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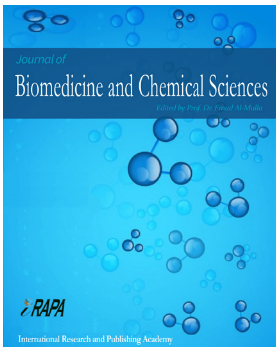

Detection of bla OXA-48 and bla IMP Resistance Genes in Escherichia coli and Klebsiellapneumoniae Isolated from Children with Urinary Tract Infections

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ABSTRACT

The pathogens Escherichia coli and Klebsiella pneumoniae are often involved in urinary tract infections (UTIs), which are common, especially in children. Due to their association with antibiotic resistance, which makes treatment tactics more difficult, the presence of the bla OXA-48 and bla IMP genes in these bacteria is essential. To detect two resistant genes *bla* OXA-48 and *bla* IMP in *Klebsiella pneumonia* and *Escherichia coli* and to detect the variant genes by sequencing for each bacterium. In all, one hundred urine samples from children with UTIs were investigated. Using molecular techniques and gene sequencing, the isolates were tested for antibiotic resistance, ESBL phenotypic detection, and *bla* OXA-48 and *bla* IMP gene identification. The SPSS software was used to do the statistical analysis. *K. pneumoniae* and *E. coli,* the two most common isolates, exhibit significant rates of resistance to commonly used antibiotics. Genes*bla* OXA-48 was present in 92.3% of *E. coli* isolates and 100% of *K. pneumoniae* isolates, bla IMP was present in 43.1% of E. coli isolates and 25.7% of *K. pneumoniae* isolates. The amplified *bla*IMP-1 sequences of *E. coli* and *K. pneumoniae* did not show any nucleic acid variation in the samples under analysis. While the results of the alignment of the amplified *bla* OXA samples with *K.pneumoniae*and *E. coli* revealed the existence of two nucleic acid variations referring to reference nucleic acid sequences of *K. pneumoniae* and *E. coli*, respectively. The study shows that *K. pneumoniae* and *E. coli* both have significant levels of the *bla* OXA-48 and *bla* IMP genes. To prevent the spread of bacteria that cause antibiotic-resistant UTIs in children's populations, our results emphasise the need of increased surveillance and monitoring of antibiotic regimens.

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) (Bagnola, 2018). The most common causes of UTIs that are contracted in medical facilities like hospitals are *Proteus mirabilis*, *Staphylococcus* species, *Saprophyticus* species, *Escherichiacoli*, and *Klebsiella* species. Gram-negative bacteria, facultative anaerobic fermentation, encapsulation, and other characteristics define the Enterobacterales order, which contains *K. pneumoniae* and *E. coli* (Mancuso, et al., 2023). In addition to the human nasal and digestive systems, they may infect the bloodstream, lungs, urinary tract, wounds, and surgical sites (Chang, et al., 2021). 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 (Mohammed & Anwar, 2022). The increasing incidence of *K. pneumoniae* UTI and ESBL-producing *E. coli*

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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 (Jones, 2023).

When it comes to bacteria like *Escherichia coli* and *Klebsiellapneumoniae*, 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 (Rahman, et al., 2018). 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 (Ballén, et al., 2021). Additionally, it has been shown that a number of strains of *Klebsiellapneumoniae* 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 *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 (Banerjee, et al., 2017). 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 (Logan & Weinstein, 2017). Bla OXA-48 and *bla* IMP are two kinds of carbapenemase genes that may confer resistance to carbapenems in *Escherichia coli* and *Klebsiellapneumoniae*. 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 (Wang, et al., 2021). 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 (Li, et al., 2022).

METHODS

A Sample Collection

Children under the age of eighteen who were diagnosed with urinary tract infections had a total of 100urine 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. 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 the environment. To make agar, 38 grammes 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 *Klebsiellapneumoniae* 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 urin samples in accordance with 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 analysed 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 in relation to 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, the amino acid sequences of the targeted proteins were obtained.

RESULTS & FINDINGS

Conventional DNA Extraction and Polymerase Chain ReactionsPCR 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). 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 analysed. 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

Only three samples were used in the bla research from the present bla IMP-1 sequences. A3 amplified the same locus inside the E. coli sequences, whereas the previous two samples (A1 and A2) were screened to partly amplify the blaIMP-1 sequences of K. pneumoiniae. In both loci, metallo beta-lactamase is encoded. After running an NCBI blastn for these PCR amplicons, the sequencing reactions revealed the precise identification. About the amplicons of the samples A1 and A2, the targeted reference *bla*IMP-1 sequences of *E. coli* (GenBank acc. KT345947.1) and the sequenced samples A1 and A2 showed a 100% complete sequence similarity. In terms of the amplicons of sample A3, the NCBI BLASTn engine revealed a 100% overall sequence similarity between the sequenced samples A3 and the targeted reference *bla* IMP-1 sequences of *E. coli* (GenBank acc. LC169568.1) (Figure 3A). Only three samples were used in the bla research from the present *bla*OXA sequences. The *bla*OXA sequences of *K. pneumoiniae* were partly amplified in the first two samples (B1 and B2), and the same locus within the *E. coli* sequences was amplified in the third sample (B3). OXA-48 family class D beta-lactamase is encoded by both loci. After running an NCBI blastn for these PCR amplicons, the sequencing reactions revealed the precise identification.

The NCBI BLASTn engine revealed the maximum sequence similarity of 99% between the sequenced samples and the targeted reference bacterial target sequences of *bla*OXAamplicons of *K. pneumoiniae*(GenBank acc. MT463291.1). Regarding the amplicon of sample B3, the NCBI BLASTn engine revealed the best sequence similarity (99%) between the sequenced samples and the specified reference *bla*OXA target sequences of *E. coli* (GenBank acc. OL872167.1). For each of the two targeted loci, the precise locations and other information of the recovered PCR fragments were determined (Figure 4B). The locations of the generated PCR fragments were also verified within the most similar bacterial reference sequences, and the total lengths of the targeted loci were localised in the NCBI server. The alignment results of the amplified similar *bla* IMP-1 sequences of *K.pneumoniae* and *E. coli* revealed the absence of any nucleic acid comparison variation in the analysed A1, A2, and A3 samples in comparison with the most similar *bla* IMP-1 sample sequences of *E. coli* (Figure 5A)

Consequently, the full sequence homology between the investigated samples and the corresponding bacterial locus was verified. While the alignment results of the amplified *bla*OXA sequences with *K. pneumoniae* and *E. coli* revealed the presence of two similar nucleic acid variations in the analysed B2 and B3 samples in comparison with the most *K. pneumoniae* and *E. coli* reference nucleic acid sequences, respectively (Figure 6A). In the B2 sample, nucleic acid G was converted to C at position 262 (262G>C), but in the B3 sample, nucleic acid T was converted to C at position 353 (353T>C). To determine if these alterations may result in changes to the corresponding positions in the translated products, further analysis was done on the observed nucleic acid variations. Using the Expasy translate suite (https://web.expasy.org/translate/), all nucleic acid sequences from A1 to A3 and B1 to B3 were translated to their corresponding amino acid sequences.

Regarding the *bla*IMP-1 gene, it was found that the amplified products of this gene spanned 79 amino acid residues inside the Metallo beta-lactamase (Figure. 7A). Regarding the *bla*OXA gene, it was found that the amplified products of this gene had covered 145 amino acid residues in the beta-lactamase of the OXA-48 family class. The results of the direct nucleic acid translation of the discovered variants of 262G>C and 353T>C showed various effects on the protein. The 262G>C variation that was discovered has one missense effect of p.171C >T on the protein. On the other hand, 353T>C variants have silently affected the protein via p.G201=. The results were displayed in the amplicons' and the whole protein's present positions (Figure 8B).

Distribution of bacteria according to sex groups

The ratio of E. coli distribution in females to men was 6:11 to 1:2, as shown in Figure 9. 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).

Sensitivity patterns of E. coli and K. pneumoniae Isolates to Antibiotics

In Tables 3, the results of the antibiotic susceptibility test for isolates of *E.coli* and *K. pneumoniae* are shown. The antibiotic resistance profiles for oxacillin, ciprofloxacin, tobramycin, ticarcillin, piperacillin, imipenem, and meropenem in *E. coli* and *K. pneumoniae* are (92.3%) and (100%) respectively.

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 *Klebsiellapneumoniae* isolates in this study. For *E. coli* and *Klebsiellapneumoniae*, 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 *Klebsiellapneumoniae*and *E. coli* was 100% and 92.3%, respectively. The outcome was comparable to a study conducted in Iraq by Gurung et al. (2020) 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 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* (Wang, et al., 2021). 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 (Singh, et al., 2023). 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. While 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 analysed 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 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). In study conducted by Poirel et al. 2012, Some variants of *bla*OXA-48 are silent mutations, which do not affect the protein structure or function, while others aremissense mutations, which change the amino acid sequence of the enzyme. Chen et al. (2014) found that *K. pneumoniae* and *E. coli* that isolate from Taiwan, which had a substitution of serine for arginine at position 228 in the blaIMP gene.

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 daa . The investigation of Rizwan et al. (2018) had similarities to this one. Who found that *E. coli* and *K. pneumoniae* isolates were more common in female (82.35%) compared to male (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 faecal matter contamination (Minardi, et al., 2011).

Antimicrobial Susceptibility Testing of E. coli and Klebsiellapneumonia

The present study tested the in vitro susceptibility of isolates of *K.pneumoniae* and *E. coli* to 13 antibacterial agents. Like this, in a study by Minardi et al. (2011) extremely resistant to Oxacillin (92.3% and 100%, respectively), moderately resistant to C iprofloxacin (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 Karki (2023) 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 *Klebsiellapneumoniae.*

Ipenem and meropenem activity against *E. coli* and *Klebsiellapneumoniae* 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 and Khadija (2019) found that the 100% activity rate of imipenem and meropenem is higher. 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., (2016), the resistance rate for gentamicin was comparatively higher. As a result, the impact of amikacin is higher than that of gentamicin, which is similar with the study conducted by Zhang, et al., (2021).

During the study period, 68.3 % and 31.7% in *E. coli* and *Klebsiellapneumoniae* 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 previous study published from Baghdad (Jalil, et al., 2022). Cefepime, a fourth-generation cephalosporin, has superior activity against Enterobacteriae and greater stability against β-lactamases' enzymatic destruction (Agbor, et al., 2011). 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 C efepime was 60% and 45.7%. Similar results were found in a Chinese study by Wang et al. (2021), which indicated that K. pneumoniae and E. coli had cefepime resistance rates of 45.7% and 56.7%, respectively. 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. Considering this, these MDR and XDR bacteria at tract attention to the necessity of making plans to regulate the process of bacteria misuse and overuse, which are regarded the most tract reasons that lead to the occurrence of antibiotic resistance in nation.

CONCLUSION

It was concluded that compared to phenotypic approaches, the molecular approach using PCR was more accurate in identifying isolates of *Klebsiellapneumoniae*and *E. coli* that were resistant to carbapenem. When compared to *bla* IMP a ligand, the percentage of bacteria that produce *bla* OXA-48 is greater. Oxacillin is very resistant to both bacteria. *K. pneumoniae*is less resistant to Ciprofloxacin than *E. coli.* Sequencing of *bla* OXA-48 two nucleic acid variations in each bacteria sample.

Competing Interest

The authors had no competing interests.

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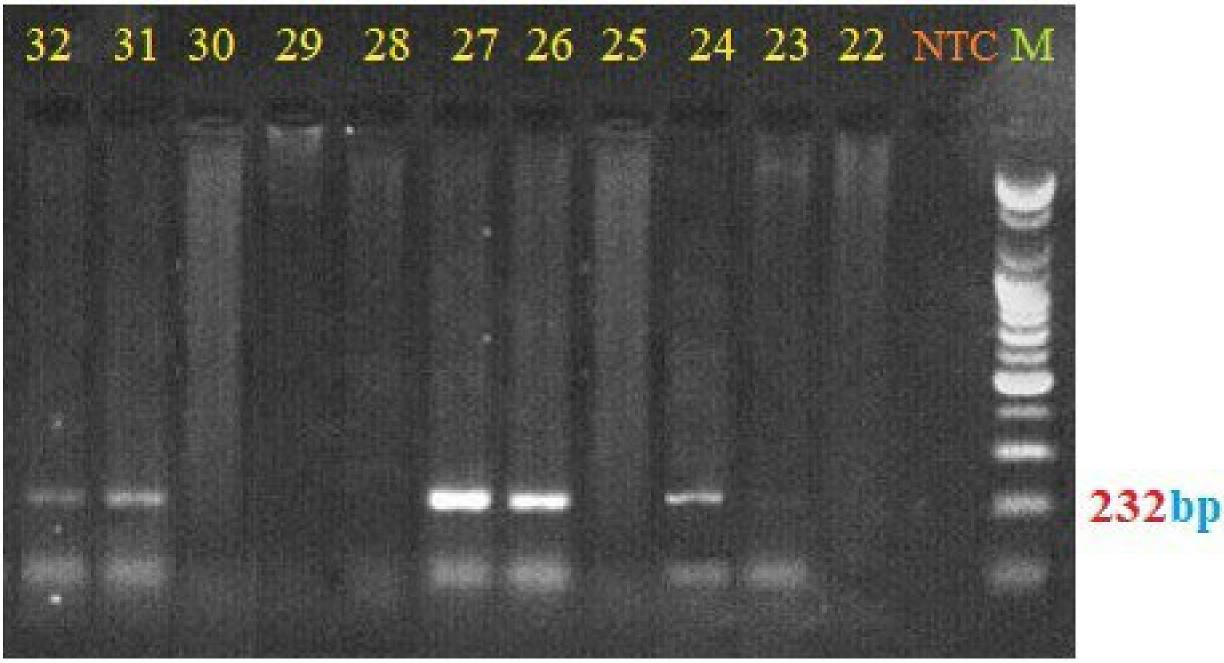


Fig. 1. Gel electrophoresis Amplified DNA for detection of MBL*bla*IMP Gene (232bp) using PCR with specific primers ; (1.5% agarose, 100v/mAmp, 1hr.) M: 100bp ladder marker, NTC: non template control, Bands of 232bp

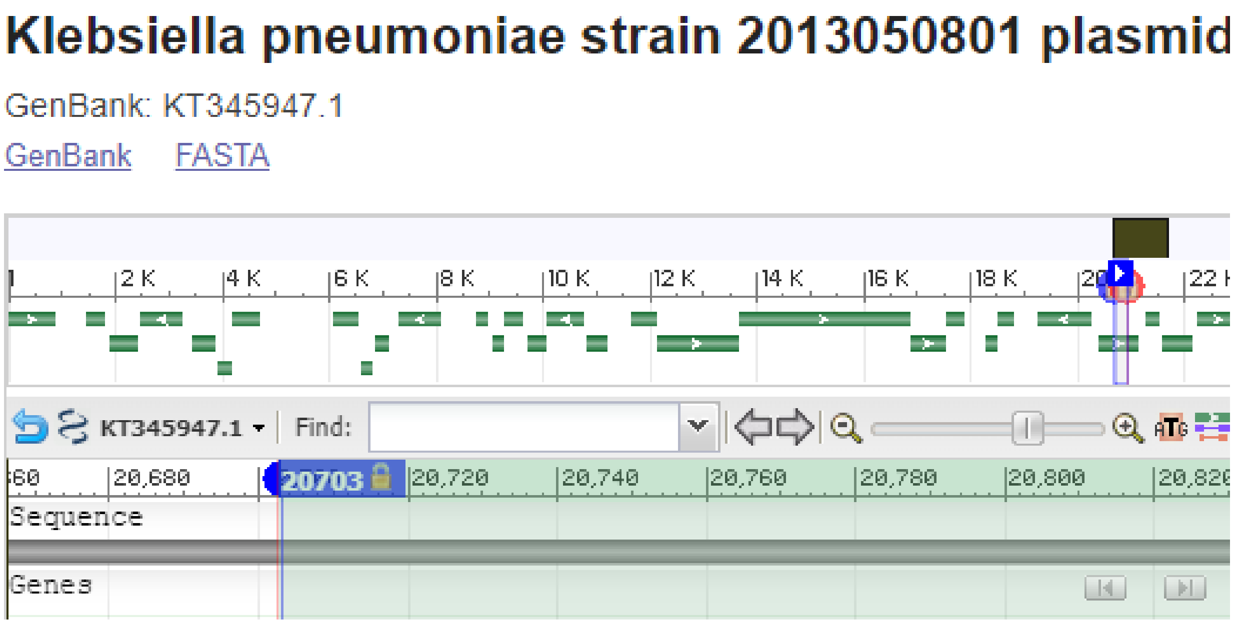
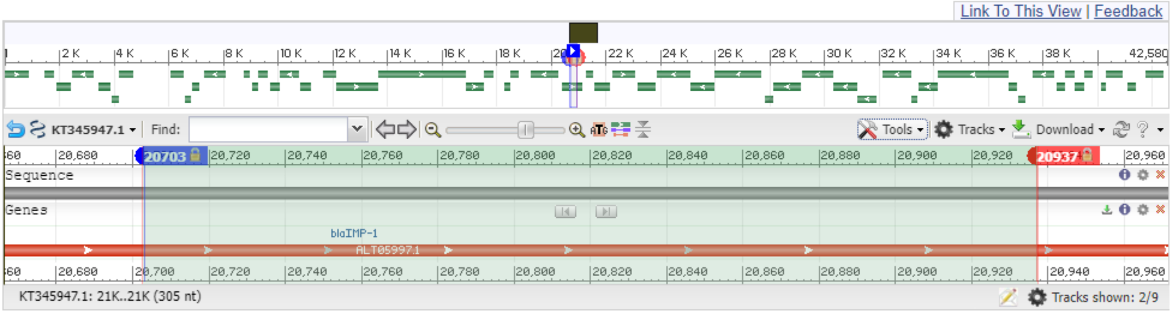


Fig. 2. Gel electrophoresis Amplified chromosomal DNA for detection of MBL *bla*-OXA-48 Gene (232bp) using PCR with specific primers ; (1.5% agarose, 100v/mAmp, 1hr). M: 100bp ladder marker, NTC: non template control, Bands of 438bp.

Klebsiella pneumoniae strain 2013050801 plasmid p0801-IMP, complete sequence

GenBank: KT345947.1

GenBank FASTA

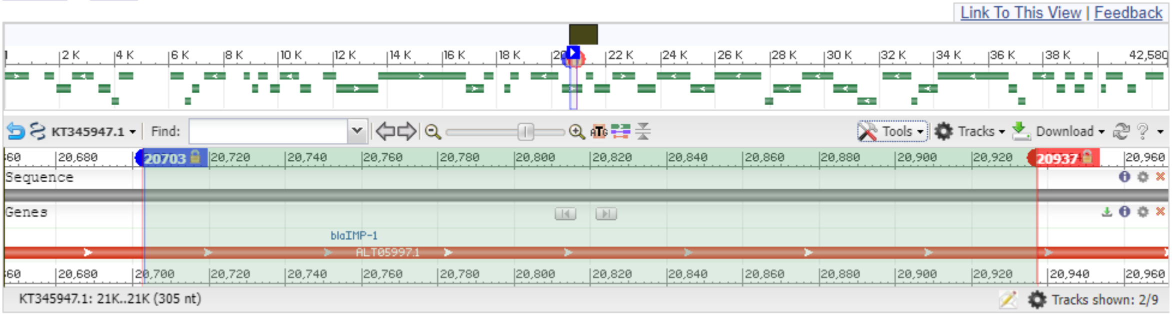


235 bp PCR amplicon length

Klebsiella pneumoniae strain 2013050801 plasmid p0801-IMP, complete sequence

GenBank: KT345947.1

GenBank FASTA

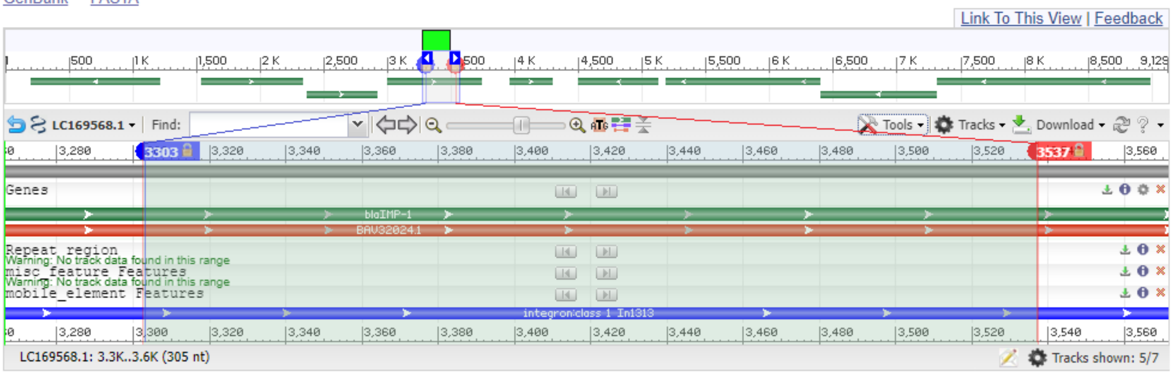


235 bp PCR amplicon length

Escherichia coli integron:class 1 DNA, strain: MBL1-07

GenBank: LC169568.1

GenBank FASTA



235 bp PCR amplicon length

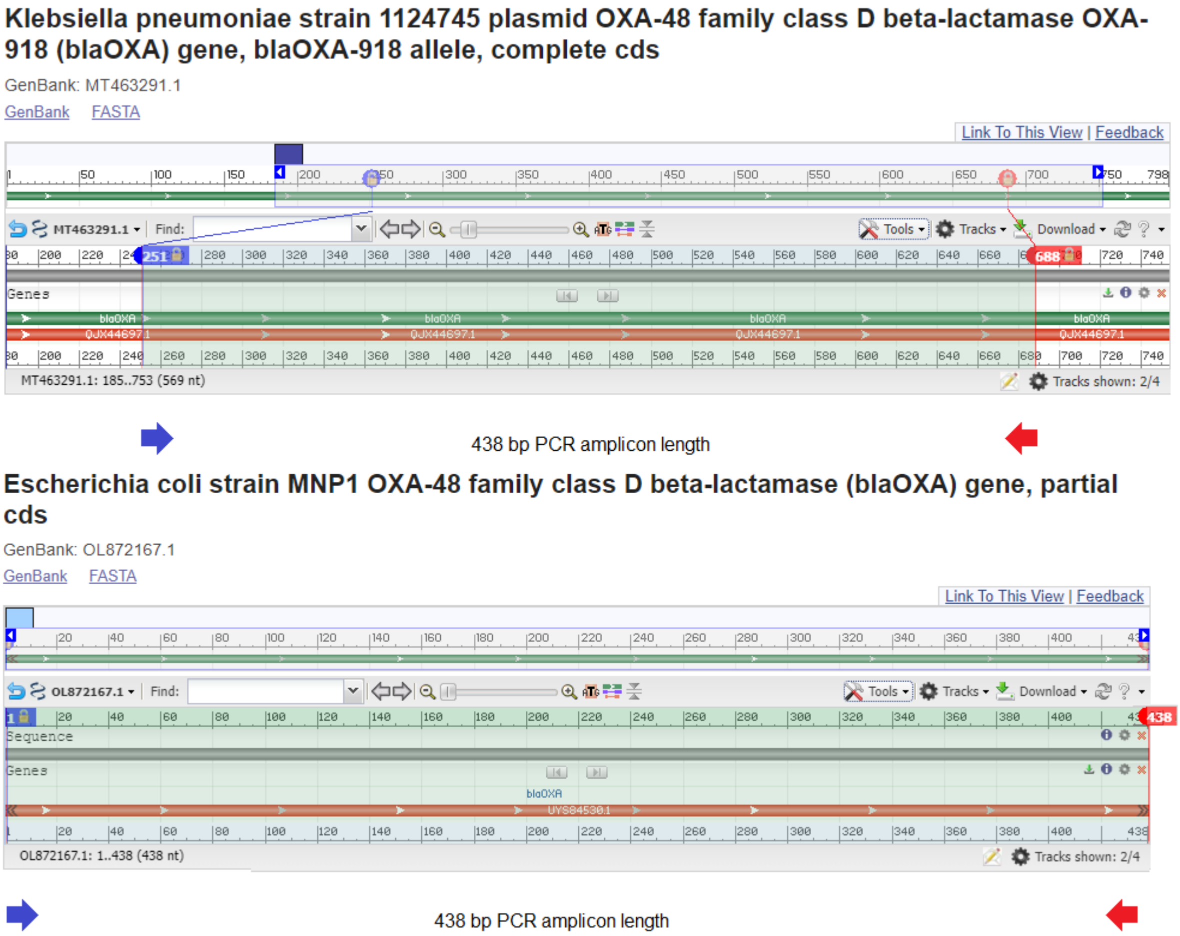


Fig. (3A-4B). The exact position of the retrieved PCR amplicon partially covered the *blaIMP*-1 gene within *K. pneumoniae* and *E. coli* (in branches A) and the *blaOXA* gene within *K. pneumoniae* and *E. coli* sequences (in branches B).

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 1A

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*	GGAATAGAGTGGCTTAATTCTCGATCTATCCCCACGTATGCATCTGAATTAACAAATGAACTGCTTAAAAA AGACGGTAAGGTTCAAGCCACAAATTCATTTAGCGGAGTTAACTATTGGCTAGTTAAAAATAAAATTGAA GTTTTTTATCCAGGCCCGGGACACACTCCAGATAACGTAGTGGTTTGGCTGCCTGAAAGGAAAATATTATT CGGTGGTTGTTTTATTAAACCGT	235bp
A)*E. coli– A3*	GGAATAGAGTGGCTTAATTCTCGATCTATCCCCACGTATGCATCTGAATTAACAAATGAACTGCTTAAAAA AGACGGTAAGGTTCAAGCCACAAATTCATTTAGCGGAGTTAACTATTGGCTAGTTAAAAATAAAATTGAA GTTTTTTATCCAGGCCCGGGACACACTCCAGATAACGTAGTGGTTTGGCTGCCTGAAAGGAAAATATTATT CGGTGGTTGTTTTATTAAACCGT	235bp
B) *K. pneumoniae– B1, B2*	GCGTGGTTAAGGATGAACACCAAGTCTTTAAGTGGGATGGACAGACGCGCGATATCGCCACTTGGAATCG CGATCATAATCTAATCACCGCGATGAAATATTCAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTG GCGAGGCACGTATGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGGGCAATGTAGA CAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGCAAATCAGCTTTTTAAGAAAGCTGTATC ACAATAAGTTACACGTATCGGAGCGCAGCCAGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGG TGACTATATTATTCGGGCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTGGCTGGTGGGTCGGTT GGGTTGAACTTGATG	438 bp
B) *E. coli – B3*	GCGTGGTTAAGGATGAACACCAAGTCTTTAAGTGGGATGGACAGACGCGCGATATCGCCACTTGGAATCG CGATCATAATCTAATCACCGCGATGAAATATTCAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTG GCGAGGCACGTATGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGGGCAATGTAGA CAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGCAAATCAGCTTTTTAAGAAAGCTGTATC ACAATAAGTTACACGTATCGGAGCGCAGCCAGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGG TGACTATATTATTCGGGCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTGGCTGGTGGGTCGGTT GGGTTGAACTTGATG	438 bp

Fig. (5A-6B). 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).

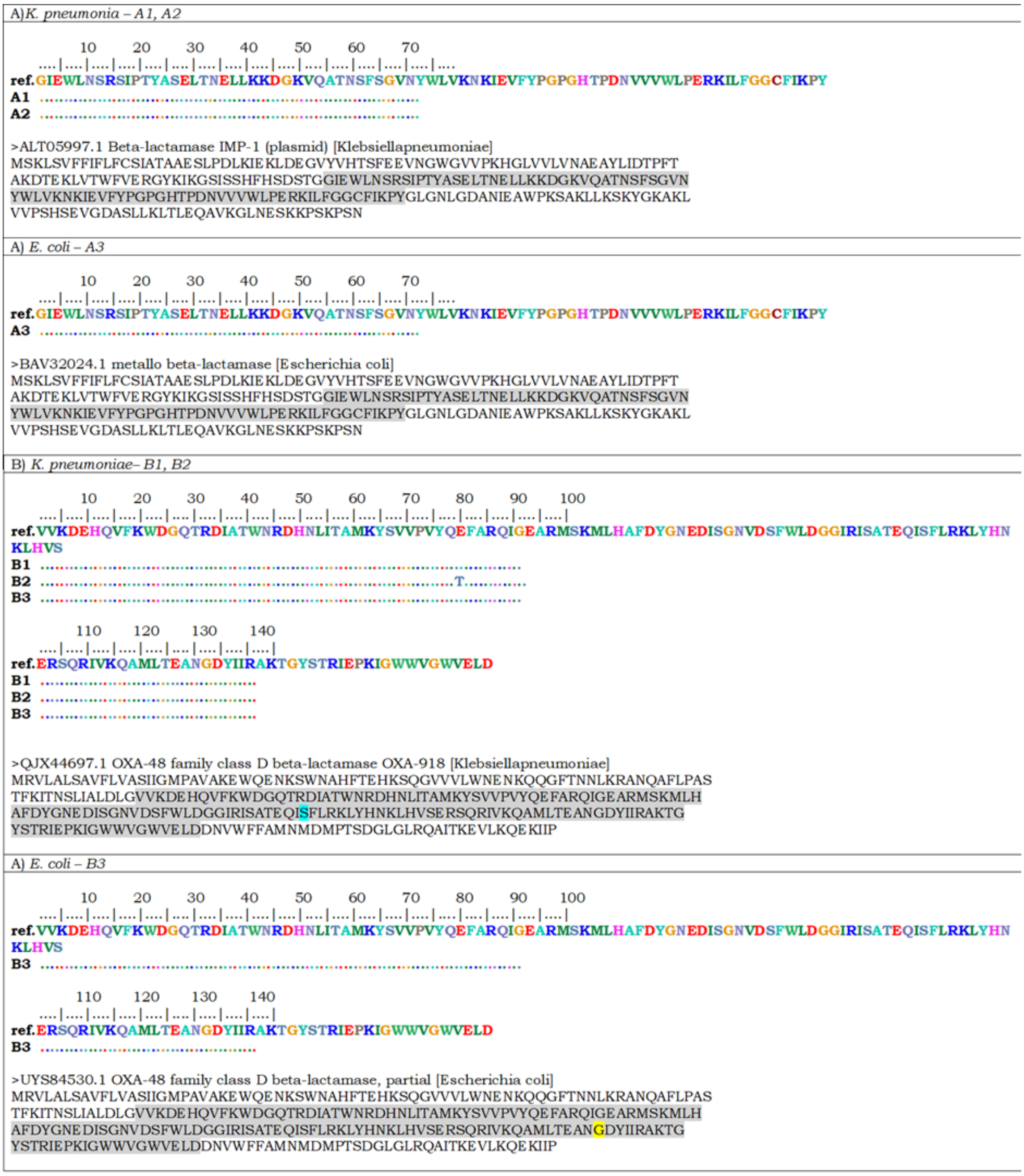


Fig. (7A-8B). Amino acid residues alignment of the detected variations of the metallo beta-lactamase (branch A) and OXA-48 family class D beta-lactamase OXA-918 (branch B) samples, respectively. The grey highlights refer to the amplified region of the enolase. The cyan and yellow colours refer respectively to the amino acid missense and silent variations in the entire protein sequence

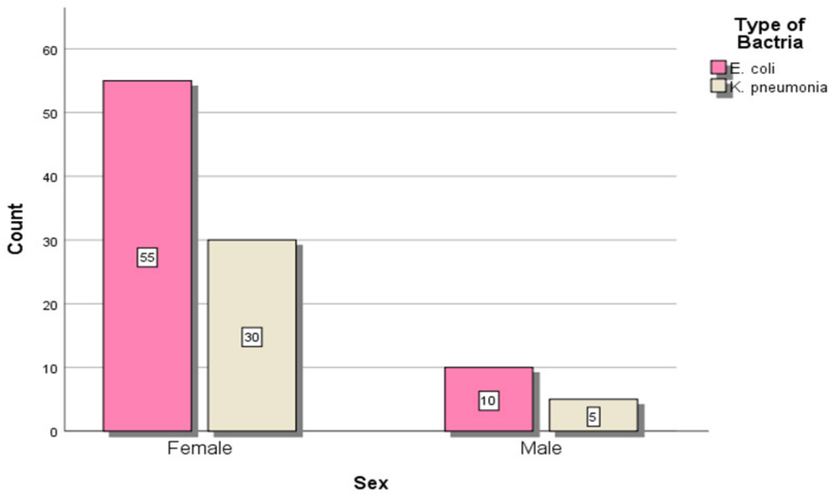


Fig. 9. Distribution of *K. pneumoniae* and *E. coli* according to sex group

Table 2

Comparison between *E. coli* and *K. pneumoniae* Patients in sex.

			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	

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)