may appear mucoid. Some strains are haemolytic.

MacConkey agar and CLED agar: *E. coli* ferments lactose, producing smooth pink Colonies on MacConkey agar and yellow colonies on CLED agar. Some strains (e.g. inactive strains) are late or non-lactose fermenting.

Sorbitol MacConkey agar: *E. coli* (VTEC) 0157 is non-sorbitol fermenting, producing colourless colonies. Most other *E. coli* strains and other enterobacteria ferment sorbitol. *E. coli* (VTEC) 0157 can be identified by testing the colonies using 0157 latex reagen.

XLD and DCA agar: Yellow colonies are produced on XLD agar.

Growth of *E. coli* is usually inhibited on DCA agar.

KIA (Kligler iron agar): Most strains of *E. coli* produce an acid deep and an acid slope with gas production and no H₂S blackening (similar to other lactose fermenting coliforms).

Biochemical reactions

Most strains of *E. coli* give positive results to Glucose and lactose fermentation, Indole, motility, Lysine decarboxylase, urine nitrite test. and negative results to Citrate, urease and H₂S.

2.2.2 *E.coli* and UTIs:

E. coli accounts for more than 90% of the more than 7 million cases of cystitis and 250,000 of pyelonephritis estimated to occur in otherwise healthy individuals every year in the United States. UTIs are more common in women, 40% of whom have an episode in their lifetime, usually when they are sexually active. The reservoir for these infections is the patient's own intestinal *E. coli* flora, which contaminate the perineal and urethral area (Kenneth *et al.*, 2014).

2.2.3 Antimicrobial resistance in *E. coli* isolates:

Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antimicrobial drugs which have favoured the emergence and survival of resistant strains of micro-organisms (Cheesbrough , 2006).

Escherichia coli is the bacterium most frequently isolated in community- and hospital-acquired urinary tract infections (CA-UTIs and HA-UTIs, respectively), and the extensive use of β -lactam antimicrobial drugs has led to the emergence of resistant strains worldwide, β -lactam resistance is mostly mediated through acquisition of β -lactamase genes located on mobile genetic elements such as plasmids or transposons (Etienne *et al.*, 2009).

Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (Kibret and Abera , 2011).

A rise in bacterial resistance to antibiotics complicates treatment of infections. In general, up to 95 % of cases with severe symptoms are treated without bacteriological investigation. Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations as well as significant differences in various populations and environments (Kibret and Abera , 2011).

The prevalence of multidrug-resistant *E. coli*, (i.e., *E. coli* isolates resistant to more than three classes of antimicrobial agents) has been increased worldwide in the past decades. The emergence and worldwide dissemination of fluoroquinolone resistant *E. coli* isolates, that are also resistant to newer β -lactams due to the production of extended-spectrum β -lactamases (ESBLs) particularly CTX-M-type enzymes, is a significant challenge to antibiotic treatment and infection control policies (Woodford *et al.*, 2011).

2.3 Back ground studies:

- In similar study in Amsterdam, (Casper *et al.*, 2012), reported that:

Within the period from January to June 2012, Their study revealed that the antimicrobial resistances of the urinary *E. coli* strains were: amoxicillin: 34%, amoxicillin-clavulanic acid: 17%, trimethoprim: 30%, SXT: 28%, norfloxacin: 14%, ciprofloxacin: 14%, and nitrofurantoin: 0%.

- Also (Hoban *et al.*, 2011) in Globally study reported that:

E. coli isolates from UTIs between 2009 and 2010 found to be resistant to third-generation cephalosporins in 17.9% overall, with the highest rate of 27.7% in isolates from the Asia/Pacific region.

-Another study in UK , by (Bean *et al.*, 2008) reported that:

In 1999 the percentage of *E. coli* bacteraemias resistant to quinolones reported to the UK health protection agency was < 5% and there were no cases of *E. coli* bacteraemia resistant to both quinolones and third-generation cephalosporins. By 2007 the proportion resistant to quinolones had risen to > 20% and > 10% were now resistant to both quinolones and third-generation cephalosporins .

CHAPTER THREE

Materials and Methods

3. Materials and Methods

3.1 Study type and design:

This was descriptive and cross sectional study.

3.2 Study area and duration:

This study was conducted in AL-Ribat University Hospital - khartoum, from January to May 2016.

3.3 Study population:

Three hundred thirty six patients with UTIs symptoms.

3.4 Ethical consideration:

Approval was taken from Research Ethical Committee of Sudan University of Science & Technology and verbal consent had been taken from each patient.

3.5 Data collection:

Data was collected by direct interviewing questionnaire (appendix I).

3.6 Experimental work:

3.6.1 Collection of specimens:

Early morning midstream clean catch urine (MSU) was collected in sterile, dry, wide-necked, leak proof container by patient (10–20 ml of urine), and the patients were adviced as follow:

-Female patients: wash the hands, Clean the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

-Male patients: wash the hands before collecting a specimen (middle of the urine flow).

Then the container was labeled with the date, the name and number of the patient, and the time of collection.

3.6.2 Microscopical examination:

Mixed uncentrifuged urine samples were examined microscopically as a wet preparation to detect the significant pyuria which indicated by presence of three or more pus cell /HPF.

3.6.3 Culture and identification:

One microliter of the mixed specimen was taken using standardized loop and inoculated on CLED medium (HIMEDIA, India) and then incubated at 37 °C over night incubation.

Significant growth is indicated by growth of hundred colony (or more) which indicate the presence of 10^5 CFU/ml of urine.

-Culture inspection:

Lactose fermenting colonies were observed and from them smears were prepared and fixed by heat.

-Gram's stain method:

1 The dry fixed smears were covered with crystal violet stain for one minute, and then washed with clean water.

2 Then the smears were covered with Lugol's iodine for one minute, and washed.

3 The smears were decolorized rapidly (few seconds) with acetone–alcohol, and then washed immediately with clean water.

4 The smears were covered with safranin stain for 2 Minutes, and then washed.

5 Then the slides were placed on a draining rack to air-dry, and then Examined microscopically with the oil immersion objective to report the bacteria and cells.

Gram negative bacilli (pink) were identified via biochemical tests.

Biochemical tests:

Using sterile straight loop The lactose fermenting colonies were touched and inoculated on (kliger iron agar, tryptophan peptone water, semisolid media, simmon's citrate agar, christensen's urea agar) (HIMEDIA, India), and then incubated at 37 °C over night incubation, then interpreted after adding kovac's reagent to tryptophan peptone water medium .

All *E.coli* isolates were glucose and lactose fermenters (give yellow butt and yellow slope on KIA), and they give positive indole test (red ring), and they were motile (diffuse growth on the semisolid media).

And they not grow on simmon's citrate agar (green colour) and give negative urease test (yellow colour).

3.6.4 Antimicrobial susceptibility testing:

Using disc diffusion method according to Kirby Baur , 1962.

-Three to five well isolated colonies of the organism were touched using sterile straight wire loop and emulsified in sterile distilled water and adjusted to 0.5 Macfarland's standard (Appendix II).

- Sterile cotton swab was dipped into suspension optimally several times and pressed firmly into the inside the wall of the tube above the fluid level to remove the excess inoculums from the swab.

-The dried surface of Muller Hinton agar plate (HIMEDIA, India) was inoculated by streaking the swab over the entire sterile to more times rotating the plate approximately 60 each time to ensure distribution of inoculums.

- After five minutes, antibiotic disks [Ciprofloxacin (5 mcg), Cephalexin (30 mcg), Trimethoprim Sulfamethoxazole (1.25 mcg), Nitrofurantoin (300 mcg), and Gentamicin (10 mg)] were applied using sterile forcep.

-Then the plates were incubated at 37 °C over night incubation.

-The diameter of the zone of inhibition around each disk was measured by using ruler.

3.6.5 Control of sensitivity tests:

Escherichia coli ATCC 25922 was used to test the performance of the method.

3.6.6 Interpretation of zone size

The zone of inhibition of each antimicrobial disc was measured and compared with the interpretative chart according to NCCLS and the test organism then reported as sensitive, intermediate or resistant [Table (I), Appendix(V)].

3.7 Data analysis:

Data was analysed by using Statistical Package for Social Science Program (SPSS) version (11.5).

CHAPTER FOUR

Results

4. Results

A total of 336 patients suffering of UTIs, who attending AL-Ribat University Hospital - khartoum during January to May 2016, were enrolled in this study.

Most of study population were females 239 (71%) compared with males, their age ranged from 1 to 92 with mean 31 years, and most of them were belong to 20 – 40 age range.

The over all results revealed that 134 (39.8%) of specimens were significantly positive for uropathogens, and *E. coli* was the major isolated pathogen 100 (74.6%) as indicated in Figure (1).

The frequency of *E. coli* isolates was higher in females 82 (82%) compared with males as indicated in Figure (2) and also the most of them were isolated from 21-40 years age group as indicated in Table (2).

The collected data revealed that fourteen patients were on treatment and their culture results show no growth, and also revealed that six patients with recurrent UTIs, and they were sensitive only to Gentamycin and Nitrofurantoin.

Clinical remarks was also observed, most of study population were suffered from back pain (65%), followed by burning sensation (60%), and statistical analysis showed significant relation between the UTIs and the symptoms (back pain, burning sensation, fever, poly uria), and the P value equal 0.00 , 0.040 ,0.043, 0.016 respectively, while it was insignificant with nausea and vomiting as indicated in Table (2)

Regarding antimicrobial susceptibility testing, most of *E. coli* were resistant to Trimethoprim Sulfamethoxazole (54%), and lowest resistant was against Nitrofurantoin (9%) as listed in Table(1).

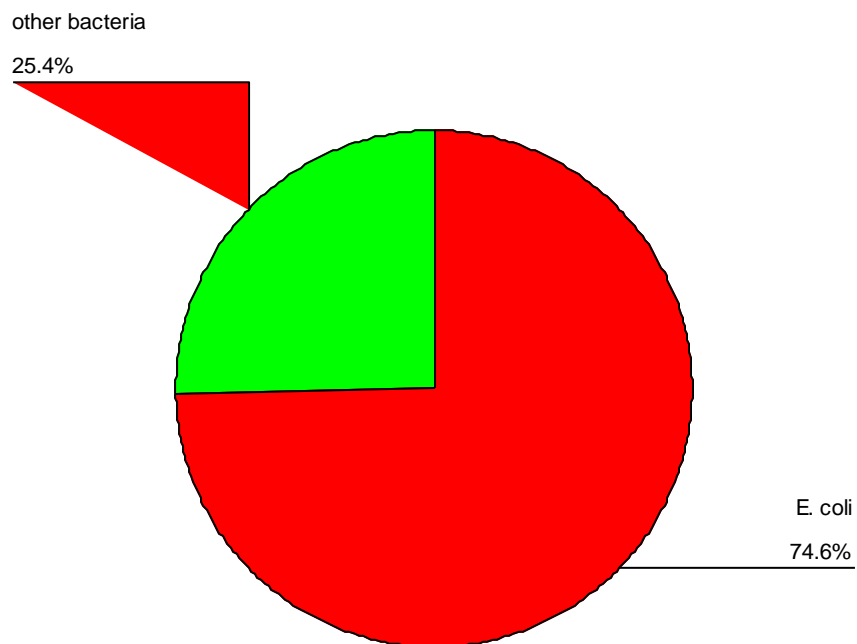


Figure 1 Frequency of *E. coli* among patients with significant UTIs (n=134)

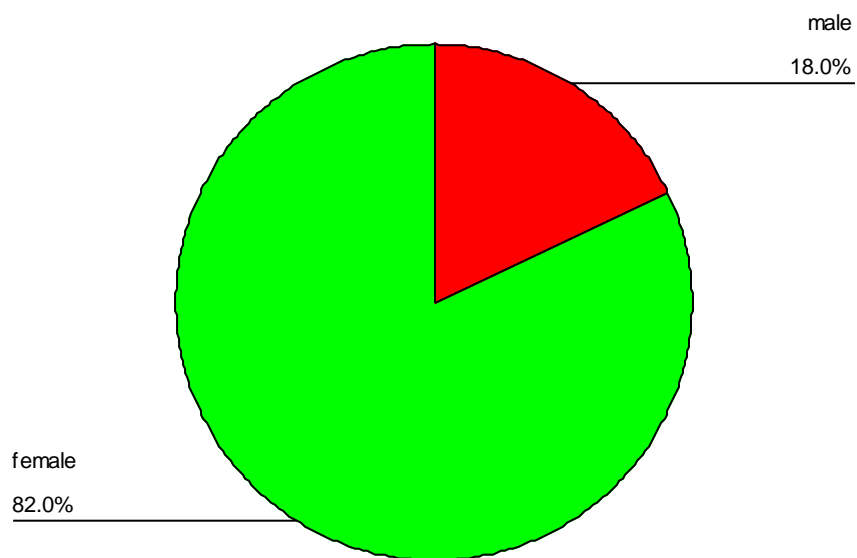


Figure 2 Distribution of *E. coli* (n=100) according to gender

**Table 1: Distribution of the UTIs among patients (n=134)
according to their age**

Age range in years	NO	The frequency
1-20	35	26%
21-40	67	50%
41-60	17	13%
61-80	12	9%
81-100	3	2%
Total	134	100%

Table 2 : Antimicrobial Susceptibility testing results of *E. coli* (n=100) isolated from UTIs patients:

The antibiotic / con (mg)	Sensitive Isolates N (%)	Resistant isolates N (%)	Intermediate isolates N (%)
TMP-SMX	37 (37%)	54 (54%)	9 (9%)
Cephalexin	56 (56%)	37 (37%)	7 (7%)
Ciprofloxacin	66 (66%)	29 (29%)	5 (5%)
Gentamicin	86 (86%)	12 (12%)	2 (2%)
Nitrofurantoin	88 (88%)	9 (9%)	3 (3%)

TMP-SMX = Trimethoprim sulfamethoxazole

Table 3 Relation between different symptoms among study population with UTIs (n=336)

Symptoms	Significant bacteruria		In Significant bacteruria	Total	P value
Back pain	Yes	94	97	191	0.000
	No	40	105	145	
	Total	134	202	336	
Burning sensation	Yes	71	84	155	0.040
	No	63	118	181	
	Total	134	202	336	
Fever	Yes	91	115	206	0.043
	No	43	87	130	
	Total	134	202	336	
Poly urea	Yes	83	150	233	0.016
	No	51	52	103	
	Total	134	202	336	
Headache	Yes	55	94	149	0.323
	No	79	108	187	
	Total	134	202	336	
Nausea	Yes	53	88	141	0.467
	No	81	114	195	
	Total	134	202	336	
vomiting	Yes	58	85	143	0.828
	No	76	117	193	
	Total	134	202	336	

CHAPTER FIVE

Discussion

5. Discussion

5.1 Discussion

The urinary tract represents the most common site of bacterial infection in both hospital and community settings (Foxman, 2010), and 60-90% of the infections are caused by *E. coli* (Cheesbrough , 2006).

Antimicrobial resistance in *E. coli* has increased worldwide and its susceptibility patterns show substantial geographic variation as well as differences in population and environment (Kibret and Abera , 2011).

In the present study 336 participant were enrolled, 134 of specimens contain uropathogens in significant number, and the result show *E. coli* responsible for 100 (74.6%) of the infections and this percentage is near to other researches (Rezaee M and Abdinia B, 2015)(71.4%) ,(Ronald A , 2003)(80%).

E. coli were isolated and The prevalence of *E. coli* was higher in females 82% compared with males, this is due to shorter urethra in female and these findings are in conformity with reports by other researchers (Schaeffer *et al.*, 2001), (Cyprien *et al.*, 2015).

Most of the *E. coli* was isolated in the age group (20-40) (sexually active) compared with younger and older age groups, because the sexual intercourse is a precipitating factor of urinary tract infection (Nicolle *et al* , 1982).

The rate of resistance were highest for trimethoprim sulfamethoxazole (54%), cephalexin (37%), and Ciprofloxacin (29%), and lowest for gentamycin (12%) and nitrofurantoin (9%).

The present study results show rate of resistance higher than that of (Casper *et al* ., 2012) in Nitrofurantoin, Ciprofloxacin, Trimethoprim sulfamethoxazole and lower than (Yilmaz *et al* ., 2016) in Gentamycin and Ciprofloxacin, and this is due to geographic variation as well as differences in population and environment.

In Sudan in peripheral health centers where is no microbiology labs, the physicians usually give the patients of urinary tract infections: Ciprofloxacin as first line, Cephalexin for pregnant women, and this study explain to them that the follow up is necessary to sure there is no resistance occur.

The symptoms (back pain, burning sensation, fever, poly urea), are closely associated with UTIs, so they show significant P value, while other symptoms are non specific to UTIs.

5.2 Conclusion

This study concluded that the rate of resistance in uropathogenic *E. coli* is highest for trimethoprim sulfamethoxazole, cephalixin, and Ciprofloxacin, and lowest for gentamycin and nitrofurantoin.

5.3 Recommendations

- Further study recommended on many hospitals in different states.
- In the next studies, recommended to add others culture media to CLED and incubate in different incubations temperature and atmosphere.

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Appendix I - questionnaire

Sudan University of Science and Technology

College of Graduate studies

Isolation and Antimicrobial Susceptibility Patterns of *Escherichia coli* in Patients with Urinary Tract Infections in AL-Ribat University Hospital - Khartoum

By: Abdellateef Abdalla Hassan

Supervised by: Dr. Wafa Ibrahim Elhag

Name.....

Date:

Index number:

Age:

Gender:

Symptoms:

1-fever

☐

2- back pain

☐

3-burning sensation

☐

4-headache

☐

5- Nausea

☐

6- vomiting

☐

7-polyurea

☐

Any treatment received?

Yes

☐

No

☐

Previous diagnosis of UTI

Yes

☐

No

☐

Culture result

Sensitivity result

Signature:

Appendix II

Reagents and Stains

1. Gram Stain

Most bacteria can be differentiated by their Gram reaction due to differences in the cell wall structure into Gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol and Gram negative which after being stained with crystal violet lose their color when treated with acetone or ethanol and stain red with safranin.

1.1 Requirements

1.1.1 Crystal violet Gram stain (Hi Media)

To make 1 liter:

Crystal violet.....20 g
Ammonium oxalate.....9 g
Ethanol or methanol, absolute.....95 g
Distilled water..... to 1 liter

1.1.2. Iugol's iodine (Hi Media)

To make 1 liter:

Potassium iodide.....20 g
Iodine.....10 g
Distilled water..... to 10 liter

1.1.3. 70% alcohol

Absolute alcohol.....70 ml
Distilled water.....30 ml

1.1.4 Safranin (HiMedia)

1.2 Method of Preparation

- The dried smear was fixed by heat.
- The fixed smear was covered with crystal violet for 30-60 minutes.
- The stain was washed off with clean water.
- All water was tipped and the smear covered with lugol's iodine for 30-60 minutes.
- The stain was washed off with clean water.
- 70% alcohol was rapidly applied for 10-20 seconds for decolourization and then washed rapidly with clean water.
- The smear then covered with safranin stain for 2 minutes.
- The stain was washed off with clean water, back of slide was cleaned.
- After air-dry, smear was examined microscopically by using X 100 lens.

1.3 Results

E. coli appear as Gram negative rods.

2. Preparation of Turbidity Standard

- 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water. Mix well.
- 1.17% w/v solution of barium chloride was prepared by dissolving of 2.35g of dehydrated barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 200 ml of distilled water.
- To make the turbidity standard 0.5 ml of barium chloride solution was added to 99.4 ml of the sulphuric acid solution. Mix well.

- A small volume of the turbid solution was transferred to screw-caped bottle of the same types as used for preparing the test and control inoculate (Mackie and MaCarenny, 1996).

Appendix III – Culture media

Preparation of Media (Chemie , 2014)

1.CLED Agar (Cystine Lactose Electrolyte Deficient)

Formula in grams per liter (PH 7.4)

Lactose.....	10,00
Gelatin Peptone	4,00
L-Cystine	0,128
Bacteriological Agar	15,00
Casein Peptone	4,00
Beef Extract.....	3,00
Bromothymol Blue	0,02

Preparation

Suspend 36 grams of the medium in one liter of distilled water. Soak 10-15 minutes and mix well. Heat slowly while stirring frequently boil for a minute. Sterilize in the autoclave at 121°C (15 lbs. of sp.) for 15 minutes. Pour into Petri dishes. When the medium is solidified, invert the plates to avoid excess moisture.

2.Kligler Iron Agar

Formula in grams per liter

Peptone mixture	20,00
Sodium Chloride.....	5,00
Ferric Ammonium Citrate	0,50
Phenol Red	0,025
Lactose	10,00
Dextrose	1,00

Bacteriological Agar15,00

Sodium Thiosulfate.....0,50

Preparation

Suspend 52 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation. Boil for one minute. Dispense into tubes and sterilize at 121° C (15lbs. pressure) for 15 minutes. Allow to cool in a slanted position so as to obtain butts of 1½-2 cm. Depth. For greater accuracy, Kligler Iron Agar should be used on the day of preparation or melted and solidified before use.

3.Tryptophan Culture Broth

Formula in grams per liter (PH 7.5)

Casein Peptone 10,00

L-Tryptophan 1,00

Sodium chloride.....5,00

Preparation

Suspend 16,0 grams of medium in one liter of distilled water. Heat to boiling agitating frequently. Distribute in test tubes, 3 ml each. Close the tubes with cotton or with a plastic or metallic cap. Sterilize at 121° C (15 lbs. sp.) for 15 minutes.

4.Simmons Citrate Agar

Formula in grams per liter (PH 7)

Ammonium Dihydrogen Phosphate1,00

Dipotassium Phosphate..... 1,00

Sodium Chloride.....5,00

Sodium Citrate 2,00

Magnesium Sulfate.....	0,20
Bacteriological Agar.....	15,00
Bromthymol Blue.....	0,08

Preparation

Suspend 24,3 grams of the medium in one liter of distilled water. Mix well and heat with frequent agitation until completely dissolved. Dispense in tubes and sterilize in the autoclave at 121°C (15 lbs sp.) for 15 minutes. Cool the tubes in a slanted position so that the base is short (1-1,5 cm. deep). Alternatively, the media can be poured into petri plates.

5. Christensen's Urea Agar

Formula in grams per liter (PH 6.9)

Gelatin Peptone.....	1,00
Dextrose	1,00
Sodium Chloride.....	5,00
Monopotassium Phosphate	2,00
Urea	20,00
Phenol Red.....	0,012

Preparation

Dissolve 29 grams of the medium in 100 ml. of distilled water. Sterilize by filtration. Separately dissolve 15 grams of agar in 900 ml. of distilled water by boiling. Sterilize in autoclave at 121°C (15 lbs.sp) for 15 minutes. Cool to 50°C and add to the 100 ml. of the sterile Urea Agar Base. Mix well and dispense aseptically in sterile tubes.

Leave the medium to set in a slanted position so as to obtain deep butts. At a pH of 6.8 to 7.0 the solidified medium should have a light pinkish yellow colour. Do not remelt the slanted agar.

6. Mueller-Hinton Agar

Formula in grams per liter (PH 7.4)

Beef, infusion	300.0g
Cas amino acids.....	17.5 g
Starch.....	1.5g
Agar	17.0g
Distilled water.....	1000ml

Preparation

38.0 g of media was suspended in 100 ml distilled water. Sterilized by autoclaving at 15lb pressure (121°C) and poured in sterile petri dishes.

Appendix IV

Zone Size Interpretative Chart for Applied Antibiotics

The antibiotic	Disc content (mcg)	Resistant mm or less	Intermediate sensitive	Sensitive mm or more
Ciprofloxacin	5	15	16-20	21
Cephalexin	30	14	15-17	18
TMP-SMX	1.25	10	11-15	16
Nitrofurantoin	300	14	15-16	17
Gentamycin	10	12	13-14	15

Mcg = Micrograms

Appendix V

Biochemical reactions of most strains of *E. coli*

The test	The result
Motility	+ve
Indole	+ve
Citrate	-ve
Urease	-ve
H ₂ S production	-ve
Lysine decarboxylase	+ve
Urine nitrite test	+ve