Molecular detection of some β-lactamase genes among K. pneumonia 258

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Abstract

In this cross-sectional investigation, researchers gathered 100 samples from patients at Hilla Teaching Hospital and Marjan Medical City. Using morphological, microscopic, biochemical, and Vitek II system testing, researchers were able to identify 40 distinct strains of K. pneumonia from 100 samples collected from a wide range of infection sites. Standard PCR was performed on the DNA from all of the probable K. pneumonia isolates to amplify the pilv-l gene using the sequences; the findings showed that only 16 (40%) of the 40 K. pneumonia isolates were connected to K. pneumonia258 by sharing the same 320 bp DNA fragment with the allelic ladder. Some virulence genes were detected in 16 K. pneumoniae258 isolates and compared with local K. pneumonia, the results showed that, BlaOXA-48 gene were detected in 13/16(81.2%) were positive to blaOXA-48 gene of K. pneumoniae258 isolates while 8/16(50%) K. pneumoniae isolates were found at (428bp). However, blaTEM gene were detected in 13/16(81.2%) were positive to blaTEM gene of K. pneumoniae258 isolates while 9/16(56.2%) K. pneumoniae isolates were found at (1080bp). blaSHV gene were detected in 12/16(75%) were positive to blaSHV gene of K. pneumoniae258 isolates while 10/16(62.5%) K. pneumoniae isolates were found at (930bp), blaCTX-M gene were detected in 15/16(93.7%) were positive to blaCTX-M gene of K. pneumoniae258 isolates while 8/16(50%) K. pneumoniae isolates were found at (585bp).

Keyword: *K. pneumoniae258, β-lactamase genes, blaCTX-M gene, blaSHV gene, BlaOXA-48 gene.*

INTRODUCTION

Klebsiella pneumoniae has developed resistance to carbapenems due mostly to the presence of carbapenemases that were not present in the strains that were originally susceptible. Class A (KPC, GES), B (VIM, IMP), and D (OXA-48) carbapenemases are all examples. The K. pneumoniae carbapenemases are the most prevalent class A carbapenemases in this pathogen (KPCs) (El-Badawy et al., 2020).

First identified in the United States in 2001, KPC-producing K. pneumoniae strains have now been found in Europe, Israel, South America, and China. In 2010, bronchial aspirates from a patient in the intensive care unit (ICU) in Korea were the source of the first KPC-2-producing K. pneumoniae strain in the country. Nevertheless, because KPC-producing K. pneumoniae also make VIM or CTX-M, it is challenging to choose the right medications to treat infections caused by these bacteria. Patients infected with KPC-producing isolates have a much greater mortality rate than those infected with imipenem-resistant isolates (Ben Tanfous et al., 2017).

During the course of the last few decades, sequence type 258 (ST258) CRKP strains have proliferated all over the world. The

presence of plasmid-encoded K. pneumoniae carbapenemases in this genetic lineage has been linked to multiple hospital-associated outbreaks, making it a public health concern (KPCs). Infections caused by multidrug-resistant Enterobacteriaceae have fewer treatment options because KPC enzymes hydrolyze all β-lactam drugs (Yang et al., 2021).

The persistence of CRKP ST258 and the dissemination of KPCs to other hospital infections are both facilitated by the ease with which plasmids encoding KPCs are horizontally transferred and recombine in the hospital setting. Carbapenemases can spread even when carbapenems aren't being selected for because of extra antibiotic resistance genes on KPC plasmids. ST258 is a "high-risk" CRKP lineage because of its worldwide prevalence, epidemic character, and capacity for rapid dissemination of numerous antibiotic resistance determinants (Paul et al., 2022).

Depending on the capsular and plasmid gene content, ST258 strains can be roughly classified into two separate clades. Plasmid-encoded KPC-2 is more common in clade I ST258 isolates, while plasmid-encoded KPC-3 is more common in clade II ST258 genomes (Marsh et al., 2019). Resistance to colistin therapy and ceftazidime-avibactam therapy has been reported in several recent studies conducted at our institution, primarily among clade I and clade II ST258 isolates. Further illustrating the danger posed by this family in healthcare facilities, we have previously identified several outbreaks linked to ST258-affected devices (Giddins et al., 2018).

Klebsiella pneumoniae, an opportunistic pathogen linked to severe infections in hospitalized patients, especially those who are immune-compromised due to other, more serious conditions, is the most prevalent bacteria found to produce ESBLs (Costa, 2019). Community- and ventilator-acquired pneumonia, urinary tract infections, abdominal diseases, and infections acquired through central venous catheters are all potential entry points for Klebsiella pneumoniae into the bloodstream (Roy, 2018).

ESBL-producing Enterobacteriaceae are common in both the community and in healthcare settings. The true prevalence of ESBL-producing microbes is likely underestimated because of the difficulty of accurately identifying them in clinical laboratory. Infections caused by these species are best treated with carbapenems (Al-Kaaby, 2016).

The epidemiology and therapy of bacteria that produce extended-spectrum beta-lactamases, as well as the many types of and methods for detecting these enzymes. There are other articles that go into greater detail about the clinical manifestations and diagnosis of infections caused by ESBL-producing organisms (Rakotovao-Ravahatra et al., 2020).

Antibiotics are rendered useless when the beta-lactam ring is broken by enzymes known as beta-lactamases. In the 1960s, scientists in Greece found the first plasmid-mediated beta-lactamase in gram-negative bacteria. The patient (Temoniera) from whom it was first isolated inspired its name, TEM (Tanko et al., 2020).

Beta-lactamases can be organized into two different categories. The Bush-Jacoby-Medeiros classification divides proteins into four functional categories (Groups 1–4), while the Ambler classification divides proteins into four groups (A–D) based on their amino acid sequence similarity (Amraei et al., 2022). They would be classified as serine beta-lactamases if they had a serine radical, or as metallo-beta-lactamases if they contained a zinc ion in their active site (Messasma et al., 2021).

Bacterial resistance to β-lactams likely developed early in the evolution of bacteria, but has only recently become a beneficial and

consequently chosen feature due to the widespread use of β-lactam antibiotics. Using a Darwinian approach, these medications selected for resistance by killing vulnerable bacteria while leaving resistant strains alive (Pereira et al., 2022). Resistance to these antibiotics may be innate to certain species. It's also possible to pick up the trait through natural mutation or a genetic exchange. Resistance to β-lactams can arise for a number of different functional reasons, including the generation of -lactamases, impermeability, efflux, and target alteration. It is possible for these modalities to occur alone or in many permutations (Darby et al., 2022).

Objective

The aim of present study is to detection of K. pneumoniae 258 β-lactamase genes at molecular level.

Materials and methods

Study Design

One hundred clinical specimens were collected from patients at Hilla teaching hospital and Marjan medical city for this cross-sectional study. From now until April of the next year, so late as 2022.

Ethical Approval

It is crucial to secure the necessary ethical approval from the hospital's ethical review board, patients, and their supporters. Before any samples are taken from participants, they are all given a verbal explanation of what will happen, and their permission to participate in the study and have their results published is obtained.

Clinical specimens

Sample collection procedures for a bacteriological research are outlined below. It was great care in collecting these samples to rule out the possibility of contamination (Collee et al., 1996).

Identification of K. pneumoniae with Vitek2 System

The Vitek 2 is an automatic identification (ID) tool utilized in the field of medical microbiology.

DNA Extraction

This procedure was developed using the manufacturer Geneaid's optional genomic DNA purification Kit (Korea).

Diagnosis of K. pneumoniae 258 by specific primer gene

K. pneumoniae 258 was diagnosed with PCR by using the primer specific for pilv-l gene (Table 1).

Table 1: The sequence of primers that used this study.

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp)
pilv-l	F	5'- TGATGCTGATGGCAGACTGA - 3'	60.6	50	320
	R	5'- TGTAGTCACACCCTGCCCA - 3’	64.4	58	

Detection of K. pneumoniae 258 β- lactamase virulence gene

DNA (extract from bacterial cells) was used as a template in specific PCRs for the detection of β- lactamase virulence gene. DNA was purified from bacterial cells by using the Geneaid DNA extraction Kit. The primers used for the amplification of a fragment gene were listed in Table (2, 3).

Table 2: the primers, sequences, and PCR conditions

Primer	Sequence	Primer sequence5'- - 3'	Tm (°C)	GC%	Size of Product (bp
blaOXA-48	F	TTGGTGGCATCGATTATCGG	59.9	50	428
	R	GAGCACTTCTTTTGTGATGGC	58.5	48	
blaTEM	F	ATA AAA TTC TTG AAG ACG AAA	48.4	24	1080
	R	GAC AGT TAC CAA TGC TTA ATC	25.3	38	
blaSHV	F	GGG TTA TTC TTA TTT GTC GC	52.8	40	930
	R	TTA GCG TTG CCA GTG CTC	59.3	56	
blaCTX-M	F	GCT ATG TGC AGT ACC AGT AA	54.8	45	585
	R	ACC AGA ATG AGC GGC GC	63	65	

Table 3: The optimum condition of detection of virulence genes

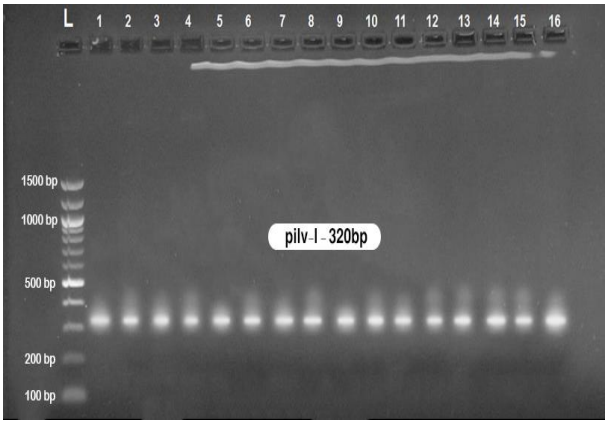
No.	Name of gene	Phase	Tm (°C)	Time	No. of cycle
1.	*blaTEM*	Initial Denaturation	95°C	2 min	1 cycle
		Denaturation -2	95°C	30 Sec	30 cycle
		Annealing	60°C	30 Sec	
		Extension-1	72°C	50 Sec	
		Extension -2	72°C	5 min.	1 cycle
2.	*blaSHV*	Initial Denaturation	95°C	2 min	1 cycle
		Denaturation -2	95°C	30 Sec	30 cycle
		Annealing	60°C	30 Sec	
		Extension-1	72°C	50 Sec	
		Extension -2	72°C	5 min.	1 cycle
3.	blaCTX-M	Initial Denaturation	95°C	2 min	1 cycle
		Denaturation -2	95°C	30 Sec	30 cycle
		Annealing	57.5°C	30 Sec	
		Extension-1	72°C	1 min	
		Extension -2	72°C	7 min.	1 cycle
4.	*blaOXA-48*	Initial Denaturation	95°C	7 min	1 cycle
		Denaturation -2	95°C	30 sec	33 cycle
		Annealing	53°C	1 min	
		Extension-1	72°C	1 min	
		Extension -2	72°C	7 min.	1 cycle

Results and Discussion

One hundred clinical specimens were gathered from patients at Hilla Teaching Hospital and Marjan Medical City for this cross-sectional study. For this study, each bacterial isolate of K. pneumoniae was analyzed using a battery of morphological, Microscopical, biochemical, and Vitek II system tests. When the Citrate utilization test was performed on the K. pneumoniae isolates, it was obvious that they had the ability to use citrate as their sole carbon source. Moreover, urease enzyme was created by the isolates, which caused the yellow Urea agar slant to change to pink. Infections caused by Klebsiella are identified by the presence of urease enzyme synthesis (Yaqoob et al., 2022). Because it catalyzes the formation of kidney and bladder stones or to encrust or obstruct indwelling urinary catheters, this enzyme is also considered one of the most significant virulence factors of K. pneumoniae, which has been involved in the pathogenesis of several diseases, including

pyelonephritis and the development of infection-induced urinary stones. They were not mobile enough to pass the motility test. (Yuan et al., 2021). DNA polymerase can manufacture a new strand of DNA that is complementary to the supplied template strand, allowing PCR to make billions of copies of the target sequence (Amplicon). DNA was extracted from all the potential K. pneumonia isolates and put through a conventional PCR for pilv-l gene primer amplification using the sequences and software in Table (1). The findings of the gel electrophoresis study are depicted in Figure (1); only 16 (40%) of the 40 K. pneumonia isolates were connected to K. pneumonia258 by sharing the same 320 bp DNA segment with the allelic ladder.

Figure (1): Agarose gel electrophoresis (1.5%) of RCR amplified of pilv-l gene (320bp) of K. pneumonia258 for (55) min at (70) volt L: ladder (DNA marker). Number (1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16) positive K. pneumonia258 isolates.



Polymerase chain reaction (PCR) amplification of a specific gene or section of the genome allows for molecular identification of K. pneumoniae 258, using specific primers, as found by Mendes et al., (2022), was confirmed by these findings. According to Gato et al., (2020), two genes, 16S rRNA and pilv-l genes, were commonly used for molecular identification of K. pneumoniae258. As Reyes Chacón, (2019) discovered, the primers used to amplify this gene were exclusive to K. pneumoniae 258 and did not amplify any other bacteria. To confirm that the bacterium is indeed K. pneumoniae258, we will compare the sequencing of the amplified product to that of known K. pneumoniae258, which has been sequenced before. Sequencing the amplified product can provide significantly more information about the bacterial genetic make-up, which can benefit in epidemiological investigations into the mechanisms of virulence and antibiotic resistance in K. pneumoniae 258 (Shankar et al., 2020). Using a molecular biology technique centered on the pilv-l gene to identify Klebsiella pneumoniae strain 258 in a biological sample (Mandras et al., 2020). Bacterial adherence to host cells and biofilm formation are facilitated by the type IV pilus protein expressed by this gene. The pilv-l gene was identified as being exclusive to K. pneumoniae strain 258, making it easy to spot. Moore, (2017) found that DNA extraction from a biological material (such blood, urine, or tissue) and subsequent amplification using polymerase chain reaction (PCR) or other molecular techniques can be used to detect K. pneumoniae 258. The presence of K. pneumoniae 258 can be confirmed using gel electrophoresis or sequencing of the amplified DNA. BlaOXA-48 gene were detected in all 16 K. pneumoniae258 isolates and compared with 16 K. pneumonia isolates. The results showed that 13/16(81.2%) were positive to blaOXA-48 gene of K. pneumoniae258 isolates while 8/16(50%) K. pneumoniae isolates were found. PCR product was roughly (428bp) in size, the results were shown in Figure (2). However, blaTEM gene were detected in all 16 K. pneumoniae258 isolates and compared with 16 K. pneumonia isolates. The results showed that 13/16(81.2%) were positive to blaTEM gene of K. pneumoniae258 isolates while 9/16(56.2%) K. pneumoniae isolates were found. PCR product

was roughly (1080bp) in size, the results were shown in Figure (3). In addition, blaSHV gene were detected in all 16 K. pneumoniae258 isolates and compared with 16 K. pneumonia isolates. The results showed that 12/16(75%) were positive to blaSHV gene of K. pneumoniae258 isolates while 10/16(62.5%) K. pneumoniae isolates were found. PCR product was roughly (930bp) in size, the results were shown in Figure (4). So, blaCTX-M gene were detected in all 16 K. pneumoniae258 isolates and compared with 16 K. pneumonia isolates. The results showed that 15/16(93.7%) were positive to blaCTX-M gene of K. pneumoniae258 isolates while 8/16(50%) K. pneumoniae isolates were found. PCR product was roughly (585bp) in size, the results were shown in Figure (5). A results of Abrar et al., (2019) found that K. pneumonia revealed blaCTX gene in rate 76% followed by blaOXA gene in rate 52%, blaTEM gene in rate 28% and blaSHV gene in rate 21%. Abrar et al., (2016) found that, the genes were most prevalent among K. pneumonia blaCTX gene in rate 65%, blaOXA gene in rate 78% and blaTEM gene in rate 57%, and Sonda et al., (2018) found blaCTX gene in rate 75%, blaOXA gene in rate 49% and blaTEM gene in rate 34% isolates among K. pneumonia. The blaOXA-48, blaTEM, blaSHV and blaCTX genes were a type of beta-lactamase genes that encodes an enzyme that can hydrolyze and inactivate certain types of antibiotics, including penicillins, cephalosporins, and carbapenems. This gene was commonly found in some bacteria, including Klebsiella pneumoniae, which is an important opportunistic pathogen associated with healthcare-associated infections (Hansen, 2021). PCR-based assays have been developed that can detect the presence of the blaOXA-48, blaTEM, blaSHV and blaCTX genes in clinical isolates of K. pneumoniae with high sensitivity and specificity.

Figure (2): Agarose gel electrophoresis (1.5%) of RCR amplified of blaOXA-48 gene (428bp) of K. pneumonia258 for (55) min at (70) volt L: ladder (DNA marker). Number (1,2,4,5,6,7,9,10,11,12,14,15,16) positive K. pneumonia258 isolates, while number (17,18,20,21, 25,28,29,30) positive K. pneumonia isolates.

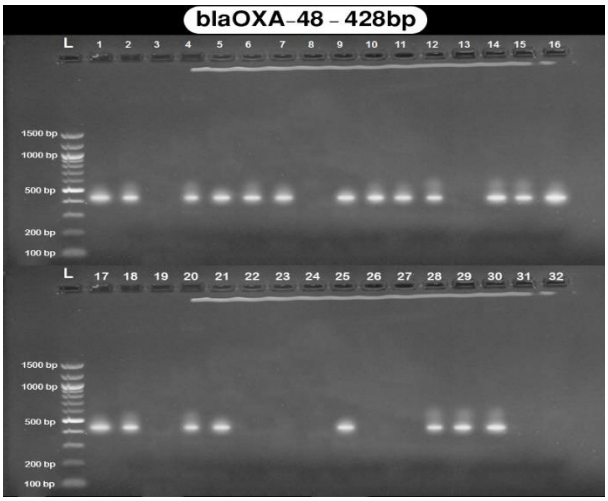


Figure (3): Agarose gel electrophoresis (1.5%) of RCR amplified of blaTEM gene (1080bp) of K. pneumonia258 for (55) min at (70) volt L: ladder (DNA marker). Number (1,3,4,5,7,8,9,11,12,13,14,15,16) positive K. pneumonia258 isolates, while number (17,18,19,21,22,23,25,27,32) positive K. pneumonia isolates.

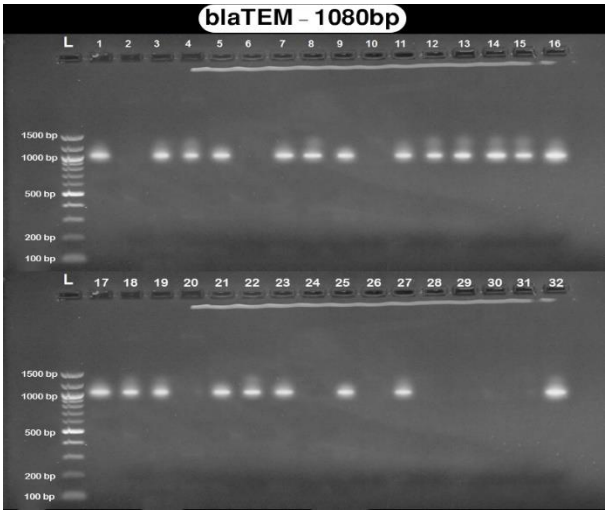


Figure (4): Agarose gel electrophoresis (1.5%) of RCR amplified of blaSHV gene (930bp) of K. pneumonia258 for (55) min at (70) volt L: ladder (DNA marker). Number (1,2,3,5,6,7,8,9,11,13,14,16) positive K. pneumonia258 isolates, while number (17,18,19,21,23,24,28,30,31,32) positive K. pneumonia isolates.

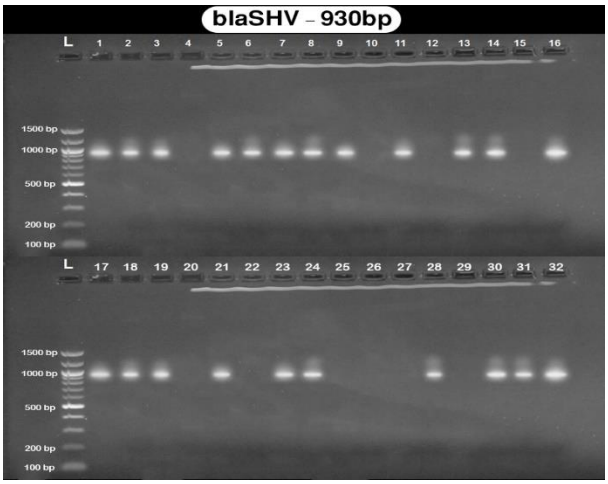
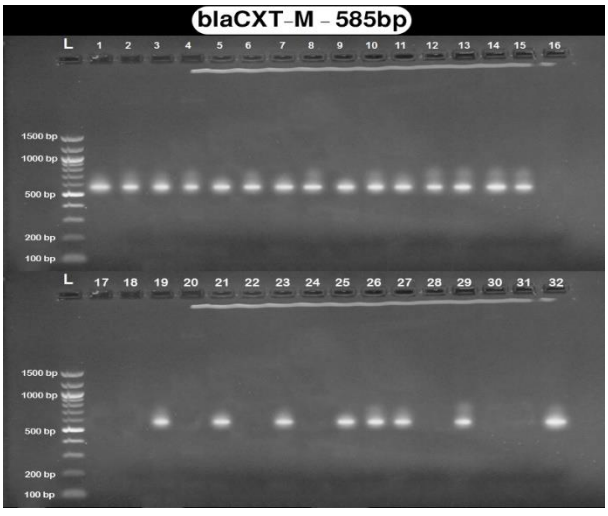


Figure (5): Agarose gel electrophoresis (1.5%) of RCR amplified of blaCTX-M gene (930bp) of K. pneumonia258 for (55) min at (70) volt L: ladder (DNA marker). Number (1,2,3,4,5,6,7,8,9,10,11,13,14,15) positive K. pneumonia258 isolates, while number (19,21,23,25,26,27,29,32) positive K. pneumonia isolates.



Rocha et al., (2019) found PCR-based assays could also be used to detect other types of beta-lactamase genes, such as blaKPC, blaNDM, and blaIMP, which are also associated with antibiotic resistance in K. pneumoniae. In addition, phenotypic tests, such as disk diffusion and minimum inhibitory concentration (MIC) assays, can be used to detect resistance to specific antibiotics, including carbapenems, which may indicate the presence of the blaOXA-48, blaTEM, blaSHV and blaCTX genes or other beta-lactamase genes.

Overall, the detection of the blaOXA-48, blaTEM, blaSHV and blaCTX genes in K. pneumoniae were an important step in identifying antibiotic-resistant strains of this pathogen and guiding appropriate treatment decisions. The spread of blaOXA-48, blaTEM, blaSHV and blaCTX-positive K. pneumoniae strains was a growing concern in healthcare settings, as they could be transmitted between patients and was associated with increased morbidity and mortality (Mpelle et al., 2019).

Pillai & Kalasseril, (2022) found that, the presence of the these genes alone does not necessarily indicate antibiotic resistance, as other factors such as the expression of the gene and the presence of additional resistance mechanisms can also contribute to antibiotic resistance. Therefore, antibiotic susceptibility testing is still necessary to determine the most effective treatment options for K. pneumoniae infections.

Conclusion

Detection of K. pneumoniae258 as the causative agent of an infection and the subsequent demonstration of the spread of a beta-lactamase gene across this strain more than local K. pneumoniae isolates.

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