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Prevalence of blaOXA-10, blaCTX-M-3 and SHV Genes among ESBL-Producing Escherichia coli and Klebsiella pneumoniae Isolated from Clinical samples in Basra city, Iraq

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Abstract

The current study was conducted to determine the prevalence of extended-spectrum β-lactamase (ESBL) in 78 drug-resistant clinical isolates (25 *Klebsiella pneumoniae* and 53 *Escherichia coli* strains) using phenotypic and molecular methods. The phenotypic method was performed using a double-disk synergy test (DDST), while the genotypic method screened for the *blaSHV*, *blaCTX-M13U*, and *blaOXA-10* genes using specific primers. The phenotypic results showed that out of 53 tested strains of *E. coli*, 17 (32.07%) produced ESBL. Similarly, out of 25 tested strains of *K. pneumoniae*, 8 (32%) produced ESBL. Genotypic detection showed that in *E. coli*, the most abundant gene was *SHV*, present in 24 strains (45.28%), followed by *blaOXA-10* in 23 strains (43.39%) and *CTX-M-3* in 8 strains (15.09%). In *K. pneumoniae*, *SHV* was detected in 12 strains (48%), followed by *OXA-10* and *CTX-M-3*, each found in 5 strains (20%).

Keywords: Klebsiella pneumoniae, Escherichia coli, ESBL, blaOXA, blaSHV and blaCTX-M3

Introduction

Gram-negative bacteria such as, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* are the most important reservoirs of resistance genes which give them a significant advantage in resisting the antibiotic, such as AmpC genes, metallo-lactamase (MBL) (Ejikeugwu et al., 2021), and Extended-spectrum β -lactamases (ESBLs) (Gales et al., 2023). Gram-negative bacteria that develop resistance to the β -lactam group of antibiotics is usually related to the production of β -lactamases, including carbapenemases and Extended-spectrum β-lactamases, that belong to various molecular classes (Bush & Bradford, 2019).

Beta-lactamases are the most common mechanism of resistance to beta-lactam antibiotics which provides a serious challenge to modern drugs (Lima et al., 2020). β-lactamases deactivate β-lactam drugs as a result of hydrolyzing a particular site in their ring structure, leading it to open, such open-ring drugs are unable to link to the target PBP proteins (Tooke et al., 2019). β-lactamases are widely distributed with different groups containing enzymes that can inactivate all current β-lactam drugs. The four main categories of β-lactams are penicillins, carbapenems, cephalosporins, and monobactams, each contains a four-membered azetidinone ring in its basic structure (De Rosa et al., 2021).

In particular, class D blactamases are responsible for widely known clinical therapy failures (Rajguru et al., 2023). The largest number of Class D β-lactamases belong to the *OXA* family, which includes 14 families of enzymes (Yoon & Jeong, 2021). The genes for class D β-lactamases are regularly detected in the chromosome as an intrinsic resistance determinant in

environmental bacteria, and a few of these are located in movable genetic components in clinically significant pathogens (Yoon & Jeong, 2021). Transposons and plasmids tend to be movable and can be spread through *staphylococcus* (Firth et al., 2018), additionally Gramnegative bacteria such as *Klebsiella* species and *Enterobacter cloacae* has plasmid origin beta-lactamases (Mahazu et al., 2022). *OXA* β-lactamases, including *OXA-11*, *OXA-14*, and *OXA-20*, have been associated to ESBL phenotype (Poirel et al., 2010). *OXA*-type enzymes have increased in importance in recent years as a result of their ability to hydrolyze some carbapenem membranes (Rima et al., 2024). *OXA*-type enzymes that belong to the carbapenemase spectrum involve *OXA-48* as well as associated enzymes such as *OXA-162*, *OXA-181*, *OXA-163*, *OXA-204* and *OXA-232*, which have spread throughout different Enterobacteriaceae (Oueslati et al., 2015).

Extended-spectrum β-lactamases are classified to Ambler class A (functional group 2be) are serine β-lactamases. This class contains *TEM*, *SHV* and *CTX-M* families are now among the most clinically important b-lactamases, and in addition, they were able to hydrolyze not just penicillins, but additionally broad-spectrum monobactams and cephalosporins (Singh et al., 2022). Currently, the *CTX-M* family of class A b-lactamases is the most common set of ESBL enzymes worldwide, with the ability to efficiently hydrolyze extended-spectrum cephalosporins such as cefotaxime (Castanheira et al., 2021).

The development of multidrug-resistant bacteria, such as Enterobacteriaceae producing extended-spectrum β-lactamase (ESBL), generated worries about successful infection treatment. The present study includes a phenotypic and genotypic investigation into ESBL characteristics in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*, providing valuable information on the epidemiology of these bacteria and potential risk factors associated with them.

Methods

Clinical bacterial samples collection

A total of 234 samples were collected from Al-Sader Teaching. Of these, 95 were urine samples, 96 were skin samples, and 43 were blood samples. The samples were collected from patients with a variety of infections, including urinary tract, skin and blood infections. The samples were sent to the laboratory for testing.

Bacterial isolation, identification, and antibiotic susceptibility testing

All the isolates used in the present study were previously identified and tested for antibiotic susceptibility in our laboratory (Mshari et al., 2024). Briefly, the clinical isolates of *E. coli* and *K. pneumoniae* were first identified based on their phenotypic characteristics through various biochemical tests. The identification was then confirmed by PCR using species-specific primers (*mal*B for *Escherichia coli* and *rpo*B for *Klebsiella pneumoniae*). The identified bacteria were then subjected to antibiotic susceptibility testing using disc diffusion and the VITEK2 system. The antibiotics tested included Cefoxitin (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefepime (10 µg), Amoxicillin/clavulanic acid (30 µg), Piperacillin/sulbactam (20 µg), Piperacillin/tazobactam (110 µg), Aztreonam (30 µg), Imipenem (10 µg), Meropenem (10 µg), Colistin (10 µg), Gentamicin (10 µg), Amikacin (30 µg), and Ciprofloxacin (10 µg).

Detection of the resistance genes (ESBL)

Phenotypic identification

The Phenotypic identification of ESBL was performed by a double-disk synergy test (DDST). Bactria suspensions were prepared from each bacteria (*E. coli*, and *K. pneumoniae*). The

bacterial suspension (adjusted with McFarland 0.5 tubes) was distributed on the surface of Mueller-Hinton agar in a sterile L-shape. Disks of aztreonam, cefotaxime, cefepime, and ceftazidime (30 µg each) are placed at a distance of 30mm (center to center) from an amoxicillin 20 µg–clavulanic acid 10 µg disk. The plates were incubated at 37 °C for 24 hours. An increase in zones of inhibition toward amoxicillin-clavulanic acid antibiotic disks is indicative of the presence of ESBL (Jarlier et al., 1988).

Detection of ESBL Genotypes by PCR Amplification

Specific primers were used to identify the resistance genes *SHV*, *CTX-M3*, and *OXA*-10 in *E. coli* and *K. pneumoniae*, as shown in Table 1. The components of the PCR reaction mixture were prepared with specific primers (25 µl) for the amplification of target DNA (resistance genes). The PCR amplification program was as follows: an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 15 seconds, an annealing step at 52°C for 30 seconds, an extension step at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. All PCR amplification products were separated on 1.5% agarose gels and visualized by staining with ethidium bromide using a UV light transilluminator.

Table 1. Sequences of primers for detection of resistance genes

Primers target	Primer sequences (5'- 3')		Primer Length	Amplification size (bp)	References
CTX-M-3, M13	F	GGTTAAAAAATCACTGCGTC	**20**	863	(Jiang et al., 2006)
	R	TTGGTGACGATTTTAGCCGC	**20**		
SHV	F	TGGTTATGCGTTATATTCGCC	21	867	
	R	GCTTAGCGTTGCCAGTGCT	19		
OXA-10	F	GTCTTTCG(A)AGTACGGCATTA	21	699	
	R	ATTTTCTTAGCGGCAACTTAC	21		

Results and Discussion

The bacterial profile and antibiotic resistance pattern

Out of 234 clinical samples, 98 isolates of *K. pneumoniae* and *E. coli* (n = 25 and 53, respectively) were retrieved, and their antimicrobial resistance profiles against 15 different antimicrobial agents were tested. The antibiotic susceptibility testing revealed different profiles of bacterial resistance. Among the antibiotics tested, *E. coli* showed high resistance to cephalosporins (2nd, 3rd, and 4th generation), monobactam, and piperacillin/sulbactam, while *K. pneumoniae* showed lower resistance levels to the same antibiotics (Table 2).

Table 2. Antibiotic resistance pattern of the isolated bacteria

Antibiotics	*E. coli* (No=53)	*K. pneumoniae* (No=25)
	Resistant isolates %	Resistant isolates %
Cefoxitin	92.45	56
Ceftriaxone	79.25	56
Cefotaxime	79.25	52
Ceftazidime	77.36	60
Cefepime	75.47	64
Amoxicillin/clavulanicacid	50.94	64
Piperacillin/Sulbactam	81.13	68
Piperacillin/tazobactam	49.06	44
Aztreonam	86.79	56
Imipenem	15.09	28
Meropenem	24.53	24

Gentamicin	22.64	36
Amikacin	3.77	20
Ciprofloxacin	43.40	28
Colistin	-	-

Phenotypic Detection of ESBL

The conventional double-disk synergy test (DDST) was used to test all strains for the production of Extended Spectrum Beta-Lactamase (ESBL). The results showed that, out of the 53 *E. coli* strains, 17 isolates (32.07%) were positive for ESBL production. Meanwhile, out of the 25 *K. pneumoniae* isolates, 8 isolates (32%) were positive for ESBL production.

Genetic detection *blaOXA*-10, *blaCTX-M*-3 and *SHV*

All 78 tested bacteria (25 *K. pneumoniae* and 53 *E. coli* strains) were screened for the *SHV*, *CTX-M-3*, and *OXA-10* genes using specific primers (Figures 2, 3, and 4). Among *E. coli*, the most abundant gene was *SHV*, found in 24 strains (45.28%), followed by *blaOXA-10* in 23 strains (43.39%) and *CTX-M-3* in 8 strains (15.09%). In *K. pneumoniae*, *SHV* was found in 12 strains (48%), followed by *OXA-10* and *CTX-M-3*, each found in 5 strains (20%) (Table 3).

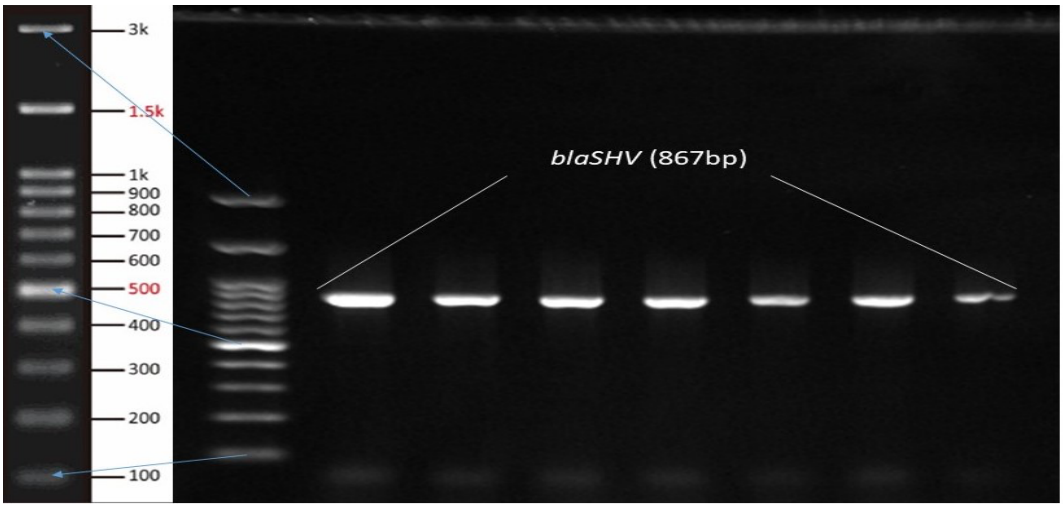

Figure 1: Gel electrophoresis for PCR products of *SHV* gene of representative isolates, Ladder (100-3000bp)

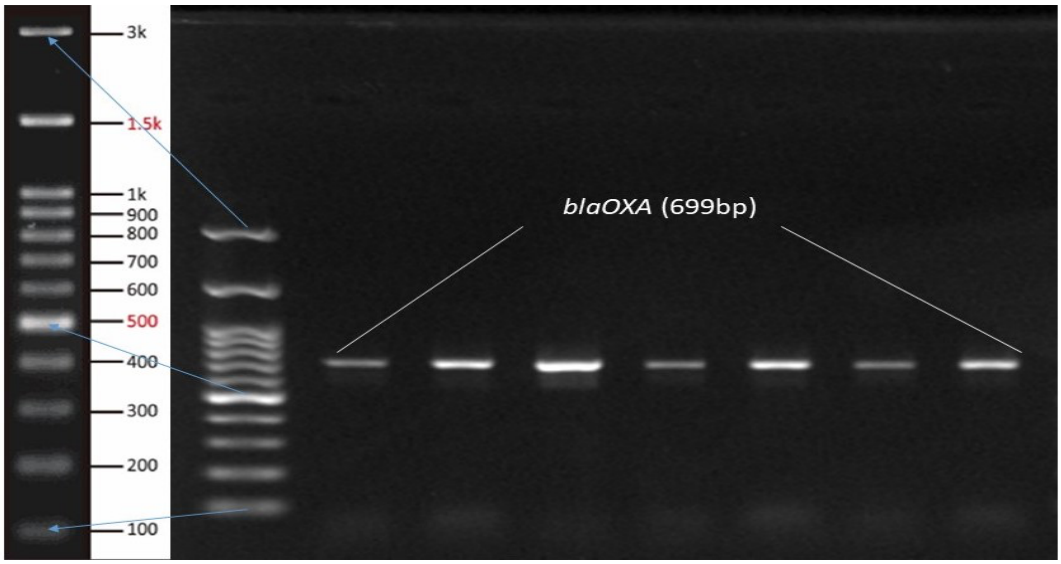

Figure 2. Gel electrophoresis for PCR products of *OXA* gene, Ladder (100-3000bp)

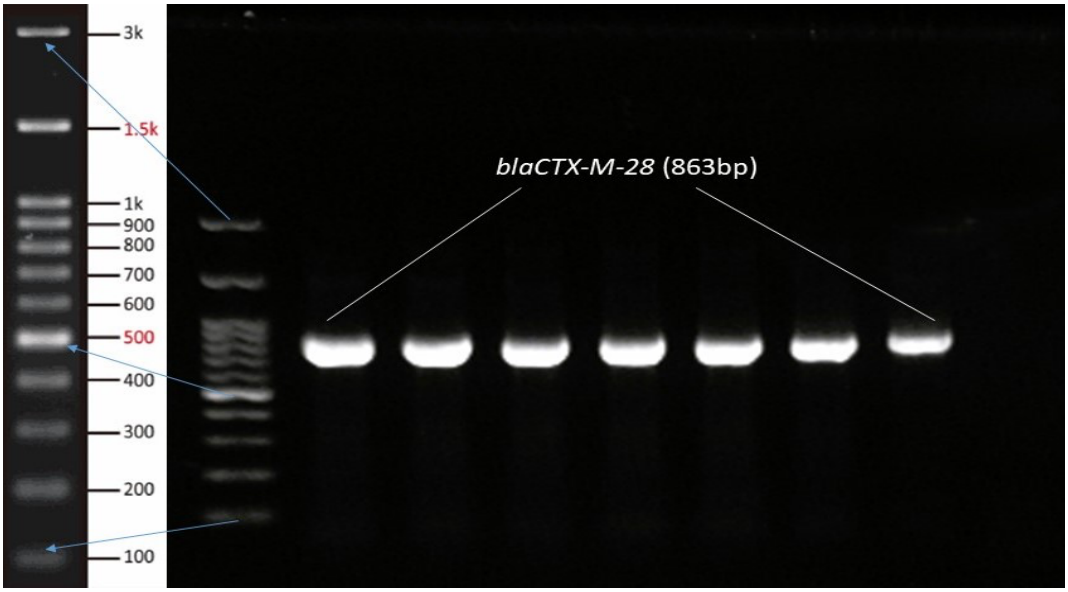

Figure 3. Gel electrophoresis for PCR products of *CTX* gene, Ladder (100-3000bp).

Table 3. The resistance genes encoding in some strains of *E. coli,* and *K. pneumoniae*

Ambler group	***E. coli* NO. (%)**	***K. pneumoniae* NO. (%)**
SHV	24(45.28)	12(40)
CTX-M-3	8(15.09)	5(20)
OXA	23(43.39)	5(20)

The tested isolates (*E. coli* and *K. pneumoniae*) exhibited high resistance to cephalosporins, monobactams, and penicillin groups. This phenomenon was discussed in detail in our previous study (Mshari et al., 2024) .

The phenotypic detection of β-lactamases, such as extended-spectrum β-lactamases (ESBLs) in clinical isolates of bacteria is essential for managing the development of antibiotic resistance bacteria. The phenotypic test in our current study showed that 32.07% of *E. coli* produced ESBLs. Meanwhile, 32% of *K. pneumoniae* produced ESBLs. A previous study in Iran, ESBLs were detected at a rate of 35.4% (Kazemian et al., 2019), while in India, the rates were 52.3% for ESBLs (Salvia et al., 2022). Furthermore, previous studies reported higher rates of ESBLs in *K. pneumoniae* compared to our study. For instance, in Iran, detection rates were 40% for ESBLs (Bajpai et al., 2019), while in India, rates were 76.5% for ESBLs (Salvia et al., 2022).

While, in genotypic detection we identified the likely resistance genes carried by the tested isolates through amplification targeting *blaOXA*-10, *blaCTX-M*-3 and *SHV* resistance genes. A previous study on the detection of resistance genes in *E. coli* in Iraq reported higher percentages than our results for the detection of *SHV* and *CTX-M*, which were 86.67% and 80.0%, respectively. Conversely, another study in India reported lower percentages than ours, with 1.82% for *CTX-M-2*, 10.9% for *SHV*, and 32.78% for *OXA-1*(Verma et al., 2023) . Moreover, a previous study in Iraq on *K. pneumoniae* reported higher results for SHV (100%) and *CTX-M* (100%) compared to ours (Hasan et al., 2022). Conversely, a study in India reported lower results, with percentages of 0.96% for *CTX M-2*, 13.5% for *SHV*, and 25.9% for *OXA* (Verma et al., 2023).

Antibiotic resistance in bacteria is primarily driven by enzymes such as ESBLs, which effectively neutralize β-lactam antibiotics, commonly used to treat bacterial infections. Genetic detection offers valuable insights into the intricate relationship between bacteria and antibiotics, informing strategies to combat antibiotic resistance and safeguard public health.

Our findings underscore the importance of integrating both phenotypic and genotypic detection methods for comprehensive characterization of bacterial resistance patterns. This integrated approach empowers clinicians to select the most appropriate antibiotics for timely and effective treatment of infectious diseases, thereby mitigating the spread of antibiotic resistance.

Conclusion

Understanding the antimicrobial resistance patterns and resistance genes of bacterial pathogens in a specific region is crucial for monitoring and controlling antibiotic resistance. The findings of this study showed a high resistance to cephalosporins, monobactams, penicillin groups. Additionally, colistin, carbapenems and Amikacin were identified as the most effective antimicrobial agents in vitro. The results also indicated that *SHV* and *blaOXA*-10 were the most common ESBL-encoding genes among the isolates.

Suggestion

It is expected that the head of the puskesmas will make team work, play a role in every implementation of puskesmas management, and carry out a leadership style that can influence subordinates to work optimally.

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