

## Relation between resistance of *Klebsiella pneumoniae* to certain antibiotics and ESBL/PBP genes

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**Abstract.** Al-Hadeithi ZSM, Jasim SA, Salahdin OD. 2022. Relation between resistance of *Klebsiella pneumoniae* to certain antibiotics and ESBL/PBP genes. *Biodiversitas* 23: 3902-3906. One of the most effective antibiotics on microbes or in treating infections caused by these most resistant microbes, as it belongs to the beta-lactam group such as cephalosporin and carbapenems, which are considered among the most important antibiotics that have wide activity compared to the rest of the other antibiotics and their effectiveness against two types of negative and positive bacteria. Antibiotic resistance is a major public health concern because it happens when antibiotics are used too much or not in the right way. The improved resistance of *Klebsiella pneumoniae* against the antibiotics because of the virulence factors make *K. pneumoniae* the most prevalent pathogenic bacteria behind nosocomial infections. Finding demonstrates that  $\beta$ -lactamase is implicated in *K. pneumoniae* antimicrobial resistance to  $\beta$ -lactam antibiotics. The purpose of this study was to look into the relation among Extended-spectrum beta-lactamase (ESBL), and the molecular biological mechanisms of antibiotic resistance in *K. pneumoniae*. Through the period from February to July 2021 a total of 100 out of 350 isolates were found to be *K. pneumoniae*. Cultural, morphological, and biochemical studies were used to identify growth in blood agar and MacConkey agar. The results revealed that the tested isolates were highly resistant to Imipenem (63%) and Amikacin (24%), while Amoxi-clav exhibited poor resistance (2%). The qualitative real-time approach was used to detect *Bla*R1 and *Bla*I genes for positive isolates that possess gene/ resistant isolate, and the findings revealed that 2 (16%), 4 (33%), 16 (88%), 7 (38%), 24 (100%), 20 (83%), 2 (66%), 2 (100%), 2 (100%), 60 (95%), and 57 (90%) for CIP, CRO, AK, TMP, AMC, and IMP respectively.

**Keywords:** Antibiotic resistance, *Bla*I gene, *Bla*R1 gene, ESBL, *Klebsiella pneumoniae* resistance, qualitative RT-PCR

### INTRODUCTION

One of the health problems that the world suffers from are bacterial infections, especially Gram-negative bacteria that produce beta-lactamase, which is of great importance in the resistance to beta-lactam antigens (extended-spectrum beta-lactamase production (ESBL). In recent decades, people have overused and abused antibiotics, which has led to more antibiotic resistance, which is now a major public health problem (Leylabadlo et al. 2017; Lim et al. 2015). Beta-lactam antibiotics, such as other antimicrobial penicillins, include carbapenems (Cephalosporin and Monobactams). The majority of antibiotics are used internationally. Antibiotics that are beta-lactams share a common molecular structure (Watkins and Bonomo 2013). Antibiotic resistance is a growing problem that is causing major public health problems and a rise in infections caused by pathogens that are resistant to antibiotics around the world (Nölvak et al. 2016; Stogios et al. 2016). Because of this increase in resistance, the World Health Organization (WHO) has directed that priority be given to discovering new alternatives that have an impact on these pathogens, particularly carbapenem resistance (Aslam et al. 2018). *Klebsiella pneumoniae* is a common source of hospital- and community-acquired illnesses throughout the world (Aljanaby et al. 2017; Motaweq 2022). Resistance to various antimicrobial medications is related to higher irregular antibiotic use, which has resulted

in drug resistance of virulence factors, development of intra-hospital cross infection, and even greater clinical treatment problems (Abdullah et al. 2021). In a previous study, it was shown that there is a link between antimicrobial drug resistance, biofilm formation, and ESBL lactamase made by *K. pneumoniae*. *Klebsiella pneumoniae* has become more resistant to drugs in recent years, which has made it hard to treat pneumonia (Neupane et al. 2016; Chen et al. 2017). Antibiotic-resistant genes come from microorganisms that make antibiotics and then become part of the genome of other pathogens through transduction and/or transformation (Boggio and Roveri 2003; Al-Awsi et al. 2022). Researchers have found that membrane-bound macromolecules called penicillin-binding proteins (PBPs) are very important in the process of making the cell wall. Also, zinc finger nuclease is a new way to beat resistance to lactam antibiotics (Macheboeuf et al. 2006; Shahbazi et al. 2016).

Furthermore, based on clinical sepsis observation, *K. pneumoniae* bacteria exhibit transferrable multidrug resistance (Aminul et al. 2021). Additionally, the infectious bacteria that can do resistance to different antibiotic classes mediated by the formation of ESBL, antimicrobial drugs susceptibility of *K. pneumoniae* has drawn interest. Nevertheless, no specific molecular biological mechanisms underpinning *K. pneumoniae* effective antimicrobial resistance have been identified (Nirwati 2019). Investigated based have been carried out to understand the process that

can cause the break down  $\beta$ -lactam antibiotics (Tooke 2019). Perhaps due to the mechanism of resistance is the failure of the antibiotic to reach its sites of action, by narrowing the openings in the wall through the purine channels (Ghai 2018). Among the important resistance mechanisms are efflux pumps, which have a major role in removing the antibiotic from its work site before it starts working. As these pumps cross through the inner and outer membranes of the negative bacteria, these bacteria resist antibiotics by increasing gene expression (Abdi et al. 2020). Among the important issues that must be addressed are mutations that can have a major role in drug resistance through modification at a site on the bacterial chromosome where mobile genetic elements have a major role in this (Pérez-Varela et al. 2019). Another mechanism that can develop antibiotic resistance is to change the target of the antibiotic. Resistance to streptomycin, quinolones, rifampin and other groups of antibiotics is caused by a series of mutations that can occur in the gene encoding the target protein and the protein involved in drug transport and/or pharmacological activation. Changing the antibiotic target is another mechanism that can develop antibiotic resistance, as the resistance of pathogens to some types of antibiotics is caused by a group of mutations that can occur in the protein-coding gene that is concerned with drug activation (Kamoshida et al. 2020). The best example of this mechanism is penicillin resistance in *Streptococcus pneumoniae*, which is obtained by the genes of the mosaic penicillin-binding protein (PbP), which has a major role in encoding penicillin-insensitive enzymes. In terms of the target site, each antagonist has its own target site, by correlating with its inhibitory effect (Chen et al. 2021). According to previous study, the evolutionary links among  $\beta$ -lactamases and antibiotic-producing bacteria are rather conservative (Santos-Lopez et al. 2019).

## MATERIALS AND METHODS

### Isolates

All experiments took place at the Baghdad medical city, and all protocols were approved by the Baghdad medical city and the Ethics Committee of the Ministry of Health and Medical City in Baghdad. 350 samples of urine and sputum have been collected during the period of February 2021 to July 2021 from Baghdad medical city, the collection of samples are subjected to the declaration of Helsinki of handling and practice of isolates then isolated from the collected samples then have been identified by biochemical methods.

### Determination of antibiotic resistance

According to the standards set by the Clinical and Laboratory Standards Institute, an antibiotic susceptibility test was determined. A number of antibiotics were used to detect the effectiveness of these antibiotics against *K. pneumoniae* (Afhami et al. 2020).

The susceptibility of all purified *K. pneumoniae* isolates to different antibiotic including Ciprofloxacin (CIP), Ceftriaxone (CRO), Amikacin (AK), Trimethoprim (TMP),

Amoxi-clav (AMC), and Imipenem (IMP) was evaluated by the disc-diffusion method. A particular volume of the bacterial strain suspension with the McFarland standard ( $1.5 \times 10^8$  CFU/mL) was inoculated and distributed over Müller-Hinton agar medium to evaluate the antibiotic susceptibility test (Merck Germany). The disks were then sequentially arranged on the plate at predetermined spacing. Finally, the inhibitory zone diameter was evaluated after a 24-hour incubation period, and the results were interpreted as sensitive, intermediate, and resistant forms.

### ELISA technique

Immunological techniques, like ELISA, are based on the antigen-antibody-specific interaction, which, if the antigen is present in the sample, causes a visible reaction in the test medium. Due to their benefits, which include quick analysis times, ease of use, high specificity, and relatively inexpensive equipment, these techniques are frequently employed to detect bacteria. In various European nations, ELISA kits and strips have become widely used for routine testing as a result of the development of commercially available versions (Ferone et al. 2020; Law et al. 2014).

To use an ELISA kit, researchers investigated PBP's affinity for penicillin (cat. no. MBS3802151, Mybiosource, U.S.A). The manufacturer's procedures were followed while performing the ELISA assays.

### Primer preparation

Forward and reverse primers that were in the lyophilized state were first dissolved and diluted in free nuclease D.S.D.W. (amount as per manufactured company's recommendation) to get 100 pico-mol/ $\mu$ L, and this is regarded as a stock solution; it can then be kept in the deep freezer. Before being employed in a PCR combination, this sample was diluted with free nuclease D.W. to obtain roughly 10 pico-mol/ $\mu$ L. This method worked with every primer used in this study. It was provided as a lyophilized suspension by Macrogen. The stock solution was dissolved in nuclease-free water to create a lyophilized 100 pmol/mL solution of the primers. By mixing 10  $\mu$ L of the primer solution with 90  $\mu$ L of non-nuclease water, a workable solution of these primers with a final concentration of 10 pMol was created (cooled to 2°C).

### Detection of ESBL/PBP genes

In order to detect the genes ESBL/PBP, the DNA have been extracted first by using Commercial extraction kit (Thermo Fisher Scientific, European Union) that is based on the solid phase isolation procedure. Then 3  $\mu$ L of eluted DNA has pipetted to a new sterile PCR tube then 10  $\mu$ L of sybr green, 0.5  $\mu$ L of each primer and 6  $\mu$ L of the nuclease free water was added, then the tube placed into the real time-thermal cycler, then the device programmed to amplify the target sequence, the program included 95°C for 10 min, followed by step 2: 95°C for 20 sec, 61°C for 20 sec then 72 for 1 min, the steps from 2 to 4 have been repeated 35 times. Sequence of specific primer pairs the sequence of BlaR1 gene Forward 5' TCTAGAGGATCAT ATTACAATACCGAGCTC 3' and Reverse 5' GAGCTCG

GTATTGTAATATGATCCTCTAGA 3, and the sequence of *BlaI* gene F- 5 CGCTTAATTCAGCACTAAAC 3 and R – GAGCTCGGTATTGTAATATGATCCTCTAGA 3 (Jiang et al. 2020).

## RESULTS AND DISCUSSION

### Bacterial isolates

From February 2021 to July 2021, a total of 100 nonduplicated *K. pneumoniae* isolates from 350 samples were collected from the different wards of Baghdad medical city, Iraq) the number of bacteria isolated according to each clinical specimen included the sputum (38%) and urinary tract (62%) collected from (44 females and 56 males).

### Antibiotic susceptibility

The medications Imipenem, Amikacin, and Ceftriaxone had the most resistance to antibiotics, with 63, 24, and 18%, respectively, according to the findings of this study. Amoxi-clav resistance was observed at 2%, which is a low level of resistance. As seen in the table 2.

The distribution of the resistance pattern and its relation to gender are summarized. In our study, we found a significant difference in the resistance and sensitivity patterns of *K. pneumoniae* among the male and female patients. Here we observed *K. pneumoniae* was found to be the most resistant (39/61.9%) to Imipenem among the female patients, while the percentage was (24/38.1%) in the male patients. Furthermore, 15/62.5% of the female and 9/37.5% of the male patients, 11/61.1%, 7/38.9 resistance for female and male patients respectively to Ceftriaxone. In contrast, 10/83.3% female resistant to Ciprofloxacin, 2/16.7% male patients. In case of sensitivity, Amoxi-clav and Trimethoprim had shown a sensitivity to *K. pneumoniae* for male, and resistance to 2%, 3% for females respectively.

Following the sensitivity test utilizing the disk diffusion method (DDM), the isolates with antibiotic resistance were identified, and qualitative real-time identification of *BlaR1*

and *BlaI* genes for (positive isolates that's possess gene/ resistant isolate). Table 3 and figure (1) *BlaR1* and *BlaI* genes were found to be 2 (16%), 4 (33%), 16 (88%), 7 (38%), 24 (100%), 20 (83%), 2 (66%), 2 (100%), 2 (100%), 60 (95%), and 57 (90%) for CIP, CRO, AK, TMP, AMC, and IMP, respectively.

### Molecular epidemiology

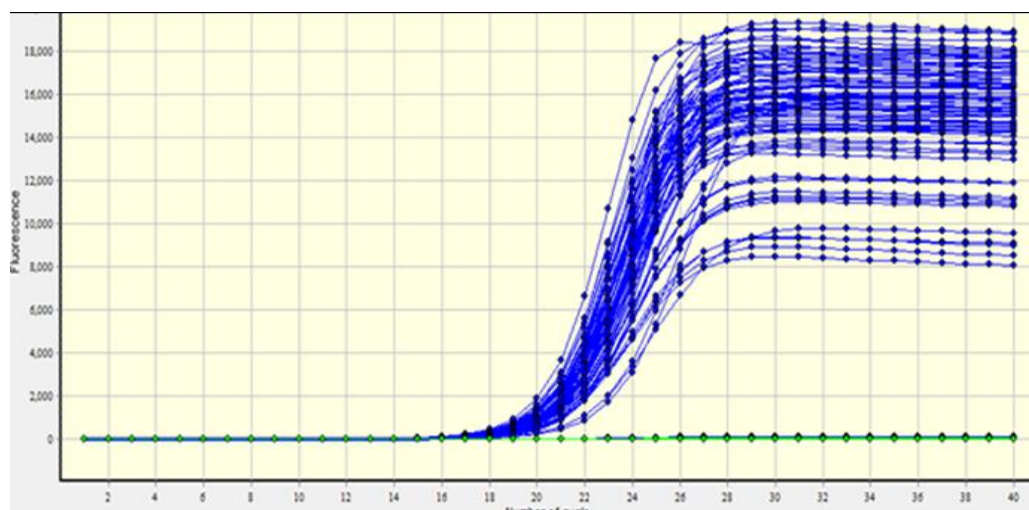
The probable molecular mechanism of antibiotic resistance of *K. pneumoniae* in Iraqi patients was examined in this study. Despite the fact that a prior study proposed a primary explanation of antibiotic resistance, the dominant point of view focused on lactamase signaling (Sasirekha and Shivakumar 2012). Other researchers in Baghdad, Al-Muhannak 2010, (15.7%), and Wang et al. 2013 (75%), and Rahim all found high incidence of *K. pneumoniae* in sputum samples (42.3%) (Rahim and Mohamed 2014).

**Table 2.** Antibiotic resistance of *Klebsiella pneumoniae* isolates

Antibiotic (abbreviation)	Number of resistant isolates (%)
<i>Ciprofloxacin (CIP)</i>	12(12%)
<i>Ceftriaxone (CRO)</i>	18(18%)
<i>Amikacin (AK)</i>	24(24%)
<i>Trimethoprim (TMP)</i>	3(3%)
<i>Amoxi-clav(AMC)</i>	2(2%)
<i>Imipenem (IMP)</i>	63(63%)
Total (%)	100 (100)

**Table 3.** Antibiotic resistance isolates revealed the presence of the *BlaR1* and *BlaI* genes

Antibiotic/ abbreviation	Genes	
	<i>BlaR1</i>	<i>BlaI</i>
<i>Ciprofloxacin (CIP)</i>	2 (16%)	4 (33%)
<i>Ceftriaxone (CRO)</i>	16 (88%)	7 (38%)
<i>Amikacin (AK)</i>	24 (100%)	20 (83%)
<i>Trimethoprim (TMP)</i>	2(66%)	2 (66%)
<i>Amoxi-clav(AMC)</i>	2 (100%)	2 (100%)
<i>Imipenem (IMP)</i>	60(95%)	57 (90%)



**Figure 1.** The result of qualitative real-time amplification of *BlaR1* and *BlaI* genes

One of the biggest problems facing Clinicians around the world is nosocomial infection, especially in underdeveloping countries. One of the most important pathogens is *K. pneumoniae*, where antibiotic resistance has developed, which has made it difficult to speed up recovery and consequently the cost of care. Despite the many studies on the genes of *Klebsiella pneumoniae*, its study is still not clear about the properties of this microbe for resistance in various hospitals in Iraq. After conducting several studies on the resistance of *K. pneumoniae*, different levels of resistance and the genes associated with it appeared in hospitals.

In clinical patients, antibiotic resistance in *K. pneumoniae* is linked to a high rate of morbidity and mortality (Koczura et al. 2011). To overcome the antibiotic resistance problem and reduce the mortality of patients infected with *K. pneumoniae*, researchers must first understand the mechanisms related in the bacteria's drug resistance. The ESBL and target mutation of gene-coding PBPs are the two primary processes that generate -lactam antibiotic resistance (Lee et al. 2016). Therapy with beta-lactam antibiotics may continue in the continuation of resistance to these factors on the increase, while there are more effective agents for clinical use, as it is one of the most prominent challenges faced by the Health Organization, especially after the emergence of resistant types of the carbapenem group (Aurilio et al. 2021; Aurilio et al. 2022).

Jiang et al. (2000) discovered that antibiotic resistance in *K. pneumoniae* was linked to the development of  $\beta$ -lactamase (Padilla et al. 2006). Most  $\beta$ -lactam medications are resistant to ESBL-producing bacteria (Wragg et al. 2017) which is consistent with our findings. Resistance genes are found in ESBL-producing *K. pneumoniae* with genetic diversity, posing a serious hazard to public health (Charrouf et al. 2014). It is necessary to continue treatment with antibiotics belonging to the beta-lactam group is necessary and therefore because of its importance in the mechanism of action by inhibiting the synthesis of the wall, especially the peptidoglycan layer, The main reason for the resistance of these bacteria to these beta-lactam antigens may be due to their failure to reach the main target or by destroying the antigen in an enzymatically (Giurazza et al. 2021).

In conclusion, one of the serious problems that is of great concern to the World Health Organization is carbapenem resistance through the production of carbapenemases by *K. pneumoniae*, where there must be many studies to control this spread and resistant organisms. Amoxi-clav low resistance was observed, and Imipenem high resistance to antibiotics, also the molecular biological mechanisms of antibiotic resistance in *K. pneumoniae*, by qPCR found Imipenem (IMP) 60 (95% and 57 (90%) for of BlaR1 and BlaI genes respectively.

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