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Detection of *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ genes among the Extended-Spectrum β-Lactamases (ESβLs) producing *Enterobacteriaceae* isolated from hospital-acquired infections and community in Egypt.

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ABSTRACT

Background: Bacteria are resistant to an antibiotic such as *Escherichia coli, Klebsiella pneumonia,* and *Enterobacter* sp. responsible for morbidity in worldwide. ESβLs are a group of plasmids encoded enzymes that have the efficacy to hydrolyze β-lactams antibiotics. The spread of (ESβLs) representing a serious problem and threatening the ability to treat an infection.
Aim of the study: This study aimed to investigate ESβLs-producing *Enterobacteriaceae* sp. isolated from patients and healthy individuals and detect the resistant genes *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$.
Patients and Methods: Two hundred bacterial isolates were recovered from patients and healthy individuals rectal swab samples. These isolates were screened for producing ESβLs and identified using both standard bacteriological methods and VITEK2 compact system). The antibiotics resistance of *Enterobacteriaceae* was assessed by the disk diffusion method and detection of *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ genes by multiplex PCR.
Results: Two hundred *Enterobacteriaceae* screening for-producing ESβLs showed that 56% (112/200) produce ESβLs. One hundred and twelve ESβLs-*Enterobacteriaceae* identified as following, *Klebsiella pneumonia* 51.73% (58/112), *Escherichia coli* and 46.40% (52/112), and *Enterobacter cloacae* 1.80% (2/112). The antibiotic resistance patterns of *Enterobacteriaceae* showed high resistance to ciprofloxacin, levofloxacin, and amikacin with the ratio of (71.76%), (60.72%) and (60.72%), respectively. Furthermore, ESβLs *Enterobacteriaceae* harbored genes *bla*$_{CTX-M}$ (78.6%), *bla*$_{TEM}$ (73.2%) and *bla*$_{SHV}$ (68.75%). The *bla*$_{TEM}$ was found the predominant gene in *E. coli* isolates 80.8%, while *bla*$_{CTX-M}$ in *Klebsiella pneumonia* 81%.
Conclusion: The present study showed a significant distribution of multidrug-resistant ESβLs-producing *Enterobacteriaceae* in patients in the hospital- and community-acquired rectal infection. ESβLs-producing *Enterobacteriaceae* species harboring co-existence resistant genes.

Keywords: *Enterobacteriaceae; ESβLs; resistance genes.*

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INTRODUCTION

Bacteria are resistant to an antibiotic such as *Escherichia coli, Klebsiella pneumonia,* and *Enterobacter* sp. responsible for morbidity in worldwide[1]. Antibiotics resistance due to extended-spectrum β-lactamases (ESβLs) was firstly recorded in 1979 in Europe[2]. ESβLs are a group of plasmids encoded enzymes that have the efficacy to hydrolyze β-lactams antibiotics[3]. It was firstly reported that ESβLs producers are predominantly belonging to *E. coli* and *Klebsiella* [4]

Among several enzymes' linkage with ESβLs activity, ESβLs class A include on cefotaximase (CTX-M), Temoneira (TEM), and SHV (to sulfhydryl variable active site)[3]. These genes are commonly in *Klebsiella pneumonia* and *E. coli* [5]. Investigation of these genes is important not only for their ability to hydrolyze β-lactam antibiotics but also because the plasmids responsible for ESβLs production regularly harboring genes encoding resistance to other antibiotic groups like aminoglycosides and fluoroquinolones. A previously

published study by Hassan *et al*.,2012 reported 98% of 65 *Klebsiella pneumonia* isolates obtained from Egyptian patient samples harbor SHV gene while 11% harbor CTX-M gene [6]. In another study conducted in Egypt highlighted that the CTX-M gene is the predominant resistance gene in ESβLs *Enterobacteriaceae* [7]. To date, few published studies were concerned with the assessment of ESβLs resistant genes in *Enterobacteriaceae* strains of the hospital and community setting. Therefore, this study aimed to isolate *Enterobacteriaceae* producing ESβls species from rectal swab samples, antibacterial resistance pattern and determine the predominate ESβLs resistance gene in isolates of hospital settings versus the presence of those genes in rectal isolates from community settings

PATIENTS AND METHODS

Samples collection

One hundred rectal swab samples were collected from 50 patients (two samples taken from each patient, one upon admission and second after 48h of admission) at Abu El-Reesh Pediatric Hospital, Cairo University Hospital, Egypt, and one hundred rectal swab samples from healthy individuals duration period extending from December 2016 to December 2019.

Cultivation and isolation of bacterial species

Rectal swab samples were cultivated on MacConkey media and incubated for 24 hours at 37^{o}C aerobically. Colonies with positive lactose fermentation (Pink colonies) were collected. The pure cultures were identified based on morphological, physiological, and biochemical characteristics using microbiological methods 8th, [8] Bergey's Manual of Systematic Bacteriology [9]. Isolates identification was confirmed by the VITEK2 compact system (Biomerieux Inc., Marcy I'Etoile, France).

Screening of (ESβLs) production

The antibiotics synergy of ESβLs producing bacteria was detected by the double-disk synergy test (DDST) [10]. Bacterial colonies from MacConkey agar equivalent to 0.5 McFarland are cultured on Mueller-Hinton agar media. The following antibiotic discs are used, cefotaxime 30µg/ml, ceftazidime 30µg/ml (third-generation cephalosporins), and amoxicillin/clavulanate 20/10µg/ml. Culturing plates were incubated at 35^{o}C for 24h. ESβLs production activity is confirmed if there is an extension of the inhibition zone between any of the cephalosporins and amoxicillin-clavulanate disk (D-shape or keyhole shape) [11, 12].

Antibiotic resistance pattern

Antibacterial sensitivity testing was carried out by disc diffusion method and the results were expressed as resistant, intermediate, or susceptible according to CLSI guidelines [10]. The antibiotics used in this study belonging to four groups antibiotics carbapenems include on (imipenem10µg and meropenem10µg), aminoglycosides (gentamicin 10µg/ml and amikacin 30µg/ml), fluoroquinolones (ciprofloxacin 5µg/ml and levofloxacin 5µg/ml), and polypeptides (colistin10µg/ml and polymyxin B 300U/ml).

Molecular detection of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes using PCR.

A total of 120 ESβLs producing *Enterobacteriaceae* were investigated for detecting three genes (*bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$) using multiplex PCR and specific primers (Table 1). The detection methods were designed according to the methods Randall *et al*., 2009 [13].

Gene type	Primer sequence (5'-3')	Gene product length (bp)	Reference
TEM F	TCGTGTCGCCCTTATTCCCTTTTT	426	[19]
TEM R	GCGGTTAGCTCCTCCGGTCCTC		
SHV F	GTGGATGCCGGTGACGAACAGC	212	
SHV R	TGGCGCAAAAAGGCAGTCAATCCT		
CTX-M F	CGCTTTGCGATGTGCAG	551	[8]
CTX-M R	ACCGCGATATCGTTGGT		

Table 1:. Primer sets used in PCR runs for tested isolates.

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) by statistical package SPSS v17. The mean difference comparison between the treatments was analyzed by the Tukey HSD test at a significance level of $P \leq 0.05$.

RESULTS

Screening of ESβLs producing *Enterobacteriaceae*

A total of 200 *Enterobacteriaceae* were screening for producing ESβLs, the results obtained showed that 112 (56%) bacterial isolates producing ESβLs. Thirty-two isolates (64%) from patients upon admission, 40 (80%) from the same patients after 48h of admission, and 40 (40%) from healthy individuals.

identification of ESBLs *Enterobacteriaceae*

The results revealed that 112 bacterial isolates producing ESβLs (32 isolates from patients upon admission, 40 from same patients after 48h of admission, and 40 from healthy individuals) were

included to identify. The results obtained from morphological, physiological, and biochemical tests revealed that 100% of bacterial isolates are Gram-negative, rod shape, motile, ferment lactose sugar in MacConkey agar, and positive results in the triple sugar iron (TSI) test. Only 46.4% (52/112) from bacterial isolates have the ability to utilizing tryptophan and forming indole and produce decarboxylase enzyme, Moreover, 1.8% (2/112), 51.73% (58/112), and 53.5(60/112) can produce H_2S, urease, and citrate utilization respectively (Table 2**)**. According to results obtained from the identification of 112 ESβLs - producing *Enterobacteriaceae* species, the most common species was found *Klebsiella pneumonia* followed by *E. coli* and *Enterobacter cloacae* with percent 51.8% (58/112), 46.4% (52/112), and 1.8% (2/112), respectively. This result was confirmed by VITEK-2 with an echelon ratio of 99%. In this study, The identification of bacterial species isolated from healthy individuals revealed that the most common species *E. coli* 22.3% (25/112) followed by *Klebsiella pneumonia* 13.38% (15/112), comparable isolates from patients the most common species were found *Klebsiella pneumonia* 38.35% (43/112) followed by *E. coli* 24% (27/112) and 1.8% (2/112) *Enterobacter cloacae.*

Bacterial strains	Health	patient		Total	%	Biochemical test						
		1st samples	2nd samples			motility	TSI	H_2S	Urease	Citrate utilization	Indole	Decarboxylase enzyme
E. coli	25	15	12	52	46.40%	+	+	-	-	-	+	+
K. pneumonia	15	16	27	58	51.73%	+	+	-	+	+	-	-
Enterobacter cloacae	0	1	1	2	1.80%	+	+	+	-	+	-	+
Total	40	32	40	112	100%	--	--	--	--	--	--	--

Table 2: Identifications of 112 ESβLs producing *Enterobacteriaceae* species isolated from patients and healthy individuals.

Resistance patterns of *Enterobacteriaceae*

The antibiotic profile of *Enterobacteriaceae* isolated from patients showed the highest resistance to ciprofloxacin, levofloxacin, and amikacin (71.76%), (60.72%) and (60.72%), while, they were sensitive to colistin, polymyxin, meropenem, and imipenem (95.22%), (95.22%) (44.16%), and (44.16%), respectively. Moreover, it was noted that bacterial species isolated from patients after 48h of admission highly resistant to bacterial species isolates from the same patients upon admission and healthy individuals (Table 3). *E. coli* isolated from patients after 48hours of admission showed the highest resistance to ciprofloxacin, levofloxacin 91.63%, while, isolates from patients upon admission 66.6%. However, *E. coli* isolated from healthy individuals exhibit a low resistance level (Table 4). In *K. pneumoniae,* from patients after 48hours of admission the highest resistance was observed against and ciprofloxacin, levofloxacin 81.4% with a low resistance level to polymyxin B and colistin 3.70%. (Table 5). *Enterobacter cloacae* recorded the highest resistance to ciprofloxacin, levofloxacin with 100%. *K. pneumoniae* showed the resistance to meropenem and imipenem with 53.32%, 51.6%, followed by *Enterobacter cloacae* 50.0% and *E. coli* with 23.04%, respectively (table 4 and 5). Overall, *K. pneumoniae* showed the highest resistance level from *E. coli* isolates (Table 6).

Prevalence of *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ genes in *Enterobacteriaceae* species

In our study, 88/112 (78.5%) isolates it has *bla*$_{CTX-M}$ resistant gene, 82/112(73.2%) *bla*$_{TEM}$ gene and 77/112 (68.70%) *bla*$_{SHV}$ (table 7) Figure 1A, 1B, 2A and 2B.

The statistical analysis revealed that no significant statistical difference between the presence resistant genes *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ in healthy individuals isolates comparable with isolates collected from patients either upon admission or after 48h. of admissions, Also, the difference between the presences of the three genes in bacterial isolates collected from patients cases either upon admission or after 48h of admission was statistically insignificant (Table 8). Interestingly, the predominant resistant gene in bacterial species isolated from patients and healthy individuals is the *bla*$_{CTX-M}$ gene followed by *bla*$_{TEM}$ and *bla*$_{SHV}$ (Table 8). *bla*$_{CTX-M}$ was the predominant gene in *Klebsiella pneumonia* isolates (81.0%) while it was the second common in *E. coli* isolates (75.0%). The *bla*$_{CTX-M}$ resistance gene was the predominant gene in *E. coli* isolates (80.8%), and the least gene in *Klebsiella pneumonia* isolates (67.2%), *bla*$_{SHV,}$ a second common gene in *Klebsiella pneumonia* (79.3%) (Table 9).

Antibiotic	Inpatients (72 isolates)						Healthy individual (40 isolates		
	Upon admission (32 isolates)			After 48h of admission (40 isolates)					
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Amikacin	16 (50%)	0(0%)	16 (50%)	27(67.5%)	0 (0%)	13 (32.5%)	6 (15%)	2 (5%)	32 (80%)
Gentamicin	16 (50%)	0(0%)	16 (50%)	27(67.5%)	0 (0%)	13 (32.5%)	8 (20%)	0(0%)	32 (80%)
Ciprofloxacin	20 (62.5%)	0(0%)	12 (37.5%)	33(82.5%)	1 (2.5%)	6 (15%)	4 (10%)	2 (5%)	34 (85%)
Levofloxacin	20 (62.5%)	0(0%)	12 (37.5%)	33(82.5%)	0 (0%)	7 (17.5%)	6 (15%)	0 (0%)	34 (85%)
Polymyxin B	2 (6.25%)	0(0%)	30(93.75%)	1 (2.5%)	0 (0%)	39 (97.5%)	1 (2.5%)	0(0%)	39 (97.5%)
Colistin	2 (6.25%)	0(0%)	30(93.75%)	1 (2.5%)	0 (0%)	39 (97.5%)	1 (2.5%)	0 (0%)	39 (97.5%)
Meropenem	14(43.75%)	0(0%)	18(56.25%)	26 (65%)	0 (0%)	14 (35%)	2 (5%)	1 (2.5%)	37 (92.5%)
Imipenem	14(43.75%)	0(0%)	18(56.25%)	26 (65%)	0 (0%)	14 (35%)	3 (7.5%)	0 (0%)	37 (92.5%)

Table 3: Antibiotic resistance pattern of 112 ESβLs producing *Enterobacteriaceae* species.

Antibiotics	Patients						Healthy individuals		
	Upon admission			After 48h of admission					
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amikacin	46.62%	0.0%	53.28%	58.31%	0.0%	41.65%	12.0%	8.0%	80.0%
Gentamicin	46.62%	0.0%	53.28%	58.31%	0.0%	41.65%	20.0%	0.0%	80.0%
Ciprofloxacin	66.6%	0.0%	33.3%	91.63%	0.0%	8.33%	8.0%	0.0%	92.0%
Levofloxacin	66.6%	0.0%	33.3%	91.63%	0.0%	8.33%	8.0%	0.0%	92.0%
Polymyxin B	6.6%	0.0%	93.24%	0.0%	0.0%	100%	0.0%	0.0%	100%
Colistin	6.6%	0.0%	93.24%	0.0%	0.0%	100%	0.0%	0.0%	100%
Meropenem	26.64%	0.0%	73.26%	50.0%	0.0%	50.0%	4.0%	0.0%	96.0%
Imipenem	26.64%	0.0%	73.26%	50.0%	0.0%	50.0%	4.0%	0.0%	96.0%

Table 4: Antibiotics resistance pattern of *E. coli* R= Resistant, I= Intermediate, S= Sensitive.

Antibiotics	Patients						Healthy individuals		
	Upon admission			After 48h of admission					
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amikacin	56. 25%	0.0%	43.75%	70.4%	0.0%	29.6%	20.0%	0.0%	80.0%
Gentamicin	56. 25%	0.0%	43.75%	70.4%	0.0%	29.6%	20.0%	0.0%	80.0%
Ciprofloxacin	62.5%	0.0%	37.5%	81.4%	3.70%	14.8%	13.32%	13.32 %	73.26%
Levofloxacin	62.5%	0.0%	37.5%	81.4%	0.0%	18.5%	26.64	0.0%	73.26%
Polymyxin B	6.25%	0.0%	93.75%	3.70%	0.0%	96.2%	6.66%	0.0%	93.24%
Colistin”	6.25%	0.0%	93.75%	3.70%	0.0%	96.2%	6.66%	0.0%	93.24%
Meropenem	62.5%	0.0%	37.5%	70.3%	0.0%	29.6%	6.66%	6.66%	86.58%
Imipenem	62.5%	0.0%	37.5%	70.3%	0.0%	29.6%	13.32%	0.0%	86.58%

Table 5: Antibiotics resistance pattern of *Klebsiella pneumonia* R= Resistant, I= Intermediate, S= Sensitive.

Antibiotics	*E. col* 52 isolates			*K. pneumonia* 58 isolates			*Enterobacter cloacae* 2 isolates		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amikacin	32.64	3.84%	63.36%	53.32%	0.0%	46.44%	50.0%	0.0%	50.0%
Gentamicin	36.48%	0.0%	63.36%	53.32%	0.0%	12.04	50.0%	0.0%	50.0%
Ciprofloxacin	44.16%	0.0%	55.65%	58.48%	5.16%	36.12%	100%	0.0%	0.0%
Levofloxacin	44.16%	0.0%	55.65%	61.92%	0.0%	37.84%	100%	0.0%	0.0%
Polymyxin B	1.92%	0.0%	97.92%	5.16%	0.0%	94.6%	0.0%	0.0%	100%
Colistin”	1.92%	0.0%	97.92%	5.16%	0.0%	94.6%	0.0%	0.0%	100%
Meropenem	23.04%	0.0%	78.72%	51.6%	1.72%	46.44%	50.0%	0.0%	50.0%
Imipenem	23.04%	0.0%	78.72%	53.32%	0.0%	46.44%	50.0%	0.0%	50.0%

Table 6: Antibiotics resistance pattern of total *E. col*, *Klebsiella pneumonia*, and *Enterobacter cloacae*. R= Resistant, I= Intermediate, S= Sensitive

Resistant gene	Number of isolates containing each gene	Percentage
$bla_{\text{CTX-M}}$	88 bacterial isolates	78.6%
bla_{TEM}	82 bacterial isolates	73.2%
bla_{SHV}	77 bacterial isolates	68.75%

Table 7: Summary of prevalence $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} genes in the 112 ESβLs producing *Enterobacteriaceae* species.

Resistant genes		Patients		Healthy individuals	Total
		Upon admission	After 48h of admission		
No gene	---------	2	3	2	7
One gene	$bla_{\text{CTX-M}}$	1	0	3	4
	bla_{TEM}	3	1	3	7
	bla_{SHV}	1	1	2	4
Two genes	$bla_{\text{CTX-M}}$ + bla_{TEM}	5	4	7	16
	$bla_{\text{CTX-M}}$ + bla_{SHV}	4	5	5	14
	bla_{TEM} + bla_{SHV}	0	2	4	6
Three genes	$bla_{\text{CTX-M}}$ + bla_{TEM} + bla_{SHV}	16	24	14	54
Total		32	40	40	112

Table 8: Distribution of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} genes in ESβLs producing *Enterobacteriaceae* species.

Resistant genes	*E. coli* (total 52 isolates)		*Klebsiella pneumonia* (total 58 isolates)		*Enterobacter cloacae* (total 2 isolates)	
	Number of isolates	Percentage %	Number of isolates	Percentage %	Number of isolates	Percentage %
$bla_{\text{CTX-M}}$	39	75%	47	81%	2	100%
bla_{TEM}	42	80.8%	39	67.2%	2	100%
bla_{SHV}	30	57.7%	46	79.3%	2	100%

Table 9: Distribution of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} genes in ESβLs-producing *Enterobacteriaceae.*

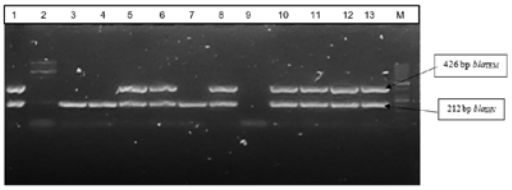

Fig 1A:. Detection of genes *bla*$_{TEM}$ and *bla*$_{SHV}$.in 12 isolates ESβLs-producing *E coli* by PCR, 426 bp PCR product of *bla*$_{CTX-M,}$ and 212 bp of *bla*$_{SHV}$. Lane M: ladder. Lanes 1,2 no *bla*$_{TEM}$ and Lane 6: *bla*$_{SHV,}$ while lanes 3, 4, 5, 7, 8,9, 10, 11 and 12 contain *bla*$_{TEM}$ and *bla*$_{SHV}$ genes

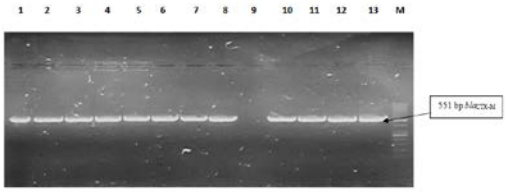

Fig 1B:. Detection of gene blaCTX-M.in 12 isolates ESβLs-producing E. coli by PCR, 551 bp PCR product of blaCTX-M. Lane M ladder, lanes 9 no blaCTX-M. while Lanes 1, 2, 3, 4, 5, 6, 7, 8, 10, 11,12 and 13 contains the blaCTX-M gene.

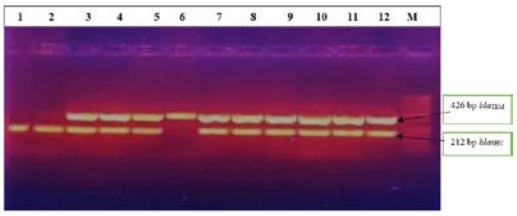

Fig 2A:. Detection of genes *bla*$_{TEM}$ and *bla*$_{SHV}$.in 12 isolates ESβLs-producing *Klebsiella pneumonia* by PCR, 426 bp PCR product of *bla*$_{CTX-M,}$ and 212 bp of *bla*$_{SHV}$. Lane M: ladder. Lanes 1,2 no *bla*$_{TEM}$ and Lane 6: *bla*$_{SHV,}$ while lanes 3, 4, 5, 7, 8,9, 10, 11 and 12 contain *bla*$_{TEM}$ and *bla*$_{SHV}$ genes.

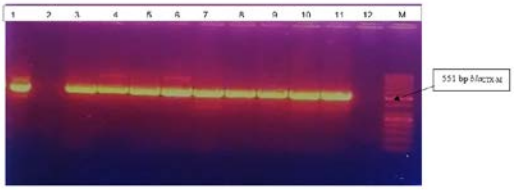

Fig 2B:. Detection of gene blaCTX-M.in 12 isolates ESβLs-producing Klebsiella pneumonia by PCR, 551 bp PCR product of blaCTX-M. Lane M ladder, lanes 2 and 12 no blaCTX-M. while Lanes 3, 4, 5, 6, 7, 8, 9, 10, 11 and contains the blaCTX-M gene.

DISCUSSION

In the last years, ESβLs producing *Enterobacteriaceae* have been increasingly recognized in hospitals in Egypt and, unfortunately, are linkage with multiple drug resistance [14]. The prevalence of producing ESβLs- *Enterobacteriaceae* was found to be (56%) 112/200, comparable with a studies from Ghana (49.3%) [15] , Ethiopia (57.6%) [16] , India (57.5%) [17], Burkina Faso (58.0%) [18] , and Uganda (62.0%) [19] . Teklu et al., 2019 recorded 57.7% (246/426) from *Enterobacteriaceae* species isolated from clinical samples producing ESβLs [20]. A current study from Turkey recorded a prevalence rate of ESβLs- *Enterobacteriaceae* carriage (34.3%) in the community [21]. The predominate ESβLs production was observed in *Klebsiella pneumonia*, these results agree with Teklu et al., 2019 [20]. In this study, the antibiotics resistance patterns of ESβLs-producing *Enterobacteriaceae* species isolated from patients showed that highly resistant level to ciprofloxacin, levofloxacin, and amikacin (71.76%), (60.72%) and (60.72%), while, they were sensitive to colistin, polymyxin, meropenem and imipenem (95.22%), (95.22%) (44.16%) and (44.16%), respectively. Teklu et al., 2019 isolated *Enterobacteriaceae* producing ESβLs from clinical samples resistant to norfloxacin with ratio (58.8%), ciprofloxacin (46.3%), gentamycin (43.4%), but low resistance to meropenem (5.2%) and amikacin (13.8%)[20]. The studies were conducted in Burkina which showed that 89% of ESβLs-producer isolates non-susceptible to gentamicin and 80% to ciprofloxacin [22]. In Ghana, 91.2% of ESβLs -producer *Enterobacteriaceae* was found resistant to gentamicin and 41.1% to ciprofloxacin [23]. In central India 50% from ESβLs -producer *Enterobacteriaceae* resistant to gentamicin and 87.5% to ciprofloxacin [22]. While in Nepal 90.7% resistant to ciprofloxacin, 90.4 and, 63.12% to gentamicin [24]. In this study, *E. coli* isolates from patients after 48hours of admission showed the highest resistance rate to ciprofloxacin, levofloxacin 91.63%, while isolates from patients upon admission showed a resistance rate of 66.6%. Several studies showed that *E. coli* isolates exhibit a resistance rate to ciprofloxacin and levofloxacin 86.6% [25]. Zheng and Xiang-zhu, 2017 reported a resistance rate to ciprofloxacin and levofloxacin among *E. coli* isolates 85.08% and 80.42%, respectively [26]. In the current study *Klebsiella pneumoniae* isolated from patients after 48hours of admission showed a resistance rate to ciprofloxacin, levofloxacin 62.5%. Zheng and Xiang-zhu, 2017 reported that *Klebsiella pneumoniae* resistant to ciprofloxacin, levofloxacin with a rate of 66.86%, and 50.0%, respectively [26]. In our study *K. pneumoniae* showed the highest resistance to meropenem and imipenem 53.32%, 51.6%, followed by *Enterobacter cloacae* 50.0% and *E. coli* with 23.04%, and all producing ESβLs-*Enterobacteriaceae* showed a resistance rate to imipenem and meropenem 37.46% (42/112) and 36.57% (41/112), respectively. This finding disagrees with Teklu et al., 2019, which found producing ESβLs-*Enterobacteriaceae* resistant to meropenem 5.2%, *E. coli* (3.5%), and *K. pneumoniae* 10.7% [20].

Prevalence of resistance genes *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{CTX-M}$ in ESβLs producing *Enterobacteriaceae* species

In the current study, the predominant resistant gene was *bla*$_{CTX-M}$78.6% followed by *bla*$_{TEM}$ 73.2%, and *bla*$_{SHV}$ 68.75%. These results are in agreement with several studies [27, 28, 29] which indicate that dissemination of the *bla*$_{CTX-M}$ gene represents a

global pandemic. The multinational survey performed by Ben-Ami *et al*., 2009 concluded that the bla_{CTX-M} gene was predominant in *E. coli* while the bla_{TEM} was predominant in *Klebsiella pneumonia* [30]. This result was not matched with our study, where the bla_{TEM} was predominate in *E. coli* while bla_{TEM} in *Klebsiella*. In our study, some ESβLs producing *Enterobacteriaceae* species harboring more than one resistant gene this result agreement with Zhao *et al*., 2015 and Bajpai *et al*., 2017 [31, 32]. The presence of more than one resistant gene could be attributed to the participation of genetic elements in the mobilization of these genes [33]. A low number of ESβLs producer isolates were not harboring one resistant gene at least from bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, a similar result was reported by Bajpai *et al*., 2017 [32]. In the same regard, the statistical analysis showed that the difference in the presence of the three ESβLs encoding genes in healthy individuals versus their presence in patient samples was not statistically significant. The presence of three resistance genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) in ESβLs-producing *Enterobacteriaceae* species illustrate the genetic diversity among isolates due to horizontal gene transfer between different bacterial species.

In this study, the bla_{CTX-M} gene was predominant in bacterial species isolated from patient samples upon a and after 48h of admission with a percentage of 81.25% (26/32) and 82.5 (33/40), respectively. This result agreed with Hagel *et al*., 2019 [29], who reported that bla_{CTX-M} was represented in 81.1% of upon admission isolates and 84.1% of discharge isolates. Moreover, Pérez *et al*., [34] showed that the bla_{CTX-M} gene represents 83.17% of all isolates taken from patients upon admission. Data analysis of healthy individuals revealed that the bla_{CTX-M} resistant gene was the most common gene detected in 72.5% of bacterial isolates. Also, the bla_{SHV} gene was detected in 62.5% of the healthy individuals bacterial isolates. In the same regard, Valverde *et al*., 2004 reported that 70% of the non-hospitalized individual were colonized with ESβLs carrying the bla_{CTX-M} gene [35]. The predominance of a bla_{CTX-M} gene in *E.coli* clinical patients isolates was also reported by Ahmed *et al.*, 2014 [36]. As well as, bla_{CTX-M} encoding ESβLs gene was detectable in 96.6% of community isolates [37].

CONCLUSION

From this study we can be concluded, ESβLs producing Enterobacteriaceae species are carried by patients and healthy individuals in the community. Fecal carriage of resistant Enterobacteriaceae species represents a high risk for spread multidrug resistance bacteria. ESβLs producing Enterobacteriaceae harboring co-existence resistant genes. The best choice for the treatment of ESβLs Enterobacteriaceae is polymyxin B and colistin. To prevent further spread ESβLs producing Enterobacteriaceae, it should be motivating the ideal use of antibiotics, and antibiotic resistance should keep under surveillance in Egypt.

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