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Molecular detection of β-lactamase genes in *Klebsiella pneumoniae* and *Escherichia coli* isolated from different clinical sources

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ABSTRACT

The rising occurrence of infections generated by *Escherichia coli* and *Klebsiella pneumoniae* that produce extended-spectrum β-lactamase (ESBL) is reason for concern. Due to the recent emergence of multidrug-resistant microorganisms that develop ESBL. The purpose of this work was to detect the ESBLs in clinical isolates of *E. coli* and *K. pneumoniae*. 118 samples of *E. coli* and 63 isolates of *K. pneumoniae* were collected from clinical samples. Polymerase chain reaction was used to detect β-lactamase genes (i.e., bla_{TEM}, bla_{SHV}, and bla_{CTX-M}). Phenotypic detection revealed that 48.31% and 85.19% of *E. coli* and *K. pneumoniae* produced ESBLs, respectively. Whereas screening of ESBL genes in both bacteria employing a multiplex PCR test revealed that 24.58% of the ESBL-producing *E. coli* strains contained bla_{TEM}, 50.85% contained bla_{SHV}, and 32.2% contained bla_{CTX-M}. Nevertheless, in *K. pneumoniae*, 40.74% bla_{TEM}, 35.19% bla_{SHV}, and 64.81% bla_{CTX-M} genes were present. Antimicrobial resistance profiles of *E. coli* and *K. pneumoniae* isolates to twenty antibiotics were observed to vary significantly. Additionally, it was determined that the majority of *E. coli* and *K. pneumoniae* isolates were multidrug resistant (MDR). Additionally, 80.51% of *E. coli* isolates were resistant to the AMC antibiotic, while 0.00% were resistant to IPM and MEM. From the other hand, the resistant proportion of *K. pneumoniae* isolates was heterogeneous, ranging from 69.84% against CAZ to 0.00% against CIP and G antibiotics. The bla_{SHV} gene was the most widespread among different forms of ESBLs in *E. coli*, but the most common gene in *K. pneumoniae* isolates was bla_{CTX-M} (64.81%).

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Introduction

The emergence of drug-resistant infectious disease-causing microorganisms is a growing public health concern that has resulted in a rise in mortality and morbidity. These infections are caused by multidrug-resistant Enterobacteriaceae bacteria (1). Extended-spectrum β-lactamases (ESBLs) are enzymes that hydrolyze the ring of β-lactam antimicrobials, enabling bacteria organisms to develop resistance to penicillins (e.g., ampicillin and amoxicillin); first-generation cephalosporins (e.g., cephalexin), second-generation cephalosporins (e.g., cefuroxime), and third-generation cephalosporins (e (e.g., aztreonam) (2). As a consequence, bacteria that produce ESBLs are resistant to β-lactams, resulting in greater healthcare costs, extended hospital admissions, elimination of prophylactic protection, and elevated death. Attributed to the fact that ESBLs are incapable of hydrolyzing cephamycins (e.g., cefoxitin) and carbapenems (e.g., imipenem), these antimicrobial drugs—mainly carbapenems—are recommended for the cure of infections produced by ESBL generators (3). ESBL enzymes are expressed by genes, most frequently bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, which are carried on self-transmissible conjugative plasmids that are horizontally transferred among bacteria of the same and other species. β -lactamase blockers including clavulanic acid and tazobactam suppress ESBL formation, a feature that may be utilized to identify ESBL synthesis in Enterobacteriaceae, especially *E. coli* and *K. pneumoniae*. Molecular-based approaches, like multiplex PCR techniques, are more beneficial for ESBL detection in epidemiological comparisons and infection prevention and control applications (4).

Extended spectrum β-lactamases (ESBLs) are enzymes generated by Gram-negative microbes that metabolize or breakdown aztreonam, penicillins, and

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first, second, and third-generation cephalosporins like as ceftriaxone, cefotaxime, and ceftazidime, rendering them ineffective for the cure of bacterial-related illnesses (5). The genes generating ESBLs are typically found on extremely mobile plasmids which could really include resistance genes to various different types of antimicrobial drugs, like as plasmid-mediated quinolone resistance (PMQR) and aminoglycoside resistance genes (6). The TEM, SHV, and CTX-M genes are most frequently regulated by ESBL plasmids in *Klebsiella* species, next by *E. coli*. These ESBL genes encode enzymes that are competent of degradation of cephalosporins and monobactams with a wide range of effectiveness however are inert toward cephamycins and imipenem. CTX-M genes, especially CTX-M-15, were implicated in a variety of epidemiologic circumstances and been spread across all of continents via epidemic plasmids and strains. *E. coli* and *K. pneumoniae* that produce CTX-M have become more prevalent in urinary tract infections (7). Numerous investigators globally have observed the synthesis of ESBLs by pathogenic *E. coli* and *K. pneumoniae* strains, and their incidence is constantly increasing, extending from 6% to 8% in diverse healthcare institutions worldwide. Apart from that, some of the most often occurring genes which result in ESBLs are bla_{TEM} and bla_{CTX-M} (1). Several β-lactamase genes were discovered in *E. coli* and *K. pneumonia* strains by (7), including SHV, CTX-M, OXA, and TEM-type ESBL genes. (1) identified one and numerous bla_{TEM} and bla_{SHV} genes in *E. coli* isolates isolated from clinical samples in Ghana. The most common subclass of β-lactamases is A, that comprises the TEM, SHV, and CTX-M subclasses (8). Consequently, the most effective method for defining and identifying the existence of ESBL genes is through the use of genetic assays that allow the detection of β-lactamase genes in test organisms. Whereas there are other ESBLs, including SHV, TEM, OXA, CTX, and AmpC, the great preponderance are variations of the SHV, TEM, and CTX-M enzymes, that were first found in *E. coli* and *K. pneumoniae* pathogens, respectively. In individuals, the most prevalent ESBLs are SHV, TEM, and CTX-M. The primary goal of this research was to ascertain the preponderance of the ESBL phenotype in specimens, and also the existence of the bla_{SHV}, bla_{CTX-M}, and bla_{TEM} genes in the isolated strains under examination.

Materials and methods

Bacterial species

In sum, 181 continuous non-duplicate *E. coli* isolates (*n*=118) and *K. pneumoniae* isolates (*n*=63) were collected from samples obtained from a variety of clinical sources in Erbil, Iraq, comprising urine, wounds, stool, and burns. Inpatients and outpatients were sampled between May 2021 and July 2021, with samples acquired from both groups. For the isolation process, conventional microbiological procedures were applied. To identify the isolates, standard microbiological techniques were used in conjunction with each other. In addition, the VITEK II compact system was used to re-identify the individuals.

Antimicrobial susceptibility profile

The antibiotic resistance profiles of bacterial samples were determined employing the Kirby-Bauer disk diffusion method in accordance with CLSI recommendations (9). The test organisms was inoculated on the surface of the Mueller-Hinton agar (MHA) plates (1×10^8 CFU/mL depending on the optical density of 0.5 McFarland standards) via a sterile cotton swab for each organism. Antibiotic discs that used in this work comprising Amikacin (AK), Amoxicillin+Clavulanic acid (AMC), Cefixime (CFM), Cefotaxime (CTX), Ceftazidime (CAZ), Chloramphenicol (C), Ciprofloxacin (CIP), Doxycycline (DOX), Gentamicin (G), Imipenem (IPM), Kanamycin (KAN), and Meropenem (MEM) were employed on the Mueller Hinton agar plates surface. Following about ten minutes of dispersing the antibiotics, the plates were incubated at 37°C for 24 hrs. Following overnight incubation, zones of inhibition diameters were measured and the outcomes were translated according to interpretative criteria of the CLSI for susceptible and resistant patterns.

Validation of ESBL development using phenotypic analysis

Isolates have been obtained through subculturing on MacConkey agar dishes and incubated aerobically for 24 hrs. at 37°C. Following incubation, a single colony was employed for phenotypic assessment of ESBL releases in

accordance with CLSI recommendations (4,9). For the purpose, each testing microorganism was placed in sterile phosphate buffered saline and the optical density adjusted to the 0.5 McFarland turbidity standard before being swabbed on the whole surface of MHA plate to create uniform lawns. In less than 15 minutes, the relevant antimicrobial discs were placed: ceftriaxone 30 μg (CTR), ceftazidime 30 μg (CAZ), cefotaxime 30 μg (CTX), aztreonam 30 μg (ATM), cefpodoxime 10 μg (CPD). The MHA petri dishes of were incubated aerobically at 37°C for 18 hrs. A strain resistant to CTR (≤25 mm), CAZ (≤22 mm), CTX (≤27 mm), ATM (≤27 mm), and CPD (≤17 mm) has been confirmed to be a suspected ESBL producer.

CLSI combination disc diffusion (CLSI-CDD) method

Phenotypically, the combination disc diffusion (CDD) assay, as suggested by (9) for recognition of ESBL synthesis in *E. coli* and *K. pneumoniae* was used. Within 15 minutes, a lawn of testing bacterial suspension corresponding to the optical density of 0.5 McFarland turbidity standard solution was streaked onto the MHA plate surfaces, and then putting the CAZ 30 μg and CTX 30 μg discs with and without clavulanic acid (CA 10 μg) on the surface. Following that, all dishes were incubated aerobically at 37°C for 18 hrs. If a discrepancy in the diameter of the inhibition zones (≥5) of dual antimicrobial disks comprising clavulanic acid and a corresponding medication without clavulanic acid was identified, an isolate was morphologically confirmed as an ESBL developer.

Modified double disc synergy (MDDS) technique

In a study (4), the researchers employed a developed double-disc synergy (MDDS) assay to even further demonstrate ESBL generation. To summarize, lawns of experimented bacterial suspensions equivalent to optical density of 0.5 McFarland turbidity standard solution were streaked on the MHA plate surfaces, and follow the antibiotic discs: CAZ 30 μg, CTX 30 μg, CPD 10 μg, and FEP 30 μg were positioned side by side with amoxicillin-clavulanic acid 30 μg (AMC) at a 15-mm space. On the same MHA plate, another antimicrobial disc containing piperacillin-tazobactam 110 μg (TZP) was sown beside FEP 30 μg at a distance of 22 mm. Increased zone of inhibition of CAZ 30 μg, CTX 30 μg, CPD 10 μg, or FEP 30 μg against AMC 30/10 μg and/or improved inhibition zone of FEP 30 μg against TZP 110 μg, phenotypically proven for ESBL generation.

Detection of ESBLs associated genes by PCR

The molecular analysis of ESBL-associated genes (bla_{SHV}, bla_{TEM}, and bla_{CTX-M}) in the most of ESBL-producing *E. coli* and *K. pneumoniae* strains was performed employing standard multiplex PCRs. The defined and the sequences of the primers used in this research was listed in Table 1. The primer mixing for detecting ESBL-associated genes was made in an Eppendorf tube and 25 μL of the reaction medium were employed for the PCRs (7 μL Nuclease free water + 10 μL Mastermix + 1 μL of each primer mix + 2 μL of genomic DNA template). The PCR cycling protocols were as shown below: initial denaturation for 10 minutes at 95°C, denaturation for 30 seconds at 95°C, annealing for 90 seconds at 60°C, elongation for 120 seconds at 72°C with 40 cycles, and final elongation for 10 minutes at 72°C. The PCR cycles were performed using an Alpha PCRmax thermal cycler. Electrophoretically separated PCR amplicons with Red Safe dye on a 1% agarose gel and observed under UV light.

Statistical analysis

The statistical package for social sciences (SPSS) version 26.0 software was used to analyze the data. The χ^2 test was performed to determine whether there were statistically substantial discrepancies between phenotypic and genotypic identification of ESBL among *E. coli* and *K. pneumoniae* strains. We defined statistically significant results as a *p*-value less than 0.05.

Results and discussion

Identification of isolates

While 61.77% (181/293) of the cultured samples showed a significant growth, 38.22% (112/293) showed no growth. Of the 671 isolates, 118 (65.19%) were *Escherichia coli* and 63 (34.81%) were *K. pneumoniae*, which isolated from different clinical

specimens of patients attending to Erbil Teaching Hospitals (Table 2).

Table 1. A description of the oligonucleotide primers that were utilized in the Multiplex PCR amplification.

The gene	Oligonucleotide primer sequence	Amplicon size	References
bla_{TEM}	5'-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3' 5'-ACG CTC ACC GGC TCC AGA TTT AT-3'	445	(10)
bla_{SHV}	5'-ATG CGT TATATT CGC CTG TG-3' 5'-TGC TTT GTT ATT CGG GCC AA-3'	747	
bla_{CTX-M}	5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3' 5'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3'	593	

Identification of *E. coli* and *K. pneumoniae* were performed depending on the morphological features (including gram staining method), cultural characteristics, and standard biochemical tests revealed that the all isolates were belonged to *E. coli* and *K. pneumoniae* species and the VITEK II compact system was employed to confirm the identify all *E. coli* and *K. pneumoniae* isolates.

Table 2. Distribution of both *E. coli* and *K. pneumoniae* isolates among clinical specimens

Sources	Total samples (*n.*)	Positive growth samples (*n.*)	*E. coli* *n.* (%)	*K. pneumoniae* *n.* (%)
Urine	175	89 (50.86)	67 (75.28)	22 (24.72)
Stool	95	43 (45.26)	27 (62.79)	16 (37.21)
Wound	77	30 (38.96)	14 (46.67)	16 (53.33)
Burn	45	19 (42.22)	10 (52.63)	9 (47.37)
Total n.	392	181 (46.17)	118 (65.19)	63 (34.81)

Susceptibility testing

The percentages of sensitivity (and thereafter of non-susceptibility comprising both resistant and intermediately resistant isolates) of *E. coli* and *K. pneumoniae* strains to the spectrum of antibiotics which are routinely employed to cure infections are presented in Table 3. Overall, 181 concurrent non-duplicate of *E. coli* (*n*=118) and *K. pneumoniae* isolates (*n*=63) were collected, and their antibiotic resistant pattern towards 20 different antimicrobial drugs was examined. The present findings demonstrated that *E. coli* and *K. pneumoniae* isolates varied substantially to various antimicrobial drugs. The resistance frequencies of *E. coli* and *K. pneumoniae* isolates toward the selected antimicrobial agents were varied. It was discovered that a large percentage of isolates of the *E. coli* were multidrug resistant (MDR) in which a total of 80.51% of isolates resist to each of AMC and CFM antimicrobials, while the lowest resistant percent of isolates against IPM and MEM antibiotic was 0.00%, so although the resistant of *K. pneumoniae* strains were demonstrated the highest percentage (69.84%) was documented against CAZ antibiotic, on the other hand, the lowest resistant (0.00%) also among *K. pneumoniae* isolates were revealed against each AK, CIP, and G antimicrobials.

Indeed, antibacterial abuse was already found as a significant factor in the selection and development of antibiotic-resistant microorganisms in clinical treatment (11). In the experiment was performed (12), the findings of antibiotic resistance screening towards 15 antimicrobial drugs revealed that all 78 (one hundred percent) *E. coli* isolates tested positive for penicillin resistance (penicillin, ampicillin, and amoxicillin).

Table 3. Antimicrobial sensitivity profiles of *E. coli* and *K. pneumoniae* isolates

Antibacterial drugs	*E. coli*						*K. pneumoniae*					
	S		I		R		S		I		R	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
AK*	93.22	110	2.54	3	4.24	5	95.24	60	4.76	3	0.00	0
AMC	17.80	21	1.69	2	80.51	95	31.75	20	3.17	2	65.08	41
CFM	16.95	20	2.54	3	80.51	95	33.33	21	1.59	1	65.08	41
CTX	16.95	20	5.08	6	77.97	92	31.75	20	7.94	5	60.32	38
CAZ	37.29	44	0.85	1	61.86	73	23.81	15	6.35	4	69.84	44
C	83.90	99	1.69	2	14.41	17	87.30	55	7.94	5	4.76	3
CIP	94.92	112	0.85	1	4.24	5	98.41	62	1.59	1	0.00	0
DOX	39.83	47	0.00	0	60.17	71	95.24	60	0.00	0	4.76	3
G	93.22	110	2.54	3	4.24	5	95.24	60	4.76	3	0.00	0
IPM	100.00	118	0.00	0	0.00	0	95.24	60	0.00	0	4.76	3
KAN	62.71	74	0.00	0	37.29	44	90.48	57	0.00	0	9.52	6
MEM	93.22	110	6.78	8	0.00	0	93.65	59	3.17	2	3.17	2

*: AK: Amikacin; AMC: Amoxicillin-Clavulanic acid; CFM: Cefixime; CTX: Cefotaxime; CAZ: Ceftazidime; C: Chloramphenicol; CIP: Ciprofloxacin; DOX: Doxycycline; G: Gentamicin; IPM: Imipenem; KAN: Kanamycin; MEM: Meropenem

Resistance to aminoglycosides (gentamicin 16.7%, amikacin 21.8%) and nitrofurantoin was found to be the least common, with 23.1%. In addition, 2/78 (2.6%) of *E. coli* isolates were resistant to all 15 antibacterial drugs was experienced, and 41/78 (52.6%) of *E. coli* strains were resistant to at least half (≥8) of the antibacterial drugs were used. In totaling, four *E. coli* isolates (5.1%) are only resistant to the penicillins that have been examined so far. The 78 isolates were tested, demonstrated a number of 53 distinct resistance profiles, with penicillin, ampicillin, amoxicillin, cephalexin, cefixime, ceftriaxone, ceftazidime, cefotaxime, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, and tetracycline which is the most prevalent. The antimicrobial sensitivity pattern in urine samples were performed (13) and they found that *E. coli* isolates were highly sensitive to ertapenem (97.6%) and imipenem (96.4%) while resistant to ampicillin (87.8%), cefepime (61.5%), and ceftriaxone (61.0%). *E. coli* isolates from wound samples were highly resistant to ampicillin (100%), cefepime (100%), and ceftriaxone (94.4%), whereas they showed 100% sensitivity to ertapenem and imipenem. *E. coli* isolates from cervical samples were sensitive to ertapenem (100%) and imipenem (100%). In addition, 100% of *E. coli* isolates from blood samples were resistant to ampicillin, cefoxitin, and ceftriaxone and 75% of them were sensitive to ertapenem, ciprofloxacin, and levofloxacin. High susceptibility of *E. coli* rate was observed (14) for nitrofurantoin (90.4%), followed by ciprofloxacin (85.6%), norfloxacin (83.9%), cefotaxime (83.7%), ceftriaxone (82.4%), and gentamicin (80.8%). Ampicillin had the lowest sensitivity frequency (19.8%), next with amoxicillin-clavulanic acid (26.8%) and cotrimoxazole (31.6%).

According to the findings (15), 100% of the isolated *K. pneumoniae* from the sputum specimens were resistant to ampicillin, and 77.8% were sensitive to gentamicin. The sensitivity of the *K. pneumoniae* isolated from the wound swabs to ertapenem was estimated at 92.3%, while these isolates were extremely resistant to ampicillin (92.3%). The strains isolated from the CV line exhibited the highest resistance rates against all the antibiotics, with the exception of ertapenem. In addition, 100% of the isolated *K. pneumoniae* from the oral swabs were highly sensitive to all the common antibiotics, with the exception of ampicillin. Nirwati et al. 2019 (16) found that most of *K.*

pneumoniae were resistant to a wide range of antimicrobial agents. Among the biofilm producing isolates, *K. pneumoniae* demonstrated only moderate susceptibility to meropenem (98.60%), amikacin (95.80%), and piperacillin-tazobactam (90%). In contrary, *K. pneumoniae* strains that did not forming biofilms were extremely susceptible to meropenem, levofloxacin, amikacin, piperacillin-tazobactam, and ciprofloxacin, with 100%, 95.83%, 91.67%, 87.50%, and 86.67% of susceptibility, respectively. Antimicrobial susceptibility test was performed for 43 *K. pneumoniae* strains (17), and the results demonstrated that the antimicrobial resistance rates to amoxicillin, Amoxicillin+Clavulanic acid, cefotaxime, ceftriaxone, nitrofurantoin and ceftazidime were all high (97.67-90.69%). The moderate resistance rate was observed for doxycycline, tetracycline, gentamicin and chloramphenicol with percentage ranged from 48.83-44.18%. The lowest resistance percentages were detected for amikacin (25.58%) and imipenem (9.30%). Also, the findings of the current work proved that there were 32 strains (74.41%) of *K. pneumoniae* were MDR, 9 strains (20.93%) were XDR and 2 strains (4.65%) were PDR.

There were a total of 27384 findings of in *K. pneumoniae*, antibacterial resistance revealed from a total of 1149 persons entered to the hospital throughout the period of experiment. Thus based on their conclusions, a number of 11676 (42.63%) of the specimens constituted a secondary specimens (i.e., specimens obtained during six months after the original sample), and these specimens were excluded from further consideration. Further testing was performed on the rest of the samples (initial samples), which totaled 15708 (57.73%) and were submitted to additional testing. High resistance percentages in routinely administered antibiotics such as amoxicillin/clavulanic acid were discovered (18), who found that 72% (*n*=572) of all antibiotics tested were resistant, with a rise between 2015 (71%) and 2017 (75.8%). The antibiotic ampicillin has a 99.9% resistance percentage (*n*=685), with only one sample demonstrating sensitivity to the antibiotic. Piperacillin had a high resistance percentage as well, with 80.4% (*n*=288) of the population showing resistance. The resistance to a piperacillin/tazobactam combination, on the other hand, was lower, at 58.7% (*n*=505). A further combination (trimethoprim/sulfamethoxazole) demonstrated substantial resistance, with 67% (*n*=693) of the population showing resistance. Fluoroquinolones had lower percentages of resistance, with levofloxacin having the lowest percentage of resistance (57.7%, *n*=384) and ciprofloxacin having the highest percentage of resistance (61.1%, *n*=686). Resistance to aminoglycosides was significantly reduced, particularly with amikacin, which had a percentage of 36.3% (*n*=408) in 2018. The percentage of amikacin resistance, on the other hand, climbed from 28.9% (*n*=54) in 2015 to 39.7% (*n*=139) in 2018. In 2018, gentamicin had a total resistance percentage of 52.2% (*n*=543), with a similar improvement in resistance percentage between 2015 and 2018: 46.1% (*n*=83) in 2015 and 52.2% (*n*=179) in 2018. Aztreonam (monobactam) demonstrated a resistance percentage of 66.3% (*n*=627), with no visible differences in rates over the course of the study's five-year period of surveillance. Cephalexin had a resistance percentage of 92% (*n*=23), cephalothin had a resistance percentage of 80.8% (*n*=563), and cefazolin had a resistance rate of 78% (*n*=71) for first-generation cephalosporins. Second-generation cephalosporins had comparable results, with cefuroxime accounting for 73.8% (*n*=45) of the total. The proportion of third-generation cephalosporins in the population varied between 57.5% and 77.8%. Cefoxitin had a resistance percentage of 57.5% (*n*=413), ceftazidime had a 66.9% (*n*=743), cefotaxime had a 77% (*n*=771), and ceftriaxone had a 77.8 (n=779). Furthermore, cefepime (a fourth-generation cephalosporin) was found to be resistant in 68.4% (*n*=564) of the cases. Detection for ESBLs in *E. coli* and *K. pneumoniae*.

Fifty seven isolates (48.31%) among the 118 E. coli strains were positive for ESBL synthesis in both the first screening test for ESBL development using MDDST and the phenotypic confirmatory assay for ESBL formation. Furthermore, 46 isolates (85.19%) of the 63 *K. pneumoniae* strains were positive in both the screening assay for ESBL formation and the phenotypic confirmation test for ESBL development (Table 4). Using PCR to identify ESBL genotypes, it was discovered that a wide variety of ESBL-positive *E. coli* and *K. pneumoniae* isolates carried one or more ESBL genes that were examined in this experiment (Figure 1). (12) recognized 27 (34.6%) of the 78 E. coli isolates as ESBL formers employing the

CLSI phenotypic confirmatory investigation. Consequently, 14 strains were identified as belonging to female patients, while 13 strains were identified as belonging to male patients. Altogether, the frequency of β-lactamase genes, comprising blaCTX-M, blaSHV, and blaTEM, was 29 (37.2%), 37 (47.4%), and 12 (15.4%) in 78 E. coli isolates, respectively. According to the sexual identity of the origin of E. coli isolates, the majority of the blaCTX-M gene was 16 (30.2%) and 13 (52%), the frequency of the blaSHV gene was 25 (47.2%) and 12 (48%), and the prevalence of the blaTEM gene was 8 (15.1%) and 4 (16%), respectively, for female and male isolates. Overall, 50.85% (n=60), 32.2% (n=38), and 24.58% (n=29) of E. coli isolates were positive for blaSHV, blaCTX-M, and blaTEM genes, respectively. The multiplex PCR technique findings showed that 64.81% (n=35) blaCTX-M, 35.19% (n=19) blaSHV, and 40.74% (n=22) blaTEM genes were identified in the K. pneumoniae isolates. The total prevalence of ESBL genotypes in E. coli and K. pneumoniae isolates is represented in Figure 2. There are significant differences in the detection of blaCTX-M (p=0.0167), and blaTEM (p=0.0002) genes where compared with phenotypic detection (MDDST) within E. coli isolates while in blaSHV gene (p=0.7947) detection, the significant difference not recorded. However, among K. pneumoniae isolates, the significant differences was stated among phenotypic detection of ESBL producing isolates with both blaSHV (p<0.00001) and blaTEM (p<0.00001) genes, whenever, this difference was not fount with blaCTX-M gene (p=0.0624).

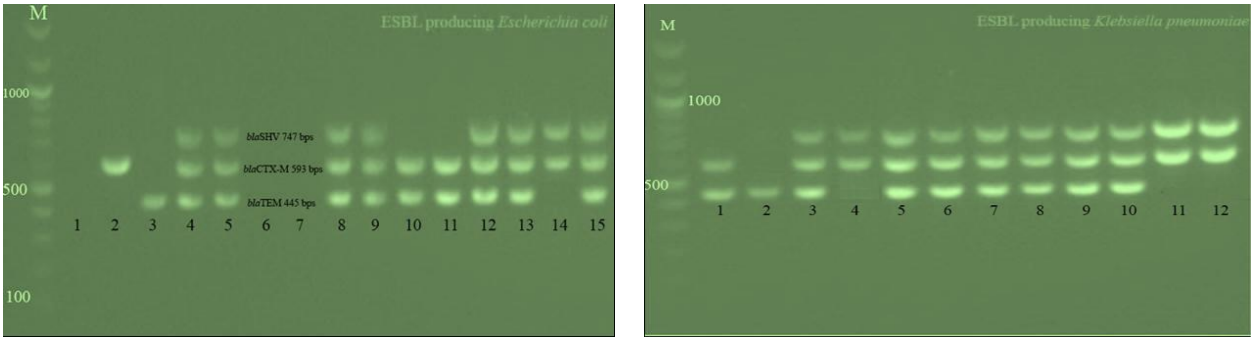
The bacteria that produce extended spectrum β-lactamase (ESBL) hydrolyze oxyimino β–lactams and monobactams, and even though have no impact on cephamycins and carbapenems are known as ESBL generating strains. ESBL forming pathogens have become increasingly resistant to antibiotics, such as clavulanic acid, sulbactam, and tazobactam, as a result of the evolution of ESBL-forming organisms (19). The prevalence and dispersion of ESBLs varies from one geographical area to another, as well as from one hospital to the next. Originally, ESBL-producing microorganisms were typically identified from healthcare-associated illnesses; however, these microorganisms are now being isolated from diseases in the general population (20). The continuous pressure applied by the employment of newer expanded-spectrum β-lactams resulted in the creation of novel blaTEM and blaSHV genes, which were later discovered. The term "ESBL" refers to a variety of enzymes that are often found in bacteria such as E. coli and *K. pneumoniae*. There are several different forms of ESBLs, including blaTEM, blaSHV, and blaCTX-M, although the vast majority of ESBLs are variants of TEM or SHV enzymes (21).

Gram negative microorganisms isolated from samples are being monitored for antimicrobial resistance. This is a significant technique for obtaining data about the frequency of ESBLs and is essential for preventing the development of antimicrobial resistance in microorganisms. The current CLSI recommendation for recognition of ESBLs in E. coli and *K. pneumoniae* contains a preliminary monitoring check with any two of the following β-lactam antimicrobials: cefpodoxime, ceftazidime, aztreonam, Cefotaxime, or ceftriaxone, followed by a second assessment exam with any two of the following β-lactam antibiotics: cefpodoxime, or ceftriaxone. Resistance to any of these antibiotics must be established phenotypically by the double disc diffusion test (DDDT), which should be performed with ceftazidime alone or with ceftazidime and clavulanic acid in combination.

Sah et al. (22) isolated and identified 44 isolates of *E. coli* among various clinical samples and employed for phenotypic and genotypic recognition of ESBL forming strains, 22 isolates were checked positive for ESBL formation using ceftazidime, whereas 24 isolates were screened positive for ESBL production using cefotaxime. In the confirmatory assay, 27.3% (12/44) were confirmed as ESBL-producing *E. coli*. Among 12 ESBL producers, the majority (66.7%; 8/12) were from females. All of the ESBL producers (*n*=12) were detected in urine samples. There was really no statistically substantial correlation among the kind of sample and the ESBL production (*p*-value=0.54). Among 12 ESBL-producing *E. coli*, 58.4% (*n*=7), 41.6% (*n*=5), and 25.0% (*n*=3) isolates were tested positive for the $bla_{\text{CTX-M}}$ gene, bla_{TEM} gene, and both $bla_{\text{CTX-M}}$ and bla_{TEM} genes, respectively.

Table 4. Comparison between phenotype and genotypic detection of ESBL producing *E. coli* and *K. pneumoniae*.

	E. coli				*K. pneumoniae*			
Tests	MDDST	*bla*$_{CTX-M}$	*bla*$_{SHV}$	*bla*$_{TEM}$	MDDST	*bla*$_{CTX-M}$	*bla*$_{SHV}$	*bla*$_{TEM}$
Positive no.	57	38	60	29	46	35	19	22
Percent	48.31	32.2	50.85	24.58	85.19	64.81	35.19	40.74
p-value		0.0167*	0.7947^{ns}	0.0002***		0.0624^{ns}	<0.00001****	<0.00001****



A B

Figure 1. Outcomes of the multiplex polymerase chain reaction (PCR) amplification of the genes for the *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{TEM}$. DNA ladder of 100 base pairs (M) A. Lanes 1 to 15 represent DNA samples from phenotypic verified ESBL isolates of *E. coli*; B. Lanes 1 to 12 represent DNA samples from phenotypic identified ESBL isolates of *K. pneumoniae*.

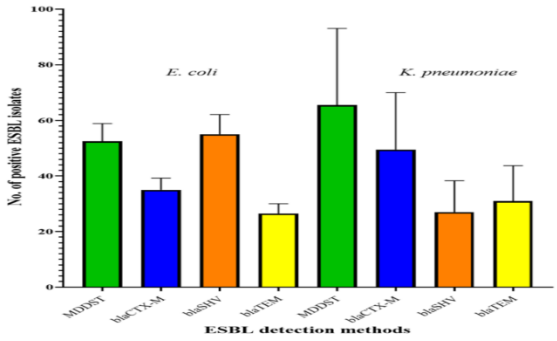


Figure 2. The total prevalence of *E. coli* and *K. pneumoniae* isolates that tested positive for ESBLs.

A total of 67 *K. pneumoniae* strains produced ESBLs phenotypically were assessed by Yang et al. 2019 (23), and among the genes encoding β-lactamases, *bla*KPC (30.2%), *bla*$_{TEM}$ (29.1%) and *bla*$_{SHV}$ (26.5%) exhibited the highest prevalence, and bla$_{NDM}$ (2.7%) revealed the lowest occurrence among all identified antibiotic resistance genes in *K. pneumoniae* strains collected from various specimens.

Kayastha et al. 2020 (24) were collected 79 *E. coli*, 18 *K. pneumoniae and 6 K. oxytoca* isolates, 28.2% (29/103) were they established as ESBL producers by combination disk diffusion assay. The highest percentage of

ESBL production was found among *K. pneumoniae* (33.3%; 6/18), followed by *E. coli* (27.9%; 22/79) and *K. oxytoca* (16.7%; 1/6). Lv et al. (25) were conducted the studies in order to identify ESBL-producing pathogens in both *E. coli* and *K. pneumoniae*, and discovered that the total percentage of ESBL-producing bacteria was 55.5% (355/640) from among 640 persons with community-onset *E. coli* bacteremia. 236 (51.0%) of the 463 patients with community-acquired infections induced by *E. coli* were infected with ESBL-producers, compared to 119 (67.2 %) of the 177 patients with healthcare-associated illness (*p*-value 0.001). While only 46 ESBL-producing bacteria were identified in the 279 individuals with community-onset *K. pneumoniae* bacteremia (16.5%). 24 (12.1%) of the 199 patients with community-acquired illness induced by *K. pneumoniae* were infected with ESBL producers, compared to 22 (27.5%) of the 80 patients with healthcare-associated infection (*p*-value 0.001)

It was conceivable to investigate 181 ESBL-producing strains using the multiplex polymerase chain reaction (PCR) procedure and to establish the existence of the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes (Figure 3 and 4). In ESBL-producing *E. coli* isolates, the presence of three genes was spotted. Enzyme-encoding genes of bla_{CTX} with bla_{SHV} and bla_{CTX-M} with bla_{TEM} types were detected in 32.2% of isolates, while in the combination of bla_{SHV} with bla_{TEM} were recorded in 24.58%. However, the genes in discussion were absent in 58 isolates (49.15%). The coexistence of ESBL genes were detected were $bla_{CTX-M}/bla_{TEM}/bla_{SHV}$ (29/118, 24.58%), as shown in Figure 3. On the other hand, the ESBL genes among *K. pneumoniae* isolates became differ and the genes not detected were 44.44%, and the presence of ESBL genes combination revealed were $bla_{CTX-M}/bla_{TEM}/bla_{SHV}$, bla_{CTX-M} with bla_{SHV}, and bla_{SHV} with bla_{TEM} (19/63, 30.16%), while in case of bla_{CTX-M} with bla_{TEM} were 34.92% (22/63).

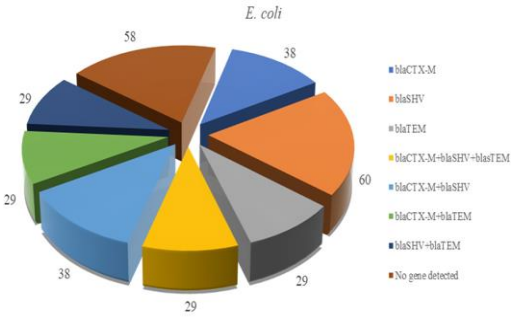


Figure 3. The prevalence of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes among the positive isolates. ESBL genes detected in *E. coli* isolates from different samples by polymerase chain reaction. The following genes were detected. bla_{CTX-M} only in 38, bla_{SHV} only 60, bla_{TEM} 29, $bla_{CTX-M+SHV+TEM}$ 29, $bla_{CTX-M+SHV}$ 38, $bla_{CTXM+TEM}$ 29, $bla_{TEM+SHV}$ 29.

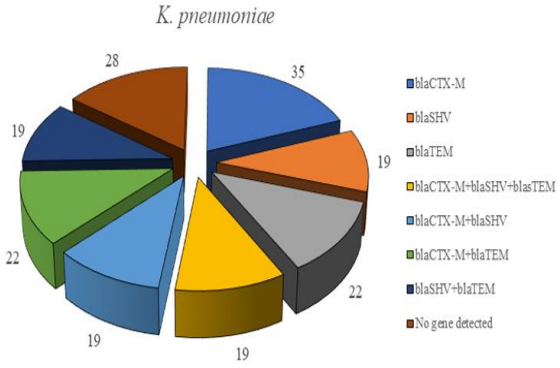


Figure 4. Distribution of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes among the positive isolates. ESBL genes detected in *K. pneumoniae* isolates from different samples by polymerase chain reaction. The following genes were detected. bla_{CTX-M} only in 35, bla_{SHV} only 19, bla_{TEM} 22, $bla_{CTX-M+SHV+TEM}$ 19, $bla_{CTX-M+SHV}$ 19, $bla_{CTXM+TEM}$ 22, $bla_{TEM+SHV}$ 19

Molecular detection has been repeatedly reported by researchers as an accurate tool for detecting bacteria (26-28). Of course, there is always polymorphism in this regard (29). For the management and monitoring of resistance to antibiotics, it is critical to understand the antibiotic susceptibility mechanisms and

resistance genes of pathogenic bacteria in a specific geographic area. According to the findings of the current investigation, MDR was extremely common among the patients. Furthermore, the Carbapenems, Amikacin, and Ciprofloxacin were shown to be the most to the least powerful antibacterial drugs in vitro, ranging from the most to the least active. According to the findings of the current investigation, TEM was shown to be significantly more frequent than the other types of ESBLs.

Interest conflict

The authors declare no conflict of interest.

Author's contribution

The authors confirm contribution to the paper as follows: study conception and design: Kamal Ismael Bakr; specimens and data collection: Sherko Muhammed Abdul-Rahman and Rebwar Muhammad Hamasalih; methodology and lab working on specimens: Kamal Ismael Bakr and Rebwar Muhammad Hamasalih; analysis and interpretation of results: Rebwar Muhammad Hamasalih and Kamal Ismael Bakr Sher; draft manuscript preparation: Kamal Ismael Bakr. All authors reviewed the results and approved the final version of the manuscript.

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