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ORIGINAL ARTICLE

Detection of Extended Spectrum Beta Lactamase Producing Strains among Clinical Isolates of Escherichia Coli and Klebsiella Pneumoniae in Alexandria using Chrom-ID ESBL Agar and Molecular Techniques

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ABSTRACT

Background: *Antibiotic resistance has become a serious global problem and affects almost every bacterial species for which treatment with antibiotics is available. The increased prevalence of Enterobacteriaceae producing ESBLs creates a great need for identifying these organisms for infection control and epidemiological surveillance.* ***Objectives:*** *In this study, the aim was to (i) evaluate different phenotypic methods for detection of ESBL producing E. coli and K. pneumoniae (ii) Evaluate the diagnostic performance of the selective chromogenic culture media, chromID ESBL, (iii) Determine the prevalence of the genes blaTEM, blaSHV, and blaCTX-M, which are responsible for ESBL production in clinical isolates of E. coli and K. pneumoniae. (iv)Identify the sequence types of the most prevalent ESBL genes among E. coli and K. pneumoniae in Alexandria.* ***Methodology:*** *One hundred isolates of ESBL producing E. coli and K. pneumoniae isolates were collected. Phenotypic characterization of ESBLs by combined disc test, double disc synergy test (DDST), and the modified double disc synergy test (MDDST) was performed simultaneously with antibiotic susceptibility testing. ChromID ESBL agar (bioMérieux) was evaluated for ESBL detection. ESBLs positive strains were tested for the presence of ESBL encoding genes by PCR. After amplification, twelve TEM, SHV and CTX-M genes were subjected to nucleic acid sequencing.* ***Results:*** *An ESBL phenotype was confirmed in 100/173 (57.8%) isolates by combined disc test. The sensitivity of DDST was 44% when the distance kept at 20 mm. Cefepime yielded the highest performance among the β-lactams, with sensitivity 70% when tested with amoxicillin-clavulanate and 86% when tested with piperacillin-tazobactam. The highest sensitivity among phenotypic methods (98%) was obtained with chromID ESBL. PCR of TEM, SHV and CTX-M genes revealed that 80% were positive for at least one of the studied genes. Sequencing of the randomly selected genes revealed that the eight* bla_{TEM} *amplification products were TEM-1, while the two* bla_{SHV} *were SHV-11 and the two* bla_{CTX-M} *genes were CTX-M-15.* ***Conclusion:*** *using only one disk combination might fail to detect ESBL production. The MDDST using cefepime and piperacillin-tazobactam gives the highest sensitivity followed by MDDST using cefepime and amoxicillin-clavulanate. Agars with chromogenic substrates could be applicable as screening methods for ESBLs producing strains.*

INTRODUCTION

Antibiotic resistance has become a serious global problem and affects almost every bacterial species for which treatment with antibiotics is available. Resistance to multiple antibiotics has developed among many common pathogens, including extended-spectrum β-lactamase (ESBL)-producing strains of *Enterobacteriaceae*, and the resistance problem is steadily increasing worldwide. [1]

Extended-spectrum β-lactamases (ESBLs)-are plasmid-mediated β-lactamase enzymes able to inactivate ß- lactam antibiotics containing an oxyimino group, such as oxyiminocephalosporins (ceftazidime, ceftriaxone or cefotaxime) or oxyimino-monobactam (aztreonam). They are not active against cephamycins (cefoxitin or cefotetan) and carbapenems. In contrast, ESBLs are readily inhibited by the beta-lactamase inhibitors (i.e., clavulanic acid, tazobactam, or sulbactam). [2, 3]

The increased prevalence of *Enterobacteriaceae* producing ESBLs creates a great need for identifying these organisms for infection control and epidemiological surveillance. In this study, the aim was to (i) evaluate different phenotypic methods for detection of ESBL producing *E. coli* and *K. pneumoniae* (ii) Evaluate the diagnostic performance of the selective chromogenic culture media, chromID ESBL, (iii) Determine the prevalence of the genes blaTEM, blaSHV, and blaCTX-M , which are responsible for ESBL production in clinical isolates of *E. coli* and *K. pneumoniae*. (iv) Identify the sequence types of the most prevalent ESBL genes among *E. coli* and *K. pneumoniae* in Alexandria.

METHODOLOGY

One hundred isolates of ESBL producing *E. coli* and *K. pneumoniae* isolated from different clinical samples obtained from patients admitted to Alexandria Main University Hospital and sent to

Routine Laboratory of Microbiology and Immunology Department of Alexandria University were collected during a period of 6 monthes.

Antimicrobial susceptibility testing

Isolates biochemically identified as *E. coli* and *K. pneumoniae* were subjected to antimicrobial susceptibility testing by the Bauer-Kirby disc diffusion technique, and interpreted in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). [4]

I. Phenotypic tests for detection of ESBL

1. ESBL screening test

According to CLSI guidelines, each *E. coli* and *K. pneumoniae* isolates were considered a potential ESBL-producer if they showed a diameter of less than 22 mm for ceftazidime, less than 27 mm for cefotaxime or aztreonam and less than 25 mm for ceftriaxone. CLSI recommended the use of more than one antimicrobial agent for screening to improve the sensitivity of detection. [4]

2. CLSI Phenotypic confirmatory test (combined disc method)

The combined disc test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for the detection of ESBLs according to the CLSI guidelines. In this test, a lawn culture of the isolates was made on the Mueller-Hinton agar plate. The cefotaxime (30µg), cefotaxime-clavulanic acid (CCT, 30µg/10µg), ceftazidime (30 µg) and ceftazidime-clavulanic acid (CCA, 30µg/10µg) discs were used. A difference in zone of inhibition greater than or equal to five mm of either ceftazidime or cefotaxime tested in combination with clavulanate versus its zone diameter when tested alone confirmed ESBL production. [4, 5]

3. Double disc synergy test

The double disc synergy test (DDST) was performed as a standard disc diffusion assay on Muller-Hinton agar (MHA). Discs of 3rd generation cephalosporin (ceftazidime 30µg, cefotaxime 30µg) and monobactam (aztreonam 30µg) were placed 30 mm apart (center to center) around an augmentin disc (20 µg amoxicillin /10 µg clavulanic acid) placed in the middle. Extension of the edge of the inhibition zone of ceftazidime, cefotaxime or aztreonam disks on the side exposed to the augmentin disk is considered positive for ESBL production. If the test was negative it was repeated placing the disks 20mm apart. [6-8]

4. Modified double disk synergy test (MDDST)

In MDDST, a lawn culture of the organisms was made on a Mueller-Hinton agar plate, as was recommended by CLSI. A disk of augmentin was placed in the center; then disks of ceftazidime (30µg), cefotaxime (30µg), aztreonam (30ug) and cefepime (30µg) were kept around it at distance ranging between 16 and 20 mm from the augmentin disk (center to center), and a disk of piperacillin-tazobactam (100/10µg) was placed at a distance ranging between 22 and 25 mm from the cefepime disk. The organisms were considered to be producing ESBL when the zone of inhibition around cefepime or any of the extended-spectrum cephalosporin disks showed a clear-cut increase towards the piperacillin-tazobactam disk or augmentin disk. [9-11]

5. Culture on chromID ESBL agar

The chromogenic chromID ESBL agar (bioMérieux, Marcy l'Etoile, France) was obtained from the manufacturer as a prepared plate medium. *E. coli* and *K. pneumonia*e isolates were suspended in 1 ml sterile 0.9% saline, and the inoculum was adjusted to a density corresponding to a 0.5 McFarland standard. A 10 µl of this suspension was streaked onto chromID ESBL agar. All plates were incubated aerobically overnight at 37°C before being read. [12] *E. coli* isolates should produce pink to burgundy coloration of beta-glucuronidase-producing colonies; while *Klebsiella* should show green and/or blue coloration of beta-glucosidase producing colonies. [12, 13]

II. Detection of ESBL resistance genes using conventional polymerase chain reaction (PCR)

All *E. coli* and *K. pneumoniae* isolates were further analyzed for the presence of the most prevalent genes responsible for ESBL production (blaTEM, blaSHV, and blaCTX-M), using conventional PCR using primers shown in table 1.

Table 1. Sequences of primers used in PCR for detection of *bla* genes encoding ESBL in isolates of *E. coli* and *K. pneumoniae*.[14]

PRIMERS	*Sequence (5`-3`)*	*Amplicon size (bp)*
TEM-F	**GTGCGCGGAACCCCTATT**	**968**
TEM-R	**TTACCAATGCTTAATCAGTGAGGC**	
SHV-F	**CTTTACTCGCCTTTATCGGC**	**982**
SHV-R	**TTACCGACCGGCATCTTTCC**	
CTX-M F	**ATGTGCAGCACCAGTAAAG**	**562**
CTX-M R	**GGTCACCAGAAGGAGC**	

The PCR reaction was performed in a final volume of 25 µL with a 50-100ng DNA template, 0.15 µM of each of the forward and reverse primers, 12.5 µL of master mix (DreamTaq Green PCR Master Mix, Thermo Scientific) and the volume was completed to 25 µL by using 9.5 µL of sterile nuclease free water. Amplification reactions were carried out under the following conditions: initial denaturation at 95ºC for 3 min, followed by 30 cycle of denaturation at 95ºC for 30 sec, annealing at 55ºC for 40 sec and elongation at 72ºC for 1 min. The final elongation step was extended to 10 min at 72ºC. The amplified products of the PCR reactions were analyzed by electrophoresis in 1.5% agarose gels in comparison to a 100 bp DNA ladder. [15,16]

Sequencing of random samples of ESBL genes

After PCR amplification of ESBL genes, twelve TEM, SHV and CTX-M genes were subjected to nucleic acid sequencing (4 *E. coli* and 4 *K. pneumoniae* carrying TEM gene, 2 *K. pneumoniae* carrying SHV gene, and 2 *E. coli* carrying CTX-M gene). The purified amplicons were sequenced with ABI 3730XL DNA analyzer (GATC Biotech, Germany).Nucleotide and deduced protein sequences were analyzed with DNAMAN Sequence Analysis Software version 4.13 and compared with sequences available at the National Center for Biotechnology Information website (https://ncbi.nlm.nih.gov/Blast)

Statistical analysis

The raw data were coded and entered into SPSS system files (SPSS package version 18). Analysis and interpretation of data were conducted. The following statistical measures were used: Univariate analyses including: Chi-Square test, Monte Carlo correction for p-value of Chi-Square test and Fisher's Exact test were used to test the significance of results of qualitative variables. Accuracy, Sensitivity, Specificity, Positive predictive value and Negative predictive value were calculated for testing efficiency of methods used. The significance of the results was at the 5% level of significance.[17]

RESULTS

An ESBL phenotype was recognized in 100 isolates out of 173 isolates (57.8%) within a period of 6 months using CLSI Phenotypic confirmatory test (combined disk test).

All isolates were tested by combined disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. A positive result in either cefotaxime or ceftazidime tested confirmed ESBL production**.** Figure 1

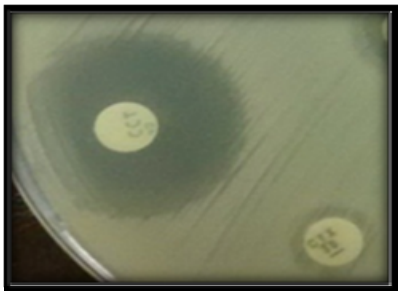

Fig. 1: Positive CLSI Phenotypic confirmatory test (combined disk method)

Table 2, shows that by employing the CLSI ESBL Phenotypic confirmatory test (the gold standard), it was found that out of 173 isolates, 100 strains phenotypically confirmed as ESBL producers by using both CTX/CCT and CAZ/CCA. In addition, CTX/CCT combination detected 98% of ESBL isolates (100% *E. coli* and 93.9% *K. pneumoniae*) in comparison with 96% (97% *E. coli* and 93.9% *K. pneumoniae*) that could be detected by CAZ/CCA combined disk.

Table 2: Comparison between the combined disks CTX/CCT and CAZ/CCA in the CLSI Phenotypic confirmatory test (combined disk method) for detection of ESBL isolates

Combined disk method	*E. coli*				*K. pneumoniae*				*All specimens*			
	ESBL Positive (n=67)		ESBL Negative (n=40)		ESBL Positive (n=33)		ESBL Negative (n=33)		ESBL Positive (n=100)		ESBL Negative (n=73)	
	No.	%	No.	%	No.	%	No.	%	*No.*	*%*	*No*	*%*
CTX/CCT												
Positive*	**67**	**100.0**	0	0.0	**31**	**93.9**	0	0.0	**98**	**98.0**	0	0.0
Negative	0	0.0	40	100.0	2	6.1	33	100.0	2	2.0	73	100.0
CAZ/CCA												
Positive*	**65**	**97.0**	0	0.0	**31**	**93.9**	0	0.0	**96**	**96.0**	0	0.0
Negative	2	3.0	40	100.0	2	6.1	33	100.0	4	4.0	73	100.0

CTX/CCT: cefotaxime/ cefotaxime-clavulanic; CAZ/CCA: ceftazidime/ ceftazidime-clavulanic; Positive*: increase in the growth-inhibitory zone around either the CCT or the CCA disks 5 mm or greater than the diameter around the disk containing CTX or CAZ alone

Figure 2, shows that among the 100 ESBL producing strains, double disk synergy test (DDST) with 30 mm distance between augmentin and β-lactam antibiotics detected only 8 (8%) ESBL producing strains. Double disk synergy test with 20 mm distance between augmentin and β-lactam antibiotics detected 44 (44%) ESBL producers. On the other hand, modified double disk synergy test (MDDST) using cefepime with amoxicillin-clavulanate detected 70 (70%) ESBL producers. Modified double disk synergy test (MDDST) using cefepime with piperacillin-tazobactam detected 86 (86%) ESBL producers.

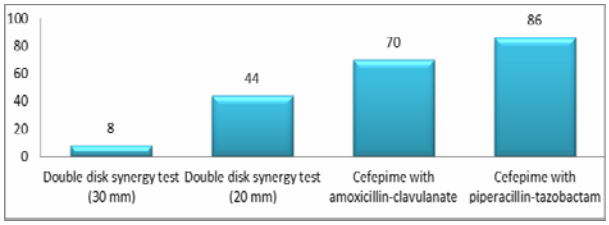

Fig. 2: Evaluation of double disk synergy test and modified double disk synergy test methods for detection of the one hundred ESBL isolates

Figure 3 shows chromID ESBL medium plated with *E. coli* and *K. pneumoniae with* characteristic chromogenic reaction for each.

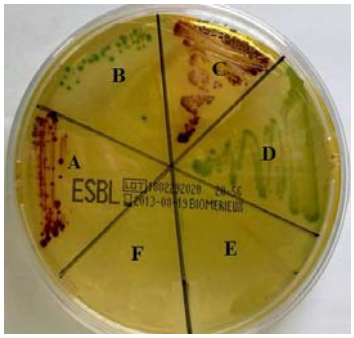

Fig. 3: ChromID ESBL agar showing: A & C: ESBL positive *E. coli* with pink to burgundy coloration. B: ESBL positive *K. pneumoniae* with green coloration. D: *K. pneumoniae* ATCC 700603 (ESBL positive). E: *E. coli* ATCC 25922 (ESBL negative). F: ESBL negative isolate.

Table 3 show that out of the 100 ESBL positive strains recognized by CLSI Phenotypic confirmatory test (the gold standard), 98 isolates (98%) were positive by chromID ESBL agar as two E. coli strains failed to be detected because they did not produce the expected-colored colony.

Table 3: Performance of chromID ESBL for the detection and presumptive identification of the one hundred ESBL-producing strains

ChromID ESBL	*E. Coli*				*K. pneumoniae*				*All* isolates			
	ESBL Positive (n=67)		ESBL Negative (n=40)		ESBL Positive (n=33)		ESBL Negative (n=33)		*ESBL Positive (n=100)*		*ESBL Negative (n=73)*	
	No.	%	No.	%	No.	%	No.	%	*No.*	*%*	*No.*	*%*
Positive	65	97.0	2	5.0	33	100.0	1	3.0	98	98.0	3	4.1
Negative	2	3.0	38	95.0	0	0.0	32	97.0	2	2.0	70	95.9

Table IV shows that out of 100 phenotypically ESBL positive isolates using CLSI ESBL Phenotypic confirmatory test, 80 (80%) were positive for at least one of the three studied genes. None of the ESBL negative isolates were harboring any of the studied genes. The most prevalent ESBL gene among the study isolates was blaTEM (76.2%) (Figure 4A). The least frequently detected genes were SHV (figure 4B) in *E. coli,* (5.6%), and CTX-M (figure 4C) in *K. pneumoniae* (25.9%). Among *E. coli* and *K. pneumoniae* isolates harboring single ESBL gene, TEM was present in 50% of isolates, CTX-M was present in 15% of isolates and SHV was present in 8.7%. Among the studied genes, TEM and CTX-M were the most common combination (18.7%) followed by TEM + SHV (5.0%). Two isolates (2.5%) harbored all the three studied genes simultaneously.

Table 4: Frequency and distribution of the studied ESBL genes among the one hundred ESBL positive isolates

***bla* genes**	***E. coli* (n=53)**		***K. pneumoniae* (n=27)**		***Total (n=80)***	
	No.	**%**	**No.**	**%**	***No.***	***%***
Any ESBL genes*						
TEM	40	75.4	21	77.7	61	76.2
SHV	3	5.6	10	37.0	13	16.2
CTX-M	22	41.5	7	25.9	29	36.2
Single ESBL genes						
TEM only**	29	54.7	11	40.7	40	50.0
SHV only	2	3.7	5	18.5	7	8.7
CTX-M only	11	20.7	1	3.7	12	15.0
Two or more ESBL genes						
TEM + SHV	0	0.0	4	14.8	4	5.0
TEM+ CTX-M	10	18.8	5	18.5	15	18.7
SHV + CTX-M	0	0.0	0	0.0	0	0.0
TEM + SHV + CTX-M	1	1.8	1	3.7	2	2.5

***** The word "any" means the presence of the indicated gene regardless of the presence or absence of the other genes
** The word "only" means the presence of the indicated gene solely

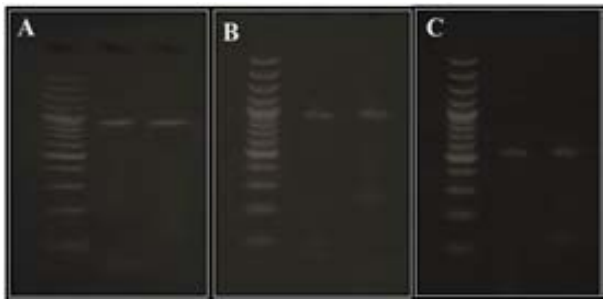

Fig. 4: Agarose gel electrophoresis of PCR products compared to a 100 bp DNA ladder and visualized by ultraviolet light showing, A: TEM gene with expected band of 968 bp length, B: SHV gene with expected band of 982 bp length, C: CTX-M gene with expected band of 562 bp length.

Table 5 shows statistical comparison of the phenotypic and genotypic methods in relation to CLSI phenotypic confirmatory test (gold standard)for detection of ESBL producing strains. The DDST at 30 mm showed sensitivity 8%, that was increased to 44% when the distance was reduced to 20 mm. In MDDST, Cefepime yielded the highest performance among the β-lactams, with sensitivity 70% when tested with amoxicillin-clavulanate and 86% when tested with piperacillin-tazobactam. The highest sensitivity among the phenotypic methods (98%) was obtained with chromID ESBL. The sensitivity of genotypic method was 80% for the 3 studied genes. All phenotypic methods showed 100% specificity except chromID ESBL which gave 95.9% specificity in detecting ESBL producing isolates. For genotypic method, the specificity was 100%.

Table 5: Statistical comparison of the phenotypic and genotypic methods for detection of the one hundred ESBL producing strains

Method of diagnosis	***All isolates [n=173]***				
	Sensitivity	**Specificity**	**PPV**	**NPPV**	**Accuracy**
DDST (30 mm)	8.0	100.0	100.0	44.2	46.8
DDST (20 mm)	44.0	100.0	100.0	56.6	67.6
MDDST: FEP with AMC	70.0	100.0	100.0	70.9	82.7
MDDST: FEP with TZP	86.0	100.0	100.0	83.9	91.9
ChromID ESBL	98.0	95.9	97	97.2	97.1
Genotypic method	80.0	100.0	100.0	78.5	88.4

The basic local alignment search tool (BLAST) for sequencing of the twelve randomly selected isolates revealed that all the eight bla_{TEM} amplification products were TEM-1, while the two bla_{SHV} were SHV-11 enzyme and the two $bla_{CTX\text{-}M}$ genes were CTX-M-15.

DISCUSSION

Extended spectrum beta lactamases (ESBLs) have recently become a significant problem because they are commonly plasmid-encoded, facilitating a high rate of horizontal transfer between different bacterial species. Furthermore, such plasmids typically carry resistance

genes to other drugs such as the aminoglycosides, thus narrowing treatment options.[18]

Early and correct identification of ESBL- producing *Enterobacteriaceae* in due time is mandatory not only for optimal patient management but also for immediate establishment of appropriate infection control measures to prevent the spread of these pathogens and also to prevent hospital-acquired (nosocomial) infections and outbreaks in the community. Since *E. coli* and *Klebsiella pneumoniae* are the most predominant ESBL-producing members among *Enterobacteriaceae* especially in the clinical setting, it is more beneficial to estimate the prevalence of ESBL-producers among them. [19, 20]

In the present study, the prevalence of ESBL-producing isolates among the 173 *E. coli* and *K. pneumoniae* isolates using combined disk method was 57.8%. This result was comparable to other Egyptian studies conducted at Assiut University hospital [21] and Banha University hospital, [22] they reported prevalence rates of 52.9% and 53.3%, respectively. In addition, other studies in Egypt conducted at Theodor Bilharz Medical Research Institute, [23] and Assiut University Hospitals [24] reported higher prevalence of ESBL producing Escherichia coli and K. pneumoniae (64.7% and 65.8% respectively) . However other studies in Egypt, Fam et al,[25] and Elsayed et al[26], reported that ESBL producing Enterobacteriaceae isolated from clinical specimens represented 29.9% and 36% respectively. This difference in the prevalence rates might be attributed to different antibiotic policies which may aid in selection of certain antibiotic resistant pathogens than another, and/or strict implementation of infection control measures.

The most effective ß-lactam/ß-lactamase inhibitor combination against *E. coli* was piperacillin/tazobactam (70.1% of the isolates were susceptible) followed by cefoperazone-sulbactam (49.3% of the isolates were susceptible); while amoxicillin/clavulanic acid and ampicillin/sulbactam combinations had poor susceptibility (only 4.5% of isolates were susceptible for both). While *K. pneumoniae* showed poor response to ß-lactam/ß-lactamase inhibitor combinations with resistance rate ranging from 81.8% to 100%. These results were in accordance to other local and global studies. [27-29]

The ESBL production coexists with the resistance to several other antibiotics. The ESBLs are encoded by plasmids which also carry resistant genes for other antibiotics.[8] In the present study, antibiotic susceptibility testing of ESBL-positive isolates of *E. coli* and *K. pneumoniae* expressed coresistance to two or more non-beta lactam antibiotics including trimethoprim-sulfamethoxazole (92.5%, 75.8%), ciprofloxacin (88.1%, 87.9%), levofloxacin (85.1%, 87.9%), Gentamicin (70.1%, 51.5%) tobramycin (73.1%, 97%). This finding is in accordance with data reported from other studies.[11,28,30,31]

Given the popularity of fluoroquinolones in treating a variety of infections including respiratory, gastrointestinal and urinary tract infections, the two drugs tested in the present study (ciprofloxacin and levofloxacin) fared poorly with >85% of the isolates showing resistance to both. This is not surprising among ESBL producers and this phenomenon is well described in the literature. [30, 31]

Carbapenems are usually used for antibiotic treatment of infections with ESBL producers. Most of the ESBL-positive isolates in the present study were susceptible to imipenem and meropenem. *E. coli* isolates were fully susceptible to meropenem (100%), and showed 95.5% susceptibility to imipenem. Isolates of *K. pneumoniae* showed 72.7% and 87.9% susceptibility respectively. This finding is in line with data reported from other studies. [27, 30-32]

Imipenem-susceptible, meropenem-resistant *Klebsiella* (ISMRK) is a recently described *K. pneumoniae* phenotype showing an unusual susceptibility to carbapenems. This phenotype is susceptible to imipenem (IPM) but resistant to other carbapenems. The ISMRK may be falsely categorized as susceptible to carbapenems if imipenem is used to screen carbapenem resistance. [33, 34]

In the present study different phenotypic methods to detect ESBL production by *E. coli* and *K. pneumoniae* were compared. Considering CLSI Phenotypic confirmatory test (combined disk method), 100 isolates were phenotypically confirmed to be ESBL producers. Two combinations with clavulanic acid were tested, cefotaxime/cefotaxime-clavulanic (CTX/CCT) and ceftazidime/ceftazidime-clavulanic (CAZ/CCA), and have been found that the maximum ESBLs detection (98%) of strains was by using cefotaxime/cefotaxime-clavulanic (CTX/CCT) combination which correlates with previous studies.[35, 36] However other studies reported that CAZ/CCA was better than CTX/CCT for detection of ESBL producing *E. coli* and *K. pneumoniae* strains.[37, 38]

In the present study, the sensitivity of double disk synergy test (DDST) was 8% at a single interdisk width of 30 mm; increased to 44% by decreasing the interdisk width to 20 mm. This correlates well with other studies. [39, 40]

Kaur[41] and Helmy (2014)[42] reported that the ESBL detection may be masked by high-level production of AmpC. Further, clavulanate may act as an inducer of high-level AmpC resulting in false negativity in ESBL detection by increasing resistance to the screening drugs. As a solution to this problem, tazobactam and sulbactam which are much less likely to induce AmpC β-lactamases are preferred as inhibitors for ESBL detection, and fourth generation cephalosporin i.e. cefepime is a better choice as an indicator drug. Cefepime, a fourth-generation cephalosporin, is a more reliable detection agent for ESBLs in the presence of the AmpC β-lactamases, as this drug is stable to AmpC β-lactamase. Thus, it will demonstrate the synergy which arises from the inhibition of ESBL by clavulanate in the presence of the AmpC enzyme.

In this study original double disk synergy test was compared with modified double disk synergy test (MDDST) using cefepime as indicator drug and

piperacillin-tazobactam as inhibitor for ESBL detection. Cefepime showed synergism with amoxicillin-clavulanate in 70 (70.0%) isolates and in 86 (86.0%) isolates with piperacillin-tazobactam. This result was in accordance with other previous reports.[9, 10, 36]

The sensitivity of chromID ESBL agar for ESBL detection was 97.0% for *E. coli* isolates and 100% for *K. pneumoniae* isolates. The overall sensitivity was 98%. The specificity was 95.0 % and 97% respectively with overall specificity 95.9%. The results of the present study were similar to other studies, [13, 43, 44] and better than results obtained in some previous studies. [45-48]

In the present study, genotypic survey on 100 confirmed ESBL phenotype strains by PCR revealed 80% positive genotypes for at least one of studied genes. In a similar finding, Ahmed *et al*[49] revealed 52.3% positive genotypes among ESBL phenotype strains.

The present study illustrated the distribution of TEM, SHV and CTX-M genes among ESBL-producing isolates, where TEM was the most prevalent β-lactamase-encoding gene. These findings agree with other studies from around the world that show that ESBL genes of the TEM are dominant. [24, 50] In contrast to these findings, other studies reported that SHV was the most frequent β-lactamase-encoding gene. [49, 51] The result from this study reported a relative low occurrence of CTX-M gene, although CTX-M gene now occurs in a higher percentage in most areas of the world. [49, 52, 53]

Sequence analysis of PCR product of selected isolates showed that all studied bla_{TEM} genes were TEM-1 subtype which correlates with other studies. [52-54] The studied SHV types were characterized as SHV-11, Previous studies have shown predominance of SHV-11 type among *K. pneumoniae* isolates. [51, 55] Sequencing of selected CTX-M carrying isolates showed that they were characterized as CTX-M-15. This finding was concordant with the results of other studies carried out in Egypt and Europe. [23, 25, 52]

CONCLUSION

Using only one disk combination might fail to detect ESBL production. The MDDST using cefepime and piperacillin-tazobactam gives the highest sensitivity followed by MDDST using cefepime and amoxicillin-clavulanate. ChromID ESBL proved to be highly sensitive, specific, time saving and cost effective as it does not need unnecessary confirmatory test, so it is recommended as a reliable culture medium for screening and presumptive identification of ESBL-producing *Enterobacteriaceae*.

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