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

Ahmed Mshari¹, Najwa M. J. Abu-Mejdad², Khairallah A. S. Mohammed¹

E-mail: ahmed.mshari@stu.edu.iq

¹Southern Technical University, College of Health and Medical Technology in Basrah 
²University of Basrah, college of science

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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 & Bradförd, 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, carbapenemases, 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 Gram-negative 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 (malB for Escherichia coli and rpoB 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). Bacteria 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

<table>
<thead>
<tr>
<th>Primers target</th>
<th>Primer sequences (5'–3')</th>
<th>Primer Length</th>
<th>Amplification size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M3, M13</td>
<td>F GGTTAAAAATCATCCTGCT</td>
<td>20</td>
<td>863</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R TGGTGACGTATTTAGGC</td>
<td>20</td>
<td></td>
<td>(Jiang et al., 2006)</td>
</tr>
<tr>
<td>SHV</td>
<td>F TGTTATGCGTTATATTG</td>
<td>21</td>
<td>867</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R GCTTAAGCTGTTAGC</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-10</td>
<td>F GTCTTTTCGAAGTACGG</td>
<td>21</td>
<td>699</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R ATTTTCTTACGGCGAA</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Gentamicin</th>
<th>22.64</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>3.77</td>
<td>20</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>43.40</td>
<td>28</td>
</tr>
<tr>
<td>Colistin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**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).

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**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)
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(I 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.

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

<table>
<thead>
<tr>
<th>Ambler group</th>
<th>E. coli NO. (%)</th>
<th>K. pneumoniae NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHV</td>
<td>24(45.28)</td>
<td>12(40)</td>
</tr>
<tr>
<td>CTX-M-3</td>
<td>8(15.09)</td>
<td>5(20)</td>
</tr>
<tr>
<td>OXA</td>
<td>23(43.39)</td>
<td>5(20)</td>
</tr>
</tbody>
</table>

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

References


