

## Mechanisms of Resistance to Antibiotics in *Escherichia Coli* from Patients with Urinary Tract Infections in Egypt

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**Abstract:** Urinary tract infections considered as the most public Infectious diseases in the world. *Escherichia coli* are the most public Gram negative bacteria which causes of both community-acquired and nosocomial transmitted UTIs. **Methodology:** In present study, a total of one hundred and fifty of urine samples collected from children aged from one month to fourteen years old of urinary tract infection, and after the Positive cultures about fifty isolates were identified as *E.coli*. The mechanisms of resistance to antibiotics in *E.coli* isolates were evaluated by using the antibiotic susceptibility and the MIC which were determined through standard disk diffusion method and E-test strips; respectively. **Results:** The resulted antibiogram patterns of the isolates showed that many resistant strains possessed resistance to different group of antibiotics such as  $\beta$ -lactams, sulpha drugs, and quinolones. Genotypic determinations of the above resistance were done resulted in presence of TEM, SHV, CTX-M, Sul-1 and Gyr-A mediated genes. **Conclusion:** In this study concluded mandatory surveillance is recommended to be extended to include the community UTIs to allow gaining a better understanding of ESBL (Extended Spectrum Beta Lactamases) producing *E.coli*. And the Monitoring of antimicrobial resistance is necessary to avoid treatment failure in patients with urinary tract infections.

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**Key words:** urinary tract infection, *Escherichia coli*,  $\beta$ -lactamase resistance, antibiotic susceptibility

### 1. Introduction:

UTI is a very common infection in both the community and hospital patients [1] and uropathogens have shown a slow but steady increase of resistance to several agents over the last decade.[2] *E.coli* and other *Enterobacteriaceae* have become less susceptible to commonly used antimicrobials such as  $\beta$ -lactam Antibiotics, trimethoprim\ sulphamethoxazole (SXT) and Fluoroquinolones[3,4] It is well known that the mechanism of antimicrobial resistance could happen by enzymatic inactivation, altered receptors or by altered antibiotic transport mechanism[5], by expulsion of the antimicrobial agents from the cell via general or specific efflux pumps[6], and or by modification of the antimicrobial target within the bacteria. In general, current knowledge of antimicrobial susceptibility pattern of uropathogens is mandatory for appropriate therapy [7] and we studied these resistances which found in our isolates and studying the mechanisms of these resistances in our isolates.

### 2. Material and Methods:

#### Collection of Clinical Samples:

In present study a total of one hundred and fifty urine samples were collected using urine bag from

the Hospital of Beni-Suef University during the last 2 years. And the urine samples were collected from children aged from one month to 14 years old and in case of infant above one UTI be defined by detection of  $10^5$  cfu/ml of tested urine sample.

#### Isolation & identification:

Urine samples were plated on nutrient agar, then further procedures and the biochemical. Identifications were done according to Shohreh Farshad RR [8].

#### Antibiotic Susceptibility:

All isolates were tested against different antibiotics, These antibiotics included cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), sulphamethoxazole\trimethoprim (SXT), ofloxacin (OF), norfloxacin (NOR) and gentamycin (HLG), and using also the following combination disks for detection of  $\beta$ -lactams as cefotaxime\clavaunin (CCT), ceftazidime\clavaunin (CAC), and amoxicillin\clavaunin (AMC). Muller Hinton agar (Oxoid) was used according to Kirby Bauer method[9].

#### MIC determination:

AMC, CTX, CAZ and SXT MICs were detected using E.test (Liofilmchem) for samples which showed high different resistances among isolates in

disk diffusion methods. And the interpretations were done according to Antimicrobial Susceptibility Testing in European Committee.

#### Phenotypic Characterization of $\beta$ -Lactamases:

The phenotypic characterization of  $\beta$ -Lactamases enzymes were done according to CLSI

guidelines (2012) including double disc potentiation test and the combination tests.[10] Furthermore, nitrocefin test.[11] The discs were moistened with 1mg of nitrocefin dissolved in 100ml of dimethylsulfoxide then several colonies of *E.coli* were applied to it.

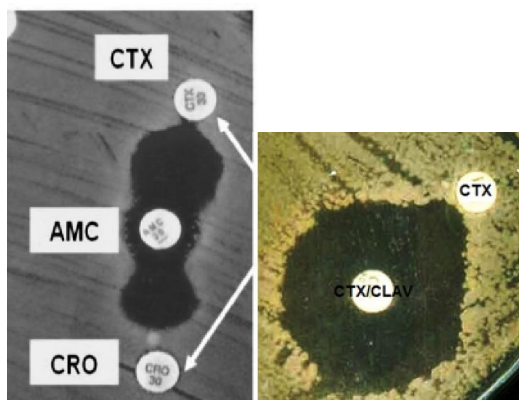


Fig (1) Pattern of Double Disk Potentiation Test

Fig (2) Combined test of  $\beta$ -Lactamases

#### Genotypic characterization:

PCR was done for the isolates of concern using for mentioned primers in Table.1.

Antimicrobial	Group	Primer name	Internal number	Sequence
Beta-lactams	SHV	TEM front P1	Primer 757	5'-GCGGAACCCCTATTTG-3'
		TEM-C-R-ny	Primer 686	5'-ACC AAT GCT TAA TCA GTG AG-3'
	CTX	ctx M U1	Primer 1354	-5'-ATGTGCAGYACCAGTAARGTKATGGC 3'
		CTX-M-U-2new	Primer 1580	5'-TGGGTRAARTARGTSACCAGAAYSAGCGG-3'
	SHV	SV OHS5	Primer1545	5'-TTATCTCCCTGTTAGCCACC-3'
SHV OS6		Primer 1546	5'-GATTTGCTGATTTTCGCTCGG-3'	
Quinolones		<i>E.coli</i> GyrAF (166 355)	Primer1662	5'-ACGTACTAGGCAATGACTGG-3'
		<i>Ecoli</i> GyrAR	Primer1663	5'-AGAAGTCGCCGTCGATAGAAC-3'
Sulfamethoxazole		Sul 1 forward	Primer319	5'-TGA GAT CAG ACG TAT TGC GC-3'
		Sul 1 backward	Primer320	5'-TTG AAG GTT CGA CAG CAC GT-3'

### 3. Results:

A number of 50 isolates conformed the study criteria. The antimicrobial susceptibility testing of all isolates towards CAZ, CTX, AMC, OF, NOR, CRO, CAC, CCT, HLG, SXT and FEB were determined by the disk diffusion method according to the clinical laboratory standards institute (CLSI, 2012). Among fifty isolates of *E.coli* showed variable resistance toward the selected antibiotics. A relatively high resistance toward AMC, SXT, CAZ, and CTX, reached 73%, 65%, 45% and 43%; respectively. And on other hand the low resistances were recorded for FEB (14%), HLG (13%), OF (8%), and NOR (8%). The most potent antimicrobial agents were CAC and

CCT as all isolates were susceptible. 45% of test organisms were regarded as ESBL producers as it confirmed by combined test. Further phenotypic tests were done using nitrocefin test for colorimetric detection of  $\beta$ -lactamase activity among the 50 isolates of *E.coli*.  $\beta$ -lactamase activity was indicated by color change from yellow to red within 1-2 min. A total of 45 of *E.coli* isolates (90%) showed positive  $\beta$ -lactamase activity. The E-test was determined for selected representative samples based on their antibiogram pattern. The results showed high resistance toward the 3<sup>rd</sup> generation cephalosporin; however SXT showed highest sensitivity as shown in table.2.

**E.test results**

		RIS		RIS		RIS		RIS		RIS		RIS		RIS
E-Test	no. 55	no. 55	no.8	no.8	no. 30	no. 30	no.37	no.37	no. 23	no. 23	no.46	no.46	no.45	no.45
CAZ	256	R	-		256	R	256	R	256	R	256	R	-	-
AMC	192	R	12	R	4	R	256	R	96	R	16	R	256	R
CTX	64	R	-		16	R	96	R	64	R	64	R	-	-
SXT	24	R	1.5	S	2	R	3	R	32	R	-	-	-	-

**The PCR results:**

Were analyzed on 1% agarose gel, stained with ethidium bromide and the bands were visualized under UV illumination and the result indicated.

No.	Primername	Sequence (5' to 3')	Positive Samples
TEM	TEM front P1 TEM-C-R-ny	GCGGAACCCCTATTTG ACCAATGCTTAATCAGTGAG	23, 30, 46
CTX	ctx M U1 CTX-M-U-2new	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYSAGCGGG	30, 45, 46, 55
SHV	SHV OS5 SHV OS6	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTTCGCTCGG	30
gyrA	E.coliGyrAF EcoliGyrAR	ACGTACTAGGCAATGACTGG AGAAGTCGCCGTCGATAGAAC	30
sul1	sul1F sul1R	TGA GAT CAG ACG TAT TGC GC TTGAAGGTTTCGACAGCACGT	23

**4. Discussion:**

Relying on phenotypic test like antibiotic susceptibility profile and combination test could estimate the broad type of enzyme Resistance toward 2nd generation cephalosporins indicates extended-spectrum  $\beta$ -lactamase (ESBL) production, ESBL production can be confirmed later with double disc synergy tests (The confirmatory tests for presence of ESBL) and combination test. Indicated that The effect of clavulanic acid on the susceptibility was high in most of isolates and showed a difference in the inhibition zone more than or equal to 5 mm between CTX, CAZ, and CTX/CLAV, CAZ/CLAV respectively. On the other hand, with the emergence of CTX-M ESBLs, it is essential to use cefotaxime in addition to ceftazidime as an indicator. Some of the isolates showed a resistance toward CTX, CAZ and CRO antibiotics and could be predicted as CTX-M producers, [10] in contrast with the TEM- and SHV-type ESBLs [16]. During the past decade CTX-M-type ESBLs have emerged and increased worldwide in proportion to the other ESBL types, in single and epidemic clinical isolates. [17] also found that *E.coli* have the high resistance to  $\beta$ -lactams antibiotics due to production of  $\beta$ -Lactamases enzymes, *E.coli* also exhibits resistance to different classes of antibiotics like quinolones and SXT. that Studies indicated on

virulence of *E. coli* that *E.coli* strains which are resistant to quinolones less virulence than *E.coli* strains which are susceptible to quinolones. And also found highly resistant to SXT.

**$\beta$ -Lactamase resistance** expression is principle mechanisms of Gram-negative resistance, found in about 45% in our isolates [18], and  $\beta$ -Lactamases considered as enzymes cause hydrolysis affect amide bond in  $\beta$ -Lactam ring and deactivated the antimicrobial before reach the site of cell wall synthesis [19]. Sometimes reduced the permeability of the outer membrane to  $\beta$ -lactams as a result of porin loss or changes in porin structure can promote resistance to these antimicrobial agents [18]. A major contribution to antibiotic resistance in Gram-negative species is the presence of broad-specificity drug-efflux pumps, and to reduce the effect of the resistance,  $\beta$ -lactamase resistant  $\beta$ -lactams compound were developed. And  $\beta$ -lactamase inhibitors, such as clavulanic acid can be administrated with susceptible  $\beta$ -lactams.

And also found high resistance SXT among the isolates of *E.coli* (73%). SXT Traditionally a front-line therapy for UTIs, their utility has decreased in certain areas due to increasing resistance. SXT inhibit dehydrate folate reductase, and dihydro pterate synthetase, respectively, and resistance to SXT can

be mediated by horizontal transfer of genes encoding resistant versions of these enzymes. A study of SXT-resistant isolates found that 73% of them may have a *Sul 1* gene encoding a sulphamethoxazole-resistant dihydro pterate synthetase. The presence of these genes on integrons and plasmids facilitates their spread among bacterial populations [20].

Finally **Quinolones**, such as NOR and OF, They are currently recommended for use as second-line agents for uncomplicated UTIs, and front-line therapy for nosocomial UTIs and pyelonephritis. Resistance to these agents is largely due to alteration in the *gyrA* gene which encoded in the gyrase enzyme the studies reported resistance rates of 10-12%, [21] in the Gram-negative organisms the DNA gyrase enzyme considered as the primary target. [22] The quinolone action can summarize in two steps: formation of DNA drug complex followed by break of DNA by the release of lethal double stranded. [23,24]

#### Conclusion:

Our findings support the hypothesis that CTX-M enzymes will become the dominant ESBLs among *E. coli* worldwide [25]. and we found that high percent of resistance of  $\beta$ -lactam group more than other groups indicated that's there is over prescribing of these antibiotics groups from the hospital physicians which result in antibiotic pressure and leading to persistence of resistance genes of  $\beta$ -lactamase in hospital microorganisms. finally Antibiotic resistance as a phenomenon is in itself, not surprising. Nor is it new. However, it is newly worrying because it is accumulating and accelerating, while the world's tools for combating it decrease in power and number.

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#### Conflict of Interest:

No conflict of interest is declared.

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