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ORIGINAL ARTICLE

Detection of class D carbapenemases (*blaoxa23* and *bla oxa48*) genes among extended-spectrum β-lactamase-producing *Escherichia coli* in Menoufia University Hospitals

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Key words:
Escherichia coli, EβSLs, carbapenemases, hospital-acquired infection

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ABSTRACT

Background: *Carbapenemases production by extended-spectrum β-lactamases (ESβLs)-producing Escherichia coli (E. coli) has been increasingly found and may be considered as a major cause of morbidity and mortality in hospital-acquired infection.* ***Objectives:*** *To determine resistance pattern and frequency of carbapenem resistance and presence of class D carbapenemases among ESβLs-producing E. coli isolated from patients in Menoufia University Hospitals.* ***Methodology:*** *Different clinical samples were obtained from 270 patients who were admitted to Menoufia University Hospitals. E. coli were isolated and identified, and their antimicrobial resistance profiles were tested by the disk diffusion and agar dilution methods. Confirmed ESβLs producers (by cephalosporin/ clavulanate combination disks and ESβL NDP tests) were further tested for carbapenemase production by phenotypic and genotypic methods.* ***Results:*** *E. coli was the most common isolate (30.4%) from clinical samples. High rate of ESβLs-producing E. coli was detected by disk diffusion (87.5%), cephalosporin/clavulanate combination disk (62.5%) and ESβL NDP test (60%).About10%, 24% and 28% of the ESβLs-producing E. coli isolates were producers of class A, B and D carbapenemases respectively. The prevalence of blashv, blaoxa23 and blaoxa48 genes among ESβLs-producing E. coli isolates was 18%, 22% and 12% respectively.* ***Conclusion:*** *Carbapenemases production by EβSLs-producing E. coli is a major challenge. A great concern should be paid to provide alternative new therapeutic agents, continuous surveillance, and effective antibiotic stewardship program.*

INTRODUCTION

E. coli is one of the most common causes of hospital-acquired infections (HAI) such as bacteremia, urinary tract infection (UTI), neonatal meningitis and sepsis[1]. *E. coli* have multiple resistance mechanisms to beta-lactam drugs and carbapenems such as production of ESβLs, overexpression of drug efflux pumps and the production of carbapenem hydrolyzing enzymes[2]. Unfortunately, spread of ESβLs-producing *E. coli* limits the therapeutic options and usually requires hospitalization that leads to financial burden to the family and society. ESβLs are capable of hydrolyzing penicillins, cephalosporins, monobactam and aztreonam, and are grouped into four classes A, B, C and D enzymes. ESβLs-producing strains are probably under-diagnosed because they are often undetected by routine susceptibility testing methods as they might show a false sensitive inhibition zone in Kirby-Bauer disk diffusion method[2].Over 200 different ESβLs genes have been described,temoneira (TEM) and sulphydryl variable (SHV) class A β- lactamases are the most common types[3].

Recently, carbapenems are considered as the antibiotics of the last resort against threatening infections caused by multidrug-resistant (MDR) *E. coli.* However, ESβLs-producing and carbapenem-resistant *E. coli* have increased globally. ESβLs-producing *E. coli* display co-resistance to carbapenems and other antibiotic types. Carbapenem-resistance in *E. coli* is mainly mediated by class D carbapenemases (OXA-type)[4]. Current knowledge of prevalence of ESβLs-producing *E. coli*, which produce carbapenemases, is necessary to prevent the spread of resistance.

The present study was conducted to determine the resistance patterns and the frequency of carbapenem-resistant strains which produce class D carbapenemases among ESβLs-producing *E. coli* isolated from patients in Menoufia University Hospitals (MUH). Our findings may help to formulate an effective antibiotic stewardship program to prevent the spread of these strains in MUH.

METHODOLOGY

Subjects:

This study was conducted at Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University during the period from April 2018 to September 2019. Clinical samples were collected

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(270) from patients (1 month-74 years old) admitted to different departments of MUH. The study protocol was approved by local ethics committee of Menoufia University. An informed consent was obtained from each patient or the guardians of unaware patients.

Specimen collection and isolation of *E. coli*:

Clinical samples (125 urine, 70 sputum and bronchial aspirate, 30 pus swabs, 22 blood samples, 15 burn swabs and 8 surgical drains) were collected, processed, and cultured on different bacteriological media. *E. coli* isolates were identified by the standard microbiological methods. *E. coli* isolates were preserved on tryptic soy broth with 16% glycerol and frozen at -80°C.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was performed by the disk diffusion method against different antimicrobial agents (Oxoid, Basingstoke, UK) as recommended by Clinical Laboratory Standard Institute (CLSI).Minimal inhibitory concentration (MIC) of imipenem (Sigma, St. Louis, Missouri, USA) was determined by agar dilution method according to CLSI guidelines[5].

***Screening for ESβLs production*:**

E. coli isolates showing zone of inhibition ≤ 21 mm for ceftazidime, ≤ 27 mm for cefotaxime, and ≤ 25 mm for ceftriaxone were considered potential ESβL-producers[5].

Phenotypic confirmation of ESβLs production:

Cephalosporins/clavulanate combination test:

E. coli isolates were considered ESβL-producers if the inhibition zone around the combined ceftazidime/clavulanic acid (30/10 µg) disk was at least 5 mm larger than that of ceftazidime (30 µg) disk alone[5].

The ESβL NDP (Nordmann-Dortet-Poirel) test:

Colorimetric detection of ESβL enzymes was performed by detection of hydrolysis of the lactam ring of cephalosporin (cefotaxime), because it generates a carboxyl group which acidifies the culture media. The change in pH is identified by the color change (from red to yellow/orange) using pH indicator (phenol red). Inhibition of ESβL activity (unchanged red color) is confirmed by adding tazobactam[6].

Phenotypic detection of carbapenemases production:

Carbapenems (imipenem, meropenem and ertapenem)-resistant ESβLs-producing *E. coli* isolates that were detected by disk diffusion were further confirmed by imipenem MIC agar dilution method and carbaNP (carbapenemase Nordmann-Poirel) test. Carbapenemase detection is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, causing change in pH which is detected by phenol red indicator[7]. Different classes of carbapenemases (class A, B and D) production were detected by inhibitor-based methods (boronic acid combined disk, imipenem/EDTA combined disk and imipenem MIC with addition of sodium chloride tests respectively). ESβLs-producing *E. coli* isolates were considered class D (oxacillinases) carbapenemases producers if there was a 4-fold decrease in imipenem MIC upon NaCl addition[8].

***Detection of bla shv gene (ESβLs gene) by PCR*:**

Bacterial DNA was extracted and purified using the gene JET™ genomic DNA purification kit (Thermo Fisher Scientific, UK). The used primers were:

F: 5`CGCCTGTGTATTATCTCC CT3`.

R: 5`CGAGTAGTCCACCAGATCCT3`.

Amplification was done by: an initial denaturation at (95°C for 2 min), followed by 35 cycles [(DNA denaturation at 95°C for 1 min), primer annealing (at 54°C for 30 sec), primer extension (72°C for 1 min), and final extension (72°C for 3min).

Detection of bla oxa23, bla oxa48 (class D carbapenemase genes) by multiplex PCR:

Bla oxa23 **F:** 5`GATCGGATTGGAGAACCAGA3`.

R: 5` ATTTCTGACCGCATTTCCAT3`.

Bla oxa48 F: 5`TTGGTGGCATCGATTATCGG3`.

R: 5`GAGCACTT CTTTTGTGATGGC3`.

Amplification was done by: an initial denaturation at (95°C for 2 min), followed by 35 cycles [(DNA denaturation at 95°C for 1 min), primer annealing (59°C for 45sec), primer extension (72°C for 45 sec), and final extension (72°C for 1 min**)**.

Electrophoresis was performed with agarose gel 1.5% (Fermentas, Lithuania) stained with ethidium bromide (Sigma, USA) for 20 minutes. The products were visualized by UV trans-illuminator and compared with a 100 bp DNA ladder (293 for *bla shv*, 501 for *bla oxa23* and 744 bp for *bla oxa48* genes)[9].

Statistical analysis:

Data were collected, tabulated and analyzed by statistical package for the social sciences (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) software. Chi-square test (χ^2) was done at 5% level of significance. Accuracy was represented using the terms sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy.

RESULTS

Bacteriological isolation from specimens:

Out of the obtained 270 samples, 247 samples (91.5%) showed monomicrobial growth, 8 samples (3%) showed mixed growth (2 isolates for each) and 15 samples (5.5%) were negative by culture. *E. coli* was the predominant isolate (30.4%), followed by *Klebsiella* spp. (19%), *Enterobacter* spp. (13%), *Staph aureus (*12%), *Pseudomonas* spp. (8%) and *Acinetobacter* spp. (5%).

Rate of ESβL production by *E. coli*:

ESβL production rate among *E. coli* was high (62.5%). ESβLs-producing-*E. coli* were mainly isolated from urine (46%), followed by respiratory secretions (28%) and pus (10%) (Fig.1), and were more frequent

in patients admitted to ICUs (46%) and oncology department (20%) (Fig. 2).

The disc diffusion method (screening test for ESβL-production) was compared with the combined disc diffusion test (CLSI, 2019) and the new ESβL NDP test (confirmatory tests of ESβL-production). The disc diffusion method was significantly ($p<0.001$) less efficient than the other 2 confirmatory tests. Both confirmatory tests had nearly the same potency in detection of ESβL-producing *E. coli* (table 1).

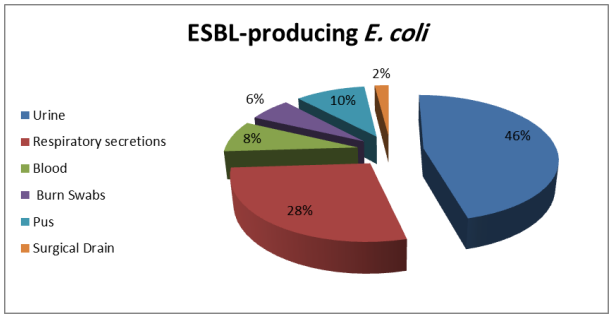

Fig. 1: Distribution of ESβL-producing *E. coli* in different clinical specimens.

Most isolates were from urine and respiratory secretions.

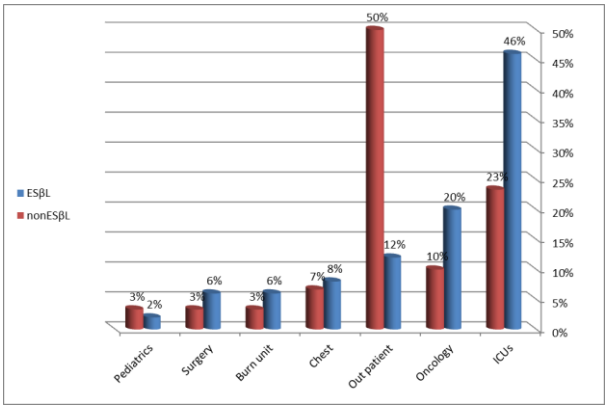

Fig. 2: Distribution of ESβL-and non-ESβL-producing *E. coli* isolates among different departments.

Most ESβL-producers were isolated from patients in ICU and Oncology department**.**

Table 1: Comparison between disk diffusion, combined disk and ESβL NDP methods for detection of ESβLs-production among *E. coli* isolates

	ESβL production											
	Disk diffusion test				**Combined disk test**				**ESβL NDP**			
	Positive		**Negative**		**Positive**		**Negative**		**Positive**		**Negative**	
Total isolates No=80	No	%	No	%	No	%	No	%	No	%	No	%
	70	87.5	10	12.5	50	62.5	30	37.5	48	60	32	40
	x^2_1 17.6				**x^2_2 0.026**							
	P_1< 0.0001				**P_2>0.05**							
	x^2_3 15.6				**P_3< 0.0001**							

1Comparison between screening test and combined disk.
2 Comparison between combined disk and ESβL NDP**.**
3Comparison between screening test and ESβL NDP

Antibiogram of ESβL-and non-ESβL-producers:

ESβLs-producing *E. coli* isolates displayed high resistance rates against most of the tested antibiotics. There was a statistically significant difference between ESβL- and non ESβL-producing *E. coli* regarding susceptibility to amoxicillin/clavulanic acid, azetreonam, imipenem, meropenem, tobramycin, ciproflxacin and tigycycline. All ESβLs-producing *E. coli* isolates were 100% resistant to cefoxitin and ceftazidime and were >90% resistant to pipracillin, amoxicillin-clavulanic acid, cefepime and ciprofloxacin. About 88%, 86%, 84% and 80% of the ESβL-producing *E. coli* were resistant to tobramycin, azetreonam, gentamycin and ceftriaxone respectively. On the other hand, 72% of ESβLs- producing *E. coli* were tigecycline-susceptible (table 2).

Table 2: Relation of antibiotic susceptibility to ESβL-production among *E. coli* isolates.

Antimicrobial agent	All *E. coli* isolates n=80									
	ESβLs-producers (no=50)				ESβLs-non producers (no=30)				X^2	p value
	S		IS+R		S		IS+R			
	No	%	No	%	No	%	NO	%		
Piperacillin	2	4%	48	96%	3	10%	27	90%	1	>0.05
Amoxicillin/clavulanic acid	3	6%	47	94%	7	23%	23	77%	5	<0.05
Pipiracillin/tazobactam	13	26%	37	74%	9	30%	21	70%	0.15	>0.05
Cefoxitin	0	0%	50	100%	0	0%	30	100%	Na	Na
Ceftazidime	0	0%	50	100%	10	33%	20	67%	19	0.0001
Ceftriaxone	10	20%	40	80%	8	27%	22	73%	0.47	>0.05
Cefepime	3	6%	47	94%	3	10%	27	90%	0.4	>0.05
Cefixime	12	24%	38	76%	13	43%	17	57%	3	>0.05
Azetronam	7	14%	43	86%	10	33%	20	67%	4	<0.05
Ertapenem	29	58%	21	42%	23	77%	7	23%	2.8	>0.05
Imipenem	28	56%	22	44%	24	80%	6	20%	4.7	<0.05
Meropenem	26	52%	24	48%	24	80%	6	20%	6	<0.05
Amikacin	9	18%	41	82%	7	23%	23	77%	0.3	>0.05
Gentamycin	8	16%	42	84%	14	47%	16	53%	8.8	<0.05
Tobramycin	6	12%	44	88%	12	40%	18	60%	8.4	<0.05
Tetracycline	11	22%	39	78%	12	40%	18	60%	2.9	>0.05
Doxycycline	13	26%	37	74%	12	40%	18	60%	1.7	>0.05
Ciprofloxacin	5	10%	45	90%	11	37%	19	63%	8.3	<0.05
Levofloxacin	6	12%	44	88%	18	60%	12	40%	20	<0.0001
Gatifloxacin	8	16%	42	84%	10	33%	20	67%	3	>0.05
Tigecycline	36	72%	14	28 %	29	97%	1	3%	7.4	<0.05

There was significant difference between ESβL-producers and non-ESβL-producers regarding resistance to amoxicillin/clavulanic acid, ceftazidime, aztreonam, imipenem, meropenem, gentamycin, tobramycin, ciprofloxacin, levofloxacin and tigecycline.

Regarding carbapenem resistance, ESβLs-producing *E.coli* isolates showed higher resistance rates to imipenem (44%) and meropenem (48%) than the non-ESβLs- producing isolates (table 2). However, there was no significant difference between the three phenotypic methods used for detection of imipenem resistance (disk diffusion, agar dilution and carba NP test) among ESβLs-producing *E .coli* isolates (table 3).

Table 3: Comparison between disk diffusion, agar dilution and carbaNptests used for detection of imipenem susceptibility among ESβL-producing *E. coli* isolates.

	Imipenem susceptibility											
	Imipenem disk diffusion				MIC (agar dilution)				Carba NP			
	S		IS+R		S		IS+R		S		IS+R	
Total isolates No=50	No	%	No	%	No	%	No	%	No	%	No	%
	28	56	22	44	30	60	20	40	33	66	17	34
	x^2_1 1.16						x^2_2 0 .164					
	P_1> 0.05						P_2>0.05					
	x^2_3 1.05						P_3>0.05					

*1 Comparison between disk diffusion and MIC methods.
2 Comparison between MIC method and Carba NP methods.
3 Comparison between Carba NP and disk diffusion methods.
There was no significant difference between the 3 studied methods.

In this work, 60% of the ESβLs-producing *E. coli* and 50% of the non-ESβL- producing *E. coli* were MDR, 24% of ESβL-producing *E.coli* and 20% of non-ESβL-producing isolates were XDR and 16% of ESβL-producing and 13% of non-ESβL-producing *E. coli* were PDR (Fig. 3).

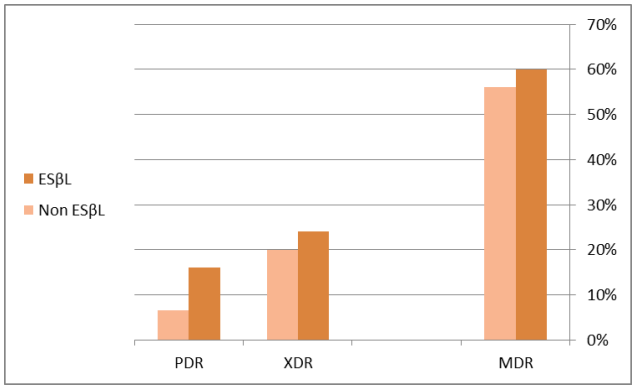

Fig. 3: Rate of MDR, XDR and PDR among ESβL-producing and non-ESβL-producing *E. coli* isolates. Multiple drug resistance was more common amongESβL-producers compared to non-producers

Rate of *blashv, bla oxa23* and *bla oxa48* genes among ESβLs-producing *E. coli:*

Molecular detection of resistance genes among ESβLs-producing *E. coli* isolates revealed that *blaoxa23*was the most prevalent gene (22%) followed, by *bla shv* (18%) then *blaoxa48* gene (12%). There was remarkable coexistence of *bla shv* + *bla oxa48*, or *bla shv* + *bla oxa23*, or *bla shv* + *bla oxa48* + *bla oxa23* genes (table 4, Fig. 4 and Fig. 5).

Table 4: Detection of *bla shv, oxa23* and *oxa48* genes by PCR among ESβL-producing *E. coli* isolates

Genotype	ESβL-producing *E. coli* (n=50)			
Single gene	**Positive**	**%**	**Negative**	**%**
bla shv	**3**	**6%**	**47**	**94%**
bla oxa23	**7**	**14%**	**43**	**86%**
bla oxa48	**3**	**6%**	**47**	**94%**
Combined genes				
bla shv +oxa 48	**2**	**4%**	**48**	**96%**
bla shv + oxa23	**3**	**6%**	**47**	**94%**
bla shv + oxa23 + bla oxa48	**1**	**2%**	**49**	**98%**
Total detected genes				
Total *bla shv*	**9**	**18%**	**41**	**82%**
Total *bla oxa23*	**11**	**22%**	**39**	**78%**
Total *bla oxa48*	**6**	**12%**	**44**	**88%**

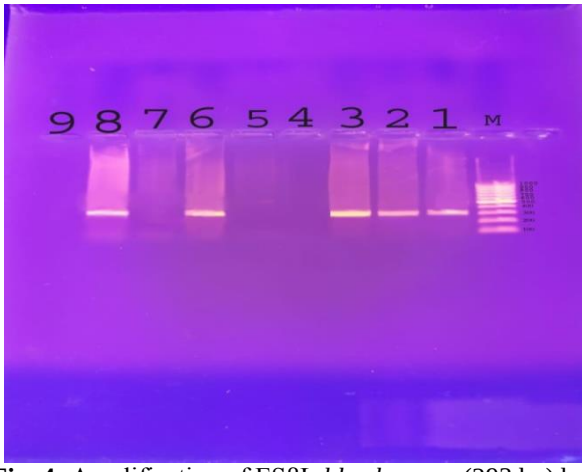

Fig. 4: Amplification of ESβL *bla shv* gene (293 bp) by PCR. Lane M: DNA marker (100bp). Lanes 1, 2, 3, 6 and 8 were positive for *bla shv* gene.

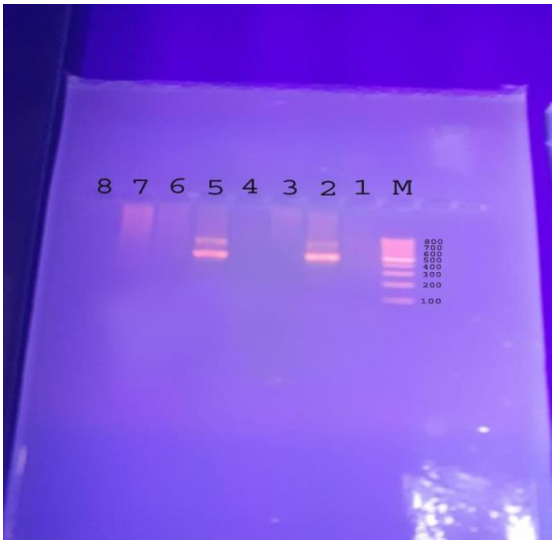

Fig. 5: Amplification of *bla oxa23* gene (501 bp) and *bla oxa48* gene (744 bp) by multiplex PCR. Lane M: DNA marker (100 bp). Lanes 2 and 5 were positive for both *bla oxa23* and *bla oxa48* genes.

Considering PCR as the gold standard, the sensitivity, specificity, PPV, NPV and accuracy of the Carba NP test in ESβL-producing *E. coli* isolates were 91%, 82 %, 59%, 97 % and 84% respectively as compared to detection of *bla oxa23* gene, and 83%, 73%, 29%, 97% and 74% respectively as compared to detection of *bla oxa48* gene (table 5).

Table 5: Sensitivity, specificity, PPV, NPP and accuracy of Carba NP in relation to PCR for detection of carbapenemases genes

	PCR		Sensitivity	Specificity	*PPV	**NPV	Accuracy
Carba NP	***bla oxa23* gene**						
	Positive (11)	**Negative (39)**					
Positive (17)	**10**	**7**	**91 %**	**82%**	**59%**	**97%**	**84%**
Negative (33)	**1**	**32**					
Carba NP	***bla oxa48* gene**						
	Positive (6)	**Negative (44)**					
Positive (17)	**5**	**12**	**83%**	**73%**	**29%**	**97%**	**74%**
Negative (33)	**1**	**32**					

DISCUSSION

ESβLs-producing *E. coli* have emerged as serious pathogens with a high prevalence rate worldwide. They represent major challenges in treatment and control of infections in the community and hospital settings[10]. In the present work, 247 out of 270 samples (91.5%) showed monomicrobial growth while only 8 samples (3%) showed polymicrobial growth. No bacterial growth was detected from 15 samples, a finding which is similarlyreported[11]. *E. coli* (30.4%) was the predominant isolate in this study, a result which is matched with *Abo-State et al.,*[12] and *Ahmed et al.,*[37] who isolated *E. coli* from 41.89% and 36% respectively and indicated that *E. coli* is a major cause of hospital infections. However, *E. coli* was the second organism ((35%), (40.1%)) after *Klebsiella* spp ((42%), (55.4%)) in other studies[13,38].

ESβLs-production has become an important resistance mechanism in hospitals worldwide. In this study, the high prevalence of ESβLs-producing *E. coli* (62.5%) was alarming. This finding is consistent with previous studies[14-16], where 61%, 66.7% and 63.8% of *E. coli* isolates were ESβL-producers respectively. Higher rate of ESβL-producing *E. coli* 84% was reported by *Essawy et al.,*[39]**.** By contrast, the lowest proportions of ESβL-producing *E. coli* were reported in Europe (< 1% in Sweden[17] and 5% in Netherlands[18]). Dissemination of β-lactamase-producing microorganisms in developing countries, including Egypt, could be attributed to lack of antibiotic policy and poor hygiene conditions in developing countries. Interestingly, *E. coli* strains that were collected from the hospital tend to be β-lactamase producers. This might be due to clonal spreading and transmission of ESβL genes between Gram-negative bacilli (GNB) in hospitals[19].

In this study, ESβLs-producing *E. coli* strains were most commonly isolated from ICUs (46%) and were most commonly recovered from urine samples (46%). Similarly, 61.1% of ESβLs-producing *E. coli* were from ICUs[14], and urine samples were reported to be the major source of *E. coli*[21,22]. *E. coli* isolates are among the most common etiological agents that cause UTI [23]. On the other hand, pus specimens were the main source of ESβL-producing *E. coli* (47.6%)[20]. It has been suggested that increased use of invasive devices and selective pressure of newer β-lactams used by patients at ICU lead to the emergence of such pathogens[19].

In the present study, the screening test of ESβL-production showed that 87.5% of *E. coli* strains were potential ESβL-producers while the combined disk test and the ESβL-NDP test showed that only 62.5% and 60% of them were ESβL-producers respectively, with no significant difference between the two confirmatory tests. Moreover, ESβL-NDP test showed 96% sensitivity and 100% specificity in relation to the combined disk test, a finding which was previously reported[24,25]. ESβL-NDP test has many advantages being rapid, sensitive and specific and the results can be interpreted on the same day. However, the requirement of microtiter plates and high-speed centrifugation at 10,000 rpm are disadvantages[26].

Most of ESβLs-producing *E. coli* isolates displayed resistance against most of the tested antibiotics, all ESβLs-producing *E. coli* strains were 100% resistant to cefoxitin and ceftazidime and were >90% resistant to pipracillin, amoxicillin-clavulanic acid, cefepime and ciprofloxacin. Moreover, there was a significant difference between ESβLs- and non-ESβLs-producing *E. coli* regarding susceptibility to amoxicillin/clavulanic acid, azetreonam, imipenem, meropenem, tobramycin, ciproflxacin and tigecycline. Similar results were previously reported[25, 27]. Our finding and that of others may be explained by the fact that ESβLs enzymes are often plasmid-mediated. Therefore, ESβLs-producing *E. coli* strains are often resistant to most antibiotic groups whose resistance genes are carried on the same plasmid[28].

Regarding carbapenem resistance, ESβLs-producing *E. coli* isolates showed higher resistance rates towards imipenem (44%) and meropenem (48%) compared to non-ESβLs-producing isolates. In agreement with our results, 41% of ESβL- producing *E. coli* were non-susceptible to carbapenems[29]. These high rates of carbapenem-resistance among ESβL-producing *E. coli* in Egypt are probably related to the overuse of

carbapenem drugs in trial therapies for the management of any febrile illness[29]. Moreover, wide spread use of third-generation cephalosporins may be the most important precipitating factor in the emergence of ESβL and carbapenemase producing *E. coli*[29].

In the present study, there was no significant difference between the three phenotypic methods used for detection of imipenem resistance. However, carba NP test showed 100% sensitivity and 85% specificity for detection of carbapenem resistance. Comparable results (100% sensitivity and specificity) were previously reported[30,31]. Moreover, the carba NP test is easier than other techniques that are time-consuming as MIC. Therefore, use of this test may contribute to a better stewardship to control carbapenemase producers worldwide [30].

In this study, 18%, 22% and 12% of ESβL-producing *E. coli* isolates carried *bla shv, bla oxa23* and *bla oxa48* genes respectively. Similar results were reported by others[32-34]. On the other hand, higher rate of *bla shv* gene detection (61.22%) was observed[29]. However, *Chaudhary et al.,*[35] detected *oxa48* gene in 32.6%, and couldn't detect *oxa23* gene in their isolates.

Considering PCR as the gold standard, the sensitivity, specificity, NPV, PPV and accuracy of carpa NP test were 91%, 82 %, 97%, 59 % and 84% respectively for *bla oxa23* detection and were 83%, 73%, 97%, 29% and 74% respectively for *bla oxa48* detection. Our results are matched with *Bakthavatchalam et al.,*[36] who reported that carba NP is valid and less expensive method for detection of carbapenem resistance.

CONCLUSION

The high prevalence of antibiotic resistance among EβSLs-producing *E. coli* isolates, especially to carbapenems, is a major challenge. A great concern should be paid to provide alternative therapeutic agents, continuous surveillance, and effective antibiotic stewardship program. Phenotypic methods for detection of EβSLs and carbapenemases represent valid and less expensive alternatives to the molecular methods.

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Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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