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Detection of Beta-lactamase TEM gene in *Escherichia coli* Isolates Collected from Urine Samples in Khartoum State – Sudan

A graduation project submitted in partial fulfillment for the requirements of B.Sc. degree in Medical Laboratory Sciences (Microbiology)

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بسم اللَّه الرحمن الرحيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا)

(سورة طه الاية 114)

DEDICATION

To our fathers, mothers,

brothers, sisters

and

our friends

we dedicate this humble work.

ACKNOWLEDGEMENT

Piously our gratitude and prayers to ALMIGHTY Allah for the mercy which followed us during the long path of this research. We owe so match to our supervisor **Us. Tanzeel Ahmed Elkhedr Hassaballa** for her close supervision, valuable advices and stimulating suggestions. We sincerely appreciate her valuable advice, guidance and assistance in practical writing of dissertation.

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ABSTRACT

Escherichia coli are the most common cause of urinary tract infections worldwide and they are the fourth leading cause of health care associated infections.

The objective of this study was detection of Beta-lactamase TEM gene in *Escherichia coli* Isolates Collected from Urine Samples in Khartoum State – Sudan.

A total of 96 of *Escherichia coli* were isolated from different hospitals in Khartoum State.

The present study showed that the percentage of infection in females (64/96) (66.7%) were more than the percentage of infection in males (32/96) (33.3%).

There was high prevalence rate of *Escherichia coli* urinary tract infection within the age group (25-44) (35.4%).

Out of 96 isolated *Escherichia coli* 76(79.2%) were positive for TEM gene.

Further studies with large number of samples and more advanced techniques are required to validate the results of the present study.

خلاصة الاطروحة

الإشريكية القولونية هي السبب الأكثر شيوعًا لعدوى المسالك البولية في جميع أنحاء العالم وهي رابع سبب رئيسي للعدوى المرتبطة بالرعاية الصحية.

الهدف من هذه الدراسة هو الكشف عن جين (تيم بيتا- لاكتميز) في معزولات الإشريكية القولونية التي تم جمعها من عينات البول بولاية الخرطوم - السودان.

تم عزل مجموع 96 من بكتيريا الإشريكية القولونية من مستشفيات مختلفة بولاية الخرطوم.

أظهرت الدراسة الحالية أن نسبة الإصابة في الإناث (96/64) (66.7٪) كانت أكثر من نسبة الإصابة في الذكور (96/32) (33.3٪).

كان هناك معدل انتشار مرتفع لعدوى المسالك البولية الإشريكية القولونية ضمن الفئة العمرية (25-44) (35.4٪).

من بين 96 معزولة 76 (79.2%) كانت موجبة لجين تيم.

يلزم إجراء مزيد من الدراسات مع عدد كبير من العينات وتقنيات أكثر تقدمًا للتحقق من صحة نتائج الدراسة الحالية.

ABBREVIATIONS

AMR	Antimicrobial resistance
AROs	Antimicrobial Resistance Organisms
CLED	Cysteine-Lactose-Electrolyte-Deficiency
CTX-M	Cefotaximase-Munich
DNA	Deoxy-Nucleic-Acid
E. coli	*Escherichia coli*
EAEC	Entero-Aggregative *Escherichia coli*
EHEC	Entero-Hemorrhagic *Escherichia coli*
EIEC	Entero-Invasive *Escherichia coli*
EIs	Extra-Intestinal Infections
EPEC	Entero-Pathogenic *Escherichia coli*
ESBL	Extended -Spectrum β-Lactamases
ETEC	Entero-Toxigenic *Escherichia coli*
EXPEC	Extra Intestinal Pathogenic *Escherichia coli*
FQ	Fluoroquinolone
GIT	Gastrointestinal Tract
GNB	Gram-Negative Bacteria
HAP	Healthcare-Associated Pneumonia
HUS	Hemolytic-Uremic Syndrome
IM	Inner Membrane
ISTM	International Society of Travel Medicine
KIA	Kligler Iron Agar
KIMCU	Kligler Iron Agar-Indole-Motility-Citrate-Urase
LPS	Lipopolysaccharide
MBL	Metallo-Beta-Lactamase
MDR	Multi Drug-Resistant
MR	Methyl-Red
OM	Outer Membrane
OMPs	Outer Membrane Proteins
OXA	Oxacillinase
PCR	Polymerase-Chain-Reaction
Qnr	Quinolone Resistance Gene
SHV	Sulfhydryl Variable

SPP	Species Pluralis
SPSS	Statistical Package Social Sciences
TBE	Tris-borate-EDTA
TEM	Temoniera
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary Tract Infection
VP	Voges-Proskauer
WHO	World Health Organization

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CHAPTER ONE

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introductions

Antibiotic resistance in bacterial pathogens is steadily increasing and recognized as one of the greatest threats to global public health. [1]

Widespread use of antibiotics is often accompanied by increased bacterial resistance, that can lead to the emergence of new antibiotic-resistant mechanisms that adversely affecting our ability to treat diseases and Increased antimicrobial resistance to antibiotics leads to longer treatment periods and higher health care costs, recently, Several reports have indicated that multi-drug resistance exists in different countries such as the United States, European countries and Asia, However, resistance to microbial agents is growing very rapidly in developing countries in Africa. [2]

Resistance to antimicrobial used to treat serious bacterial infections results in substantially increased mortality. [3]

Antimicrobial resistance (AMR) is the condition in which pathogenic strains of the bacteria develop resistance against the specific drug prescribed in response to that microorganism(s). [4]

Overuse of such drugs in humans, animals, and the environment is deemed responsible for the emergence of antibiotic resistance. [5]

Widespread excessive dispensing and irresponsible use of antibiotics has resulted in the development of resistant strains. Unfortunately, most antibiotics are available over the counter in the developing countries and can be dispensed

without prescription; therefore, patients and general public education are crucially needed. [6]

Antimicrobial resistance among gram-negative bacteria (GNB) is growing worldwide. It is a main public health problem causing both significant morbidity and mortality among hospitalized patients. A direct correlation between resistance of GNB and patient mortality, cost of patient care, and length of hospital stay has been shown. [7]

Resistance in Gram-negative bacteria is mainly mediated via the production of extended -spectrum β-lactamases (ESBL), ampC β-lactamases and carbapenemases. Infections with these multi drug-resistant (MDR) organisms will thus pose therapeutic challenges; the antibiotic pipeline is drying up, and no new antimicrobial agents are anticipated in the near future to treat infections caused by these bacteria. [8]

Gram-negative bacteria have developed resistance to one of the most effective drugs (β-lactams) by producing enzymes that can hydrolyze bonds of β-lactam rings and These enzymes are named as extended-spectrum β-lactamases (ESBLs), which possess the ability to inactivate extended-spectrum β-lactams and monobactams, except cephamycins and imipenem. [9]

Extended-spectrum beta-lactamases (ESBLs) are plasmid-encoded enzymes and are easily transferred from one bacterium to another by horizontal gene transfer which exchanges between E. coli strains and a recognized source of rapid spread of antimicrobial resistant strains. [10]

β-lactamases are classified into four molecular classes: A, B, C, and D. Classes A, C and D act via a serine-based mechanism, whereas class B (or MBL: metallo-betalactamase) β-lactamases require zinc to function. [11]

The class A beta-lactamases are most common group and large family consisting of TEM, SHV and CTX-M β- lactamases, in addition to other number of rare enzymes that often exhibit ESBL activity. [12]

The most frequently detected and clinically important ESBLs belong to the Temoniera (TEM), sulfhydryl variable (SHV) and cefotaximase-Munich (CTX-M) families. [13]

The emergence and spread of these drug-resistant genes can limit therapeutic options, increase morbidities and mortalities, prolong hospital stays, and cost massive economic loss. [14]

Escherichia coli (*E. coli*) is a common enteric organism and one of the predominant species in most bacterial infections. [15]

E. coli was classified into four major phylogenetic groups: A, B1, B2 and D. [16]

Uropathogenic *E. coli* (UPEC) were found to be gender-associated and in higher numbers in group B2. [11]

Escherichia coli antibiotic resistance is very worrying because it is the most pathogenic Gram-negative bacterium. It causes urinary tract infections and both society- and hospital acquired bacteremia and diarrhea. [17]

The emergence and spread of *E. coli* resistance to several antibiotics has been reported in several regions. [18]

Resistance to extended-spectrum cephalosporin in extra intestinal pathogenic *Escherichia coli* (EXPEC) represents a major clinical challenge and is commonly caused by the presence of extended-spectrum beta-lactamases(ESBLs). [19]

The global prevalence of bla *TEM* in clinical isolates varies and has continued to change over time. [20]

The number of organisms producing TEM enzymes continues to increase, and the antibiotic resistance conferred by TEM enzymes remains a major crisis in the treatment of infections caused by these bacteria. [21]

Extended-spectrum beta-lactamase (ESBL) producing *E. coli* strains are causing global public health threats, and They are resistant to several classes of antibiotics, which results in limited therapeutic options to treat the infections caused by these pathogens, so The rapid increase in the spread of these antimicrobial resistant organisms, coupled with the unavailability of effective antimicrobial agents has made the World Health Organization (WHO) to warn against "post-antimicrobial era", where people die because of common infections and minor injuries. [15]

Therefore, this study aimed to detect the TEM beta-lactamase gene in *Escherichia coli* isolates collected from urine sample in Khartoum state.

1.1.1 Justification

Antimicrobial resistance is a global public health issue that threatens the effective prevention and treatment of many bacterial infections. In developing countries.

Antimicrobial resistance among gram negative bacteria(GNB) is growing worldwide. It is a main public health problem causing both significant morbidity and mortality among hospitalized patients.

Escherichia coli accounts for 70–95% of community-acquired urinary tract infections (UTIs) and the rise of multidrug resistance in uropathogenic *Escherichia coli* is now a serious challenge encountered by healthcare professionals. especially caused by β-lactamases producing MDR strains.

1.1.2 Objectives

1.1.2.1 General objectives

To detect the TEM beta-lactamase gene in *Escherichia coli* isolates collected from urine sample in Khartoum State-Sudan.

1.1.2.2 Specific objectives

1- To determine the prevalence of plasmid TEM beta-lactamase gene in *Escherichia coli* isolates collected from urine samples.

2- To compare the occurrence of *Escherichia coli* urinary tract infection with different variables.

1.2 Literature Review

1.2.1 *Escherichia coli*

1.2.1.1 Introduction

Escherichia coli is a Gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae that was described in 1885 by a German pediatrician, Theodor Escherich in the faeces of a child suffering diarrhoea (Escherich, 1885), While many strains occur as commensal members of the microbiota in the intestinal tract of animals and humans, some strains are, however, important pathogens that cause a wide spectrum of diseases, ranging from self-limiting to life-threatening intestinal and extra-intestinal illnesses. [22]

1.2.1.2 Morphology

The genus Escherichia is a Gram-negative, non-spore forming, facultative anaerobic, rod-shaped bacteria from the family Enterobacteriaceae. [23]

1.2.1.3 Normal habitat

Escherichia coli are a group of bacteria normally found in the flora of human and animal digestive tracts and symbionts participating in digestion and synthesis of certain vitamins. [24]

Escherichia species provide aportion of the microbially derived vitamin K for their host. A number of the species of Escherichia are pathogenic. [23]

1.2.1.4 Transmission

Human-to-human transmission within the household is also an important pathway for the spread of AMR bacteria within the community. Also the family members can share fecal and oral bacteria, potentially resulting in pathogenic

bacteria being harbored quiescently by individuals and causing disease in cohabiting persons. [25]

Ruminants are a major source of *E. coli* O157:H7, and transmission principally occurs through consumption of contaminated food but also through direct or indirect contacts with contaminated buffalos or persons. [26]

1.2.1.5 Epidemiology

Escherichia coli (E. coli) is the most frequently isolated microorganism, in up to 80% of UTIs. [27]

Escherichia coli is responsible of 30% of nosocomial infections. [28]

E. coli O157: H7 causes an estimated 63,000 hemorrhagic colitis cases annually in the United States. Review of database and studies from 10 out of 14 world health organization's subregions showed the global incidence of *E. coli* to be 2.8 million cases per year. [29]

In low-income countries the prevalence of *E. coli* strains resistant to antibiotics such as tetracycline, ampicillin and trimethoprim/sulfamethoxazole exceeds 50%. [30]

1.2.1.6 Virulence factor

Uropathogenic *E. coli* have many virulence factors, i.e. adhesins, toxins (e.g. alpha-hemolysin, cytotoxic necrotizing factor 1, autotransporter toxins), iron/heme-acquisition systems, and iron ion transport. P, S and type 1 fimbriae are responsible, among others, for adhesion to epithelial cells of intestines, kidneys, or lower urinary tract, and for stimulating cytokine production by T cells. [31]

A characteristic feature of UPEC is the ability to multiply intracellularly. [32]

1.2.1.7 Pathology

Escherichia coli results in intestinal illness as well as infection outside of the intestine. Intestinal illness caused by *E. coli* is caused by one of five subtypes, and they are identified according to their O and H antigens. The O antigen is determined by a repeating polysaccharide chain present in the lipopolysaccharide (LPS) outer membrane, and the flagellum determines the H antigen. [33]

E. coli are involved in the urinary tract infection (UTI), hospital acquired pneumonia (HAP), sepsis, surgical site infection (SSI), gastrointestinal tract infections, hemolytic-uremic syndrome (HUS), meningitis and inflammation of the meninges. [31]

Enterhemorrhagic *Escherichia coli* (EHEC) O157:H7, as a severe enteric pathogen in human, generally causes bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). Also conventional antibiotic therapy increases the incidence of HUS due to the release of Shiga toxin into intestinal mucosa by antibiotic-mediated bacteriolysis. [34]

Patients who develop diarrheal illness are at an increased risk for dehydration, but this can often be prevented through adequate hydration and early symptomatic intervention. Long-term complications include chronic diarrhea and irritable bowel syndrome, but these occur in a small number of patients. Patients with EHEC/STEC diarrheal illness are at risk for developing hemolytic uremic syndrome, which is more common in children less than five years old and adults older than. [35]

Extraintestinal pathogenic *E. coli* (ExPEC) (Dale i Woodford) have a complex phylogenetic structure, wide range of virulence factors (VF), and considerable plasticity of the genome. [36]

These strains not only cause uncomplicated UTIs, but also bacteremia or sepsis. Mechanisms underlying the dynamics of ExPEC transmission and the selection of resistant clones are still poorly understood and require further research. [31]

The ExPEC group includes uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC), and avian pathogenic *E. coli* (APEC). [37]

E. coli is the dominant causative agent in all patient groups, causing 50–90% of all UTIs. [38]

UPEC strains infect the urinary bladder through the urethra (cystitis), and if they remain untreated, the infection can spread to the kidneys (pyelonephritis) leading to renal failure and sepsis. Unlike UPEC strains, commensal-like *E. coli* strains can colonize the urinary bladder in large numbers without symptoms. Such asymptomatic bacteriuria (ABU) strains have evolved from UPEC strains by losing the ability to express functional virulence factors. [39]

1.2.1.8 Molecular detection

The development of techniques of molecular biology enables for sequencing whole genomes of reference *E. coli* strains for example: commensal *E. coli* K-12, pathogenic strain O157:H7 that causes intestinal infections and uropathogenic *E. coli* J96. There are also known whole genetic sequences of at least 20 *E. coli* strains. [31]

Analysis of sequences of house-keeping genes that is MutliLocus Sequence Typing, MLST made possible to more accurate exploration the phylogenetic structure of *E. coli* species. [40]

1.2.1.9 Treatment

Treatment is dependent on the strain, as well as the illness. Care of the patient with an intestinal disease caused by *E. coli* begins with symptomatic management. [41]

Diarrheal illness can be extremely distressing for patients. Experts recommend rehydration and antidiarrheals as the mainstays of treatment for mild disease. Oral rehydration is recommended first-line therapy for all patients with diarrheal illness when tolerated and is equally efficacious as compared to intravenous hydration (IV). [33]

Managing UTI caused by UPEC has become challenging over the years due to increasing resistance to the commonly used antibiotics. [42]

1.2.1.10 Prevention

Illnesses caused by *E. coli* can be prevented by regular hand washing, washing fruits and vegetables, and thoroughly cooking meat. When traveling to areas with inadequate sanitation practices, such as in many developing regions, illness can be avoided through consuming purified water and thoroughly cooking food or by rinsing raw fruits and vegetables in purified water. [43]

When infection cannot be avoided, or patients are at high risk for complications of diarrheal illness (e.g., immunosuppressed), prophylactic antibiotics can significantly reduce disease. The ISTM recommends travelers at risk for contracting diarrheal illnesses who require antibiotic prophylaxis should take rifaximin or bismuth-subsalicylate for chemoprophylaxis. [44]

Reducing the risk of extraintestinal infections is disease-specific but includes interventions such as reducing the use of indwelling medical devices to prevent catheter-associated urinary tract infections. Developing ICU protocols to reduce aspiration risks, including elevating the patient's head of the bed to 30 degrees, leads to lower rates of ventilator-associated pneumonia. [45]

Clinicians trained in travel medicine can identify candidates for chemoprophylaxis to traveler's diarrhea and help initiate appropriate therapy. [46]

1.2.2 Beta-lactam

B-lactam are a large group of important antibiotics, because their effectiveness and generally low toxicity and there are four main groups of B-lactam antibiotics including penicillins, cephalosprins, carbapenems and monobactams which are arranged according to structure. [26]

β-Lactam antibiotics such as penicillin, cephalosporin, and carbapenem inhibit the synthesis of bacterial peptidoglycan cell walls. The third and fourth generation of cephalosporins like ceftazidime and cefepime. [47]

1.2.2.1 Beta-lactamase

Since the first β-lactamase (an enzyme that provides resistance to β-lactam-based antibiotics) was discovered in *E. coli* in 1940 >2,000 similar enzymes have been discovered, including extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases (AmpCs) and carbapenemases (such as KPC, NDM, IMP, VIM and OXA. [48]

ESBL enzymes are plasmid borne and they have evolved from point mutations which altered the configuration of the active site of the original and long known β-lactamases, which have been designated as (TEM-1, TEM-2, and SHV-

1). Although most of the ESBLs are mutants of the TEM and the SHV enzymes. [49]

Since the first description of plasmid-mediated extended spectrum beta-lactamase (ESBL)in 1983, The ESBL producing gram-negative organisms have posed a significant threat to hospitalized patients due to their hydrolyzing activity against extended spectrum cephalosporins often employed in the treatment of hospital-acquired infections. Detection of organisms harboring ESBLs provides clinicians with helpful information. [50]

Treatment of infections caused by ESBL producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing. [51]

The ESBLs are derivatives of common β-lactamases (TEM and SHV β-lactamases) that have undergone one or more amino acid substitutions near the active site of enzyme, thus increasing their affinity and the hydrolytic activity against third generation cephalosporins and otherβ-lactam antibiotic. Extensive use of newer generation cephalosporins has been the strong factor for the evolution of newer B-lactamases such as ESBLs. The later are encoded by transferable conjugative plasmids, which often code resistance determinants to other antimicrobial agent such as aminoglycoside. These conjugative plasmids are responsible for the dissemination of resistance to other member of Gram negative bacteria in hospital and in the community. [52]

ESBLs are heterogeneous group of enzyme that confer resistance to penicillin; 3and 4 generation cephalosporins and monobactams. [53]

Also ESBLs are undergoing continuous mutation, causing the development of new enzyme showing expanded substrate profile, at present there are more than 300 different ESBL variants. [54]

ESBLs encoded by gene located on large plasmids, which also carry gene for resistance to other antimicrobial agent such as aminoglycosides, trimethoprim, sulfonamide, tetracycline, and the chloramphenicol. [55]

Due to the noticeable geographical differentiation of bla genes among ESBL-producers, examination of the prevalence of these genes among ESBL-producing strains is of great importance for clinical care and for developing optimal infection control measures in hospital. [56]

The class A beta-lactamases are most common group and large family consisting of TEM, SHV and CTX-M β-lactamases, in addition to other number of rare enzymes that often exhibit ESBL activity. The best way to define and identify the presence of a β-lactamase gene is by genetic methods PCR and sequencing which are standard methods for the determination of specificβ-lactamase genes in bacterial isolates. [12]

Bla-TEM was first isolated in 1965 from *E. coli*, a disease in Athens, Greece. They then appeared in 1969 in P. aeruginosa, in 1973 in Vibrio cholerae, and in 1974 in Haemophilus and Neisseria species, also bla-TEM has the ability to hydrolyze ampicillin more than Carbenicillin, Oxacillin and Cefalotin, and has little activity against broad-spectrum Cephalosporin and inhibited by clavulanic acid. TEM-1, TEM-2 and TEM-3 have similar hydrolytic profiles. [57]

1.2.3 Enterobacteriaceae resistance

Enterobacteriaceae family such as *Escherichia coli*, klebsiell spp, and Enterobacter spp. is the major cause of urinary tract infection (UTI), blood-stream

infection, hospital, and healthcare-associated pneumonia. Resistance is mainly related to the production of ESBLs, but other mechanisms of resistance are also emerging, leading to multidrug-resistance (MDR). [54]

1.2.3.1 Enterobacteriaceae-3rd Generation Cephalosporin-Resistan

Enterobacteriaceae resistance to third-generation cephalosporins is aresult of the production of β-lactamases. For example, ESBLs can hydrolyze broad-spectrum cephalosporins, monobactams, and penicillins. Enzymes of classA β-lactamases, like TEM-1, TEM-2, and SHV-1 are responsible for the resistance to ampicillin, amoxicillin, and early generation cephalosporins. [54]

Enterobacteriaceae resistance to third generation cephalosporin's is now above 10%, and 2-7% for carbapenem. This is because of the rapid spread of extended-spectrum β-lactamase (ESBL) producing strains. Carbapenem resistance rates for klebsiella pneumonia is above25% while 20 to 40% is for P. aeruginosa and 40 to 70% ICU acquired infections being carbapenem-resistant. [58]

1.2.3.2 *Escherichia coli* antibiotic resistance

E. coli antibiotic resistance is very worrying because it is the most pathogenic Gram-negative bacterium. It causes urinary tract infections and both society-and hospital-acquired bacteremia and diarrhea. [17]

The emergence and spread of *E. coli* resistance to several antibiotics has been reported in several regions. [18]

Among the *E. coli* strains, the principal mechanism of resistance to β-Lactams is the production of β-lactamase enzymes, all of which differ from each other based on their substrate profile, inhibitor profile, and sequence homology. [59]

Several risk factors associated with ESBL-producing *E. coli* infections have been described in the community, including: previous use of antibiotics (especially quinolones and third- or fourth-generation cephalosporins), recurrent *E. coli* infections, recent hospitalization (within the prior year), artificial nutrition, previous admissions to intensive care units (ICUs), foster home stays, and receiving hemodialysis. [60]

Strains of ESBL-producing *E. coli* are important antimicrobial-resistant organisms (AROs). In 2017, the WHO defined a list of priority AROs for research purposes, among which ESBL-producing Enterobacteriaceae are in priority group one. [61]

CHAPTER

TWO

CHAPTER TWO

MATERIAL AND METHODS

2. Materials and Methods

2.1 study design

This is a descriptive cross -sectional study.

2.2 Study Area

The study was conducted in Khartoum state during the period from August 2021 to December 2021.

2.3 Study period

This study was conducted during the period from August 2021 to December 2021.

2.4 Study population

The study was confined on patients who suffer from urinary tract infection caused by *Escherichia coli.*

2.5 Sampling Technique

Simple random sample.

2.6 Sample Size

A total of hundred (n=96) bacterial strains of gram-negative specimens identified as *Escherichia coli* were collected from different clinical laboratories.

2.7 Inclusion criteria

All urine samples isolates identified as *Escherichia coli.*

2.8 Exclusion criteria

Any CLED media will be contain more than two organisms per urine samples.

2.9 Data collection

Personal data were obtained by instruction questionnaire.

2.10 Data analysis

The data that collected from questionnaire and laboratory results were analyzed by Statistical Package Social Sciences (SPSS) version 20 computerized program.

2.11 Ethical Considerations

The study was approved by the Ethical Board of Napata College -Khartoum state, and the permission to collect the isolates were obtained from authorities of the different hospitals allocated in Khartoum. After explaining the study and its goal, a verbal consent was obtained and the information collected will not been use for any purpose other than this study.

2.12 Material

2.12.1 Instruments

- Autoclave
- Hot air oven
- Microscopes
- Sensitive balance
- Centrifuge
- Vortex
- Automated pipette

- Gel Documentation System
- Gel Electrophoresis
- Thermal cycler
- Incubator
- Refrigerator
- Microwave

2.12.2 Glass ware

- Bottles
- Cover glass
- Flask
- Microscope
- Slides
- Petri dishes
- Measuring cylinders
- Test tubes
- Beaker

2.12.3 Other

- Cotton
- Bunsen burner
- Forceps
- Slide racks
- Plastic sack
- Tube racks
- Wire loop
- Straight wire
- Spatula

- Holder
- Cotton swabs
- Eppendorf tube
- Tips and tips racks
- Pasture pipette
- Plain container
- Gloves
- Wooden sticks
- Wire loop
- Straight wire

2.12.4 Sterilization

2.12.4.1 Red heat

It was used to sterilize wire loop by holding it over Bunsen burner until become red hot.

2.12.4.2 Hot air oven

It was used to sterilized glass ware such as test tubes, glass Petri dishes, flasks, and forceps the holding period was one hour, the temperature was 160°c.

2.12.4.3 Moist heat autoclave

Autoclaving at 121°c for 15minutes was used for sterilization of media.

2.12.5 Reagents and stain

2.12.5.1 Alcohol

Alcohol was used as disinfectant and dissolving agent.

2.12.5.2 Cedar oil

Was used for oil immersion lens.

2.12.5.3 Gram stain

- **Crystal violet**

It was prepared by dissolving grams' crystal violet and ammonium oxalate in 95ml absolute methanol mixing with distilled water then it was labeled.

- **Lugol's iodine**

It was prepared by dissolving 20g potassium iodine at 100ml distilled water and 10g iodine is added then mixing until iodin dissolved then was labeled and used for gram stain.

- **Alcohol fixative solution:**

It was prepared by mixing 180ml of ethanol with 10ml of glacial acetic acid in al ml distilled water then it was labeled used in gram.

- **Safranine:**

It was prepared by mixing 0.5 grams of safranine with 100ml distilled water then it was labeled used in gram stain.

2.12.6 Preparation of media

The following media were used for isolating studying and maintenance of *Escherichia coli* isolated from different clinical specimens.

2.12.6.1 CLED agar

This medium was provided by (Scharlau), It was prepared by adding 36 grams of powder to 1000 ml of distilled water and it was mixed well to be dissolved then it was autoclaved for 15 minutes at 121°c.

2.12.6.2 MacConkey agar

This medium was provided by (Himedia), it was prepared by adding 51.55 g of MacConkey agar to 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c.

2.12.6.3Nutrient agar

This medium was provided by (Micro master), it was prepared by adding 28 gram of media in 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c. It was used for storage of isolates.

2.12.6.4 Mueller Hinton agar

This medium was provided by (Himedia), it was prepared by adding 38 gram of Mueller Hinton powder to 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c.

2.12.6.5 Kligler Iron Agar

This medium was provided by (Micro master), it was prepared by adding 57.52 gram of media in 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c. Allow the tube to cool in slanted position to form slopes with about 1inch butt. It was used for biochemical tests.

2.12.6.6 Simmon`s Citrate Agar

This medium was provided by (HIMEDIA), it was prepared by adding 24.28 gram of media in 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c. Allow the tube to cool in slanted position to form slopes with about 1inch butt. It was used for biochemical tests.

2.12.6.7 Motility Test Medium

This medium was provided by (HIMEDIA), it was prepared by adding 20.00 gram of media in 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c. Allow the tube to cool in upright position. It was used for biochemical tests.

2.12.6.8 Indole Test

This medium was provided by (HIMEDIA), it was prepared by adding 20.00 gram of peptone media in 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c. Allow the tube to cool in upright position. It was used for biochemical tests.

2.12.6.9 Urea Agar

This medium was provided by (Scharlau), it was prepared by adding 24 gram of media in 950 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121°c.Let it cool to 50-55°c add 0.4g of urea crystal for each 50ml of urea sterile solution and mix well. Distribute aseptically in tubes and let them solidify slanted. It was used for biochemical tests.

2.12.6.10 Agarose Gel

This Agarose was provided by(Intron), it was prepared by adding 1.5gram of agarose in 80ml of distilled water and 20ml of TBE buffer. It was mixed well to be dissolved, then it was heating by use microwave to complete dissolving. After that allow the flask cool down to 55°C and then add 5µl of ethidium bromide and mix. It was used for separation of DNA.

2.13 Method

2.13.1 Collection of samples

The samples were *Escherichia coli* isolates were collected in a CLED media.

2.13.2 Culturing of samples

For culture CLED media was used. The sample was inoculated on CLED and incubated at 37°C. Then observed after 24hr. Colonies were identified by the colony characters and by gram’s stain.

2.13.3 Microscopical examination

Gram's staining was done for the isolates.

2.13.4 Gram's stain

Smears were made from the clinical sample on a clean and grease free glass slide, then heat fixed by just passing the glass slide over the flame. Then the smear was stained by Gram’s method and observed under oil immersion objective and looked for the presence gram negative bacilli.

2.13.5 Biochemical tests

All Gram-negative bacilli were further identified by using standard conventional biochemical tests using set of KIMCU.

2.13.5.1 Urease test

The test organisms were cultured in slope of urea based medium contain phenol red as indicator and incubated at 37 °C overnight. Positive results were given pink color and in negative result no change in the color of the media.

2.13.5.2 Citrate utilization test

The test organisms were cultured in slope of citrate based medium contain bromothymol blue as indicator and incubated at 37 °C overnight. Positive results were given blue color and growth but in negative result no change in the color of the media.

2.13.5.3 Motility test

The test organisms were cultured in tube contain semi solid media and incubated at 37 °C overnight. Positive results were given diffuse growth and in negative result no diffuse growth of the media.

2.13.5.4 Indole test

The test organisms were cultured in broth of peptone water with tryptophan and incubated at 37 °C overnight and add Kovacs reagent as indicator. Positive results were given red ring and in negative result no change in the color of the Kovacs reagent.

2.13.5.5 KIA test

The test organisms were cultured in slope of kligler iron agar medium contain phenol red as indicator and incubated at 37 °C overnight. Positive results were given yellow butt yellow slope, gas without H2S production of the media.

2.13.6 The storage of samples

Each *Escherichia coli* isolates were sub-culture on nutrient agar slope in plain container and preserved at 4ºC till the further processing.

2.13.7 Molecular detection of beta-lactamase TEM gene

2.13.7.1 The DNA extraction

DNA was extracted using the boiling method. Two ml of an overnight growth of bacteria on peptone water were picked. The peptone water was put in a plain container. The plain container was then centrifuged at 5,000 rpm for 3 minutes after that discharge the supernatant and transfer the sediment to new Eppendorf tube and centrifuged at 12,000 rpm for 2 minutes. Then discharge the supernatant from Eppendorf tube. After that add 100 µl of injection water in each Eppendorf tube. This was then boiled for 30 minutes in a water bath and then put in ice quickly to shock for 10 minutes and then centrifuged for 3 minutes at 12,000 rpm.

The supernatant containing DNA was then transferred into a new Eppendorf tube and stored at -20^{0}C. Five microliters of the supernatant were used for the PCR.

2.13.7.2 PCR

The beta lactamase TEM gene coding for resistance was detect by the conventional polymerase chain reaction(PCR)technique.

The DNA amplification of the beta lactamase TEM gene was carry.the primer sequences below:

*Forward: CTTCCTGTTTTTGCTCACCCA

*Reverse: TACGATACGGGAGGGCTTAC

The PCR was carried in 50µl PCR reaction volumes containing 5µl of template DNA, 1µl of each primer, 13µl of distill water and 30 Taq PCR Master Mix.

The amplification of DNA was performed by using the thermo-cycling condition in thermo-cycler will be as the following condition:

* Initial denaturation was performed at 94°C for 5 minutes.

* Denaturation at 94°C for 45 seconds.

* Annealing at and 59°C for 45 seconds.

* Elongation for 60 seconds at 72°C.

* Final elongation for 5 minute at 72°C.

2.13.7.3 Gel electrophoresis

The amplify products(5µl) were separated by electrophoresis on 1.5% agarose gel and visualized by staining with ethidium bromide using a UV gel documentation system. A971bp PCR products will amplify above the beta lactamase TEM gene specific-primers.

CHAPTER THREE

CHAPTER THREE

RESULTS

3.1 Results

In the present study a total of (96) various isolates of *Escherichia coli* were collected from nine hospitals, Military hospital 25(26%), Baraha Medical city 17 (17.7%), East Nile Hospital 17(17%), Fedail hospital 9(9.4%), Almoalem Medical City 7(7.3%), Police's Hospital 7(7.3%), Alzaytouna Specialist Hospital 7(7.3%), Al-amal Hospital 5 (5.2%), Royal Care Hospital 2(2.1%), (Table1).

According to gender out of isolated *Escherichia coli* 32(33.3%) were isolated from males and 64(66.7%) were isolated from females (Table 2).

According to the age group the isolated *Escherichia coli* were found 16(16.7%) within the age group (5-24), 34(35.4%) within (25-44), 31(32.3%) within (45-64) and 15(15.6%) within (65-84) (Table 3).

Out of 96 isolated *Escherichia coli* 76(79.2%) were positive for TEM gene while 20(20.8%) were negative for TEM gene (Table 4).

Table.1 Distribution of isolates according to the hospital

Hospital	Number	Percentage %
Military hospital	25	26%
Baraha Medical city	17	17.7%
East Nile Hospital	17	17.7%
Fedail hospital	9	9.4%
Almoalem Medical City	7	7.3%
Police's Hospital	7	7.3%
Alzaytouna Specialist Hospital	7	7.3%
Al-amal Hospital	5	5.2%
Royal Care Hospital	2	2.1%
Total	96	100%

Table.2 Distribution of isolates according to the gender

Gender	Number	Percentage%
Male	32	33.3%
Female	64	66.7%
Total	96	100%

Table.3 Distribution of isolates according to age groups

Age group	Number of isolates	Percentage %
5-24	16	16.7%
25-44	34	35.4%
45-64	31	32.3%
65-84	15	15.6%

Table.4 Distribution of isolates according to the TEM gene

TEM gene	Number	Percentage%
Positive	76	79.2%
Negative	20	20.8%
Total	96	100%

CHAPTER FOUR

CHAPTER FOUR

DISCUSSION

4.1 Discussion

Extended spectrum beta-lactamases (ESBL) are rapidly evolving group of beta-lactamase which share the ability to hydrolyse third generation cephalosporin. [62]

The present study aimed to Detect of Beta-lactamase TEM gene in Escherichia coli Isolates Collected from Urine Samples in Khartoum State – Sudan. Ninety-six *Escherichia coli* isolates were obtained from different hospitals.

The *Escherichia coli* isolates were more from female (66.7%) than male (33.3%) patients which is similar to a study in by Shafiq *et al.*, reported that Escherichia coli in female is more than male (68%) (32%) respectively. [63]

In the present study it was found that *Escherichia coli* can cause UTI at all ages and in both gender. The youngest in our study was 5 years old while the oldest was 84 years. The majority of the patients were in the age group of (25-44years) (34%) which is related to the age group (21-40 was (30%) of a study in Pakistan by Shafiq *et al.* [63]

In the present study 76(79.2%) of total isolate were found to be positive for TEM gene. This result agreed with study in Egypt by Noha *et al.,* (75%) and in Nigeria by Habeeb *et al.,* (73.3%) and higher than the percentage of Varun *et al.,* study which was (48%) in India. [64] [65] [66]

4.2 Conclusion

– In the present study TEM gene was positive for the most of *Escherichia coli* isolates species (79.2%).

– The study showed that the number of isolated *Escherichia coli* species in females (66.7%) was more than males (33.3%).

– The highest isolation of *Escherichia coli* was found within age group (25-44).

4.3 Recommendation

– Health educational programs must be improved to facilitate the prevention and control of *Escherichia coli* urinary tract infections.

– Antibacterial treatment should be done on the base of sensitivity test to reduce the development of resistance strains.

– Studies in herbal medicine for treatment of urinary tract infection.

– Phenotypic and Genotypic detection of other B-lactamase resistance gene.

– Further studies in different geographical locations with large numbers of samples and more advanced techniques such as sequencing are required to validate the results of the present study.

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Appendices

Figure (1): MacConkey agar shows Lactose fermenting colonies

A

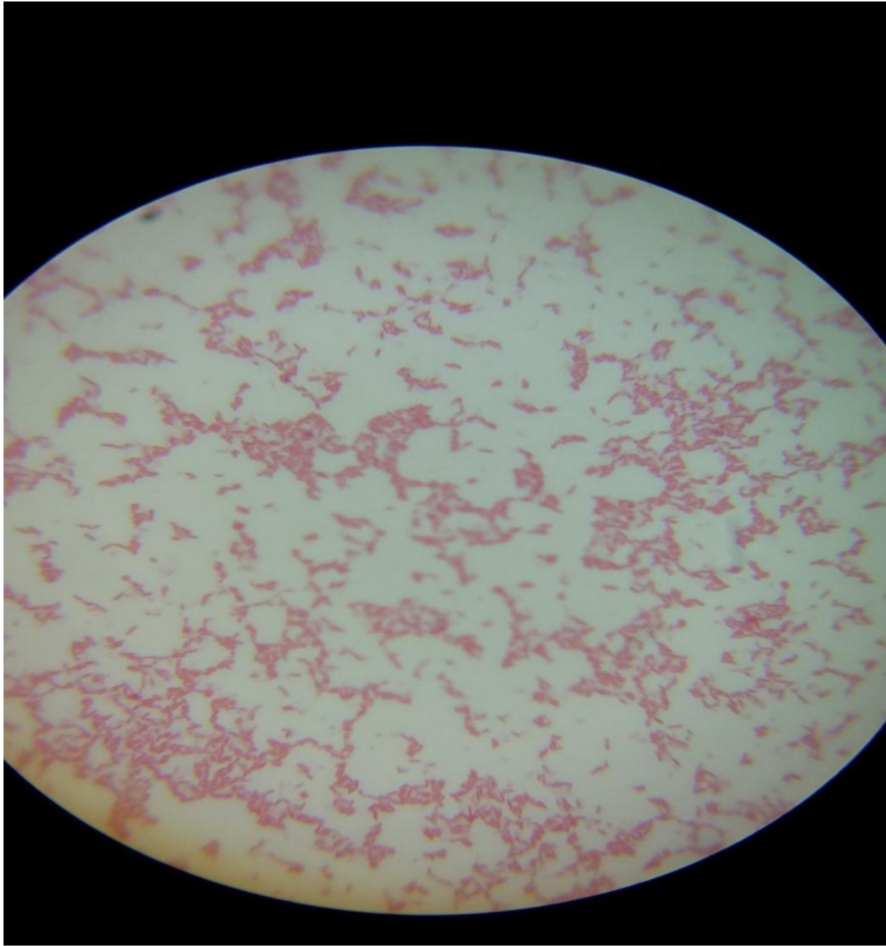

Figure (2): Gram stain shows Gram negative bacilli

Figure (3): Sterile Biochemical tests

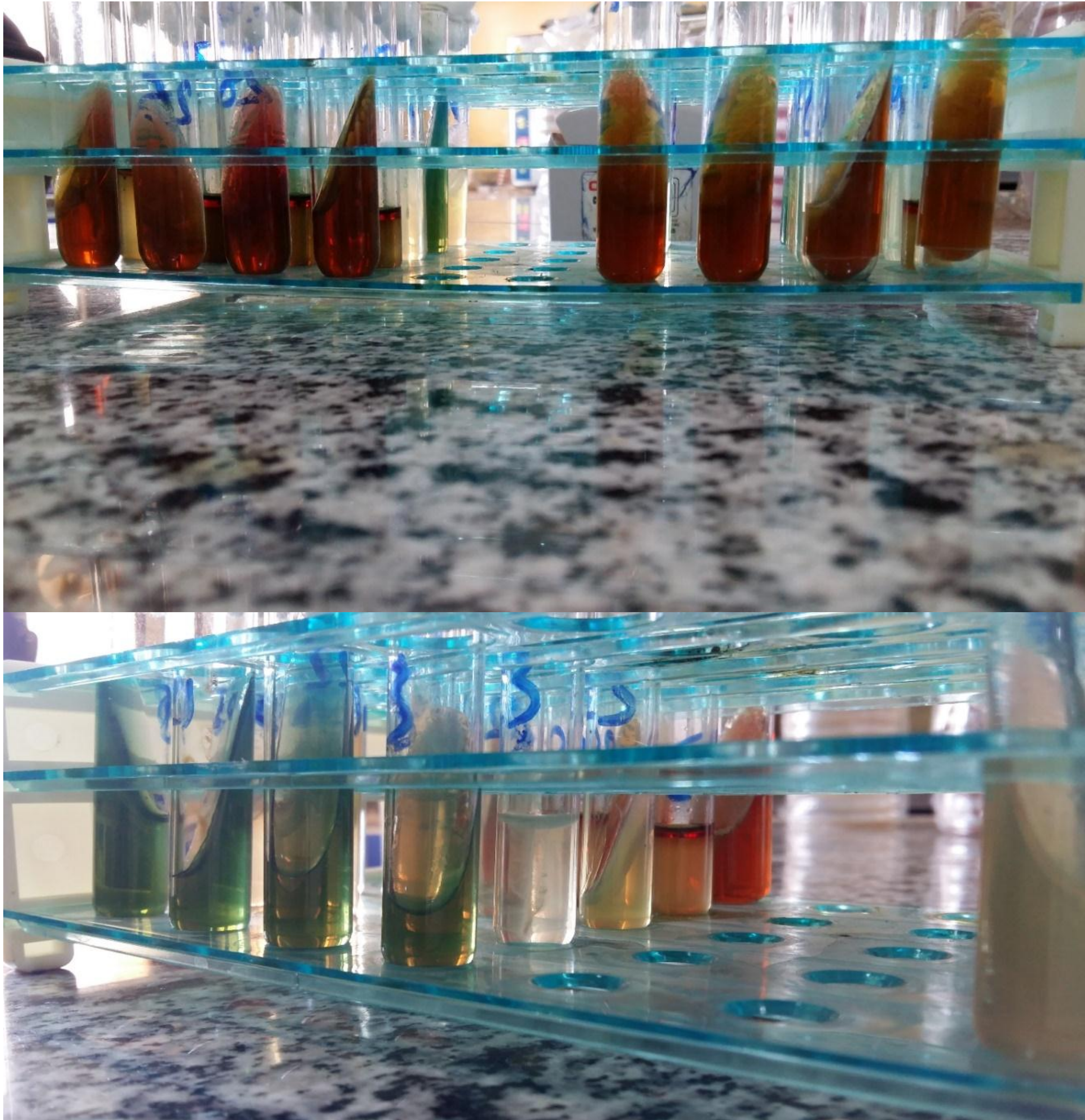

Figure (4): Result of Biochemical test identical for *Escherichia coli*

Figure (5): Thermocycler

Figure (6): Gel documentation System

Figure (7): Result of Gel Electrophorresis for PCR products of blaTEM gene (971 bp).

Lane 1: DNA marker.

Lane 2,3,4, 5, 7,8,9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 26, 27, 28, 30, 31, 32, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47: Positive samples.

Lane 6, 13, 14, 23, 29, 33, 35: Negative samples.

Lane 24: Positive Control.

Lane 25: Negative Control.

بسم الله الرحمن الرحيم

NAPATA COLLAGE

Faculty of Medical Laboratory Science

Department Of Medical Microbiology

Isolation of *Escherichia coli* from urine sample

NO ( )

1/ Your information will keep strictly confidential:

Name:…………………………………………………….

Date:…………………………………………………….

Age:……………………………………………………..

Gender Female ( ) Male ( )

2/ Do you massively use oral antibiotic without control :

Yes ( ) NO ( )