

## Phenotypic detection and Antibiogram of ESBL producing *Escherichia coli* isolated from Urinary Tract Infected patients at Khartoum state, Sudan

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### Abstract

*Urinary tract infection (UTI) is one of the most common bacterial infections and E. coli is the most common cause of UTI, and considered one of the common Multi-drug resistant Bacteria, especially those producing Extended-Spectrum Beta-Lactamases (ESBLs) have been reported worldwide. The aim of this study was to evaluate the Antibiotics sensitivity Profile and to screen and detect ESBL producing Escherichia coli from clinical isolates Among UTI patients in Khartoum, Sudan. A Ninety samples were isolated from patients Attending Elribat University Hospital, Military Hospital and Royal Care International Hospital, the isolates were purified and preserved and then were used for characterization and susceptibility testing. ESBL detection was performed on all the isolates by Ceftazidime Screening test, those that tested positive were subjected to ESBL confirmatory test by Double disk test. A total of 90 E. coli isolates were tested, comprising 39 (43.3%) of isolates from males and 51 (56.7%) isolates from female subjects. The most sensitive antibiotics Tested against isolated E. coli were Meropenem and Nitrofurantoin (97.8% and 82.2%, respectively) followed by Amikacin (77.8%), Ciprofloxacin (21.1%) and Cefepime (19%). All the E. coli isolates were*

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*tested for ESBL production, only 40 (44.4%) were found to be ESBL Producers. This study revealed the high prevalence of beta-lactamase producing E. coli, which has created a therapeutic challenge for the clinicians and microbiologists. Simple Disk method can be routinely employed to detect these common resistance mechanisms which will reduce the mortality and also reduce the spreading of such resistant Strains.*

**Key words:** Urinary tract infection (UTI), Extended-Spectrum Beta-Lactamases (ESBLs), clinical isolates Antibiotics sensitivity, Khartoum, Sudan.

## INTRODUCTION

Urinary tract infections (UTIs) most commonly affect either the lower urinary tract (infection of the urethra or bladder) or, less frequently, the upper urinary tract (acute pyelonephritis, or infection of the kidney). UTIs are termed “uncomplicated” when there is no underlying condition that increases the risk of infection, such as obstruction or urologic dysfunction. Most patients with UTI have uncomplicated cystitis, which is one of the most common infections in the United States, especially in sexually active women [1]

*Escherichia coli* is the most common cause of uncomplicated cystitis and pyelonephritis (70 to 95 percent of infections). Fecal contamination can lead to entry of an organism such as *E. coli* (one of the most common facultative organisms found in stool) into the urethra. These bacteria then move up into the bladder (and sometimes ascend into the kidney), producing infection. UTIs occur much more frequently in women as a result of the proximity of the urethral opening to the anus and the shorter length of the urethra before it opens into the bladder in women [1].

The increase of drug resistance among these organisms has made therapy of UTI difficult and has led to greater use of expensive broad spectrum antibiotics such as third generation of cephalosporin. Therefore, systematic monitoring of such resistance at local, national and international levels is recognized as an integral part of the control

strategy by most national and international organizations including World Health Organization.[2]

For a long time, the widespread use of antibiotics to treat *E. coli* infectious disease has rapidly increased the multidrug resistance (MDR) of *E. coli* [3, 4] especially with those strains producing ESBL [5, 6]

The appearance of ESBL stated in the 1980s and widely distributed in the world [7, 8] and conferred increased resistance to beta-lactams except carbapenems and cephamycins [9, 10]. ESBLs are plasmid mediated and the genes encoding these enzymes are easily transferable among different bacteria [11]. Most of these plasmids not only contain DNA encoding ESBLs but also carry genes conferring resistance to several non- $\beta$ -lactam antibiotics [12]. The presence of ESBL in clinical isolate has been documented as a very serious problem and a significant trait to: quick survival of patients in the hospital, high economic burden, loss of hours in life's activities and high treatment failure [13]. The phenotypic methods are currently the gold standard in determination of susceptibility or resistance of clinical isolates. The most widely used methods to screen ESBL are E-test, or double-disk synergy test (DDST) [14]. Several reports have described the prevalence of ESBLs in the Middle East North Africa region and most of the Gulf Cooperation Countries [15]. However, there is insufficient scientific data on the prevalence of ESBLs available from the States of Sudan.

This study aimed to determine the prevalence of ESBL production among *E. coli* isolates from Urinary Tract Infected patients at Khartoum state, Sudan.

## **MATERIAL AND METHODS**

### **Study design**

This was a descriptive cross-sectional study to assess the prevalence of extended spectrum beta-lactamase producing *Escherichia coli* isolated from UTI patients in Khartoum state-Sudan.

### **Study area and duration**

The samples of this study were collected from Elribat University Hospital, Military Hospital and Royal Care International Hospital,

Khartoum-Sudan, and the practical work was conducted in Microbiology lab, Faculty of Medical Laboratories Sciences, International University of Africa, Sudan. During the period from January to May 2017.

#### **Inclusion criteria**

All patients with Urinary Tract infection attending to Elribat University Hospital, Military Hospital and Royal Care International Hospital during study period were included in this study.

#### **Exclusion criteria**

All healthy people or those without Urinary Tract infection were excluded.

#### **Data collection**

A structured questionnaire was used; demographic data and other behavioral information were recorded from each patient.

#### **Ethical Consideration**

This study was approved by the ethical committee of the International University of Africa, Faculty of Medical Laboratory Sciences. Permission from Medical director Elribat University Hospital, Military Hospital and Royal Care international Hospital was applied and verbal consent was obtained from all subjects enrolled in the study.

#### **Sample size**

About 90 *Escherichia coli* isolated from patients who had Urinary Tract infection attending Elribat University Hospital (n=55), Military Hospital (n=7) and Royal Care international Hospital (n=28), during the study periods.

#### **Sample collection**

All 90 strains of *E. coli* were collected by Sub-cultured on sterile freshly prepared MacConkey agar for refreshment and further studies.

#### **Laboratory examination**

*Escherichia coli* isolates were re-identified by sub-cultured on sterile fresh prepared MacConkey Agar, and incubated at 37c overnight. After incubation period the growth look for pink color or lactose fermenting colonies. Isolated colonies were identified on basic result of gram-negative biochemical tests [16].

### **Biochemical tests:**

The biochemical activities of the purified isolates were then studied for identification and confirmation of these organisms. The biochemical tests carried out include:-

#### **Kligler Iron agar tests:**

Both the butt and the slant were streaked, to determine fermentation of glucose, lactose and to detect the production of hydrogen sulfide and gas. Inoculated test was incubated aerobically at 37°C for 24hr [16].

#### **Methyl red tests:**

It is used to detect the ability of some bacteria to produce significant amounts of acidic substances due to fermentation of glucose using methyl red- Voges- Proskauer medium, colour changes from yellow (pH6.2) to red (pH4.2) after addition of Methyl red reagent indicate acid production. Inoculated test was incubated aerobically at 37°C for 24hr [16].

#### **Citrate utilization test:**

It is based on the ability of some organisms to utilize citrate as the sole carbon and energy source for growth, and an ammonium salt as the sole source of nitrogen. Test organism was inoculated in Simon's citrate medium and incubated at 37°C for 24hr, the colour of the incorporated bromothymol blue indicator changes from green to blue, due to citrate utilization and production of alkaline pH [16].

#### **Indole production test:**

It is based on the ability of certain bacteria to oxidize the side chain of the amino acid Tryptophan with the production of indole. Test organism was inoculated in peptone and incubated for 24 hr at 37 °C, after incubation period, few drops of Kovac's reagent was added, a red colour developed indicate the production of indole [16].

#### **Urease test:**

It is based upon the presence of enzyme urease in the cells of certain bacteria. Urease enzyme catalyses the decomposition of urea with the production of ammonia. The test was carried out by growing the test organism in presence of urea and testing for ammonia production by means of suitable pH indicator e.g phenol red, colour changes from yellow to pink with ammonia production, indicating positive urease test [17].

### **Motility test:**

To determine whether the organism is motile or not it was stabbed with a straight inoculating needle, making a single stab about 1–2 cm down into the medium.

Motility was indicated by the presence of diffuse growth (appearing as clouding of the medium) away from the line of inoculation after incubation at 37°C for 24hr [18].

### **Sources of collection of antibiotic disc**

The antibiotic susceptibility testing discs was procured from local market manufactured by OXOID and Himedia.

### **Antimicrobial susceptibility testing**

The antimicrobial susceptibility testing of all identified isolates of *E. coli* samples was done according to the criteria of the Clinical and Laboratory Standards Institute method (CLSI) [19]. From a pure culture a loopful bacterial colonies was taken and transferred to a tube containing 5 ml of normal saline and mixed gently until it forms homogenous suspension. The turbidity of the suspension was then adjusted to the density of McFarland 0.5g in order to standardize the inoculum size [19].

A sterile cotton swab was then deep into suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Muller Hinton agar. The inoculated plates were left at room temperature to dry for 3-5 minutes [19].

With the aid of sterile forceps the following concentration of antibiotic disc were put on the surface of Muller-Hinton agar (Oxoid) include:

Meropenem (10mcg/disc), Ciprofloxacin (5mcg/disc), Nitrofurantoin (300mcg/disc), Cefepime (30mcg/disc), and Amikacin (30mcg/disc) [19].

The plates were then incubated at 37C for 24hours. Diameters of the zone of inhibition around the discs were measured using a digital caliper, and the isolates were classified as sensitive and resistant according to the standardized table supplied by CLSI 2014.

## **Phenotypic detection of ESBL producer's strains:**

### **ESBLs Screening:**

This test was done along with susceptibility testing of each isolate. All *E. coli* isolates were screened for ESBL production by using Cefotaxime (CTX 30ug), Ceftazidime (CAZ 30µg), and Ceftriaxone (CRO 30µg). Each *E. coli* isolates which showed resistant to one or more of these antibiotics were confirmed For ESBL production by double disk synergy test (DDST) as recommended by the CLSI guidelines [20].

### **ESBLs confirmation by DDST:**

Standardized inoculums of bacterial suspension equivalent to 0.5 McFarland standard turbidity of each isolate was inoculated on Mueller-Hinton agar plates (Himedia) by using a sterile cotton swab, then with sterile forceps the disk of Amoxicillin-clavulanic acid (MAC 30ug) was placed at centre of plate and the disks of Cefotaxime (30ug), Ceftazidime (30µg), and Ceftriaxone (30µg) were placed (centre to centre) at distance 20 mm from MAC 30ug disk. After incubation at 37 °C for 18hours aerobically, a clear extension of the edge of the inhibition zone of cephalosporin towards MAC 30ug disk was interpreted as positive for ESBL production(12). *E. coli* strains ATCC 25922 were used as negative controls and *E. coli* strains known as ESBLs positive by phenotypic and genotypic method (PCR and DNA sequencing) were used as a positive control [21].

### **Data analysis**

By using statistical packaged for social science (SPSS) software version 20.

## **RESULT**

A total of 90 *E. coli* strains were isolated from patients who suffer from UTI referred to three hospitals in Khartoum State, Elribat University Hospital (n=55), Military Hospital (n=7) and Royal Care international Hospital (n=28) as shown in **Table (1)**.

All isolated *E. coli* (n=90) were introduced to Antibiotics sensitivity test against Ciprofloxacin, Meropenem, Nitrofurantoin, Amikacin, Cefepime by using Kirby-Bauer disc diffusion method.

The most sensitive antibiotics tested against isolated *E. coli* were Meropenem and Nitrofurantoin (97.8% and 82.2%, respectively) followed by Amikacin (77.8%), Ciprofloxacin (21.1%) and Cefepime (19%) as described on **Table (2)**.

All *E. coli* isolates were tested for screening and confirmatory tests, 60 (66.6%) were positive ESBL and 30 (33.3%) were negative ESBL for screening test, while 40 (44.4) were positive ESBL confirmatory test and 50 (55.6) were negative ESBL production confirmatory test as shown in **Figure (1)** and **Table (3)**.

### **Prevalence of ESBL *E. coli* according to demographic characteristic of the studied patients**

The demographic characteristic of the studied patients is summarized in **Table (4)**. Out of the 90 *E. coli*, 39 (43.3%) were isolated from male patients, and 51 (56.7%) from female patients. However, the distribution of ESBL producers based on gender indicates that female had a higher prevalence rate of 49% than male, 38.5% ( $P= 0.9$ ). There was insignificant difference between the gender distributions. Otherwise, samples were collected from patients ranging in age from 2 year to over 61 years. The highest prevalence (52.4%) of ESBL was observed among the age group 21-40 years followed by the age group of less than 20 years (50%), age group 41-60 years (45.8%), age group more than 61 (33.3%) ( $P=2.129$ ). **Table (5)**

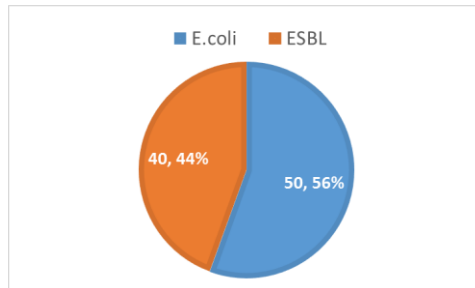
Low prevalence of ESBL *E. coli* was observed in Military Hospital with 4.4%; as compared to Royal Care International Hospital and Elribat University Hospital (11.1%) and (28.9%), respectively. This difference was significant among the three hospitals **Table (1)**. Antibiotic susceptibility of ESBL producers were evaluated for 5 antimicrobial agents. The majority of ESBL producers were resistant to Ciprofloxacin (70%), Followed by Cefepime (67.5%) as in **Table (6)**.



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**Table (1): Total of *E. coli* isolated from clinical Hospitals:**

Hospitals	<i>E. coli</i> N (%)	ESBL N (%)
Royal Care International Hospital	28 (31.1)	10 (11.1)
Elribat University Hospital	55 (61.1)	26 (28.9)
Military Hospital	7 (7.8)	4 (4.4)
<b>Total</b>	<b>90 (100)</b>	<b>40 (44.4)</b>



**Figure (1): ESBL producers among clinical isolates *E. coli*.**

**Table (2): antibiotic sensitivity test of selected antibiotics against isolated *E. coli*:**

Antibiotic	Conc. (µg)	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikacin	30	70 (77.8)	1 (1.1)	19 (21.1)
Cefepime	30	17 (19)	12 (13)	61 (68)
Ciprofloxacin	5	19 (21.1)	6 (6.7)	65 (72.2)
Nitrofurantoin	300	74 (82.2)	8 (8.9)	7 (7.8)
Meropenem	10	88 (97.8)	2 (2.2)	-

**Table (3): Distribution of ESBL strains according to screening and confirmation test:**

Test	Positive ESBL		Negative ESBL		Total	
	No	(%)	No	(%)	No	%
ESBL screening	60	66.6	30	33.3	90	100
ESBL DDST	40	44.4	50	55.6	90	100

**Table (4): Frequency and percentage of ESBLs producer and non-ESBLs producer *E. coli* isolates among gender:**

Gender	ESBLs		Total (%)	P. Value
	Positive (%)	Negative (%)		
Male	15 (38.5)	24 (61.5)	39 (100)	0.9
Female	25 (49)	26 (51)	51 (100)	
<b>Total</b>	40 (44.4)	50 (55.6)	90 (100)	

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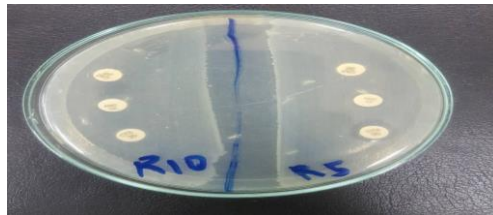
**Table (5): Frequency and percentage of ESBLs producer and non-ESBLs producer *E. coli* isolates among age group:**

Age group	ESBLs		Total (%)	P. Value
	Positive (%)	Negative (%)		
Less than 20	9 (50)	9 (50)	18 (100)	2.129
21-40	11 (52.4)	10 (47.6)	21 (100)	
41-60	11 (45.8)	13 (54.2)	24 (100)	
More than 61	9 (33.3)	18 (66.7)	27 (100)	
Total	40 (44.4)	50 (55.6)	90 (100)	

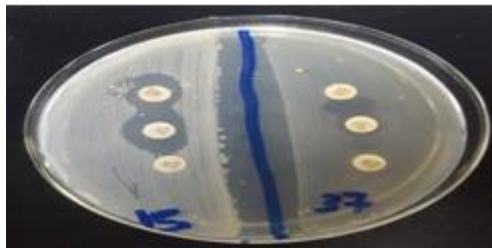
**Table (6): Antibiotic sensitivity profile of selected antibiotics against ESBL producing strains:**

Antibiotic	Conc. (µg)	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikacin	30	30 (75)	1 (2.5)	9 (22.5)
Cefepime	30	7 (17.5)	6 (15)	27 (67.5)
Ciprofloxacin	5	11 (27.5)	1 (2.5)	28 (70)
Nitrofurantoin	300	34 (85)	3 (7.5)	2(5)
Meropenem	10	40 (100)	-	-

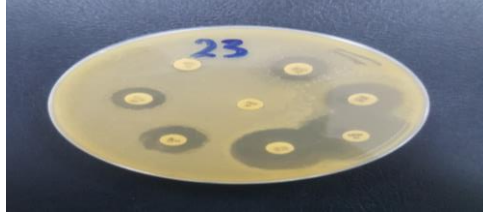
**Figure (2): ESBLs producing *E. coli* image inoculated on Mueller Hinton agar (MHA) media by Double Disk test.**



**Figure (3): Non-ESBLs producing *E. coli* image inoculated on Mueller Hinton agar (MHA) media detected by Double Disk test.**



**Figure (4): Antibiotic sensitivity test of selected antibiotics against *E. coli* isolate, inoculated on Mueller Hinton agar (MHA) media.**



## DISCUSSION

Urinary tract infections are the most common bacterial infection[22]. *Escherichia coli* is the most common organism causing urinary tract infection (UTI). Extended spectrum beta - lactamases (ESBLs) are on the rise in hospital settings across the globe [23]. The antimicrobial resistance patterns of organisms-causing UTI are changing over the years, including resistance due to ESBL producing pathogens. Correct identification of ESBL producing organisms in due time is necessary not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organism[24].

In this study the ESBLs producing uropathogenic *E. coli* isolates were 44.4%. This finding is a little bit higher than those obtained from the studies done by Al mugadam [25] and Mutasim [26] from Sudan where ESBL were produced by 35% and 30.2% respectively. Another study conducted by Datta [27], Dugal and purohit,[28], Dissanayake [29] and Singh [30] who reported 21.4%, 24.4%, 29% and 82.6% ESBL producing *E. coli* isolates respectively. The result of this study is much less than Mohanty [31] in India, where they observed ESBL production in 71.5% of the Gram-negative bacilli. other studies such as Mahesh [32] ;Chaudhary and Murthy [33] who reported 56.2% and 54.5% ESBL production in *E. coli* isolates respectively.

However our study reports ESBL producer is (49%) in female and (38.5%) males respectively. This finding are higher than result obtained by studies done by Al mugadam [25] that report ESBLs producer are 30% in male, and 38% in female, and by Rajan and

Prabavathy, [34] who report ESBLs 13.9% in male, and 20.8% in female. And lower than studies done by Vidhya and Sudha,[35] who report ESBLs 47.22% in male, and 52.77% in female.

This study showed that all ESBLs producer were sensitive to Meropenem (100%); and most sensitive to Amikacin (75%), while highly resistant to Ciprofloxacin (70%), Cefepime (67.5%)

This result agree with other study which done by Al mugadam [25] who report that all ESBLs producer were sensitive to Nitrofurantoin (85%); and the resistant to Ciprofloxacin (70%).

Our findings also agree with another study which done by Shafaq [36] who report that most of ESBL producers were resistant to ciprofloxacin (85%).

## CONCLUSION

The present study revealed the high prevalence of ESBL producing *E. coli* among collected isolates (44.4%) confirming the increasing spread of such resistant strains. Among isolated *E. coli* the highest sensitivity result was given by Meropenem followed by Nitrofurantoin, Amikacin, Ciprofloxacin and Cefepime.

The distribution of ESBL producers based on gender and age groups indicates that the highest prevalence of ESBL was observed among female than male, and among the age group 21-40 years than other groups.

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