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# Prevalence of blaOXA-1 and blaOXA-48 genes in Klebsiella pneumoniae Isolates

**Rand Hatam Kalo [1]* , Wafaa Dawood Ahmed[2]**

1 Al-Mahmoodiya hospital, Ministry of Health and Ecology, msc.randhatam@gmail.com, Baghdad, Iraq

*Correspondence author. E-mail: msc.randhatam@gmail.com

# إنتشار جينيات أوكسا-1 و أوكسا-48 في عزلات *Klebsiella pneumonia*

**رند حاتم كلو*[1]، وفاء داود أحمد[2]**

[1] مستشفى المحمودية, وزارة الصحة والبيئة، msc.randhatam@gmail.com ، بغداد, العراق

[2] القسم الطبي، وزارة النفط ، wafaadawood074@gmail.com، بغداد, العراق

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## ABSTRACT

**Background:** *Klebsiella pneumoniae* is bacteria gram-negative opportunistic pathogen that causes a variety of infectious diseases.

**Aim of the study:** The present study aimed to isolate *K. pneumoniae*, and finding out the extent of the spread of the $bla_{OXA}$ *-1* and *blaOXA* -48 genes responsible for antibiotics resistance.

**Materials and methods:** the detection of *Klebsiella pneumoniae* on morphological and biochemical tests and then assured with VITEK-2 system, and genotyping of *blaOXA-1* and *blaOXA-48* genes in isolates was done by PCR technique.

**Results:** One hundred and fifty-eight samples were collected randomly from adults who suffers from respiratory tract infection, urinary tract infection, Inflamed burns and wounds including both sexes from different hospitals in Baghdad especially Teaching Laboratories of Medical City, Al-Mahmodia health centre, Al-Yarmouk hospital,and Al-Mahmodia hospital during the period from September to November 2020.Shown the results by VITEK-2 system for all isolates that 50 (31.6%) isolate from the total of isolates were *K. pneumonia* .Molecular test was detected via Polymerase Chain Reaction (PCR) conventional proved to be effective for the detection of diffuse $bla_{OXA}$ -1 gene at 66.7% and the present results demonstrated a high significant presence at $P \leq 0.0001$ among isolates. While the prevalence of 5 isolates of the $bla_{OXA}$ -48 gene was 16.6% showed significant difference between the isolate types with p value $\leq 0.01$.

**Conclusions:** *K. pneumoniae was* the most bacterial species isolated from patient with pneumonia and our results indicate the spread of the *OXA* genes.

**Keywords***: K. pneumoniae*; Clinical Samples; $bla_{OXA}$ *-1;* $bla_{OXA}$ -48; *OXA* genes.



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## INTRODUCTION

An opportunistic bacteria known as *K. pneumoniae* is linked to a number of community-acquired illnesses, including as wound infections, septicemia, pneumonia, and urinary tract infections. The most frequent *Klebsiella* species causing infections in humans is one of the three most prevalent pathogens of worldwide concern that were found in the 2014 World Health Organization global study on antimicrobial resistance surveillance**[1]**. *OXA* enzymes are prevalent among several Gram-negative bacteria. The spread of plasmids, transposons, and integrons among bacteria and species contributes to so-called gene epidemics. Integrons have an alarming ability to enroll, propagate, and express resistance genes, and surveys indicate that they are widespread among gram-negative bacteria **[2]**. The subgroup of *OXA-1* **[3]**.The *OXA-1* and *OXA-30* β-lactamases are identical enzymes that were produced due to an initial sequencing error in the *OXA-1* sequence**[4]**. at several different gram-negative bacteria, the *blaOXA-1* gene was found at integron and plasmid locations.Like the majority of OXAs, *OXA-1*-lactamase hydrolyzes amino-and penicillins significantly while weakly hydrolyzing narrow-spectrum cephalosporins**[5]**. Additionally, *OXA-1* hydrolyzes broad-spectrum cephalosporins weakly, which indicates a decreased vulnerability to cefepime and cefpirome.**[ 6]**. As a result, it can be classified as β-lactamase that applies cephalosporins either widely or narrowly**[7]**. *K. pneumoniae* isolates that are resistant to carbapenem contain *OXA-48*. CPE isolates are developing the carbapenem *OXA-48* in greater numbers**[8]**. With regard to imipenem, this enzyme has the highest known catalytic efficacy among class D-lactamases. *K. pneumoniae* contains a plasmid containing the *blaOXA -48* gene. With regard to imipenem, this enzyme has the highest known catalytic efficacy among class D-lactamases. *K. pneumoniae* contains a plasmid containing the *blaOXA -48* gene.**[ 9]**. Plasmid-mediated dispersal resulted in an increasing issue. The Appleton class D beta-lactamase *OXA-48* hydrolyzes carbapenems but has relatively little activity against cephalosporins with a longer spectrum, such as cefepime and ceftazidime**[10]**. *OXA-48* was found in isolates of *K. pneumoniae* that were resistant to carbapenem, despite the fact that these isolates were generally multidrug-resistant and combined multiple resistance mechanisms**[11]**.

## MATERIAL AND METHODS

**Isolation of bacteria:**

This cross-sectional investigation was conducted between September 2020 and November 2020 on 50 isolates from various clinical specimens, including 14 urine isolates, 5 burn isolates, 10 ear isolates, one vaginal isolate, 4 wound isolates, and 16 sputum isolates. All of the patients tested clinically positive *for K. pneumoniae* and were examined according to standard operating procedures. To avoid contamination, clean-catch midstream urine samples were taken from patients using sterile disposable glass containers (5 ml), whereas sputum was collected from each patient using sputum containers with screw covers. Additionally, clinical wound, ear, burn, and vaginal samples were collected using sterile cotton swabs.

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**Identification of bacteria:**

Clinical *K. pneumoniae* isolates were done utilizing the compact bacterial identification of the VITEK-2 system, the GN card that was used for Gram-negative bacterial species which consisted of 47 substrates (biochemical tests) .All subsequent steps were practiced according to the guidelines of the manufacturer.

**Detection of *blaOXA -1* gene and *blaOXA -48* gene in *K. pneumoniae* isolates by molecular techniques (PCR assay)**

**3.1 Preparation of Extraction DNA**

The DNA was extracted by using Presto™ Mini gDNA Bacteria Kit (Gene aid QAIC/TW/5077/Thailand). DNA was isolated from overnight cultures of carefully selected *K. pneumoniae* isolates in accordance with the manufacturing company's technique.

**3.2 Primers**

**Table 1 : The name, sequence and product size of primers used in this study.**

| Primer | Primer Sequence 5′→3′ | Amplicon Size (Bp) | Reference |
|---|---|---|---|
| ***bla*$_{OXA}$ *-1* F** | GATCGCATTATCACTTATGGC | 454 | This Study |
| ***bla*$_{OXA}$ *-1* R** | GGTTCTATTTGCTGTGAATCC | 454 | This Study |
| ***bla*$_{OXA}$ *-48* F** | AATCATCAGGGGATTCTTCAG | 481 | This Study |
| ***bla*$_{OXA}$ *-48* R** | CCATAATCGAAAGCATGTAGC | 481 | This Study |

**3.3 PCR assay (conventional PCR for detection of genes)**

**3.3.1 Preparation of *bla*$_{OXA}$ *-1***

A PCR mixture comprising 4 µl of template DNA, 5.5 µl of sterile nuclease-free water, 1.5 µl of each primer, forward and reverse, and 12.5 µl of one taq master mix was assembled in a total volume of 25 µl. The mixture was then vortexed, and the conditions for amplification were as follows: Initial denaturation takes place for 3 minutes at 94°C. Thereafter, there are 35 cycles of denaturation for 30 seconds at 94°C, primer annealing for 30 seconds at 50°C, and strand extension for 3 minutes at 70°C. The PCR products were visualized by staining with red safe stain and subjected to gel electrophoresis on a 1.0 w/v Agarose gel in 1x TAE buffer for 25 minutes (90 volts).

**3.3.2 Preparation of *bla*$_{OXA}$ *-48***

5 µl of template DNA, 4.5 µl of sterile nuclease-free water, 1.5 µl of each primer forward and reverse, and 12.5 µl of one taq master mix were added to the 25 µl PCR mixture. After the mixture was vortexed, the following criteria were met for amplification: After three minutes of initial denaturation at 94°C, there were 35 cycles of denaturation at 94°C for 30 seconds, primer

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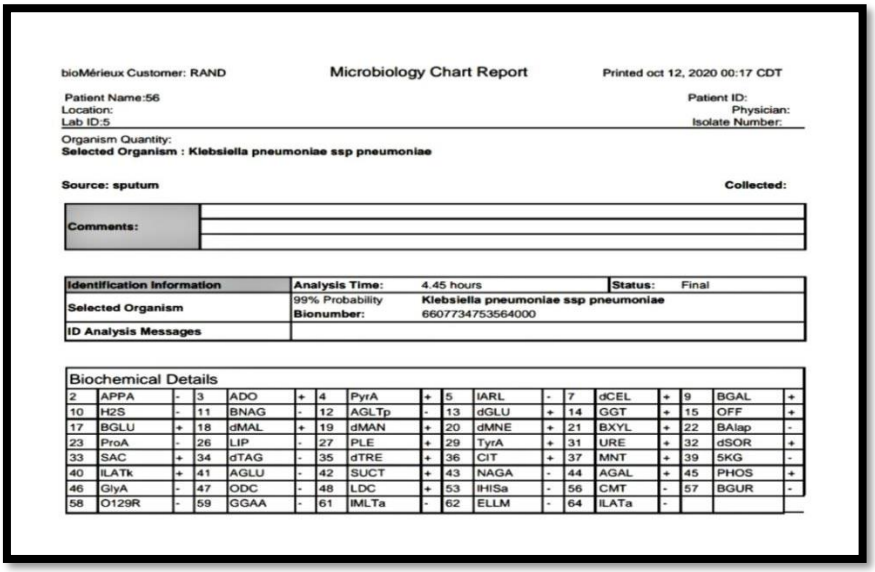
annealing for 30 seconds at 57°C, and strand extension for three minutes at 70°C. Red safe dye was used to view the PCR products after they were separated by gel electrophoresis on a 1.0 w/v Agarose gel in 1x TAE buffer for 25 minutes (90 volts).

## Statistical Analysis

Data in this study were analyzed using the Chi-square test and were reported as a frequency and percentage. To determine the impact of various factors on research parameters (percentage), the statistical analysis system, SAS, is utilized to determine P-values < 0.05 and 0.01 statistically. **[14].**

# RESULTS AND DISCUSSION

This system was used to prove a final identification of *K. pneumoniae*. The system was detected bacteria efficiently, faster, and away from the contamination that may prevent detection of the pathogen, by using Identification gram-negative Bacteria (ID-GNB) cards and showed results that 50 isolates belonged to *K. pneumoniae*. The GN card for gram-negative bacteria was used which consists of 47 biochemical tests as shown in figure 1.



bioMérieux Customer: RAND — Microbiology Chart Report — Printed oct 12, 2020 00:17 CDT

Patient Name:56 — Patient ID:
Location: — Physician:
Lab ID:5 — Isolate Number:

Organism Quantity:
**Selected Organism : Klebsiella pneumoniae ssp pneumoniae**

**Source: sputum** — **Collected:**

| Comments: | |
|---|---|

| Identification Information | Analysis Time: | 4.45 hours | Status: | Final |
|---|---|---|---|---|
| Selected Organism | 99% Probability | Klebsiella pneumoniae ssp pneumoniae | | |
| | Bionumber: | 6607734753564000 | | |
| ID Analysis Messages | | | | |

Biochemical Details

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | + | 4 | PyrA | + | 5 | IARL | - | 7 | dCEL | + | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | + | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | + | 22 | BAlap | - |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | + | 29 | TyrA | + | 31 | URE | + | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - |
| 40 | ILATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | + | 45 | PHOS | + |
| 46 | GlyA | - | 47 | ODC | - | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

**Fig. 1** Identification test by VITEK2 system (*K. pneumoniae* with probability 99%).

In study, 50 isolates of *K. pneumoniae* from the total samples 158 . The consent to participate in the study was taken. The isolates of *K. pneumoniae* were collected from different clinical samples such as 16(32%) isolates from sputum,14 (28%) isolates from urine, 10 (20%) isolates from ear swabs, 5(10%) isolates from burns swab, 4(8%) isolates from the wound, and 1(2%) isolate from Vaginal as Shown in figure 2. The percentage of *K. pneumoniae* isolation was at a high rate (32%)



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from sputum as compared with the remaining clinical samples. In modern years, *K. pneumoniae* have become becomes one of the essential bacterial causes in nosocomial infections **[12].** It is an important nosocomial pathogen involved in urinary tract infections, and hospital-acquired pneumoniae (HAP), also *K. pneumoniae* is a part of the intestinal flora, it was isolated as the causative agent in severe infections such as bacteremia **[13]**, surgical wound infection, and septicemia **[14]**.

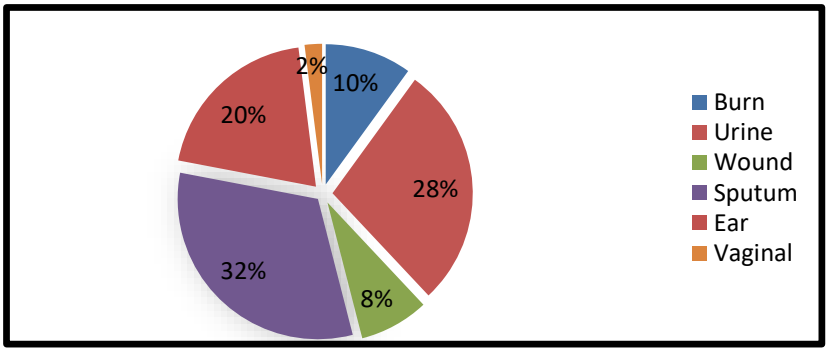


**Fig.2:** Percentages of the collected *K. pneumoniae* isolates according to the source of isolation.

The current study found the *bla*$_{OXA}$-48 gene in 5(16.66%) of the *K. pneumonia* isolates by conventional PCR (fig.3). The results showed that the existing rate of *bla*$_{OXA}$-48 gene among isolates *K. pneumonia* shown in Table 2. Amplification of β-lactamases gene by conventional PCR (fig.4) showed the presence of *bla*$_{OXA}$ -1 genes among 20 *K. pneumonia*e (66.7%) showed in Table 3.This result as in agreement with those of a local study carried by **[15].** Showed that the *bla*$_{OXA}$ -48 genes was detected in 21.4% *K. pneumoniae* isolates, and disagreement with several studies in countries of the region was reported the predominance of *bla*$_{OXA}$-48 gene among Gram-negative bacteria, in UAE, **[16].** Reported that the prevalence rate of *bla*$_{OXA}$ -48 gene was 53.3% in *Klebsiella pneumoniae* isolates, while in Turkey,**[17].** Revealed that *Klebsiella pneumoniae* were possessed *bla*$_{OXA}$ -48 gene with a rate of 86%.Gene prevalence in 30 isolates showed that 5/30 isolates (16.67%) were positive for *bla*$_{OXA}$ *-48* gene, and showed significant difference between the isolate types with p value ≤ 0.01 and the most common gene in these isolates were sputum 3 (10%) while 1 (3.33%) of the isolates were from urine and ear swabs, as shown in Table 2. Gene prevalence in 30 isolates showed that 20/30 isolates (66.67%) were positive for *bla*$_{OXA}$ -1 gene, and showed a very high significant difference between the isolate types with p value ≤ 0.01 and the most common gene in these isolates were sputum 6 (20%) followed by ear isolates 6 (20%), and urine It was 4 (13.3%) while 2 (6.67%) of the isolates were from burns and wounds swabs, as shown in Table 3. The occurrence rate of the *bla*$_{OXA}$ -1 gene in

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studied isolates varies widely. This result is in agreement with those of a local study carried by **[18]** showed that the *bla*$_{OXA}$ -1 gene have been detected in 71.7% *K. pneumoniae* isolates**.**

**Table 2:** Distribution of patients study according to sources and *bla*$_{OXA}$ *-48.*

| Sources | Positive *bla*$_{OXA}$ *-48* | Total |
|---|---|---|
| Burn | 0 (0.00%) | 4 (13.33%) |
| Urine | 1 (3.33%) | 6 (20.00%) |
| Wound | 0 (0.00%) | 2 (6.67%) |
| Sputum | 3 (10.00%) | 9 (30.00%) |
| Ear | 1 (3.33%) | 8 (26.67%) |
| Vaginal | 0 (0.00%) | 1 (3.33%) |
| Total | **5 (16.67%)** | **30** |
| Chi-Square ($\chi^2$) | 4.269 ** | 7.251 ** |
| * (P≤0.05), ** (P≤0.01) | | |

**Table 3:** Distribution of patients study according to sources and *blaOXA-1* .

| Sources | Positive *bla*$_{OXA}$ *-1* | Total |
|---|---|---|
| Burn | 2 (6.67%) | 4 (13.33%) |
| Urine | 4 (13.33%) | 6 (20.00%) |
| Wound | 2 (6.67%) | 2 (6.67%) |
| Sputum | 6 (20.00%) | 9 (30.00%) |
| Ear | 6 (20.00%) | 8 (26.67%) |
| Vaginal | 0 (0.00%) | 1 (3.33%) |
| Total | **20 (66.67%)** | **30** |
| Chi-Square ($\chi^2$) | 7.316 ** | 7.251 ** |
| * (P≤0.05), ** (P≤0.01) | | |

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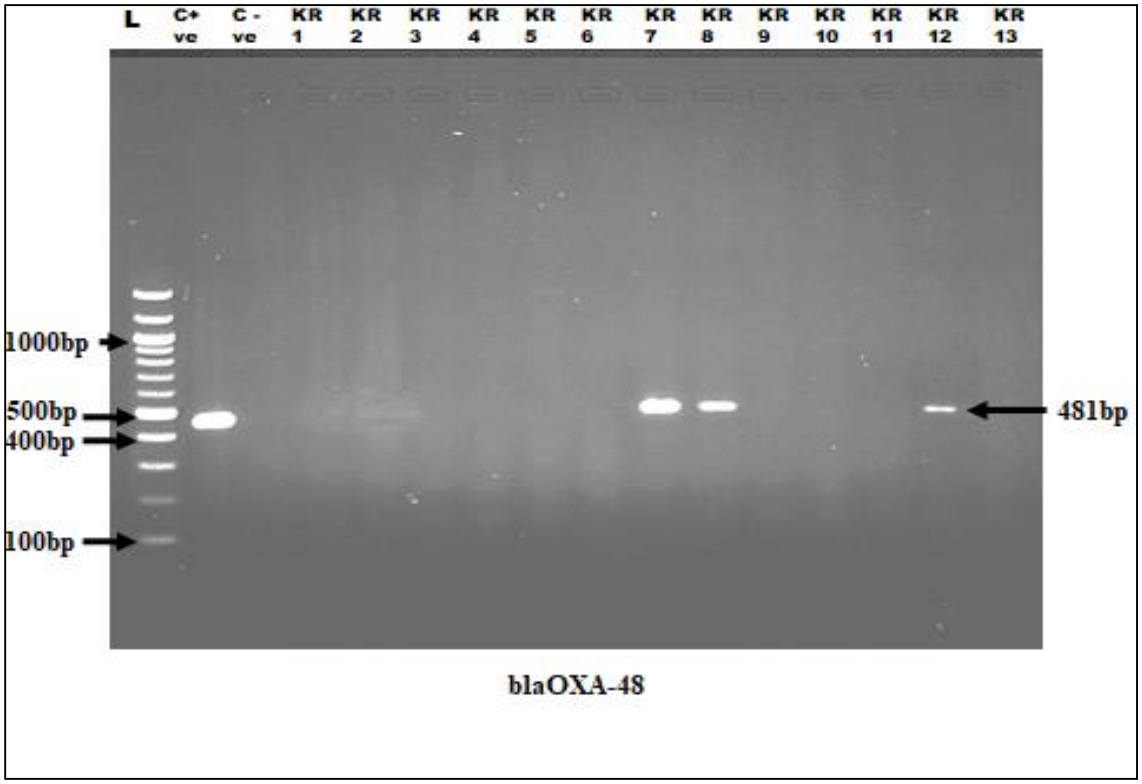


**Fig. 3** : Agarose gel electrophoresis analysis of blaOXA -48 (481bp). Ladder denotes to 1000 pb DNA ladder. Detection was accomplished on agarose gel (1.5%) at 90 V for 2 hour, stained with safety Red Stain and visualized on a UV transilluminator documentation system. L:ladder

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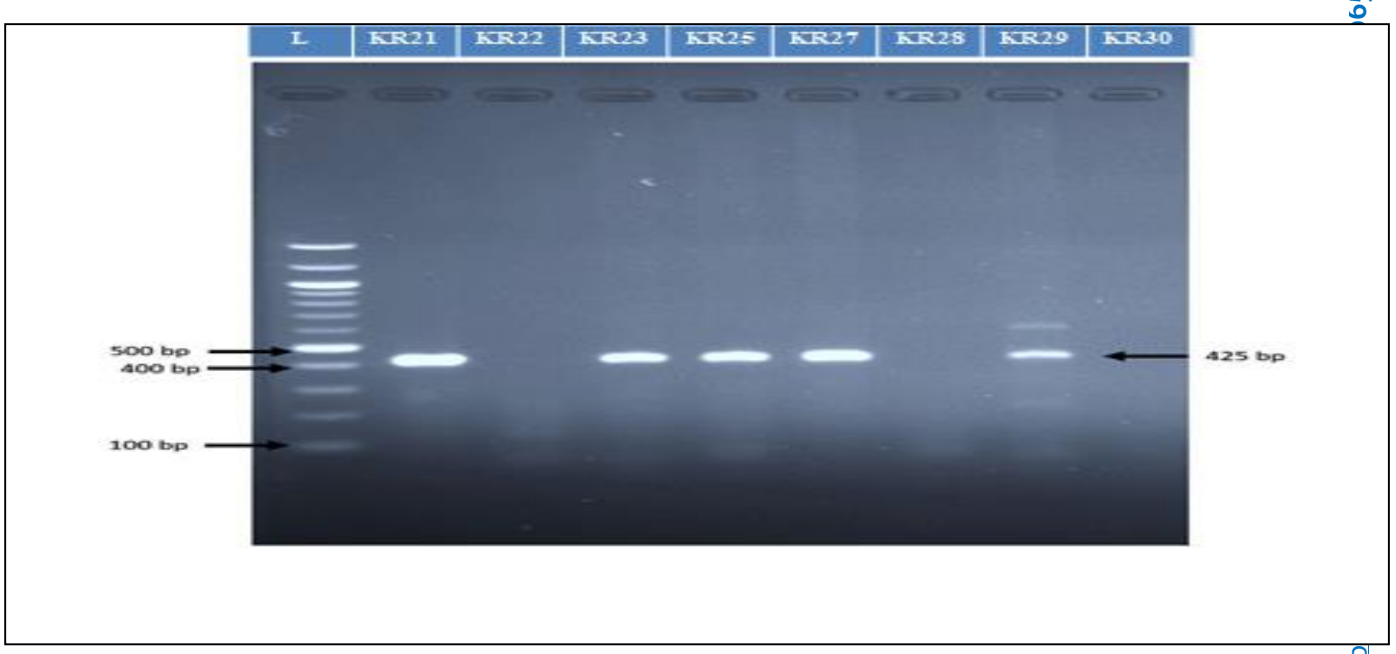


**Fig. 4**: Agarose gel electrophoresis analysis of blaOXA -1 (425bp). Ladder denotes to 1000 bp DNA ladder. Detection was accomplished on agarose gel (1.5%) at 90 V for 2 hour, stained with safety Red Stain and visualized on a UV transilluminator documentation system. L:ladder mark.

## CONCLUSION

The majority of the bacteria that were identified from the sputum samples was *K. pneumoniae* infection. Conclude that a high frequency of *blaOXA -1* and *blaOXA -48* gene occurrence was found among identified *K. pneumonia* cases, which may reflect the high level of pressure from the use of related antibiotics. Additionally, the results demonstrated that sputum had a higher prevalence of the *blaOXA -1* and *blaOXA -48* gene to isolate sources.

## Conflict of interests.

There are non-conflicts of interest.

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## الخلاصة

**المقدمة:** *Klebsiella pneumon* هي بكتريا سالبة لصبغة كرام إنتهازية تسبب العديد من الأمراض.

**الهدف من الدراسة:** تهدف الدراسة الحالية لعزل البكتريا *K. pneumon* وإيجاد مدى إنتشار جينات أوكسا-1 وأوكسا-48 المسؤولة عن مقاومة المضادات الحيوية.

**المواد وطرق العمل:** تم تشخيص البكتريا بواسطة الطرق المظهرية و البايو كيميائية وتم التاكيد على التشخيص بنظام الفايتك .وبالطريقة الجينية تم عزل جينات أوكسا-1 وأوكسا-48 باستخدام تفاعل البلمرة.

**النتائج والمناقشة:** هدفت الدراسة الحالية الى عزل البكتريا *K. pneumoniae* وإيجاد مدى إنتشار جينات أوكسا-1 وأوكسا-48 المسؤولة عن مقاومة المضادات الحيوية، وتم جمع 158 عينة سريرية جمعت بصورة عشوائية من أشخاص بالغين يعانون من التهاب المجاري التنفسية ،إلتهاب المجاري البولية، الحروق والجروح الملتهبة في كلا الجنسين من مستشفيات عدة في بغداد وخصوصا المختبرات التعليمية في مدينة الطب، المركز الصحي في المحمودية، مستشفى اليرموك ومستشفى المحمودية. في الفترة من أيلول الى تشرين الأول 2020 وأظهرت النتائج بنظام الفايتك لكل العزلات بأنه 50 (31.6%) عزلة من العزلات الكلية كانت *K. pneumoniae* والتحليل الجيني كشف من خلال تفاعل البلمرة التقليدي أثبت كفاءته في التحري عن إنتشار جين أوكسا-1في 66.7 % والنتائج الحالية أظهرت فرق معنوي عالي في $P \leq 0.0001$ بين العزلات بينما الإنتشار في 5 عزلات لجين أوكسا-48 كانت 16.6% أظهرت فرق معنوي بين أنواع العزلات $p\ value \leq 0.01$.

**الإستنتاجات:** *Klebsiella pneumoniae* كانت أغلب أنواع البكتريا المعزولة من مرضى الإلتهاب الرئوي والنتائج أظهرت إنتشار جينات الأوكسا.

**الكلمات المفتاحية:** *Klebsiella pneumoniae*، عينات سريرية، أوكسا-1، أوكسا-48، جينات أوكسا.



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