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Rapid Detection of Extended Spectrum B-lactamase (ESBL) Producing Strain of *Escherichia coli* in Urinary Tract Infections Patients in Benha University Hospital, Egypt

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Authors' contributions

This work was carried out in collaboration between the two authors. Author ESK planned and designed the study, wrote the protocol, collected the samples, performed the practical laboratory activities, participated in the interpretation of the results and analysis, drafted and critically revised the manuscript. Author HWS participated in planning and designing the study, sample collection, participated in the interpretation of the results. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: An increase in *extended spectrum β-lactamase (ESBL)*-producing Escherichia coli *(E. coli)* has been observed.
Aims: Of this study was done to detect the prevelance of ESBL, AmpC producing and ESBL and AmpC co-producing strains of *Escherichia coli (E. coli)* in urinary tract infections patients in Benha University Hospital and to evaluate the performance of CHROMagar™ ESBL media for rapid screening of ESBL producing *E. coli.*
Place and Duration of Study: This is a Six-months Cross sectional study conducted in Urology and Microbiology & Immunology departments, Benha University, Egypt.
Methodology: All patients under study were subjected to: Full history taking and clinical examination. Bacteriological study included; urine sample collection from each patient and subjected to urine analysis, urine culture on cysteine lactose electrolyte deficient agar (CLED) agar, CHROMagar™ ESBL media and MacConkey agar supplemented with 2 mg/liter ceftazidime (MCKC). ESBL detection in *E. coli* isolated on CLED agar by phenotypic screening by clinical and laboratory standards institute (CLSI) method then

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phenotypic confirmation by E. test. The presence AmpC beta-lactamase ESBL was detected by AmpC disc test and detection of AmpC beta-lactamase and ESBL co-producers by cefepime and Cefepime + Clavulanate E test.

Results: In this study out of 45 E. coli strains 24 (53.3%) ESBL producers were detected by E. test (golden method for confirmation of ESBL according to CLSI) and 21(46.7%) strains were non ESBL producers. There was no significant difference between ESBL isolation from community acquired and health care associated UTI patients; out of the 24 isolated ESBL producing *E.coli* strains 9 (37.5%) were detected in community acquired UTI patients while 15 (62.5%) were detected in health care associated UTI patients. The sensitivity of both MCKC and CHROMagar™ ESBL media were 100% (95%CL: 85.6% to 100%).While specificity were 87.5% (95%CL:67.6% to 97.2%) and 80.8% (95%CL: 60.6% to 93.4%) respectively. In our study out of 45 isolated *E. coli* strains 14 (31.1%) were AmpC producers by AmpC test, 4 (8.9%) were AmpC and ESBL co-producers by cefepime/ cefepime clavulanic E.test.

Conclusion: It is important to know the prevalence of ESBL, AmpC producing and ESBL&AmpC co-producing organisms so that judicious use of antibiotics could be done and increase awareness about the need for routine detection of AmpC and ESBL in clinical isolates. CHROMagar™ ESBL media detect ESBL producers from clinical specimen and give rapid presumptive identification by means of colony colour after 24h with good sensitivity and specificity.

Keywords: E. coli; ESBL; AmpC; CHROMagar; UTI.

1. INTRODUCTION

Microorganisms responsible for urinary tract infection (UTI) such as *E. coli* and *Klebsiella* spp. have the ability to produce ESBL in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat [1]. Enterobacteriaceae, especially *E. coli* and *Klebsiella* spp.-producing ESBLs such as SHV and TEM types, have been established since the 1980s as a major cause of hospital-acquired infections. However, during the late 1990s, several community-acquired pathogens that commonly cause urinary tract infections and diarrhea have also been found to be ESBL producers. These include *E. coli*, *Salmonella*, *Shigella* and *Vibrio Cholera* [2].

ESBLs are enzymes capable of conferring bacterial resistance to the penicillins, first, second- and third-generation cephalosporins and aztreonam. They are not active against the cephamycins (cefoxitin and cefotetan), but are susceptible to β- lactamase inhibitors (clavulanic acid) [3].

Genotypic methods based on enzyme assays, PCR and others are not suitable for routine clinical testing in most clinical diagnostic laboratories. The phenotypic confirmatory tests are highly sensitive and specific compared to genotypic confirmatory tests [4].

Phenotypic tests, which require a screening step followed by confirmation used to detect ESBL in most microbiology laboratories. The screening test is based on testing the organism for resistance to an indicator cephalosporin. Cefpodoxime is commonly used as it is hydrolysed by TEM, SHV, and CTX-M types, but other cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime are also used. To confirm the presence of an ESBL, synergy between the indicator cephalosporin and clavulanic acid needs to be demonstrated (ESBLs are inhibited by clavulanic acid) [5]. E test is a reliable method for detection of ESBL by MIC

reduction. Two different E test gradient formats have been in use based on reduction of ceftazidime or cefotaxime MICs by 3 two-fold dilutions in the presence of clavulanic acid and have been used successfully for ESBL detection [6,7].

Various selective media have been proposed in order to assess the presence of ESBL producers in stool and urine samples. Examples of such media include Drigalski agar supplemented with cefotaxime, MacConkey agar supplemented with ceftazidime or nutrient agar supplemented with ceftazidime, vancomycin and amphotericin B. Chromogenic media were developed for isolation of ESBL from clinical specimens. CHROMagar™ ESBL media allows the detection of ESBL-producing bacteria while inhibiting the growth of other bacteria. Identification of ESBL producers depend on colony colour after 24h incubation [8].

The ESBL phenotypes have become more complex due to the production of multiple enzymes which include the inhibitor-resistant ESBL variants and plasmid-borne AmpC. AmpC is normally produced in low levels by many organisms and is not associated with resistance, but it can be produced at high levels and cause resistance to all beta lactams, except carbapenems and 4th generation cephalosporins. These enzymes have spread worldwide and their prevalence varies by the geographical area. Many clinical laboratories currently test *E. coli* and *Klebsiella* spp. for production of ESBLs but do not attempt to detect plasmid mediated AmpC beta lactamases. These enzymes are typically associated with multiple antibiotic resistances, leaving a few therapeutic options [9].

The present study was done to detect the prevelance of ESBL, AmpC producing and ESBL and AmpC co-producing strains of *E. coli* in urinary tract infections patients in Benha University Hospital and to evaluate the performance of CHROMagar™ ESBL media for rapid screening of ESBL producing *E. coli.*

2. MATERIALS AND METHODS

2.1 Studied Subjects

This study was done in Urology and Microbiology and Immunology departments, Benha University, Egypt from October 2012 to March 2013. Out of the 135 urine samples collected from patients suspected to have UTI, a total of 100 were culture positive on CLED, collected from 100 patients (56 males and 44 females) who were diagnosed as UTIs patients in Benha University hospital. Their ages ranged from 20 to 68 years. The diagnosis of UTIs in urine samples was based on the presence of 10^5 CFU of microorganisms per ml in urine culture on CLED [10]. Full history taking and clinical examination with specific stress on fever, urgency, frequency, dysuria, suprapubic tenderness and costovertebral angle pain and tenderness.

2.2 Urine Sample Collection

Early morning and mid-stream urine is collected in a sterile container. If collected from indwelling catheter the wall at the juncture with the drainage tube was disinfected and sterile syringe was used for the urine specimen collection. Viable bacterial count was performed for urine samples using the pour plate method [10]

2.3 Urine Culture

The urine samples were centrifuged; cultures were done from the deposit on CLED agar, all plates were incubated at 37ºC for 48 hrs.

Culture on CHROMagar™ ESBL(CHROMagar, France) which was freshly prepared on the day of the study according to the manufacturer's instructions and poured into 90-mm-diameter petri dishes. Urine was directly inoculated onto a CHROMagar plate and streaked for colony isolation. The plates were incubated overnight at 35ºC in ambient air and then examined for any growth [11]. Interpretation of CHROMagar™ ESBL according to colony colour *E. coli* dark pink to reddish colonies, Klebsiella, Enterobacter, Citrobacter spp. Metallic blue colonies, Proteus brown halo colonies and Non Resistant Other Gram(-) strains and gram(+) strains inhibited.

Culture on Mac Conkey agar supplemented with 2 mg/liter ceftazidime (MCKC). The plates were incubated overnight at 35ºC in ambient air and then examined for any growth.

Identification of bacterial isolates by colony morphology, gram stain and biochemical reactions [12]

2.4 Testing for the ESBL Production

2.4.1 Phenotypic screening CLSI method

- *E. coli* isolates were screened for ESBL production by the disc diffusion method according to the CLSI guidelines [7]. The following antibiotics were used; cefotaxime (30µg), cefpodoxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), cefepime (30µg), aztereonam (30µg). (Oxoid, UK). This screening method based on measuring the specific zone diameters for the antibiotic discs.
- All the strains which showed a zone diameter of ≤17 mm for cefpodoxime, ≤22 mm for ceftazidime, ≤27 mm for aztreonam, ≤25 mm for ceftriaxone and ≤27 mm for cefotaxime were selected for checking the ESBL production as was recommended by CLSI M100-S21 (2010) [7]. Every isolate that showed resistance to at least one of the screening agents was tested for ESBL production. The use of more than one of these agents for screening improves the sensitivity of detection.

2.4.2 Phenotypic confirmatory test by ESBL-E-test

The ESBL-E-Test strips were obtained from (AB biodisc, Solna, Sweden) ceftazidime/ ceftazidime + clavulanic acid (TZ/TZL) and cefotaxime and cefotaxime + clavulanic acid (CT/CTL) in accordance with the manufacturer's instructions. One end of each strip contains a gradient concentration of either ceftazidime (TZ) (MIC range 0.5 to 32 µg/ml) or cefotaxime (CT) (MIC range of 0.25 µg to 16 µg). The other end of the strip with a gradient of ceftazidime plus a constant concentration of clavulanate TZ/TZL (0.064-4 µg/ml plus 4 µg/ml of clavulanic acid) or with a gradient of cefotaxime plus a constant concentration of clavulanate CT/CTL (0.25 µg- 16 µg plus 4 µg of clavulanic acid) [13]. After overnight growth, the organism was emulsified in saline solution to a turbidity of 0.5 McFarland standard. The suspension was spread on a Muller Hinton agar plate with a cotton swab. After the plates were dried for 15 min, the E-Test strips were placed on them, after incubation at 35ºC for 18 hrs. The MIC was interpreted as the point of intersection of the

inhibition ellipse with the edge of the test strip. After overnight growth, the organism was emulsified in saline solution to a turbidity of 0.5 McFarland standard. The suspension was spread on a Muller Hinton agar plate with a cotton swab. After the plates were dried for 15min, the E-Test strips were placed on them after incubation at 35ºC for 18 hrs. The MIC was interpreted as the point of intersection of the inhibition ellipse with the edge of the test.

1) ESBL positive:

   - If CT≥0.5 and CT/CTL≥8 or TZ≥1 and TZ/TZL≥8.
   - Presence of a phantom zone or ellipse deformation.

2) ESBL Negative: If CT<0.5 or CT/CTL<8 and TZ<1 or TZ/TZL<8.

3) Non-determinable (ND): CT>16 and CTL>1 and TZ>32 and TZL>4. Strains showing non-determinable (ND) results with CT/CTL and TZ/TZL strips should be further tested using PM/PML strips for detection of Amp C beta-lactamase ESBL co-producers.

2.5 Ampc Beta-lactamase Detection by Ampc Disc Test

The test is based on use of Tris EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. Cefoxitin (30µg). (Oxoid, UK) resistant *E. coli* isolates tested by AmpC disc test as following. [14].

A lawn culture of a 0.5 McFarland's suspension of ATCC *E. coli* 25922 (bioMérieux) was prepared on a Mueller-Hinton agar plate. A 30 µg cefoxitin (fox) disc was placed on the inoculated surface of the agar. A sterile AmpC discs (i.e., filter paper discs containing Tris-EDTA) obtained from (Becton Dickinson, Sparks, MD) which was inoculated with several colonies of the test organism was placed beside the cefoxitin disc, almost touching it.

After an overnight incubation at 37ºC, the plates were examined for either an indentation or a flattening of the zone of inhibition, which indicated the enzyme inactivation of cefoxitin (positive result), or an absence of distortion, which indicated no significant inactivation of cefoxitin (negative result).

2.6 Ampc Beta-lactamase ESBL Co-Producers Detection

2.6.1 Cefepime/cefepime + clavulanic acid (PM/PML) strips

All non-determinable ESBLs by TZ/TZL and CT/CTL E test were further tested using PM/PML strips for detection of AmpC beta-lactamase ESBL co-producers. [15]

One end of each strip contains a gradient concentration of cefepime (PM) (MIC range 0.25-16 µg/mL). The other end of the strip with a gradient of cefepime plus a constant concentration of clavulanate PM/PML (0.064-4 µg/mL plus 4 µg/ml of clavulanic acid). The results were interpreted as positive.

- If the MIC ratio for PM/PMLwas ≥8.
- Presence of a phantom zone, deformation or ellipse.

2.7 Statistical Analysis

Data were entered into a database using SPSS 13 for Windows (SPSS Inc., Chicago, IL). Sensitivity: the ability of the test to detect true positive cases and specificity: the ability of the test to detect true negative cases. [16].

3. RESULTS

In this study 45 patients were out-patients and 55 in-patients admitted in Urology department. Out of 55 in-patients 30 patients were catheterized and 25 were non catheterized. 105 isolates on CLED agar were obtained from the studied 100 patients urine samples, while most samples yielded only single isolate, five samples yielded two isolates. The most common isolated microorganisms were *E. coli* 45 (42.9%) followed by *Klebsiella pneumonia*e 22 (21%).

Out of *45 E. coli* strains 24 (53.3%) ESBL producers were detected by E. test (golden method) and 21 (46.7%) strains were non ESBL producers as shown in Fig. 1.

Table 1 shows that out of the 24 isolated ESBL producing *E. coli* strains 9 (37.5%) were detected in community acquired UTI patients while 15 (62.5%) were detected in health care associated UTI patients.

Table 1. Distribution of ESBL producing *E. coli* among community acquired and health care associated UTI infections

	Community acquired UTI		Health care associated UTI	
ESBL producing	NO	%	NO	%
E. coli (n=24)	9	37.5	15	62.5

Z=1.7 and P =0.08

Table 2 shows that out of 45 *E. coli* isolates 16 (35.6%) yielded no growth on any selective media and 29 (64.4%) yielded growth on MCKC, while 27 (60%) yielded growth on CHROMagar™ ESBL media. In comparison with the E. test, sensitivity of both MCKC and CHROMagar™ ESBL media were 100%. While specificity were 80.8% and 87.5% respectively.

Table 2. Identification of ESBL producer and non ESBL producer *E. coli* growth on CHROMagar™ ESBL agar and MCKC agar

Results of growth	CHROMagar™ ESBL agar		MCKC	
	NO	%	NO	%
Growth	27	60	29	64.4
No growth	18	40	16	35.6
TOTAL	45	45	45	45
Specificity	87.5% (95%CL: 67.6% to 97.2%)		80.8% (95%CL: 60.6% to 93.4%)	
Sensitivity	100% (95%CL: 85.6 % to 100%)		100% (95%CL: 85.6% to 100%)	
PPV	88. 9% (95%CL: 70.8% to 97.5%)		82.8% (95%CL: 64.2% to 94.1%)	
NPV	100% (95%CL: 83.8% to 100%)		100.00% (95%CL: 83.8% to 100%)	

Preliminary screening test by CLSI method showed that resistance of *E coli* strains to Ceftazidime, Ceftriaxone, Aztereonam, Cefpodoxime and Cefepime were 35 (77.7%), 33(73.3%),31(68.9%), 26 (57.8%), 26 (57.8%) respectively, so 35 strains (77.8%) of isolates were considered as preliminary producers of ESBLs. Comparison between CHROMagar™ ESBL agar and CLSI screening method to screen ESBL producing *E. coli* is shown in Table 3, Table 4 shows distribution of AmpC and AmpC and ESBL co-produers among *E. coli* isolates.

Table 3. Comparison between CHROMagar™ ESBL agar and CLSI screening method to screen ESBL producing *E. coli*

	CHROMagar™ ESBL agar		CLSI screening method	
	NO	%	NO	%
Preliminary ESB producers	27	60	35	77.8
Non ESBL producers	18	40	10	22.2
Total	45	100	45	100

CLSI method: Specificity: 65.6%(95%CL: 46.8% to 81.4%) Sensitivity: 100%(95%CL: 85.6% to 100%) PPV: 68.6(95%CL: 50.7% to83.1%) NPV: 100% (95%CL: 83.7% to 100%).

Table 4. Distribution of AmpC and AmpC &ESBL co-produers among *E. coli* isolates

E. coli isolates n=45							
Amp C producers		Amp C&ESBL co-producers		Pure ESBL producers		Non Amp C &ESBL producers or co-producers	
NO	%	NO	%	NO	%	NO	%
14	31.1	4	8.9	24	53.3	3	6.7

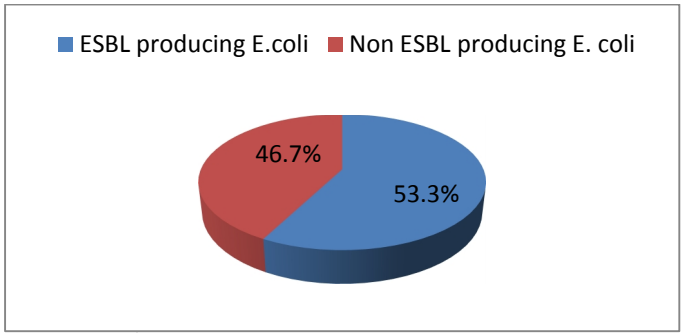

Fig. 1. Distribution of ESBL producing *E. coli* among UTI patients

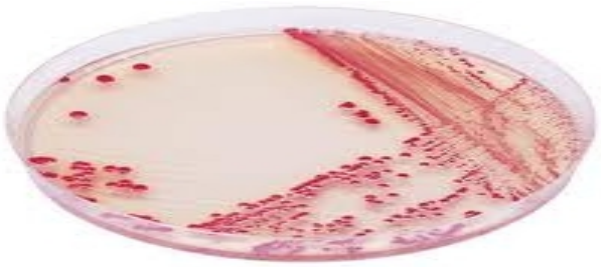

Fig. 2. Dark pink to reddish *E. coli* colonies isolated on CHROMagar™ ESBL media

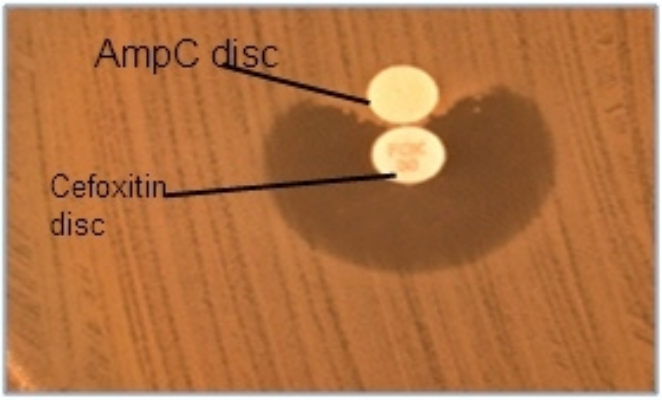

Fig. 3. Positive AmpC test (indentation of the zone of inhibition around cefoxitin disc)

4. DISCUSSION

Extended-spectrum β-lactamases (ESBLs) producing Gram-negative bacteria are large, rapidly evolving group of plasmid-mediated enzymes emerging pathogens. Clinicians, microbiologists, infection control practitioners and hospital epidemiologists are concerned about ESBL-producing bacteria because of the increasing incidence of such infections [17] In the present study out of 45 *E. coli* strains 24 (53.3%) ESBL producers were detected by E. test (golden method for confirmation of ESBL according to CLSI[7]).This is in agreement with Hasan et al. [18] who reported (57.4%) ESBL rate in uropathogenic *E. coli.* Thabit et al. [19] also reported 53% ESBL producing *E. coli by* E. test. In our study out of the 24 isolated ESBL producing *E. coli* strains 9 (37.5%) were detected in community acquired UTI patients while 15 (62.5%) were detected in health care associated UTI patients. This was in agreement with Thabit et al, [19] Who reported that ESBL producing *E. coli* among community isolates was (39.47%) while among nosocomial isolates the rate was (70%).Our results are comparable to those obtained by Bean et al. [20] who reported a community-based ESBL prevalence to be 5.7% in London. The cause of the upsurge in community-acquired infections with ESBL-producing organisms is not yet clear, but associations with foodstuffs, animal consumption of antibiotics, and frequent patient contact with health care facilities need to be explored [21]. Methods to detect ESBL-producing organisms from clinical specimens should have high sensitivity and high specificity combined with a short time to the reporting of results. In order to identify ESBL-producing gram-negative bacilli from clinical samples more easily and reliably, selective media should ideally achieve the identification of the organisms and detection of ESBL in one step. At the least, it should decrease the workload and reduce the need of unnecessary confirmations [22]. In the present study out of 45 *E. coli* isolates 16 (35.6%) yielded no growth on any selective media and 29 (64.4%) yielded growth on MCKC, while 27 (60%) yielded growth on CHROMagar™ ESBL media. In comparison with the E. test, sensitivity of the MCKC and CHROMagar™ ESBL media were 100%. While specificity were 80.8% and 87.5% respectively.Glupczynski et al, (2007) [23] reported sensitivity and specificity of MCKC 84% and 91% respectively. Regarding sensitivity and specificity of CHROMagar™ ESBL media lagace- Wiens et al. [24] reported high sensitivity (99,2%) and Specificity (89%). Also Saito et al,(2010)([25]reported sensitivity and specificity of CHROMagar™ ESBL media 100% and 93% respectively. In our study preliminary screening test by CLSI screening method showed that 35 strains (77.8%) of isolates were considered as preliminary producers of ESBLs. While preliminary producers

of ESBLs from CHROMagar™ ESBL agar were 27 (60%). 24 (100) confirmed ESBLs were detected by both CLSI screening method and CHROMagar™ ESBL agar. 11 were positive ESBLs by CLSI screening method and 3 positive ESBLs by CHROMagar™ ESBL agar, while all these strains were confirmed negative. Sensitivity of both CLSI screening method and CHROMagar™ ESBL agar were 100%. While high specificity showed by CHROMagar™ ESBL agar 100%, low specificity showed by CLSI screening method 65.6%. Also Manhas et al. [26] reported sensitivity and specificity of CLSI screening method were 99.4% and 66.1% respectively. Thabit et al. [19] reported 76.5% potential producers of ESBLs by CLSI screening method while confirmed ESBLs 53% With the spread of AmpC and ESBL producing strains all over the world, it is necessary to know the prevalence of these strains in hospitals. Use of cefepime is more reliable to detect these strains because high AmpC production has little effect on cefepime activity. In this study out of 45 isolated *E. coli* strains 14 (31.1%) were AmpC producers by AmpC test, 3 (6.7%) were AmpC and ESBL co-producers by cefepime/ cefepime clavulanic E. test, 24 (53.3%) were pure ESBL by TZ/TZL or CT/CTL E.test. This was in agreement with Singhal et al. [27] who reported AmpC enzyme production in 36% of *E.coli* isolates by AmpC test,also Sinha et al. [28] reported AmpC production in 24% and co-production of ESBL and AmpC enzymes in 8%. Stürenburg et al. [29] evaluated the performance of the cefepime clavulanate ESBL E test to detect AmpC and ESBL co-producers in an Enterobactriaceae strain collection. The ESBL E test was 98% sensitive with cefepime-clavulanate.

5. CONCLUSION

1- It is important to know the prevalence of ESBL, AmpC producing and ESBL and AmpC co-producing organisms so that judicious use of antibiotics could be done.
2- CHROMagar™ ESBL media detect ESBL producers from clinical specimen and give rapid presumptive identification by means of colony colour at 24h with good sensitivity and specificity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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