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ORIGINAL ARTICLE

Are TEM and CTX Beta-Lactamase genes common among urinary isolates from Ain Shams University Hospitals, Intensive Care Units?

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Antimicrobial susceptibility testing, blaTEM, blaCTX-M, ESBLs.

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ABSTRACT

Background: *Many extended-spectrum β-lactamases (ESBLs) variants are known among Gram-negative bacilli, and are classified into different structural families as TEM, SHV, CTX-M, and OXA.* ***Objectives:*** *To detect the distribution of bla_{TEM} and bla_{CTX-M} genes among the Gram-negative isolates collected from urine samples from patients admitted to ICUs of Ain Shams University Hospitals, Egypt.* ***Methodology****: Forty Gram-negative urinary isolates were enrolled, and subjected to microbiological identification and antimicrobial susceptibility testing. Phenotypic detection of ESBLs production was done. Detection of bla_{TEM} and bla_{CTX-M} genes was done by PCR.* ***Results:*** *Phenotypically, 8(20%) isolates were ESBL producers and 32(80%) were non ESBL producers. Bla_{TEM} gene was found in 15 isolates(37.5%) and bla_{CTX-M} gene was found in 8 isolates(20%), while both genes were detected among five isolates.* ***Conclusion:*** *Molecular methods should be used for definitive identification of ESBLs. Bla_{TEM} gene was more common than bla_{CTX-M} gene in urine specimens in our setting.*

INTRODUCTION

Multidrug resistance among bacteria is increasing worldwide, specially in Gram negative ones, causing both community and hospital acquired infections [1]. Multidrug resistant organisms can cause life threatening infections worldwide. The *Enterobacteriaceae family* and other Gram negative bacilli are mainly responsible for many cases of these antibiotic resistance, mainly by ESBLs production [2].

ESBLs were first discovered in 1983. ESBLs were derived from genes (TEM-1, TEM-2, or SHV-1) by alteration of the amino acid configuration around the enzyme active site [3]. More than 350 different natural ESBL variants are known and classified into nine distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA[4].

ESBLs are typically plasmid-mediated enzymes that hydrolyze penicillins, third generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and the monobactam aztreonam. They are not active against the cephamycins (cephoxitin and cefotetan) and carbapenems (imipenem, meropenem), but are susceptible to β-lactamase inhibitors such as clavulanic acid and tazobactam [5]. Gram negative bacilli are now considered as a major cause of urinary tract infections (UTIs) [6]. ESBL-producing *E. coli* is considered as the most multidrug resistant strain emerging worldwide [7].

A variety of ESBLs, mostly of the genotypes CTX-M, TEM and SHV types, had been reported in members of *Enterobacteriaceae* family, and are often undetectable by the current isolation and susceptibility methods[8]. For detection of ESBLs production, determination of susceptibility to cephalosporin followed by inhibition of the ESBLs activity by clavulanic acid or tazobactam is usually done [9] or by the double-disk synergy test, with 80 to 90% sensitivity and specificity respectively [10].

Molecular detection of ESBLs genes by PCR, hybridization, and sequencing is an alternative accurate method [11]. Although conventional microbiological methods for detection of antibiotic resistance are reliable, yet they need several days to be completed. Speedy detection of resistant genes using molecular methods as PCR might help in rapid patient treatment[12,13].

We hereby studied the distribution of ESBLs (bla_{TEM} and bla_{CTX-M}) genes among the Gram-negative microorganisms collected from urine samples in ICUs of Ain Shams University Hospitals.

METHODOLOGY

Bacterial isolation and identification:

The research was conducted on 40 Gram negative isolates, isolated from urine samples obtained from patients admitted to ICUs of Ain Shams University Hospital, Cairo, Egypt, from September 2018 till December 2018, who were suspected of having urinary

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tract infection (UTI). The study was performed according to the regulations of The Research Ethics Committee, Faculty of Medicine, Ain Shams University. An informed consent was obtained from each patient. The isolates were detected by conventional methods based on the colonial morphology on blood agar and MacConkey's agar, then confirmation was done using a number of biochemical assays, including triple sugar iron, indole production, citrate utilization and urease production testes [14,15]. All the media and biochemical tests were supplied by Oxoid, UK.

Antimicrobial susceptibility testing:

The antibiotic susceptibility testing for the isolated Gram-negative bacilli was done by the Kirby-Bauer diffusion method [15,16]. Antibiotics disc selection and interpretation of the results were done according to Clinical and Laboratory Standards Institute (CLSI) guidelines [17].

The antibiotics discs used were: Ampicillin (AM, 10µg), Amoxicillin/ Clavulanic acid (AMC, 20/10 µg), Piperacillin/ tazobactam (TPZ,100/10 µg), Aztreonam (ATM, 30 µg), Ceftazidime (CAZ, 30 µg), Cefotaxime (CTX, 30 µg), Cefoxitin (FOX, 30 µg), Cefepime (FEP,30 µg), Meropenem (MEM,10 µg), Gentamicin (CN,10 µg), Amikacin (AK, 30 µg), Ciprofloxacin (CIP, 5 µg), Trimethoprim-sulfamethoxazole (SXT, 25µg) (Liofilchem®, Italy).

Phenotypic detection of ESBL:

All the Gram-negative isolates were tested for ESBL production using the double disc synergy method [11,18]. Commercially prepared antibiotic discs of Ceftazidime (CAZ, 30 µg) together with Ceftazidime/ Clavulanic acid (CZC, 30/10 µg) discs were placed on the surface of Mueller-Hinton agar plates, which were inoculated with the tested isolated microorganisms and then incubated at 37°C for 24 h. A bacterial isolate was considered as ESBL producer if there was a ≥5 mm increase in the diameter of the inhibition zone of ceftazidime/clavulanic acid disc than that of ceftazidime disc alone [18] (Figure 1).

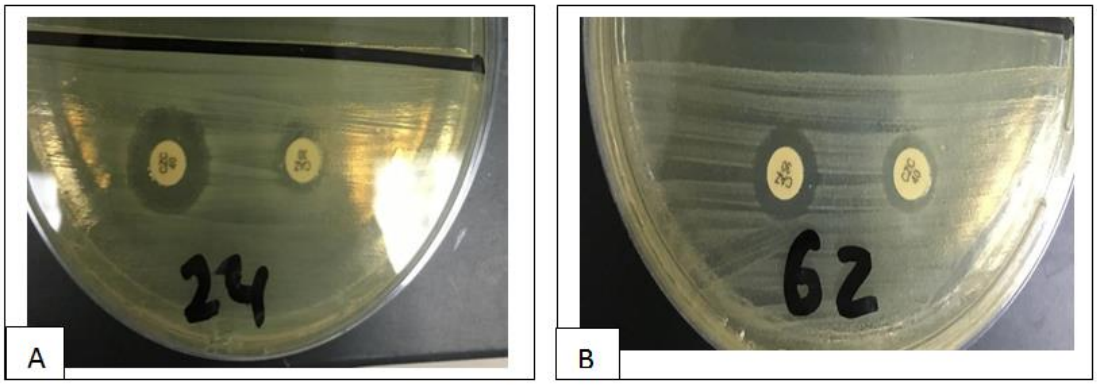

Fig. 1: ESBLs detection by double disc synergy test. (A) Positive for ESBL production, (B) Negative for ESBL production

Genetic detection of bla_{TEM}, and bla_{CTX-M} genes by PCR

Extraction of total DNA:

DNA extraction was performed using QIAGEN DNA extraction Kit® (QIAGEN, USA). Purification of DNA from the bacterial cultures were done using the spin column method, as per manufacturer's instructions.

Polymerase chain reaction (PCR):

The purified DNA or bacterial colonies were used in PCR for the detection of the presence or absence of bla_{TEM}, and bla_{CTX-M} genes. The PCR was performed in 25 µl reaction volume containing: 1X buffer (10 mM Tris-HCl pH 8.3, 50 Mm KCl, 2 mM $MgCl_2$), 250 µM for each of dGTP, dATP, dCTP and dTTP, 0.5 units of Taq DNA polymerase, 100 pmol of every primer and the DNA template. DNA amplification was performed in the thermal cycler with an initial DNA denaturation step for 5 min. at 94°C followed by 35 cycles. Each cycle consisted of: denaturation for 1 min. at 94°C, annealing for 45 sec at 50°C, extension at 72°C for 3 min., and a final extension step for 7 min. at 72°C carried out at the end of the 35 cycles before analyzing the PCR products by electrophoresis in agarose gel (figure 2). Primers used for bla_{TEM}, and bla_{CTX-M} genes detection are shown in table (1).

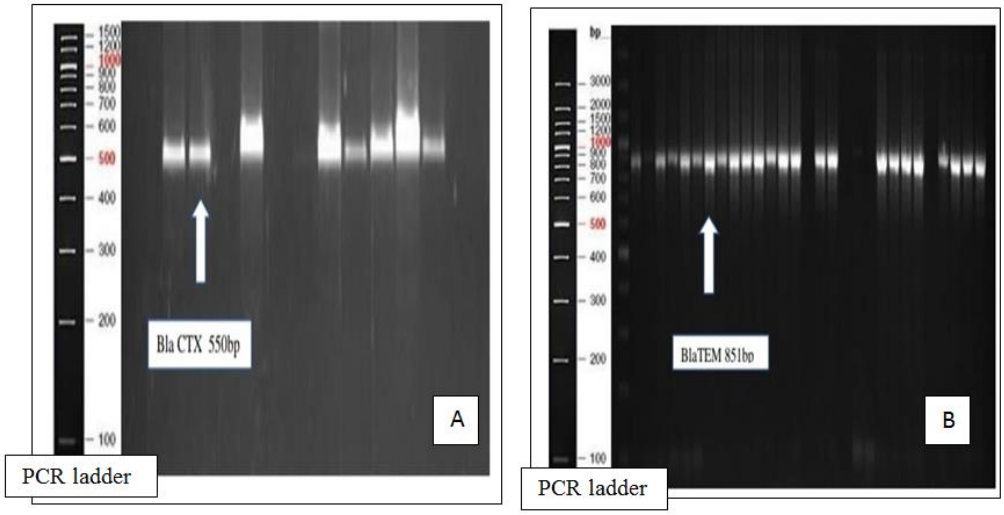

Fig. 2: Gel electrophoresis for the detection of the amplified PCR products of bla_{CTX-M} (A) and bla_{TEM} (B) genes in the urinary isolates of Gram-negative bacilli.

Table 1: Primers used for detection of ESBLs genes by PCR assay

Primers	Target genes	Oligonucleotide sequence (5` - 3`)	Size of amplicons (bp)
TEM-F TEM-R	Bla_{TEM} [19]	ATGAGTATTCAACATTTCCG TTAATCAGTGAGGCACCTAT	851 bp
CTX-M-F CTX-M-R	Bla_{CTX-M} [20]	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550 bp

Statistical analysis:

Statistical analysis was done on a personal computer using the Statistical Package for Social Sciences (SPSS) version 17 as follow:

- Descriptive statistics:
  - Frequency number and percentage for qualitative data.
- Analytical statistics:
  - Paired t-test used to compare between related samples.
  - Level of significance was considered at 0.05 i.e.: P value > 0.05 non-significant, P value ≤ 0.05 significant and P value ≤ 0.01 highly significant.

RESULTS

Out of 95 urine samples collected from the patients, 40 isolates of Gram-negative bacilli were recovered. These isolates were: *Klebsiella pneumoniae (Kl. pneumoniae):* 21 (52.5%) isolates, *E. coli*: 10 (25%) isolates (25.0%), *Pseudomonas aeruginosa*: 6 (15.0%) isolates, *Citrobacter spp.:* 3 (7.5%) isolates. The demographic data regarding the age, comorbidity conditions and antibiotics taken by the patients from who the Gram-negative isolates were isolated are analyzed in table (2).

Table 2: The demographic data of all the patients from who the 40 Gram negative isolates were recovered

Number of bacterial isolates	**No.** = 40
Age	
Mean ± SD	62.53 ±12.82
Range	35 –82
Sex	**No. (%)**
Female	21 (52.5)
Male	19 (47.5)
Clinical Manifestations	**No. of patients (%)**
Respiratory failure	7 (17.5)
Septic shock	4 (10)
Stroke	3 (7.5)
Renal failure	3 (7.5)
Diabetic ketoacidosis	2 (5)
Portal hypertension	2 (5)
Decreased conscious level	2 (5)
COPD	2 (5)
Chronic liver disease	1 (2.5)
Others	12 (30)
Antibiotics received by the patients*	**No. of patients (%)**
Ceftriaxone	22 (55)
Clindamycin	20 (50)
Ceftazidime	14 (35)
Amoxicillin- Clavulanic acid	7 (17.5)
Meropenem	6 (15)
Vancomycin	5 (12.5)
Levofloxacin	5 (12.5)
Flagyl	4 (10)
Ciprofloxacin	3 (7.5)
Cefepime	3 (7.5)
Gentamycin	1 (2.5)
Colistin	1 (2.5)
Imipenem	1 (2.5)

** Some patients were receiving more than one type of antibiotics therapy.*

Antimicrobial susceptibility testing:

The 40 Gram-negative isolates were multidrug resistant. All the isolates (100%) were omit resistant to ampicillin, cefotaxime, cefoxitin and ceftazidime. About 70-90% of the isolates showed resistance to amoxicillin, pipracillin/tazobactam, cefepime, meropenem, amikacin, cefalexin, trimethoprim/ sulfamethoxazole, aztreonam and ciprofloxacin as shown in figure (3).

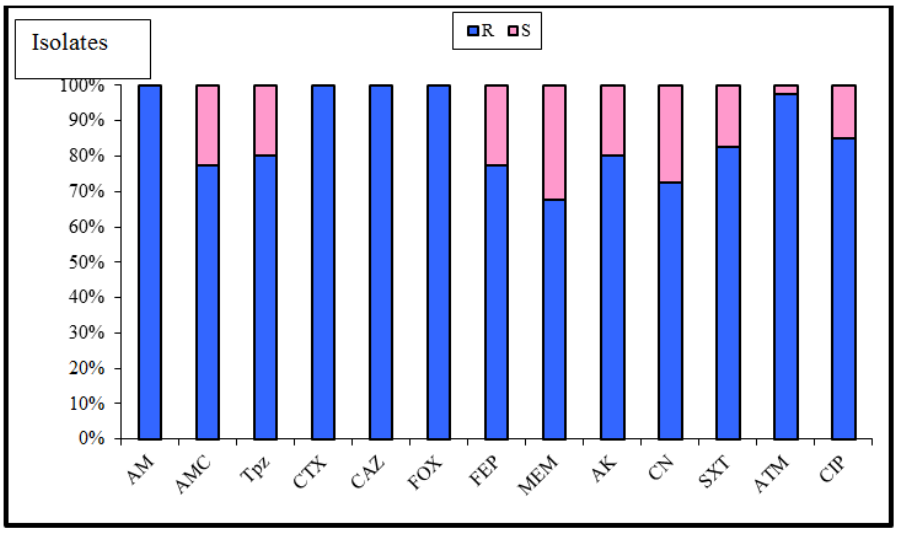

Fig. 3: Antibacterial susceptibility pattern of the bacterial isolates against the tested antibiotics. R: Resistant, S: Sensitive. AM: Ampicillin, AMC: Amoxicillin/ Clavulanic acid, TPZ: Pipracillin/Tazobactam, CTX: Cefotaxime, CAZ: Ceftazidime, FOX: Cefoxitin, FEP: Cefepime, MEM: Meropenem, AK: Amikacin, CN: Gentamycin, SXT: Trimethoprim/ Sulfamethoxazole, ATM: Aztreonam, CIP: Ciprofloxacin.

Phenotypic detection of ESBL:

There were 8 isolates (20%) that were producers of ESBL, while the other 32 isolates (80%) were non ESBLs producers by double disk synergy test. The 8 ESBLs producers isolates were 4 isolates of *KI. pneumoniae.*, 2 isolates of *E. coli* and 2 isolates of *Pseudomonas aeruginosa*. The isolates that were ESBLs producers expressed either Bla_{CTX-M}, Bla_{TEM} or both, except for 2 isolates of *Kl. pneumoniae* that did not express either genes when assessed by PCR.

Genetic detection of bla_{TEM}, and bla_{CTX-M} genes by PCR:

Bla_{TEM} gene was detected in 15 isolates (37.5%), and bla_{CTX-M} gene was found in 8 isolates (20%). Meanwhile, 5 isolates demonstrated the presence of both genes as follows: 2 isolates of *Kl. pneumoniae*, 2 isolates of *E. coli* and 1 isolate of *Pseudomonas aeruginosa*. The distribution of Bla_{TEM} and bla_{CTX-M} genes among the 40 isolates is illustrated in tables (3,4).

The Bla_{TEM} gene was detected in 15 isolates (37.5%), 9 isolates (60 %) of *Kl. pneumoniae*, 3 isolates (20.0%) of *E. coli*, and 3 isolates (20 %) of *Pseudomonas aeruginosa.* Among these 15 isolates, only 5 isolates were producers of ESBL by double disc test; however, no statistically significant difference was detected (table 3).

The Bla_{CTX-M} gene was found in 8 isolates (20%), 4 isolates (50%) of *E. coli*, 3 isolates of (37.5 %) *Kl. pneumoniae*, and a single isolate (12.5%) of *Pseudomonas aeruginosa.* Among the 8 isolates, only 3 isolates showed ESBL production by using the double disc test, with no detected statistical significance (table 4).

Table 3: The distribution of Bla_{TEM} gene among the isolates:

		Negative Bla_{TEM} Total No.25	Positive Bla_{TEM} Total No.15	Test value	P-value	Sig.
		No. (%)	No. (%)			
Organism	*E. coli*	7 (28)	3 (20)	4.160	0.385	NS
	Kl. pneumoniae	12 (48)	9 (60)			
	P. aeruginosa	3 (12.0)	3 (20)			
	Citrobacter	3 (12.0)	0 (0)			
Screening Double Disc test	Non ESBL producer	22 (88)	10 (66.7)	2.667	0.102	NS
	ESBL producer	3 (12)	5 (33.3)			

Sig.: significance, NS: non-significant

Table 4: The distribution of bla_{CTX-M} gene among the isolates:

		Negative Bla_{CTX-M} Total No.32	Positive Bla_{CTX-M} Total No.8	Test value	P-value	Sig.
		No. (%)	No. (%)			
Organism	*E. coli*	6 (18.8)	3 (20)	3.854	0.426	NS
	Kl. pneumoniae	18 (56.2)	9 (60)			
	P. aeruginosa	5 (15.6)	3 (20)			
	Citrobacter	3 (9.4)	0 (0)			
Screening Double Disc test	Non ESBL producer	27 (84.4)	5 (62.5)	2.667	0.167	NS
	ESBL producer	5 (15.6)	3 (37.5)			

Sig.: significance, NS: non-significant

Correlation between prior antibiotics intake and the presence of bla_{TEM} and bla_{CTX-M} genes:

A statistically significant association was detected between positive bla_{TEM} and prior ceftazidime intake (table 5) and there was a statistically significant association between positive bla_{CTX-M} gene and prior ceftriaxone and amoxicillin/clavulanic acid intake (table 6). No other significant associations were found with other antibiotics intake.

Table 5: Correlation between prior antibiotics intake and the presence of bla_{TEM} gene:

Antibiotic	Positive for bla_{TEM} Total No. 15 No. (%)	Negative for bla_{TEM} Total No. 25 No. (%)	Test value	P value	Sig.
Ceftriaxone	5 (33.3)	17 (68)	3.259	0.07	NS
Clindamycin	5 (33.3)	15 (60)	2.667	0.102	NS
Ceftazidime	9 (60)	5 (20)	6.5934	**0.01***	**S**
Amoxilcillin/ clavualanic acid	5 (33.3)	2 (8)	2.5974	0.107	NS
Meropenem	4 (26.6)	2 (8)	0.784	0.376	NS
Vancomycin	3 (20)	2 (8)	1.234	0.267	NS
Levofloxacin	2 (13.3)	3 (12)	0.015	0.902	NS
Metronidazole	2 (13.3)	2 (8)	0.296	0.586	NS
Ciprofloxacin	2 (13.3)	1 (4)	1.177	0.278	NS
Cefepime	1 (6.7)	2 (8)	0.024	0.877	NS
Gentamycin	1 (6.7)	0 (0)	1.709	0.191	NS
Colistin	0 (0)	1 (4)	0.615	0.433	NS
Imipenem	0 (0)	1 (4)	0.615	0.433	NS

Sig.: significance, NS: non-significant, S: significant.

Table 6: Correlation between prior antibiotics intake and the presence of bla_{CTX-M} gene:

Antibiotic	Positive for bla_{CTX-M} Total No. 8 No. (%)	Negative for bla_{CTX-M} Total No. 32 No. (%)	Test value	P value	Sig.
Ceftriaxone	7 (87.5)	15 (47)	4.2677	**0.03***	**S**
Clindamycin	4 (50)	16 (50)	0.000	1.000	NS
Ceftazidime	1 (12.5)	13 (40.6)	2.22	0.135	NS
Amoxilcillin/ clavualanic acid	6 (75)	1 (3.1)	22.9	**<0.0001***	**S**
Meropenem	2 (25)	4 (12.5)	0.784	0.375	NS
Vancomycin	2 (25)	3 (9.4)	1.428	0.231	NS
Levofloxacin	1 (12.5)	4 (12.5)	0.000	1.000	NS
Metronidazole	0 (0)	4 (12.5)	1.111	0.292	NS
Ciprofloxacin	1 (12.5)	2 (6.3)	0.360	0.548	NS
Cefepime	0 (0)	3 (9.4)	0.811	0.368	NS
Gentamycin	1 (12.5)	0 (0)	0.256	0.613	NS
Colistin	0 (0)	1 (3.1)	0.256	0.613	NS
Imipenem	0 (0)	1 (3.1)	0.256	0.613	NS

Sig.: significance, NS: non-significant, S: significant.

Antibiotic susceptibility testing of the isolates in which bla_{TEM} and/or bla_{CTX-M} genes were detected:

All the 15 (100%) bla_{TEM} positive isolates showed resistance to ampicillin, cefotaxime, cefoxitin and ceftazidime, 14 (93.3%) isolates showed resistance to aztreonam and amikacin, 12 (80%) isolates showed resistance to ciprofloxacin, 11 (73.3%) isolates showed resistance to gentamycin, 10 (66.7%) isolates showed resistance to trimethoprim/ sulfamethoxazole and pipracillin/tazobactam and 9 (60%) isolates showed resistance to amoxicillin, meropenem and cefepime as shown in (table 7).

All the 8 (100%) bla_{CTX-M} positive isolates showed resistance to ampicillin, cefotaxime, cefoxitin, ceftazidime and aztreonam, 7 (87.5%) isolates showed resistance to ciprofloxacin, 6 (75%) isolates showed resistance to trimethoprim/ sulfamethoxazole and amikacin, 5 (62.5%) isolates showed resistance to pipracillin/tazobactam, and 4 (50%) isolates showed resistance to amoxicillin, cefepime, meropenem and gentamycin as shown in (table 7).

Table 7: Antibiotic susceptibility testing of the isolates positive for bla_{TEM} and bla_{CTX-M} genes:

		Positive bla_{TEM} No.= 15 No. (%)	Positive bla_{CTX-M} No. = 8 No. (%)
Ampicillin	R	15 (100)	8 (100)
	S	0 (0)	0 (0)
Amoxicillin/ Clavulanic acid	R	9 (60)	4 (50)
	S	6 (40)	4 (50)
Piperacillin/ tazobactam	R	10 (66.7)	5 (62.5)
	S	5 (33.3)	3 (37.5)
Cefotaxime	R	15 (100)	8 (100)
	S	0 (0)	0 (0)
Ceftazidime	R	15 (100)	8 (100)
	S	0 (0)	0 (0)
Cefoxitin	R	15 (100)	8 (100)
	S	0 (0)	0 (0)
Cefepime	R	9 (60)	4 (50)
	S	6 (40)	4 (50)
Meropenem	R	9 (60)	4 (50)
	S	6 (40)	4 (50)
Amikacin	R	14 (93.3)	6 (75)
	S	1 (6.7)	2 (25)
Gentamicin	R	11 (73.3)	4 (50)
	S	4 (26.7)	4 (50)
Trimethoprim-sulfamethoxazole	R	10 (66.7)	6 (75)
	S	5 (33.3)	2 (25)
Aztreonam	R	14 (93.3)	8 (100)
	S	1 (6.7)	0 (0)
Ciprofloxacin	R	12 (80)	7 (87.5)
	S	3 (20)	1 (12.5)

R: resistant, S: sensitive.

DISCUSSION

In our research, resistance genes (bla_{TEM} and bla_{CTX-M}) were detected collectively among 45% of 40 isolates of Gram-negative bacilli. The ESBL gene that predominated among the studied isolates was the bla_{TEM} gene (15 isolates (37.5%) out of 40 isolates) followed by bla_{CTX-M} gene (8 isolates (20%) out of 40 isolates). Five isolates showed positivity for both genes concomitantly.

Our results come in accordance with other studies [21-22]. Yazdi et al. [21] noticed that the predominant gene was the bla_{TEM} gene detected in 87% of the isolates in their study, followed by SHV that was present in 70.6% of isolates. Trupti et al [22] reported that the TEM gene predominated over both the SHV and CTX-M genes responsible for ESBL production. On the other hand, Eftekhar et al[23], reported that SHV gene was predominant in 43.1% of their isolates in comparison to TEM gene which was detected in 35.2% of the isolates. Also, Shahid et al [24] reported that CTX-M gene was predominant than other genes. Also, Ahmed et al [25] reported that CTX-M gene exceeded the percentage of TEM gene. The distribution of ESBLs genes varies among different geographical regions [25-28].

Concomitant occurrence of more than one of these resistance genes was reported by Shahid et al [24] as they found many isolates of *E. coli* and *Klebsiella species* had different combinations of bla_{TEM}, bla_{CTX-M} and bla_{SHV} detected by PCR. Trupti et al [22] stated that 18 (45%) isolates had bla_{TEM} as one of the ESBL genes. Among these 18 genotypically positive isolates, 2 (11.1%) isolates carried bla_{SHV} gene and 4 (22.2%) of them carried bla_{CTX-M} gene. Sharma et el [29] also reported on the concomitant presence of other genes like bla_{TEM} and bla_{SHV} in some *E. coli* and *Klebsiella spp.* isolates. This coordinates with our findings, where 5 isolates had both genes simultaneously. Being carried on the mobile plasmids, more than one of the ESBLs genes may exist in the same bacterial cell [30].

In the present study, ESBL genes were detected among 8 and 15 phenotypically diagnosed ESBL producers and non-ESBL producers, respectively. Similar results were detected by Sharma et al [29] who reported that 52.6% genotypically positive out of 38 phenotypically non-ESBL producer isolates carried bla_{TEM} gene. Trupti et al [22] also detected that ESBL genes were found among 45% phenotypically confirmed ESBL producers and 52.5% phenotypically confirmed non-ESBL producers. This discrepancy between the phenotypic and molecular detection of ESBLs is expected, since some ESBLs fail to reach a detectable level by disk diffusion tests, but leads to treatment failure [29]. Phenotypic tests needed to be periodically evaluated, as their efficiency is affected by the introduction of new resistance enzymes. Phenotypic

detection of ESBLs only confirms whether an ESBL is produced, but cannot detect the ESBL subtype [29, 31]. Definitive identification is possible only by molecular detection methods, using specific PCR for detection of resistance genes. Molecular detection has higher specificity and sensitivity, yet it is expensive, cumbersome and requires specialized equipment [31].

In our study, we detected that there was a statistically significant positive correlation between the presence of bla_{TEM} gene and prior administration of ceftazidime, and a positive correlation between the presence of bla_{CTX-M} and prior administration of ceftriaxone and amoxicillin/clavulanic acid. The emergence and spread of resistant microorganisms could be attributed to the increased consumption of β-lactam antibiotics, especially ceftazidime [32].

Although the ESBLs β-lactamases are capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam, but not to the carbapenems, and though these enzymes are inhibited by β-lactamase inhibitors such as clavulanic acid [30], yet we found out a high resistance pattern among the genotypically confirmed ESBLs producing isolates, even to the antibiotics out of the action spectrum of ESBLs. In ESBL producing bacteria, higher prevalence of antibacterial resistance to non-β-lactam antibiotics should be put in consideration as a serious concern, mandating the rationale use of antibiotics [32].

In our study, 12 out of 15 and 7 out of 8 isolates carrying the bla_{TEM} and bla_{CTX-M} genes respectively, showed resistance to ciprofloxacin, which could have been a treatment option for ESBLs producing isolates[17,33]. Warburg et al [34] studied the resistance rate among *E. coli* isolates to fluoroquinolones. They found that the resistance to ciprofloxacin is growing, and most of these strains produce ESBLs as well.

In our study, the susceptibility pattern of the genotypically confirmed ESBL was of concern, as 66% of the bla_{TEM} positive isolates and 62.5% of the bla_{CTX-M} positive isolates showed resistance to pipracillin/tazobactam, and 60% of the bla_{TEM} positive isolates and 50% of the bla_{CTX-M} positive isolates showed resistance to meropenem, limiting the therapeutic options available to treat these ESBL producing isolates. This resistance pattern could be explained by the simultaneous presence of another type of beta lactamases, other than ESBL, in these isolates, e.g. metallo-β-lactamases, which have a broad spectrum and can hydrolyze all β-lactam antibiotics except monobactams[35]. They are not inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam [36,37]. Closely related antimicrobial susceptibility patterns of ESBLs producing Gram-negative bacteria were reported in many studies in different geographical regions [38-40].

Although carbapenems are the treatment of choice for ESBLs producers, yet the emerging resistance to it should limit its use. Other alternative treatment options may include an empirical combination therapy of amikacin with either piperacillin/tazobactam or ciprofloxacin [38].

CONCLUSION

In conclusion, bla_{TEM} gene predominates over bla_{CTX-M} gene in our setting. Phenotypic tests for detection of ESBLs only confirm whether an ESBL is produced, but cannot detect the ESBL subtype and cannot detect those genes whose expression is hidden, masked or of low undetectable level. The molecular methods in detecting ESBLs is much more accurate than phenotypic tests. The increased resistance pattern among Gram-negative bacteria and the positive correlation with prior antibiotic intake mandates the rationalization of antibiotic use, otherwise treatment options to treat such superbugs will not be available.

- 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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