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RESEARCH ARTICLE OPEN ACCESS

Phenotypic and Genotypic Detection of Extended-spectrum β-lactamase production by *Klebsiella pneumoniae* Isolated from Different Clinical Samples in Baghdad, Iraq

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ABSTRACT

The present study was conducted for the phenotyping of antibiotic resistance patterns among patients infected with *Klebsiella pneumoniae*, isolated from different clinical sites of the patients admitted to the Medical City Teaching Laboratories in Baghdad, Iraq, and to study the frequencies of the *bla*CTX-M, *bla*TEM, and *bla*OXA genes in the extended-spectrum β-lactamase (ESBL)-producing isolates. A total of 20 out of 35 (57.14%) *K. pneumoniae* isolates collected from different clinical samples were identified as ESBL producers using the combination disk test (CDT) against six types of antibiotics, as suggested by the Clinical and Laboratory Standards Institute. All *K. pneumoniae* isolates were observed for ESBL positivity using the CDT method and screened for *bla*TEM, *bla*CTX-M, and *bla*OXA genes by PCR using a specific primer. In total, 19/20 (95.0%) ESBL-positive isolates harbored the TEM genes, 18/20 (90.0%) carried CTX-M, while the *bla*OXA gene, for the first time in Baghdad city, was not reported in any of the isolates. A high occurrence of ESBL-producing *K. pneumoniae* was observed in our study based on the analysis of the TEM and CTX-M genes. Although molecular methods are more reliable in identifying ESBL production, routine clinical screening for ESBL-producing *K. pneumoniae* by phenotypic methods, such as CDT tests, must be introduced and encouraged in clinical settings because of its low cost.

Keywords: *K. pneumoniae,* blaOXA,CTX-M,blaTEM,ombination disk test (CDT)

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INTRODUCTION

Klebsiella pneumoniae is the causative agent of opportunistic diseases in humans, such as respiratory tract infection, burn inflammation, wound inflammation, septicemia, diarrhea, and liver abscesses[1]. The urinary tract is commonly infected by *K. pneumoniae,* following *Escherichia coli*[2]. The resistance of *K. pneumoniae* to antibiotics, such as beta-lactam antibiotics, is mainly caused by the production of a broad-spectrum of beta-lactamases (ESBL), which is an important problem leading to increased infections in hospitals; the resistance results from an alteration of the permeability barrier or the bacterial target site represented by a penicillin-binding protein, and by the changes in the outer membrane protein[3]. Beta-lactam antibiotics are frequently used to treat bacterial infections. The antibiotic groups in this category, including penicillins, cephalosporins, and carbapenems, are associated with the emergence of beta-lactam-mediated bacterial resistance, which subsequently results in the development of ESBL-producing bacteria. ESBLs are enzymes that mediate resistance to an extended spectrum of antibiotics, for example, third-generation cephalosporins, and monobactams, such as aztreonam[4]. ESBLs can be classified into three main types, TEM, SHV, and CTX-M[-5]. The isolates of the *Enterobacteriaceae* family showing plasmid-mediated lactamase production have the *bla*TEM gene, which hydrolyzes penicillins, first-generation cephalosporins, as well as the *bla*CTX-M gene, which preferentially hydrolyzes cefotaximes[6]. The other ESBL gene type is SHV, which is responsible for the plasmid-mediated resistance to ampicillin. Most ESBLs are SHV or TEM enzyme derivatives most frequently present in enterobacteria, such as *K. pneumoniae* and *E. coli*[7]. This study aimed to isolate and identify the types ESBLs phenotypically and genotypically produced by *K. pneumoniae* isolated from different clinical samples (urine, blood, stool, sputum, and burn) from patients admitted to the Medical City Teaching laboratories, Baghdad, Iraq. In addition, phenotyping and molecular detection of the three beta-lactam genes (OXA, SHV, and CTX-M) were performed by studying the prevalence of various ESBL genotypic patterns in ESBL-producing bacterial isolates.

Phenotypic methods for ESBL pattern determination

Thirty-five isolates of *K. pneumoniae* bacteria were previously acquired and identified from different clinical samples from patients admitted to the Medical City Teaching Laboratories, Baghdad, Iraq between November 2020 and January 2021. The isolates were phenotypically investigated for ESBL production using the CDT as a screening test, per the guidelines of the Clinical and Laboratory Standards Institute (CLSI)[8]. Resistance to 6 types of drugs at different concentrations (Bioanalyse, Turkey) (Table 2) was tested using the disk diffusion method, following the manufacturer's instructions[9].

Genotypic methods for ESBL resistance determination

Extraction of *K. pneumoniae* genome

DNA extraction was performed using the steps mentioned in Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). After extraction, the gel electrophoresis method was used to detect the presence of the *K. pneumoniae* genome. The selected genome of *K. pneumoniae* was then further investigated for the molecular detection of three types of ESBL genes, *bla*CTX-M-D1, *bla*TEM-D1, and *bla OXA-D-1*.

Polymerase chain reaction (PCR) technique for beta-lactamase encoding gene detection

PCR was performed according to the manufacturer's instructions (Bioneer, South Korea). A PCR assay, which was designed in this study and gave the different sizes of bands, was used to detect CTX-M, TEM, and OXA genes (Table 1) (Sentebiolab, Turkey). The following PCR program was used: one cycle of 3 min, initial denaturation at 94°C, followed by 40 cycles of denaturation at 93°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min, and a final extension step for 10 min at 72°C. Then, 5 ìL each of the amplified PCR products was loaded onto an agarose gel along with a standard molecular weight DNA ladder (Promega, USA) and subjected to electrophoresis as described by a previous study[10].

Statistical analysis

Data analysis were performed using MS Excel software (version 2010).

RESULTS AND DISCUSSION

Phenotypic analysis for antibiotic resistance determination

Determination using CDT

In total, 35 *K. pneumoniae* isolates were tested for ESBL production. The results showed that 20 (57.14%) isolates were ESBL positive, which was indicated by an increase in the score of the inhibition zone diameter e" 5 mm with clavulanic acid than without (Table 2), while 15 (42.85%) isolates were ESBL negative, which was indicated by a decrease in score of inhibition zone < 5 mm with clavulanic acid than without (data not shown). The results of the present study are in agreement with those of a previous study[11], in which 58.8% of the isolates produced ESBLs, as determined by CDT. The results of the present study also agree with another previous study[12], that showed that the resistance rate of ESBL-producing *K. pneumoniae* isolated from different clinical samples was 88.23%, as determined by the disk diffusion test (DDT), used as a screening test for ESBL detection. It was noted that CDT was easily performed and immediately interpreted. The specificity and sensitivity of this procedure were initially recorded to be 96% and 100%, respectively[5]. The high percentages of ESBL-producing isolates of *K. pneumoniae* detected in this study may be due to patients taking large amounts of third-generation cephalosporins, sometimes without the guidance

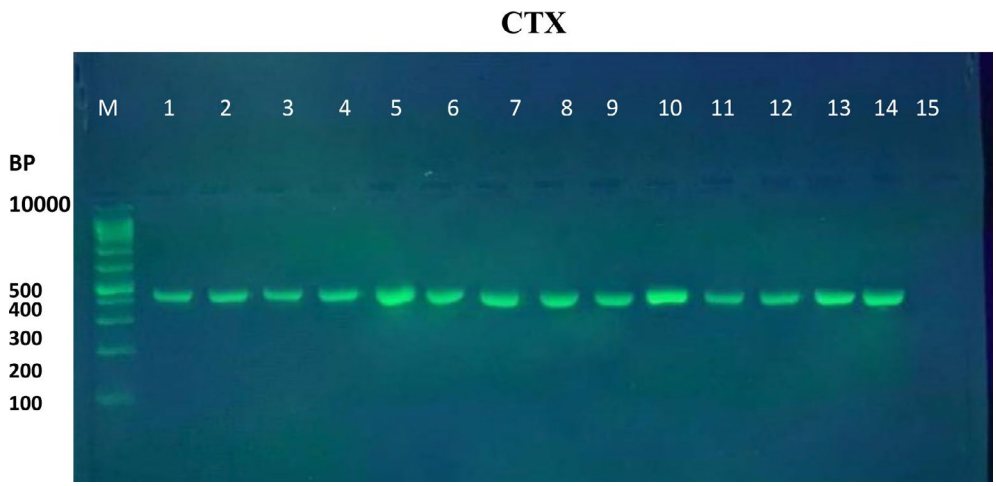

Fig. 1. Gel electrophoresis of PCR product of CTX-M gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 1 to 15 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours

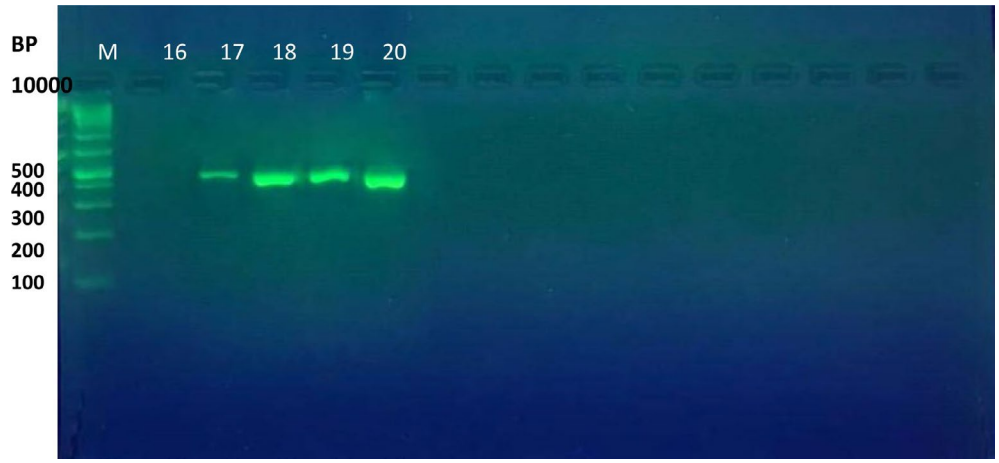

Fig. 2. Gel electrophoresis of PCR product of CTX-M gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 16 to 20 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours.

of a physician, and the widespread abuse of antibiotic administration by patients as well as physicians.

Molecular detection of the ESBL genes

The results of the ESBL phenotyping of the investigated isolates were highly correlated with the genotyping test results. Based on the

Table 1. Primer design of three ESBL genes and its product yields

Genes	Primer Sequence (5' to 3')	Size of product bp	References
TEM- F	TCCTTGAGAGTTTTCGCCCC	550	Design in this study (D)
TEM- R	TGACTCCCCGTCGTGTAGAT		
CTX-M- F	AAGCACGTCAATGGGACGAT	500	
CTX-M- R	GTTGGTGGTGCCATAGCCA		
OXA- F	TTGCACTTGATAGTGGTGTGA	no band	
OXA- R	AGTGAGTTGTCAAGCCAAAAAGT		

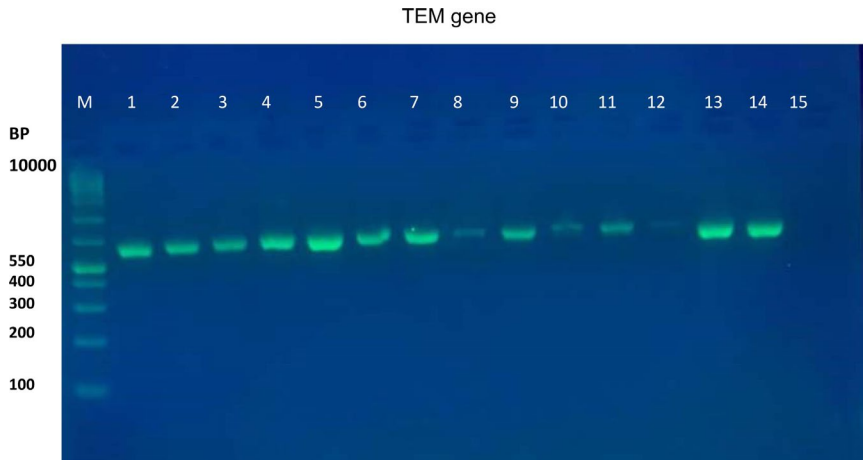

Fig. 3. Gel electrophoresis of PCR product TEM gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 1 to 15 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours

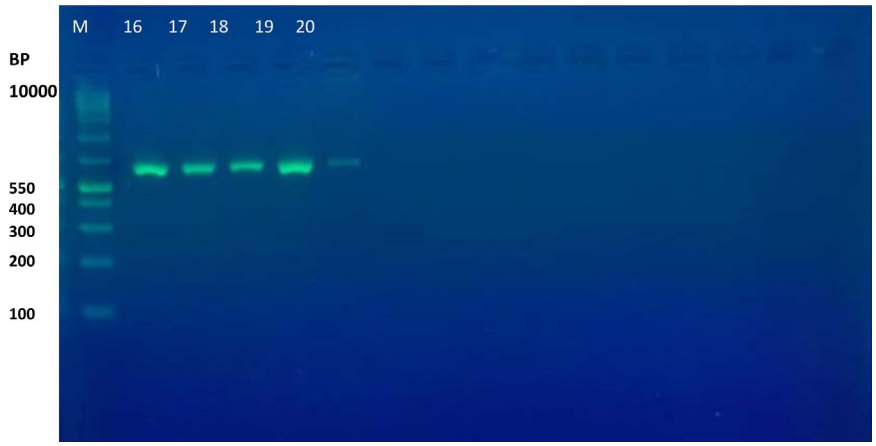

Fig. 4. Gel electrophoresis of PCR product TEM gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 16 to 20 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours

results of the CDT test, only 20 (57.14%) out of the 35 *K. pneumoniae* isolates were ESBL-producing. All three *K. pneumoniae* genes associated with ESBL production, namely the *bla*TEM, *bla*CTX-M, and *bla*OXA genes, were screened using PCR. Genotypically, 95% (19/20) of the ESBL-positive isolates produced the TEM ESBLs, whereas 90% (18/20) produced CTX-M ESBLs. In contrast, no isolate showed the production of OXA ESBLs (Table 2 and Figure 1-6). According to the type of the sample, the results of this study showed that genotypically, the highest number of resistance patterns were observed among the *K. pneumoniae* isolated from urine samples. Locally, the findings of this study were consistent with those of a study in Najaf City[13] that used PCR methods and reported that 60.1% of the isolated *K. pneumoniae* carried CTX-M genes. In another study in Erbil City, the ESBL-producing *K. pneumoniae* showed a lower resistance rate than those obtained in this study, with 64.7% harboring *bla*TEM ESBL genes while 41.1% harboring the *bla*CTX-M genes[14]. Another study performed in Najaf City focused on studying ESBL genotypic patterns among patients with urinary tract infections without kidney disease and those with chronic kidney disease. The study revealed that the percentages of the *bla*TEM- and *bla*CTX-M-harboring ESBL-positive *K. pneumoniae* isolates were 83.3%, 88.2%, 61.1%, and 6.4%[15]. Genotypically, the percentage found in the current study was higher than that found in Turkey in a previous study[12] that demonstrated that the TEM-type ESBLs were found in 73.33% of *K. pneumoniae* isolates from different clinical samples. In a study conducted in India, 115 (38%) of 304 *K. pneumoniae* isolates harbored *bla*TEM[16].

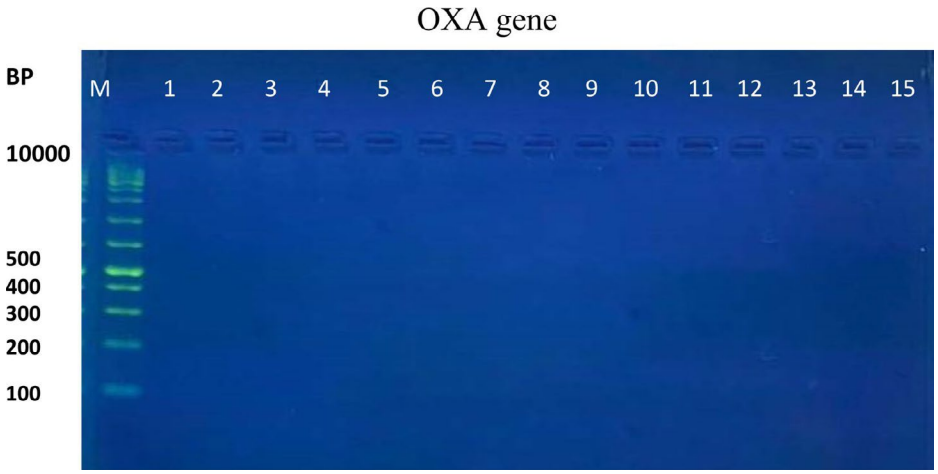

Fig. 5. Gel electrophoresis of PCR product OXA gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 1 to 15 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours

Fig. 6. Gel electrophoresis of PCR product OXA gene in K. pneumoniae isolates, M= DNA molecular size markers (100 bp), 16 to 20 represents number of isolates, 1% agarose gel at 5 Vol /cm for 1:15 hours

Table 2. Phenotyping and genotyping patterns of isolated *K. pneumoniae* from different clinical samples

No	Position	CTX	CTC	FEP	FEC	CAZ	CZC	Phenotyping patterns Interpretation	Genotyping patterns TEM	CTX	OXA
1	Urine	22	20	27	26	11	16	ESBL positive	+	+	-
2	Urine	35	21	30	29	29	31	ESBL positive	+	+	-
3	Urine	0	12	18	22	11	22	ESBL positive	+	+	-
4	Urine	17	11	26	22	0	11	ESBL positive	+	+	-
5	Urine	31	30	32	20	22	27	ESBL positive	+	+	-
6	Urine	0	15	17	21	17	23	ESBL positive	+	+	-
7	Urine	0	0	9	7	0	16	ESBL positive	+	+	-
8	stool	25	20	25	23	20	13	ESBL positive	+	+	-
9	Urine	19	10	16	10	10	R	ESBL positive	+	+	-
10	Blood	20	15	25	25	20	10	ESBL positive	+	+	-
11	Urine	15	R	14	R	R	R	ESBL positive	+	+	-
12	Sputum	25	2	2	15	15	8	ESBL positive	+	+	-
13	Urine	30	25	25	R	1	R	ESBL positive	+	+	-
14	Urine	20	15	2	2	15	R	ESBL positive	+	+	-
15	Burn	25	15	25	20	15	7	ESBL positive	-	-	-
16	Blood	20	15	20	15	14	15	ESBL positive	+	-	-
17	Urine	5	R	R	R	7	10	ESBL positive	+	+	-
18	Urine	25	20	25	20	20	20	ESBL positive	+	+	-
19	Urine	8	R	7	R	0	8	ESBL positive	+	+	-
20	Blood	25	15	22	20	17	13	ESBL positive	+	+	-
Total							N	20/20	20/19	20/18	20/0
							%	100%	95%	90%	0.0%

CTX 30; cefotaxime; CTC 40; cefotaxime / clavulanic Acid; FEP 30; cefepime; FEC40; cefepimeclavulanic Acid; CAZ 30 ; ceftazidime; CZC 40 ; ceftazidime / clavulanic Acid, R;resistance, S,sensitive

With respect to the ESBL *bla*OXA gene, our results showed that no isolate harbored the OXA genes. The production of beta-lactamases, which inactivate and hydrolyze β-lactam antibiotics, has become the most important resistance mechanism of different bacterial species, particularly among the *Enterobacteriaceae* family[17]. In this study, ESBL-positive isolates were detected using CDT and PCR. Twenty *K. pneumoniae* isolates were positive in the CDT test and 19/20 and 18/20 *K. pneumoniae* isolates were positive for the TEM and CTX genes, respectively, as determined by PCR. The observation of negative ESBLs in the two isolates using PCR confirmed the accuracy of the DDT. SHV and TEM β-lactamases are particularly present in *K. pneumoniae* and *E. coli* but may be found in other members of the *Enterobacteriaceae* family and in non-enteric microorganisms, such as *Acinetobacter* species[18]. Rapid laboratory identification of these strains is necessary because of their resistance to available antibiotics, the genes responsible for which can be passed to other strains. Molecular typing can identify the type of ESBLs that exist in each isolate. Molecular identification and detection of beta-lactamases are important for the accurate epidemiological detection of antimicrobial resistance.

CONCLUSION

In this study, the isolated *K. pneumoniae* exhibited high resistance to most antibiotics, especially the beta-lactam group, and were considered multidrug-resistant bacteria. Furthermore, *K. pneumoniae* produced the TEM and CTX-M ESBLs at high rates of occurrence, reaching 95 % and 90%, respectively. This highlights the need to adopt strict protocols related to antibiotic administration in hospitals to control the emergence of extensive antibiotic resistance in different species of bacteria. Because of the significance of ESBL-producing *K. pneumoniae* and the difficulties involved in the therapy of infections caused by these bacteria, clinical laboratories should adopt a simple test based on the suggestions of the CLSI for the rapid identification and confirmation of ESBL production in *Enterobacteriaceae*.

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CONFLICT OF INTEREST

All authors declared that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All data sets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable

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