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Detection of *bla* OXA-48 and *bla* IMP Resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolated from Children with Urinary Tract Infections

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Abstract

Background: Urinary tract infections (UTIs) frequently affect the urinary system, predominantly caused by *Escherichia coli* (E. coli) and *Klebsiella pneumoniae*. This study examines the prevalence of these bacteria and *bla* OXA-48 and *bla* IMP genes. **Objectives:** The research aims to assess the occurrence of MDR and XDR *E. coli* and *K. pneumoniae* in pediatric UTI cases, including the resistance genes *bla* OXA-48 and *bla* IMP. **Methods:** A total of 100 pediatric UTI urine samples were analyzed for antibiotic resistance, ESBL detection, and *bla* OXA-48 and *bla* IMP gene presence using molecular techniques, gene sequencing, and statistical analysis via SPSS software. **Results:** High resistance rates to common antibiotics were found in *K. pneumoniae* and E. coli isolates. *bla* OXA-48 was detected in 92.3% of *E. coli* and 100% of *K. pneumoniae* isolates, while *bla* IMP was found in 43.1% of *E. coli* and 25.7% of *K. pneumoniae* isolates. No nucleic acid variation was observed in the *bla*IMP-1 sequences of both bacteria.

Conclusion: The significant presence of *bla* OXA-48 and *bla* IMP genes in *K. pneumoniae* and *E. coli* highlights the urgent need for enhanced surveillance and careful antibiotic use to curb antibiotic-resistant UTIs in children.

Keywords: *bla* OXA-48 gene, *bla* IMP gene, Urinary tract infections, E. coli, *Klebsiella pneumoniae*.

Introduction

A child has a urinary tract infection (UTI) when bacteria enter the urinary tract and cause irritation and pain. The urinary tract consists of the kidneys, ureters, bladder, and urethra. UTIs may affect any part of the urinary tract, although they are more common in the bladder (cystitis) and kidneys (pyelonephritis) (1). The most common causes of UTIs that are contracted in medical facilities like hospitals are *Proteus mirabilis*, *Staphylococcus* species, *Saprophyticus* species, *Escherichia coli*, and *Klebsiella* species. Gram-negative bacteria, facultative anaerobic fermentation, encapsulation, and other characteristics define the Enterobacterales

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order, which contains *K. pneumoniae* and *E. coli* (2). In addition to the human nasal and digestive systems, they may infect the bloodstream, lungs, urinary tract, wounds, and surgical sites (3). They may also cause no symptoms at all.

E. coli and *K. pneumoniae* are associated with high rates of mortality and morbidity. The antibiotics to which these bacteria have shown resistance are especially excellent targets for the extended-spectrum beta-lactamases (ESBLs), which can hydrolyze cephalosporins and penicillins (4). The increasing incidence of *K. pneumoniae* UTI and ESBL-producing *E. coli* throughout the globe poses a serious threat to public health. The introduction and spread of ESBL-producing *K. pneumoniae* and *E. coli* represent a serious threat to public health, necessitating the implementation of effective infection control measures (5).

When it comes to bacteria like *Escherichia coli* and *Klebsiella pneumoniae*, which may cause infections in a variety of anatomical places, antibiotic resistance poses a severe threat to public health. Extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to most beta-lactam antibiotics, including aztreonam, cephalosporins, and penicillins. Producing these enzymes is possible for these bacteria (6). ESBL-producing *K. pneumoniae* and *E. coli* isolates are primarily found in urine, and they often exhibit resistance to ampicillin, cefazolin, cefotaxime, trimethoprim/sulfamethoxazole, and fluoroquinolones (7). Additionally, it has been shown that some strains of *Klebsiella pneumoniae* and *Escherichia coli* include genes producing extended-spectrum beta-lactamases (ESBLs) or resistance to fosfomycin, which limits the range of therapies that may be used.

Carbapenems are the recommended beta-lactam antibiotics because bacteria that manufacture extended-spectrum beta-lactamases (ESBLs), such as *K. pneumoniae* and *E. coli*, may cause infections. When treating infections associated with healthcare that carry a life-threatening risk, these antibiotics are regarded as the final resort (10). Transposons and plasmids, two different kinds of mobile genetic elements, are used by bacteria to spread the genes producing carbapenemases.

K. pneumoniae and *E. coli* have the most common carbapenemase genes, *bla* KPC, *bla* NDM, *bla* OXA-48, bla VIM, and *bla* IMP (11). Bla OXA-48 and *bla* IMP are two kinds of carbapenemase genes that may confer resistance to carbapenems in *Escherichia coli* and *Klebsiella pneumoniae*.

These genes are mobile genetic elements that may disperse across bacteria. *Bla* OXA-48, *bla* OXA-181, *bla* OXA-232, *bla* OXA-204, *bla* OXA-162, *bla* OXA-244, and *bla*OXA-370 are the seven variants of the OXA-48-like group, which also includes the *bla* OXA-48 gene (12).

The *bla* IMP gene is a member of the IMP group, which includes more than 50 variants. The *bla* IMP gene may be found on plasmids or integrons (13).

Methods

A Sample Collection

Subjects: Children under the age of eighteen who were diagnosed with urinary tract infections had a total of 100 urine samples taken. The patients were collected from Al-Imamain Al-Kadhimain Medical City, Ibn Al-Balady Children & Maternity Hospital, Imam Ali Hospital, and Fatima Al-Zahraa Hospital between August 1 and October 31, 2022, and the doctor diagnosed them as HAUTI or CAUTI. This research was approved by the institutional review board (IRB) of the Al-Nahrain University College of Medicine. The study was conducted at the Department of Microbiology of the college.

Culture: All 100 urine samples were inoculated on blood agar as well as MacConkey agar using a calibrated inoculating loop that contained 0.001 ml of urine, and the samples were then incubated for 24 hours at 37 °C in an aerobic environment. To make agar, 38 grams of powder were suspended in one

litre of distilled water, heated until the powder was completely dissolved, and then autoclaved to guarantee sterility. This medium is used for the antibiotic susceptibility test.

Antimicrobial susceptibility tests and identification of *Klebsiella pneumoniae* and *Escherichia coli* using the VITEK 2 compact system.

Molecular identification for two genes *bla* IMP and *bla* OXA 48 in each bacterium. Genomic DNA was extracted from urine samples following the manufacturer's instructions using the gSYNCTM DNA extraction kit from Geneaid. Using a primer set for *bla* IMP (forward: GGAATAGAGTGGCTTAAYTC, reverse: TCGGTTTAAYAAAACAACCACC) and set for *bla* OXA48 (forward: GCGTGGTTAAGGATGAACAC reverse: CATCAAGTTCAACCCAACCG), conventional PCR was used to identify resistance genes. The AccuPower® PCR PreMix kit (Bioneer, Korea) was used to run PCR reactions in the MyGenie 96/384 Gradient Thermal Block (Bioneer, Korea).

Analysis and Sequencing of Nucleic Acids

Using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA), the sequencing results of the PCR product of the targeted sample were edited, aligned, and analyzed as long as possible with the relevant sequences in the reference database.

Each sequenced sample's observed differences were numbered in PCR amplicons as well as in their corresponding location within the referring genome. As well as at their corresponding places within the referring genome, the observed nucleic acids were numbered in PCR amplicons.

Translation of nucleic acid variations into amino acid residues

Using BioEdit suit, the sequencing findings of the PCR products were modified, aligned, and examined with the relevant sequences in the reference database. The observed variations in each sample were numbered in PCR amplicons as well as in their corresponding places within the referring genome. From the Protein Data Bank (http://www.ncbi.nlm.nih.gov), the amino acid sequences of the targeted proteins were obtained.

Results

Conventional DNA Extraction and Polymerase Chain Reactions PCR examination of the 100 samples tested revealed that genes *bla* OXA-48 were present in 92.3% of the isolates of *E. coli* and 100% of the isolates of *K. pneumoniae*,

while genes *bla* IMP were present in 43.1% of the isolates of *E. coli* and 25.7% of the isolates of *K. pneumoniae* (Figure 1, 2).

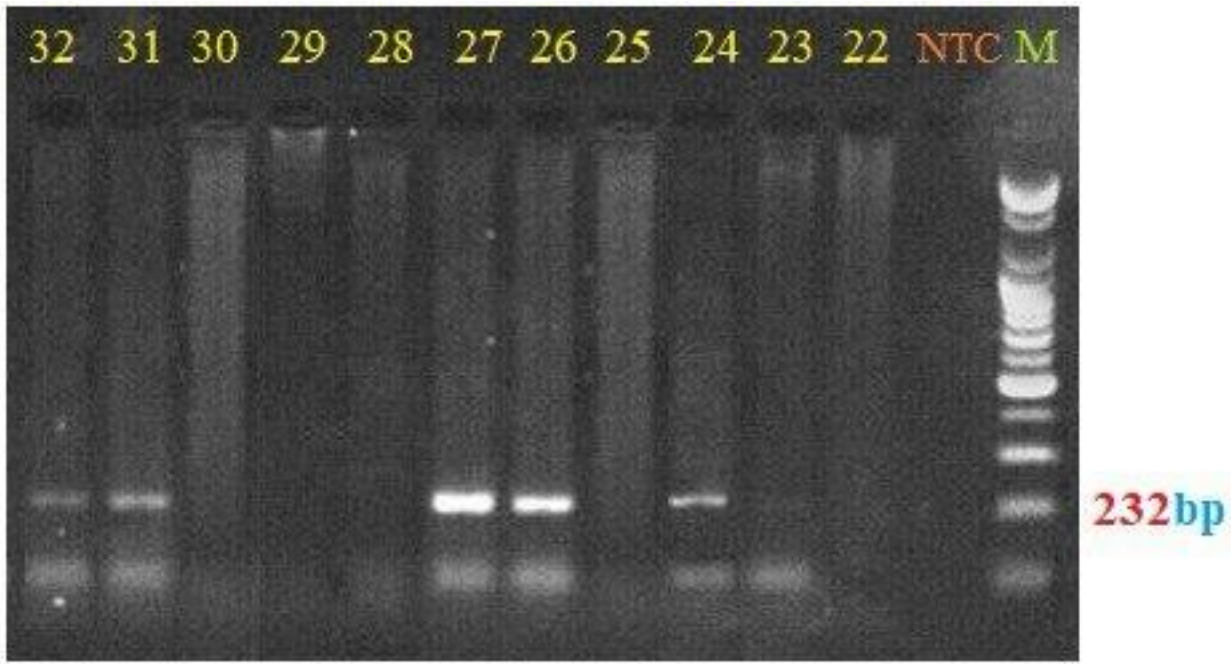


Figure 1: Gel electrophoresis Amplified DNA for detection of MBL *bla* IMP Gene (232bp) using PCR with specific primers; (1.5% agarose, 100v/mAmp, 1hr.) M: 100bp ladder marker, NTC: non template control, Bands of 232bp.

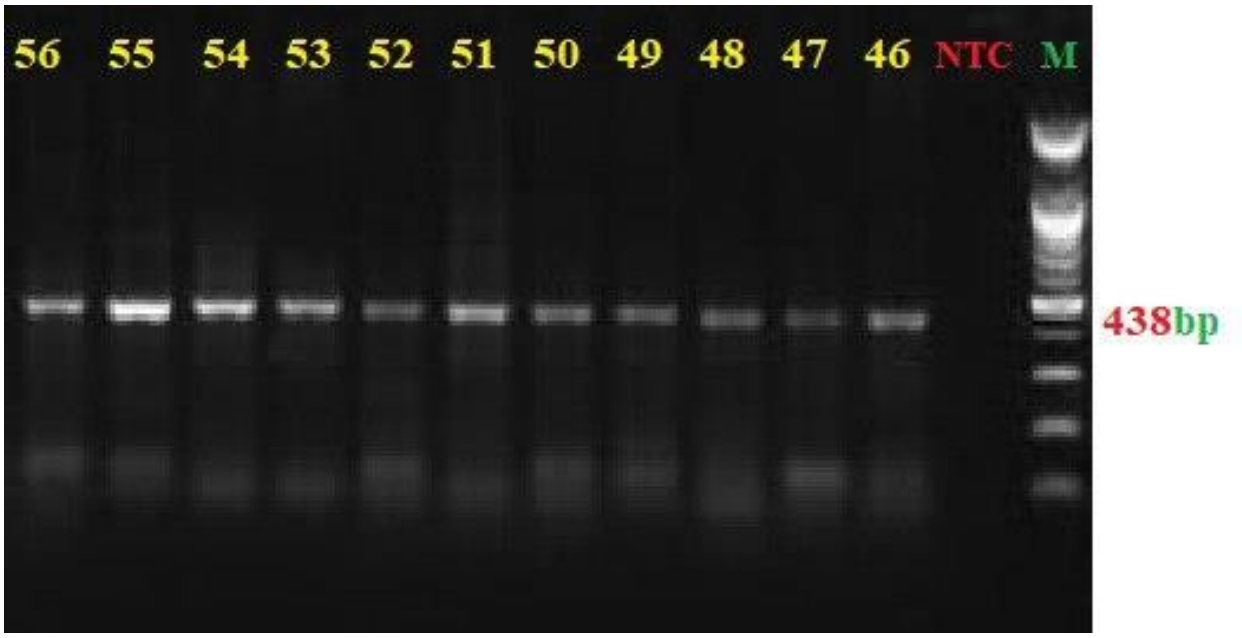


Figure (2): Gel electrophoresis Amplified chromosomal DNA for detection of MBL *bla*-OXA-48 Gene (232bp) using PCR with specific primers; (1.5% agarose, 100v/mAmp, 1hr). M: 100bp ladder marker, NTC: nontemplate control, Bands of 438bp.

There was no alteration seen in the nucleic acids of the amplified *bla* IMP-1 sequences of *K. pneumoniae* and *E. coli* in the samples analyzed.

However, two nucleic acid variations referring to the reference nucleic acid sequences of *K. pneumoniae* and *E. coli*, respectively, were found when the amplified *bla* OXA samples were aligned with *K. pneumoniae* and *E. coli*.

Sequencing Outcomes

Within the targeted *blaIMP*-1 sequences, only three samples were included in the present study. The first two samples (A1 and A2) were screened to partially amplify the *blaIMP*-1 sequences of *K. pneumoiniae*, while the third sample (A3) amplified the same locus within the sequences of *E. coli*. Both loci encode metallo beta-lactamase. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons.

Concerning the amplicons of the samples A1 and A2, the NCBI BLASTn engine showed an entire sequence similarity of 100% between the sequenced samples A1 and A2 and the targeted reference *blaIMP*-1 sequences of *E. coli* (GenBank acc. KT345947.1). Concerning the amplicons of sample A3, the NCBI BLASTn engine showed an entire sequence similarity of 100% between the sequenced samples A3 and the targeted reference *blaIMP*-1 sequences of *E. coli* (GenBank acc. LC169568.1) (Fig. 4-6A).

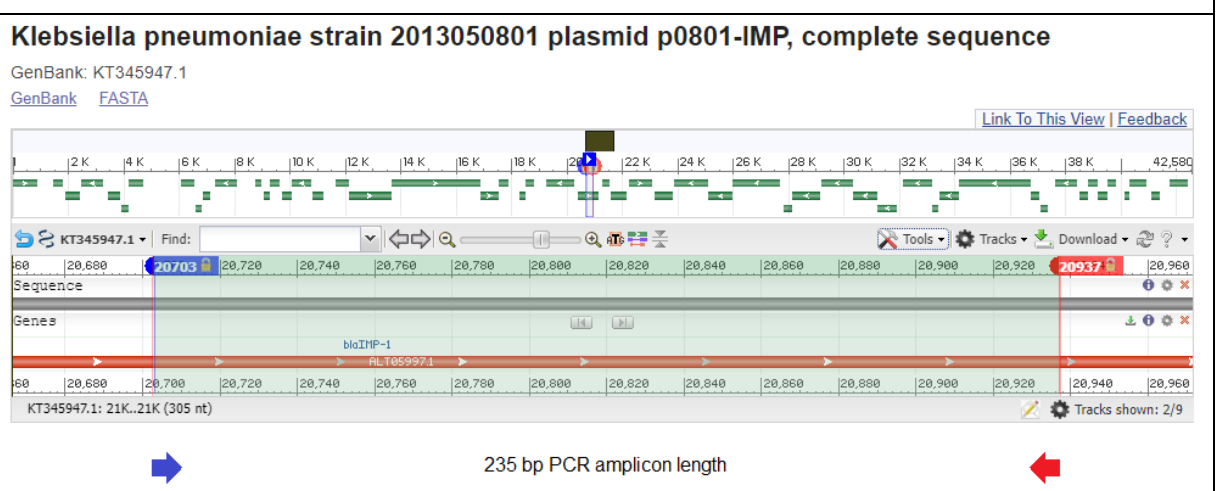
Within the targeted *bla*OXA sequences, only three samples were included in the present study. The first two samples (B1 and B2) were screened to partially amplify the *bla*OXA sequences of *K. pneumoiniae*, while the third sample (B3) amplified the same locus within the sequences of *E. coli*. Both loci encode OXA-48 family class D beta-lactamase. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons.

Concerning the amplicons of the samples B1, and B2, the NCBI BLASTn engine showed the highest sequence similarity of 99% between the sequenced samples and the intended reference bacterial target sequences of *blaOXA* sequences of *K. pneumoiniae* (GenBank acc. MT463291.1). Concerning the amplicon of sample B3, the NCBI BLASTn engine showed the highest sequence similarity of 99% between the sequenced samples and the intended reference bacterial target sequences of *bla*OXA sequences of *E. coli* (GenBank acc. OL872167.1) (Fig. 4-6B).

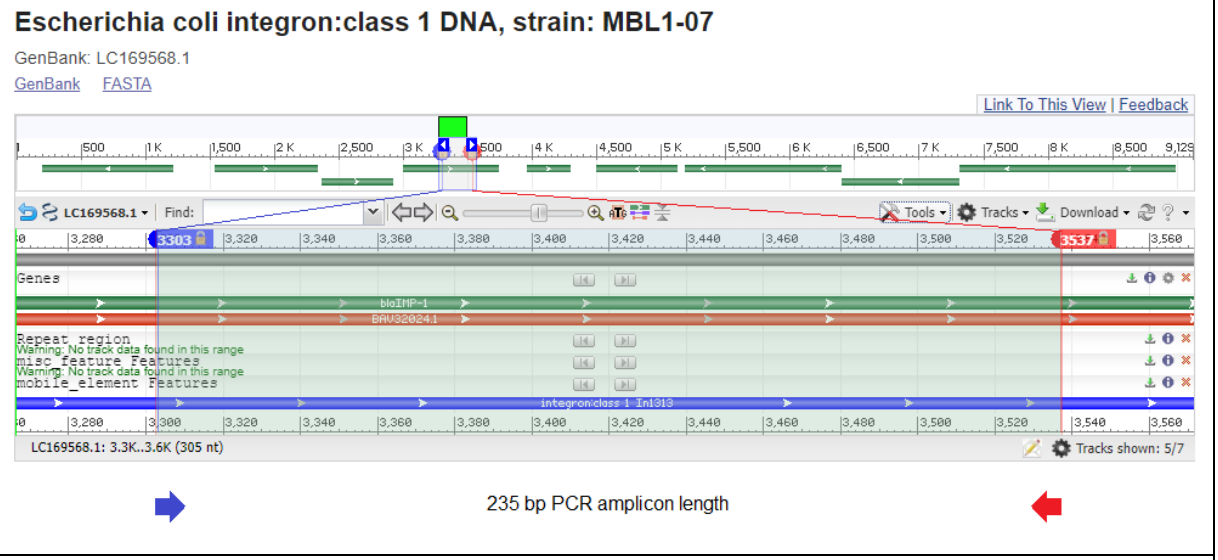
The accurate positions and other details of the retrieved PCR fragments were identified for both targeted loci. The total lengths of the targeted loci were localized in the NCBI server, and the positions of the amplified PCR fragments were also confirmed within the most homologous bacterial reference sequences.

After positioning the PCR amplicons' sequences within the *blaIMP*-1 and *blaOXA* sequences, the details of these sequences were highlighted, and the total length of the amplified amplicons was also determined (Table 4-15A, and B).

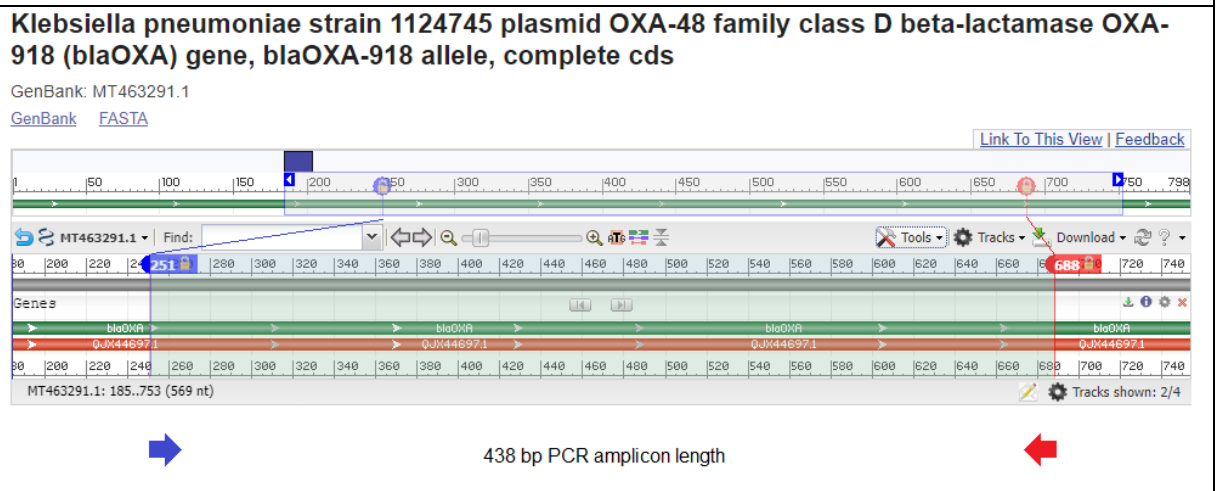
A) *K. pneumonia – A1, A2*



A) *E. coli – A3*



B) *K. pneumoniae – B1, B2*



B) *E. coli – B3*

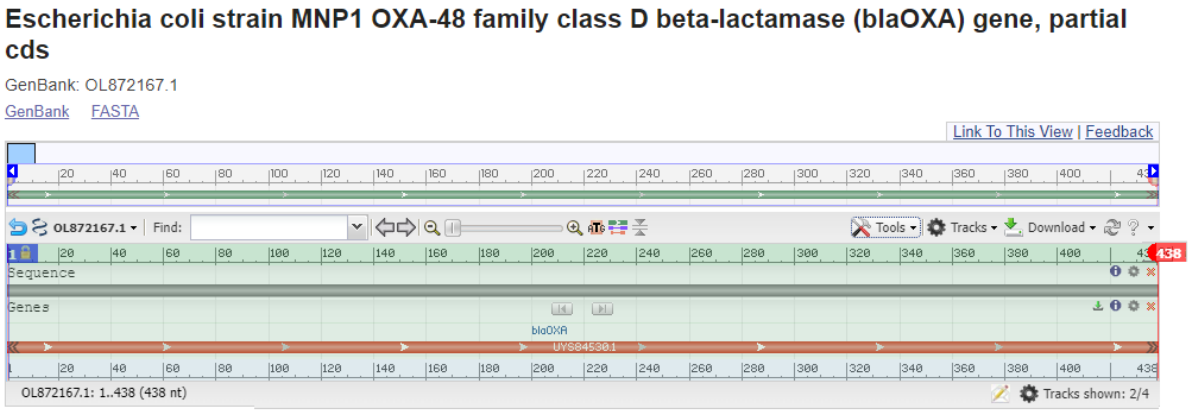


Figure (3): The exact position of the retrieved PCR amplicon partially covered the *blaIMP*-1 gene within *K. pneumoniae* and *E. coli* (in branches A) and the *blaOXA* gene within *K. pneumoniae* and *E. coli* sequences (in branches B).

Table (1): The position and length of the PCR amplicons that partially covered the *blaIMP*-1 gene within *K. pneumoniae* and *E. coli* (in branches A) and the *blaOXA* gene within *K. pneumoniae* and *E. coli* sequences (in branches B). Green and red colors refer to the positions of the forward and reverse primers, respectively.

Amplicon	Reference locus sequences (5′ - 3′)	length
A) *K. pneumonia – A1, A2*	GGAATAGAGTGGCTTAATTCTCGATCTATCCCCACGTATGCATCTGAATTA ACAAATGAACTGCTTAAAAAAGACGGTAAGGTTCAAGCCACAAATTCATTT AGCGGAGTTAACTATTGGCTAGTTAAAAATAAAATTGAAGTTTTTTATCCA GGCCCGGGACACACTCCAGATAACGTAGTGGTTTGGCTGCCTGAAAGGAAA ATATTATTCGGTGGTTGTTTTATTAAACCGT	bp 235
A) *E. coli – A3*	GGAATAGAGTGGCTTAATTCTCGATCTATCCCCACGTATGCATCTGAATTA ACAAATGAACTGCTTAAAAAAGACGGTAAGGTTCAAGCCACAAATTCATTT AGCGGAGTTAACTATTGGCTAGTTAAAAATAAAATTGAAGTTTTTTATCCA GGCCCGGGACACACTCCAGATAACGTAGTGGTTTGGCTGCCTGAAAGGAAA ATATTATTCGGTGGTTGTTTTATTAAACCGT	235 bp
B) *K. pneumoniae – B1, B2*	GCGTGGTTAAGGATGAACACCAAGTCTTTAAGTGGGATGGACAGACGCGCG ATATCGCCACTTGGAATCGCGATCATAATCTAATCACCGCGATGAAATATT CAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTGGCGAGGCACGTA TGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGGGCA ATGTAGACAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGC AAATCAGCTTTTTAAGAAAGCTGTATCACAATAAGTTACACGTATCGGAGC GCAGCCAGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGGTGACT	438 bp

	ATATTATTCGGGCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTG GCTGGTGGGTCGGTTGGGTTGAACTTGATG	
B) *E. coli – B3*	GCGTGGTTAAGGATGAACACCAAGTCTTTAAGTGGGATGGACAGACGCGCG ATATCGCCACTTGGAATCGCGATCATAATCTAATCACCGCGATGAAATATT CAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTGGCGAGGCACGTA TGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGGGCA ATGTAGACAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGC AAATCAGCTTTTTAAGAAAGCTGTATCACAATAAGTTACACGTATCGGAGC GCAGCCAGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGGTGACT ATATTATTCGGGCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTG GCTGGTGGGTCGGTTGGGTTGAACTTGATG	438 bp

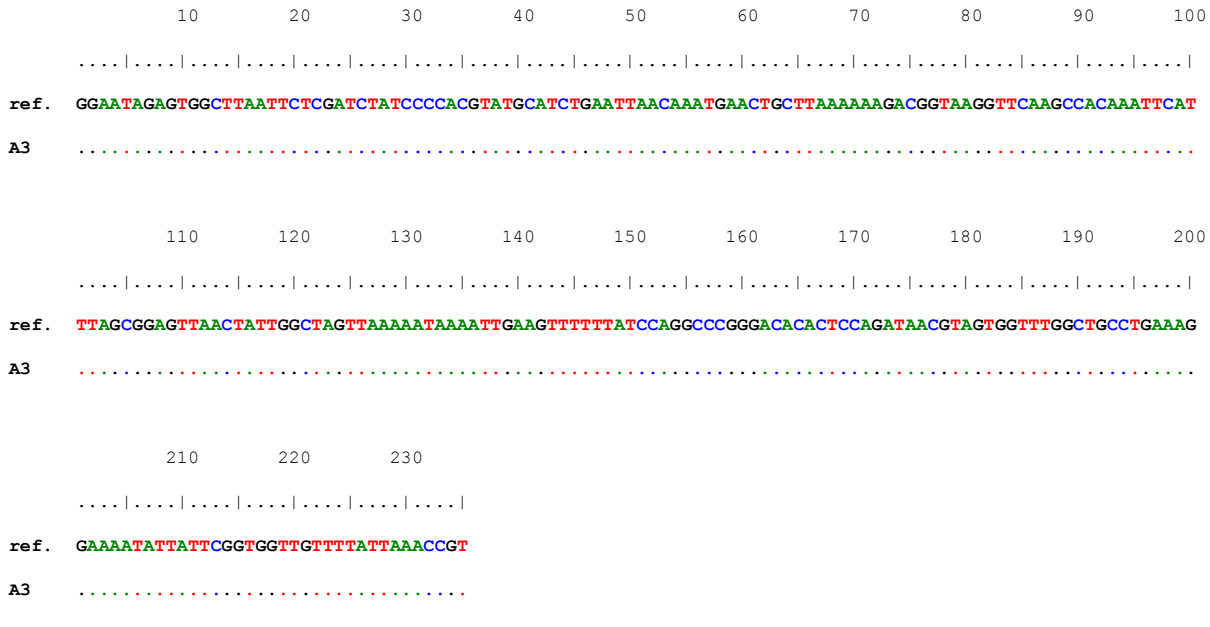
The alignment results of the amplified *blaIMP*-1 sequences of *K. pneumoniae* and *E. coli* revealed the presence of no nucleic acid variation in the analyzed A1, A2, and A3 samples in comparison with the most similar referring reference nucleic acid sequences of *E. coli* (Fig. 4A).

Therefore, the entire sequence homology was confirmed between the investigated samples with their corresponding bacterial locus. Whereas the alignment results of the amplified *blaOXA* samples with *K. pneumoniae* and *E. coli* revealed the presence of two nucleic acid variations in the analyzed B2 and B3 samples in comparison with the most similar referring reference nucleic acid sequences of *K. pneumoniae* and *E. coli*, respectively (Fig. 4B). The nucleic acid G was converted to C in the position 262 in B2 sample (262G>C), while the nucleic acid T was converted to C in the position 353 in B3 sample (353T>C).

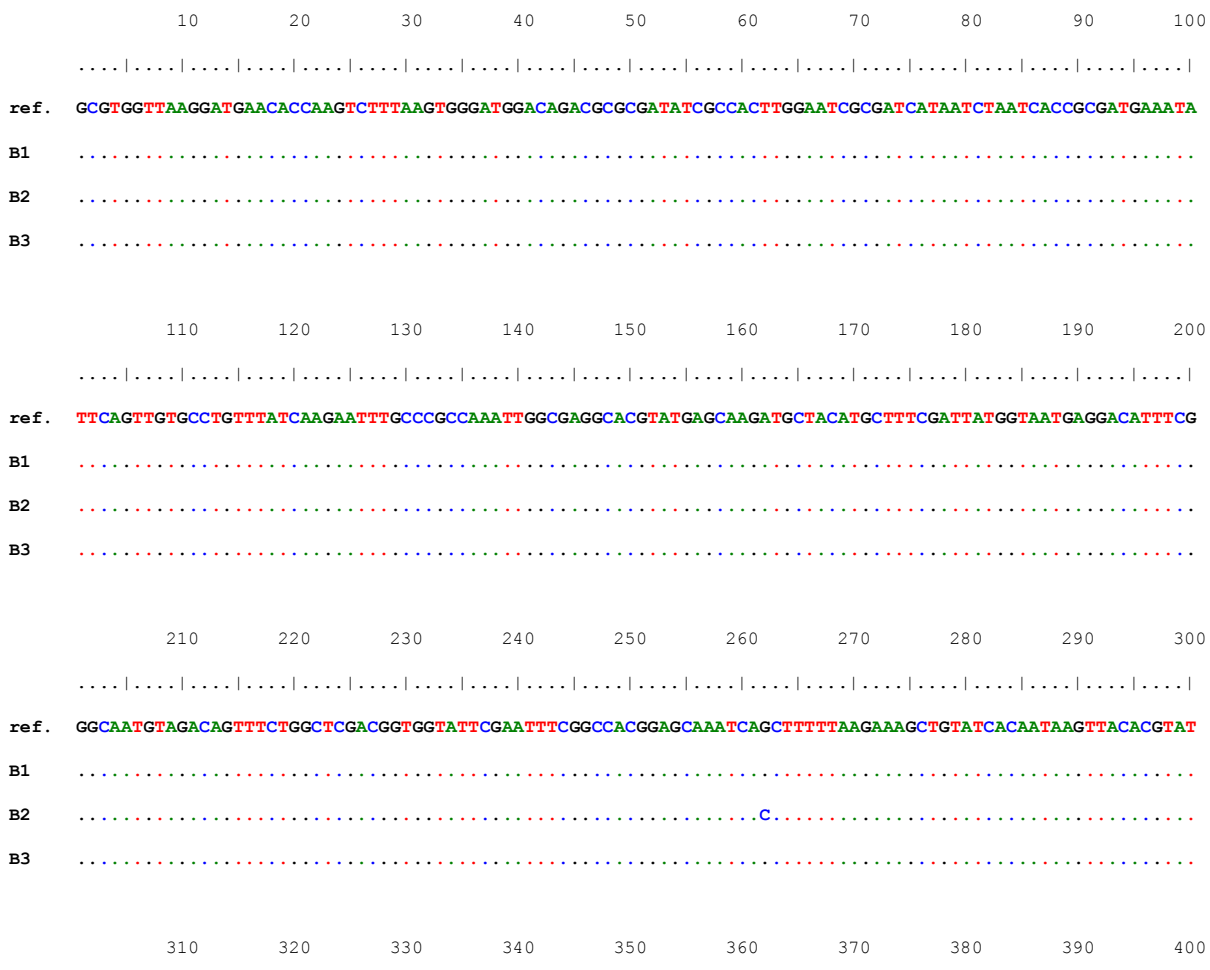
A) *K. pneumoniae – A1, A2*

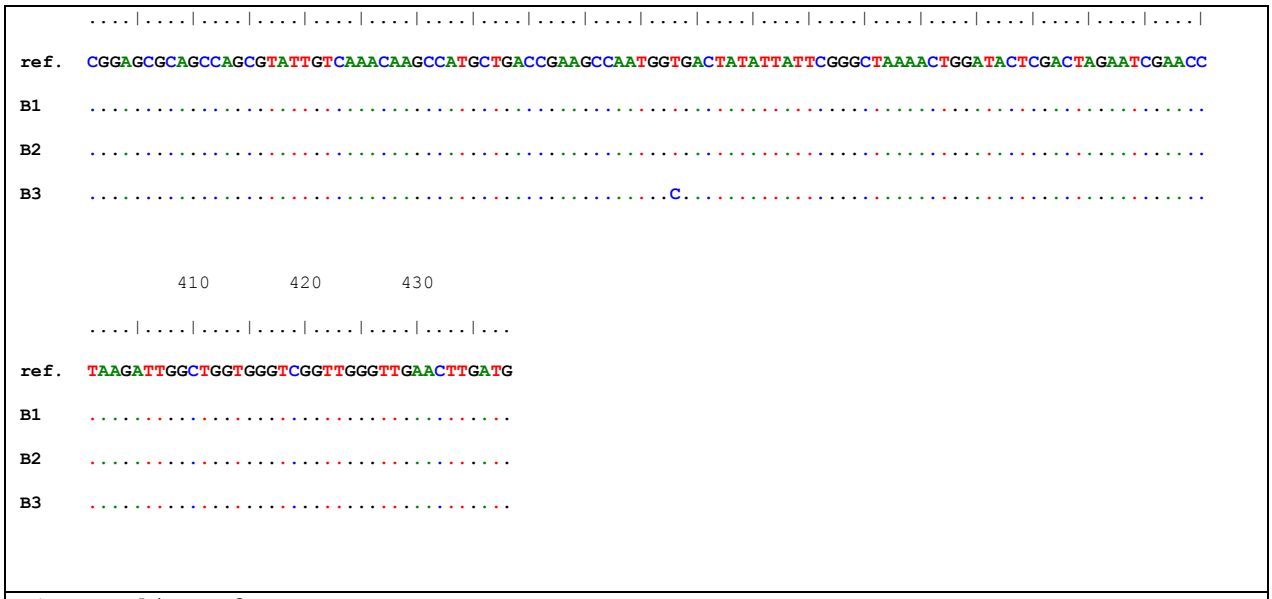
```
              10        20        30        40        50        60        70        80        90        100
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.  GGAATAGAGTGGCTTAATTCTCGATCTATCCCCACGTATGCATCTGAATTAACAAATGAACTGCTTAAAAAAGACGGTAAGGTTCAAGCCACAAATTCAT
A1    ....................................................................................................
A2    ....................................................................................................

              110       120       130       140       150       160       170       180       190       200
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.  TTAGCGGAGTTAACTATTGGCTAGTTAAAAATAAAATTGAAGTTTTTTATCCAGGCCCGGGACACACTCCAGATAACGTAGTGGTTTGGCTGCCTGAAAG
A1    ....................................................................................................
A2    ....................................................................................................

              210       220       230
      ....|....|....|....|....|....|....|
ref.  GAAAATATTATTCGGTGGTTGTTTTATTAAACCGT
A1    ...........................
A2    ...........................
```

A) *E. coli – A3*



B) *K. pneumoniae – B1, B2*





B) *E. coli – B3*

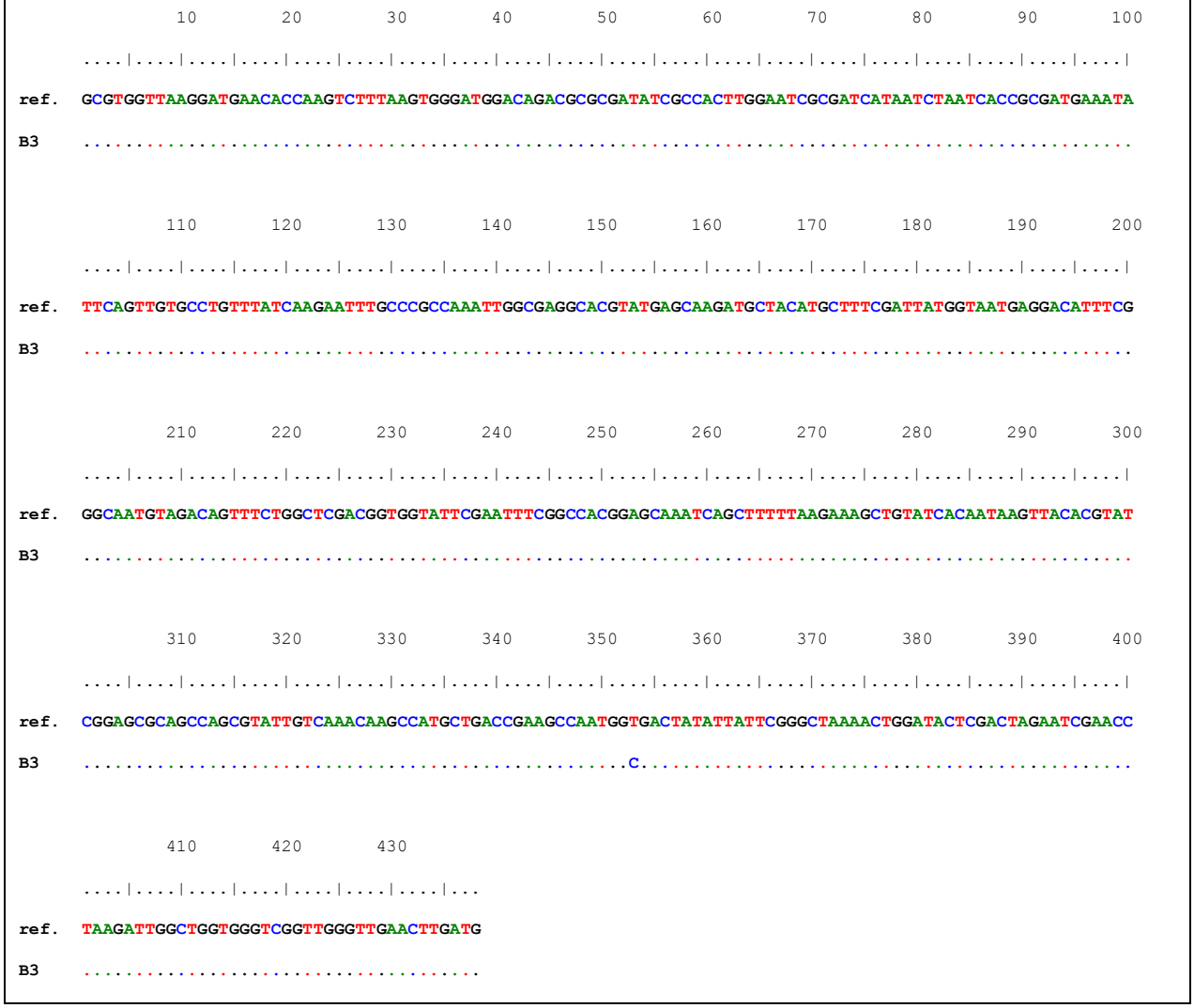


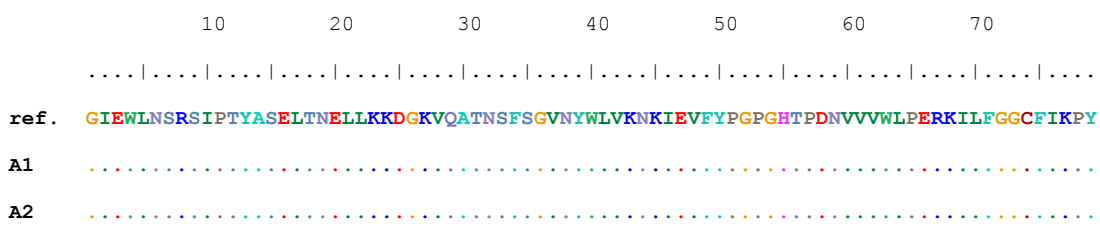
Figure (4): Nucleic acid sequences alignment of three samples with their corresponding reference sequences of the PCR amplicon that partially covered the *blaIMP*-1 gene within *K. pneumoniae* and *E. coli* (in branches A) and *blaOXA* gene within *K. pneumoniae* and *E. coli* sequences (in branches B).

The observed nucleic acid variations were further analyzed to identify whether such substitutions induce possible alteration in their corresponding positions in the translated products. All nucleic acid sequences of A1 – A3 and B1 – B3 were translated to their corresponding amino acid sequences using the Expasy translate suite (https://web.expasy.org/translate/).

Concerning the *blaIMP*-1 gene, it was found that the amplified products of this gene covered 79 amino acid residues within the metallo beta-lactamase (Fig. 4-8A).

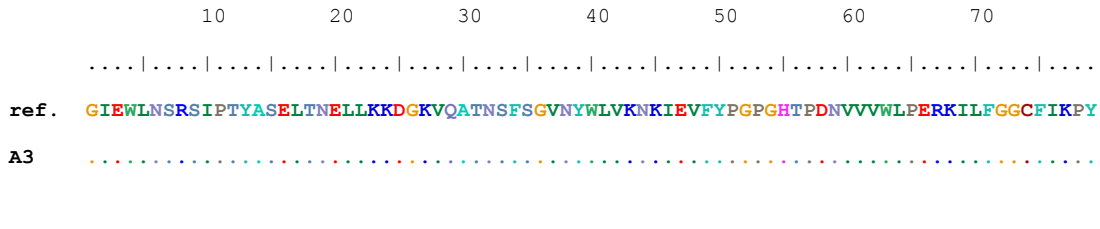
Concerning *bla*OXA, it was found that the amplified products of this gene have covered 145 amino acid residues within the OXA-48 family class D beta-lactamase. Results from the direct nucleic acid translation of the identified variants of 262G>C and 353T>C showed different impacts on the protein. The identified 262G>C variant has exhibited one missense impact of p.171S>T on the protein. Whereas 353T>C variants have exerted a silent impact of p. G201= on the protein. The results were represented in their positions within the amplicons and the entire protein (Fig. 5).

A) *K. pneumonia – A1, A2*



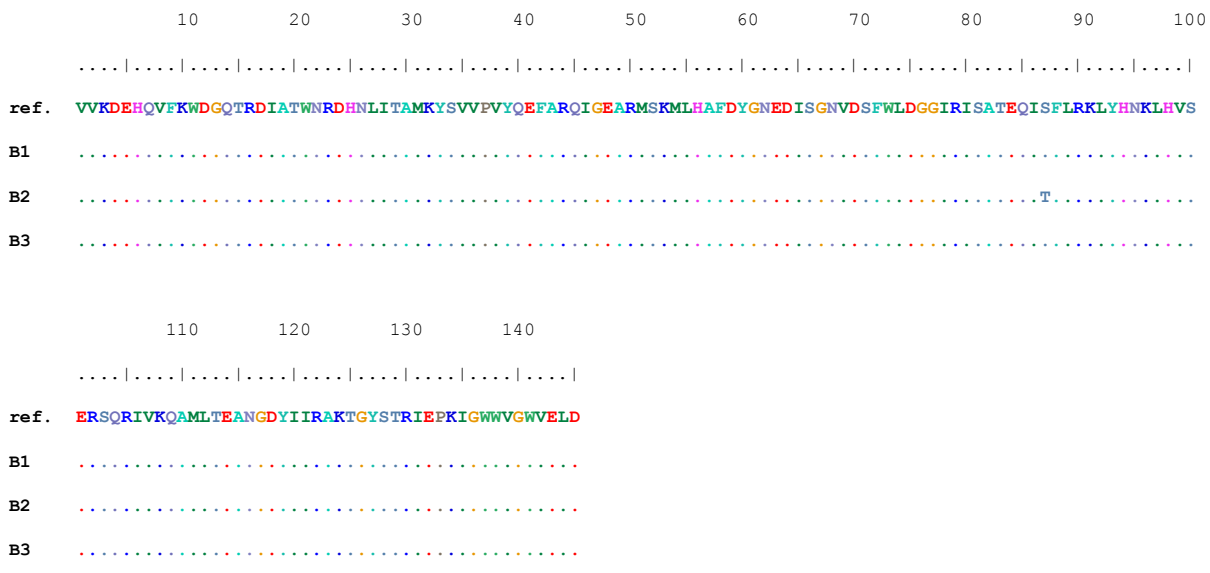
>ALT05997.1 Beta-lactamase IMP-1 (plasmid) [Klebsiella pneumoniae]
MSKLSVFFIFLFCSIATAAESLPDLKIEKLDEGVYVHTSFEEVNGWGVVPKHGLVVLVNAEAYLIDTPFT
AKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSRSIPTYASELTNELLKKDGKVQATNSFSGVN
YWLVKNKIEVFYPGPGHTPDNVVVWLPERKILFGGCFIKPYGLGNLGDANIEAWPKSAKLLKSKYGKAKL
VVPSHSEVGDASLLKLTLEQAVKGLNESKKPSKPSN

A) *E. coli – A3*



>BAV32024.1 metallo beta-lactamase [Escherichia coli]
MSKLSVFFIFLFCSIATAAESLPDLKIEKLDEGVYVHTSFEEVNGWGVVPKHGLVVLVNAEAYLIDTPFT
AKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSRSIPTYASELTNELLKKDGKVQATNSFSGVN
YWLVKNKIEVFYPGPGHTPDNVVVWLPERKILFGGCFIKPYGLGNLGDANIEAWPKSAKLLKSKYGKAKL
VVPSHSEVGDASLLKLTLEQAVKGLNESKKPSKPSN

B) *K. pneumoniae – B1, B2*



>QJX44697.1 OXA-48 family class D beta-lactamase OXA-918 [Klebsiella pneumoniae]

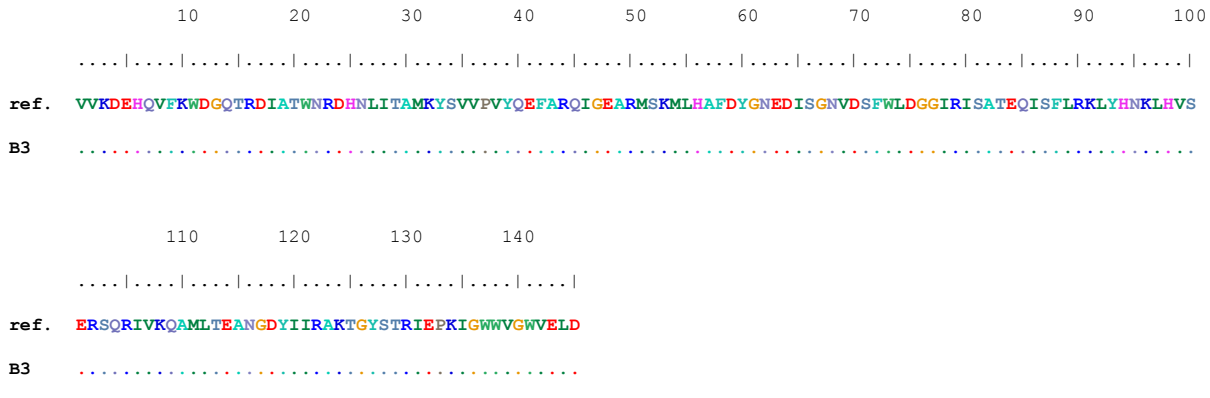
MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPAS

TFKITNSLIALDLGVVKDEHQVFKWDGQTRDIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLH

AFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEANGDYIIRAKTG

YSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP

A) *E. coli – B3*



>UYS84530.1 OXA-48 family class D beta-lactamase, partial [Escherichia coli]
MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLKRANQAFLPAS

TFKITNSLIALDLGVVKDEHQVFKWDGQTRDIATWNRDHNLITAMKYSVVPVYQEFARQIGEARMSKMLH
AFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNKLHVSERSQRIVKQAMLTEANGDYIIRAKTG
YSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP

Figure (5): Amino acid residues alignment of the detected variations of the metallo beta-lactamase (branch A) and OXA-48 family class D beta-lactamase OXA-918 (branch B) samples, respectively. The grey highlights refer to the amplified region of the enolase. The cyan and yellow colors refer respectively to the amino acid missense and silent variations in the entire protein sequence**.**

Patient characteristics and demographics

The frequency of *E. coli* and *K. pneumoniae* isolates was observed to be greater in females (84.6%, 85.7%) compared to men (15.4%, 14.3%) respectively according to demographic data, as shown in Figure (6). According to the p-value of 0.8, there is no statistically significant variation in the prevalence of bacterial infection between the sexes (Table 2).

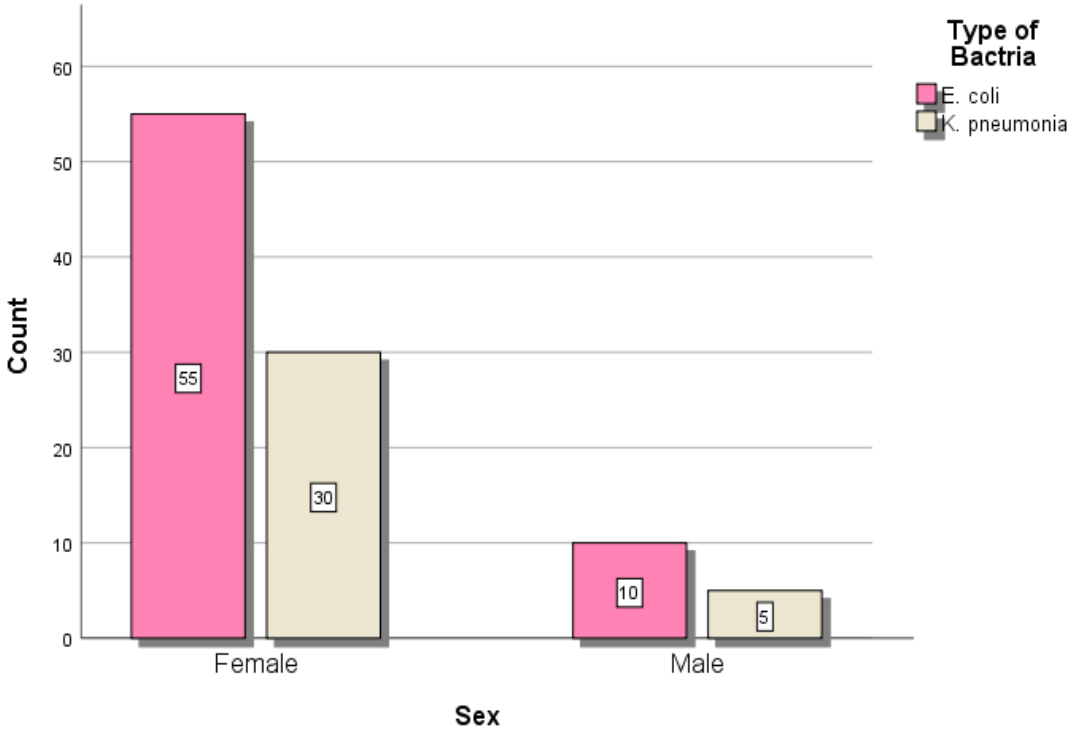


Figure (6): Distribution of *K. pneumoniae* and *E. coli* according to gender group

Table (2): Comparison between *E. coli* and *K. pneumoniae* Patients in gender.

			Type of Bactria		Total	p-value
			K. pneumoniae	*E. coli*		
Sex	Male	Count	5	10	15	0.8 NS
		% within Type of Bactria	14.3	15.4	15.0	
	Female	Count	30	55	85	
		% within Type of Bactria	85.7	84.6	85.0	
Total		Count	35	65	100	
		% within Type of Bactria	100.0	100.0	100.0	

Sensitivity patterns of *E. coli* and *K. pneumoniae* isolates to antibiotics:

In Table (3), the results of the antibiotic susceptibility test for isolates of *E. coli* and *K. pneumoniae* are shown.

E. coli exhibits 49.2% resistance to Imipenem and 41.5% to Meropenem, while *K. pneumoniae* shows slightly higher resistance at 51.4% and 54.2%, respectively. Both bacteria have significant Ciprofloxacin resistance, with *E. coli* at 63% and *K. pneumoniae* closely behind at 62.8%. Ticarcillin and Piperacillin resistances are notably high in *K. pneumoniae* at 74.2% but Piperacillin resistances in *E. coli* at 73.8%, respectively. Tobramycin and Ceftazidime resistances are also more pronounced in *K. pneumoniae* at 68.5% and 71.4%. Conversely, Minocycline and Gentamicin show lower resistance rates, particularly Minocycline in *K. pneumoniae* at only 2.8%. Notably, *E. coli* is highly sensitive to Nitrofurantoin at 89.2%, while *K. pneumoniae* is almost completely resistant to Oxacillin.

Table (3): Susceptibility of *E. coli* and *K. pneumoniae* to different antibiotics

		Type of Bactria	
		E. coli	***K. pneumonia***
		Count (%)	**Count (%)**
Imipenem	**R**	32(49.2)	18(51.4)
	S	33(50.8)	17(48.5)
Meropenem	**R**	27(41.5)	19(54.2)
	S	38(58.5)	16(45.7)
Ciprofloxacin	**I**	4(6.2)	0
	R	41(63.0)	22(62.8)
	S	20(30.8)	13(37.1)
Ticarcillin	**R**	42(64.6)	26(74.2)
	S	23(35.3)	9(25.7)
Cefepime	**I**	3(4.6)	3(8.5)
	R	39(60)	16(45.7)
	S	22(33.8)	17(48.5)
Piperacillin	**R**	48(73.8)	22(62.88)
	S	17(26.1)	13(37.1)
Tobramycin	**R**	28(43.0)	24(68.5)
	S	37(56.9)	11(31.4)
Minocycline	**I**	2(3.1)	5(14.2)
	R	20(30.7)	1(2.8)
	S	43(66.2)	29(82.8)
Gentamicin	**R**	23(35.3)	16(45.7)
	S	42(64.6)	19(54.2)
Nitrofurantoin		58(89.2)	19(54.2)
	I	0	7(20)
	R	1(1.5)	0
	S	6(9.2)	9(25.7)
Oxacillin	**R**	60(92.3)	35(100)
	S	5(7.7)	0
Amikacin	**I**	19(29.2)	7(20)
	R	1(1.5)	58(165.7)
	S	6(9.2)	9(25.7)
Ceftazidime	**I**	1(1.5)	0
	R	35(53.8)	25(71.4)
	S	29(44.6)	10(26.5)

Discussion

Molecular (genotypic) Detection of Carbapenemases Production

Using the PCR technique, two resistance genes (*bla* IMP and *bla* OXA-48) were found in *E. coli* and *Klebsiella pneumoniae* isolates in this study. For *E. coli* and *Klebsiella pneumoniae*, the current study revealed that the percentage of the bla IMP gene by conventional PCR was 43.1% and 25.7%, respectively. In contrast, the percentage of *bla* OXA-48 in *Klebsiella pneumoniae* and *E. coli* was 100% and 92.3%, respectively.

The outcome was comparable to a study conducted in Iraq by Al-Charrakh et al., 2018 (14) who found that the percentage of *bla*OXA-48-positive isolates was 92.3% in *E. coli* and 100% in *K. pneumoniae* among the carbapenem-resistant isolates.

In an investigation by Wang *et al*. (2021), the *bla* OXA-48 was detected in 0.5% of isolates, *bla* IMP was more common in *K. pneumoniae* than in *E. coli*, while *bla* OXA-48 was only found in *E. coli* (15). Another study by Singh *et a.* (2023) reported that *bla*OXA-48 was detected in 40% of *E. coli* and *K. pneumoniae* isolates from North India (16). The justification is that genes are responsible for resistance to carbapenem.

Gene sequencing

In the present research alignment results of the amplified *bla* IMP-1 sequences of *K. pneumoniae* and *E. coli* revealed the absence of nucleic acid variation in the examined A1, A2, and A3 samples in comparison with the most similar *bla* IMP-1 sample sequences of *E. coli*. Consequently, the full sequence homology between the studied samples and the corresponding bacterial locus was verified. The alignment results of the amplified *bla* OXA samples with *K. pneumoniae* and *E. coli* revealed the presence of two similar nucleic acid variations in the analyzed B2 and B3 samples in comparison with the most similar *K. pneumoniae* and *E. coli* reference sample sequences, respectively.

The nucleic acid G was converted to C in position 262 in the B2 sample (262G>C), while the nucleic acid T was converted to C in position 353 in the B3 sample (353T>C).

In a study conducted by Poirel *et al.* 2012(17), Some variants of *bla* OXA-48 are silent mutations, which do not affect the protein structure or function, while others are missense mutations, which change the amino acid sequence of the enzyme.

Chen *et al* 2014 found that *K. pneumoniae* and *E. coli* that isolated from Taiwan, which had a substitution of serine for arginine at position 228 in the blaIMP gene (18).

Patient characteristics and demographics

The frequency of E. coli and K. pneumoniae isolates was observed to be greater in females (84.6%, 85.7%) compared to men (15.4%, 14.3%), respectively, according to the demographic data .

Rizwan et al.'s (19) investigation had similarities to this one. Who found that *E. coli* and *K. pneumoniae* isolates were more common in females (82.35%) compared to males (17.64%). This may be due to the structural variations between the sexes; for example, bacteria have a shorter path to reach the bladder since the female urethra is shorter than the male urethra. Additionally, the urethra and the anus, where these bacteria often live, are closer in females. This may raise the possibility of fecal matter contamination (20).

Antimicrobial susceptibility testing of *E. coli* and *Klebsiella pneumonia.*

The present study tested the in vitro susceptibility of isolates of *K. pneumoniae* and *E. coli* to 13 antibacterial agents .

Similar to this, in a study by Al-Masaudi et al. (21) extremely resistant to Oxacillin (92.3% and 100%, respectively), moderately resistant to Ciprofloxacin (63% and 62.8%, respectively), and variable resistance to the other antibiotics. Imipenem (50% and 40%, respectively) and Meropenem (not tested for *E. coli* and 20% for *K. pneumoniae*) had the lowest observed resistance rates. Results for Oxacillin (77% for *E. coli* and 80% for *K. pneumoniae*), Ciprofloxacin (80% for *E. coli* and 40% for *K. pneumoniae*), and Piperacillin (80% for

E. coli and 99% for *K. pneumoniae*) were observed in 2022, while the present results did not match with the study conducted by Smith et al.

This was further supported by Karki et al., who said that the resistance rates to oxacillin and piperacillin were (95.65%), making this antibiotic an unsuitable option for treating infections caused by *E. coli* and *Klebsiella pneumoniae*.

Ipenem and meropenem activity against *E. coli* and *Klebsiella pneumoniae* was found to vary in this study (the percentage of them was 49.2% and 51.4% resistant in *E. coli* and 41.5% and 54.2% in meropenem, respectively), which is inconsistent with the percentage found in Erbil by Pishtiwan *et al*. 2019 found that the 100% activity rate of imipenem and meropenem is higher (24). All strains were responsive to imipenem, and previous research from Hillah showed that the resistance rate against meropenem was 4.86%. Furthermore, as compared to previous studies by Najjuka et al., the resistance rate for gentamicin was comparatively higher (25). As a result, the impact of amikacin is higher than that of gentamicin, which is like the study conducted by Zhang *et al*. (26).

During the study period, 68.3 % and 31.7% of *E. coli* and *Klebsiella pneumoniae* respectively of the isolates were found to be resistant to piperacillin. Noteworthy, present results revealed that the ratio of resistance to this antibiotic was high in comparison with a previously published study from Baghdad (27).

Cefepime, a fourth-generation cephalosporin, has superior activity against Enterobacteriae and greater stability against β-lactamases' enzymatic destruction (28). In comparison to other cephalosporins, it has a better capacity to cross the membrane of Gram-negative cells and a higher stability against ESBL enzymes. In E. coli and Klebsiella pneumonia, the percentage of resistance to Cefepime was 60% and 45.7% .

Similar results were found in a Chinese study by Wang et al. 2020(30), which indicated that K. pneumoniae and E. coli had cefepime resistance rates of 45.7% and 56.7%, respectively. While Zhang et al. 2020 (31) found that 90% and 70.7% of Klebsiella pneumoniae and E. coli showed resistance to cefepime, respectively, the results were inconsistent.

Overall, the isolates' antibiogram results showed extraordinary resistance to more than two antibiotics, which may suggest that the MDR and XDR bacteria were the primary cause of the infection in the individuals from whom they were isolated. In light of this, these MDR and XDR bacteria attract attention to the necessity of making plans to regulate the process of bacteria misuse and overuse, which are regarded as the most tract reasons that lead to the occurrence of antibiotic resistance in the nation.

Conclusions

For this study, it was concluded that:

1-Compared to phenotypic approaches, the molecular approach using PCR was more accurate in identifying isolates of *Klebsiella pneumoniae* and *E. coli* that were resistant to carbapenem.

2- When compared to *bla* IMP a ligand, the percentage of bacteria that produce *bla* OXA-48 is greater.

3- Oxacillin is very resistant to both bacteria

4-*K. pneumoniae* is less resistant to Ciprofloxacin than *E. coli*

5- Sequencing of *bla* OXA-48 two nucleic acid variations in each bacteria sample.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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