Molecular Detection of multidruge resistance carbapenemase genes *blaKPC, blaOXA 48* in *Klebsiella pneumoniae*.

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ABSTRACT

Klebsiella pneumoniae is a pathogenic Gram-negative bacterium belonging to the Enterobacteriaceae family. Multidrug-resistant (MDR) bacteria are a significant global issue in human medicine due to the high occurrence of these organisms, making them a major economic issue. Carbapenems are the last line of defense against infections caused by Multi Druge Resistance (MDR) Klebsiella pneumoniae isolates, but overuse can result in the emergence of antimicrobial resistance isolates. The carbapenemase enzymes (KPC) and (OXA 48), which produce from bacteria K. pneumoniae today, have emerged as one of the β-lactamase enzymes that can inactivate the last line of carbapenems. The study aims to detect blaKPC, blaOXA48, genes from Klebsiella pneumonia which isolated from different clinical samples in Kerbala province. Sixty-eight Klebsiella pneumoniae isolates were collected from pateints with different infections, and isolates were identified by Vitek 2 automated system. Antibiotic susceptibility was measured using the disc diffusion method. Following phenotypic detection of multidrug resistance, and finally the blaKPC and blaOXA48 genes were detected. All isolates of K. pneumoniae which used in this study based on by susceptibility testing with used 10 antimicrobial, K. pneumoniae isolates were highly resistant to Carbapenem and Cephems antibiotics, intermediate to Cefipeme, and sensitive to Sulfonamide.

Key Words: *Klebsiella pneumoniae, Multi Druge Resistance, carbapenems, blaKPC, blaOXA 48.*

الكشف الجزيئي عن جينات الكاربنيمز المقاومة متعددة الجراثيم في عزلات بكتريا الكلبسيلا الرئوية المعزولة من مصادر سريرية مختلفة

Klebsiella pneumoniae هي بكتريا ممرضة سالبة لصبغة كرام تنتمي الى عائلة Enterobacteriaceae وتعد هذه البكتريا متعددة المقاومة لادوية (MDR) قضية عالمية مهمة في الطب البشري بسبب الانتشار الكبير لهذه الكائنات ، مما يجعلها مشكلة اقتصادية كبيرة. الكاربابينيمات هي خط الدفاع الأخير ضد العدوى التي تسببها عزلات *Klebsiella pneumoniae* المقاومة للأدوية المتعددة (MDR) ، ولكن الإفراط في استخدامها يمكن أن يؤدي إلى ظهور عزلات مقاومة لمضادات الميكروبات. ظهرت إنزيمات carbapenemase (KPC) و (OXA 48) ، التي تنتج من بكتيريا كلبسيلا الرئوية اليوم ، كواحدة من إنزيمات β-lactamase التي يمكن أن تعطل السطر الأخير من carbapenems. تهدف الدراسة إلى الكشف عن جينات *blaKPC* و *blaOXA48* من الالتهاب الرئوي Klebsiella والتي تم عزلها من عينات سريرية مختلفة في محافظة كربلاء. جمعت ثمانية وستون عزلة من نوع *Klebsiella pneumoniae* من pateints المصابة بالتهابات مختلفة ، وتم التعرف على العزلات بواسطة نظام Vitek 2 الآلي ، وتم قياس الحساسية للمضادات الحيوية باستخدام طريقة الانتشار القرصي. بعد الكشف عن النمط الظاهري لمقاومة الأدوية المتعددة ، وفي النهاية تم اكتشاف جينات *blaKPC* و*blaOXA48* جميع عزلات *K. pneumoniae* التي استخدمت في هذه الدراسة بناءً على اختبار الحساسية باستخدام 10 مضادات للميكروبات حسب تعليمات CLSI 2022 ، كانت عزلات *K. pneumoniae* شديدة المقاومة للمضادات الحيوية Carbapenem و Cephems ، وسيطة لـــ Cefipeme ، وحساسة للسلفوناميد. قضية عالمية مهمة في الطب البشري بسبب الانتشار الكبير لهذه الكائنات ، مما يجعلها مشكلة اقتصادية كبيرة. الكاربابينيمات هي خط الدفاع الأخير ضد العدوى التي تسببها عزلات *Klebsiella pneumoniae* المقاومة للأدوية المتعددة (MDR)، ولكن الإفراط في استخدامها يمكن أن يؤدي إلى ظهور عزلات مقاومة لمضادات الميكروبات. ظهرت إنزيمات carbapenemase (KPC)و (OXA 48)، التي تنتج من بكتيريا .Kالرئوية اليوم ، كواحدة من إنزيمات β-lactamase التي يمكن أن تعطل السطر الأخير من carbapenems. تهدف الدراسة إلى الكشف عن جينات *blaKPC* و *blaOXA48* من الالتهاب الرئوي Klebsiella والتي تم عزلها من عينات سريرية مختلفة في محافظة كربلاء. جمعت ثمانية وستون عزلة من نوع *Klebsiella pneumoniae* من pateints المصابة بالتهابات مختلفة ، وتم التعرف على العزلات بواسطة نظام Vitek 2 الآلي ، وتم قياس الحساسية للمضادات الحيوية باستخدام طريقة الانتشار القرصي. بعد الكشف عن النمط الظاهري لمقاومة الأدوية المتعددة ، وفي النهاية تم اكتشاف جينات *blaKPC* و*blaOXA48* جميع عزلات *K. pneumoniae* التي استخدمت في هذه الدراسة بناءً على اختبار الحساسية باستخدام 10 مضادات للميكروبات)حسب تعليمات (CLSI 2022، كانت عزلات *K. pneumoniae* شديدة المقاومة للمضادات الحيوية Carbapenem و Cephems، وسيطة لـ Cefipeme، وحساسة للسلفوناميد.

1.INTRODUCTION

Klebsiella genus is a member of Enterobacteriaceae family Gram-negative, facultatively anaerobic, rod-shaped, nonsporulating, and immobile. It is named after German scientist Edwin Klebs (1834–1913)[1]. Is classified as rendering to the following. It is also known as Friedlander's bacillus, a German pathologist who hypothesized that this bacterium was the cause of tuberculosis etiological factor for pneumonia[2]. In the late 19th century, *K. pneumoniae* was first isolated and was initially termed Friedlander's bacterium. Gram-negative bacteria, particularly Enterobacteriaceae, are frequently responsible for both community- and hospital-acquired infections, including those of the bloodstream, lower respiratory tract, and urinary tract. These microbes can obtain genes encoding several strategies for resisting antibiotics, including extended-spectrum β-lactamases

(ESBLs), ampCs, and carbapenemases[3]. In the pre-antibiotic era, it was an important cause of community-acquired pneumonia, especially in alcoholics and diabetic[4].

Multi-drug resistant (MDR) *K. pneumoniae* became established in hospitals as a prominent cause of healthcare-associated infections during the antibiotic era. *K. pneumoniae* has a large auxiliary genome made up of plasmids and chromosomal gene loci[5]. Since the mid-1980s, it has been identified as the source of extremely persistent community-acquired illnesses, accounting for about one-third of all Gram-negative infections[6]. It has acquired multidrug resistance by horizontal transfer of antimicrobial resistance genes mediated by mobile genetic components such as integrons. Multiple nosocomial epidemics caused by *K. pneumoniae* with multiple treatment resistance have been observed globally[7] . Over the last 20 years, the incidence of *K. pneumoniae* infections has increased considerably, coinciding with extended-spectrum beta-lactamase (ESBL) spread, which is attributed to the pathogen's pathogenicity and virulent factors[8]. Pathogenicity refers to the capability of a pathogen to cause disease, while virulent factors refer to the degree of pathogenicity; generally. These lesions caused by *K. pneumoniae* may be attributed to the pathogen's pathogenicity and virulent factors[9]. This organism accounts for about one-third of all Gram-negative infections for instance, urinary tract infections, cystitis, pneumonia, surgical wound infections, endocarditis and septicemia[10]. MDR was defined as the acquisition of resistance to at least one drug from three or more antibiotic classes[11]. The rise of carbapenem resistance in gramme-negative bacteria has been documented globally in recent years. The primary carbapenem-hydrolyzing β-lactamases in Enterobacteriaceae are the Ambler class A β-lactamases (KPC) and the class D β-lactamase OXA-48[12].Carbapenems are becoming increasingly important in treating serious infections, but care must be taken to avoid overuse to avoid emerging resistance[13].

2. MATERIALS AND METHODS

Two hundred and seventy-five specimens were isolated from various clinical samples (blood, sputum, urine, wounds, burns, high vaginal swab (HVS), pharyngeal swab, and ear swab) from patients in distinct Karbala hospitals. All collected specimens grown, separately, on MacConkey agar and blood agar after that incubated for 24 hours at 37°C[14] . After incubation and depending on their colonies morphology (shape, size, color and texture), isolates suspected to belonging to Klebsiella genus were taken for identification[15]. Mucoid growth Lactose fermenting colonies on MacConkey agar and non-hemolytic mucoid colonies on sheep blood agar are gray, white[16] and we used vitik-2 system for identification. The susceptibility of *K. pneumoniae* to 10 antibiotics agents for 7 classes Carbapenem antibiotics including Meropeneme and Imipenem, Cephems group including Cefipeme and Cefotaxime, beta lactam antibiotics group including Ceftazidime, Aminoglycoside including Gentamicin, Monobactam including Aztreonam, Fluoroquinolone including Levofloxacin and Ciprofloxacin, Sulfonamide (folate pathway) including Trimethoprim-sulfamethoxazole ,it was determined by the Kirby-Bauer disk diffusion method according to (CLSI 2022)[17] instructions. Activation of isolates was performed using MacConkey Agar plate culture for 24 hours at 37°C and the growth was transferred to a tube containing 3 ml of normal saline, the turbidity was adjusted to 0.5 McFarland tube equal to 1.5×10^8 CFU/ml standard, and then spread on Muller Hinton agar by a sterile cotton swab[18] . The inhibition zone diameter was read and interpreted as a sensitive, intermediate or resist. Carbapenem resistant *K. pneumoniae* isolates were defined as carbapenem non-susceptible [19]. The carbapenemase gene bla_{KPC} and bla_{OXA48} were detect by using the Polymerase Chain Reaction PCR were performed to amplify the target DNA using particular primer pairs. Single plex was used in this study. 12.5 µl of master mix, 2 µl of each primer, 5 µl of template DNA and 3.5 µl of Nuclease free water mixed in 25 µl of total reaction volume.

3. RESULTS AND DISCUSSION

3.1 Bacterial isolates: Sixty-eight *K. pneumonia* isolates (blood, burn, urine, sputum, wound, urine, HVS , pharynx swab and ear swab) were collected from various clinical sources in Karbala during the period from September 2022 to December 2022. Females had a higher isolation rate than males (35:33). *Klebsiella pneumoniae* isolates were identified based on morphological characteristics of the colonies on MacConkey agar and Blood agar. The VITEK 2 automated identification system was used to confirm Klebsiella identification at the species level and to eliminate variability in biochemical test findings[20]. Sixty-eight of *K. pneumonia* isolates were isolated from different clinical samples such as 22/68(32.35%) isolates from sputum, 16/68(23.52%) isolates from urine, 8/68(11.76%) isolates from wound, 5/68(7.35%) isolates from burns, 6/68(8.82%) isolates from blood, 6/68(8.82%) isolates from higher vagina swab (HVS), 3/68(4.41%) isolates from pharynx,2/68(2.94%) isolates from ear swab as shown in figure. The survey validated the prevalence of *K. pneumoniae* in Karbala hospitals.

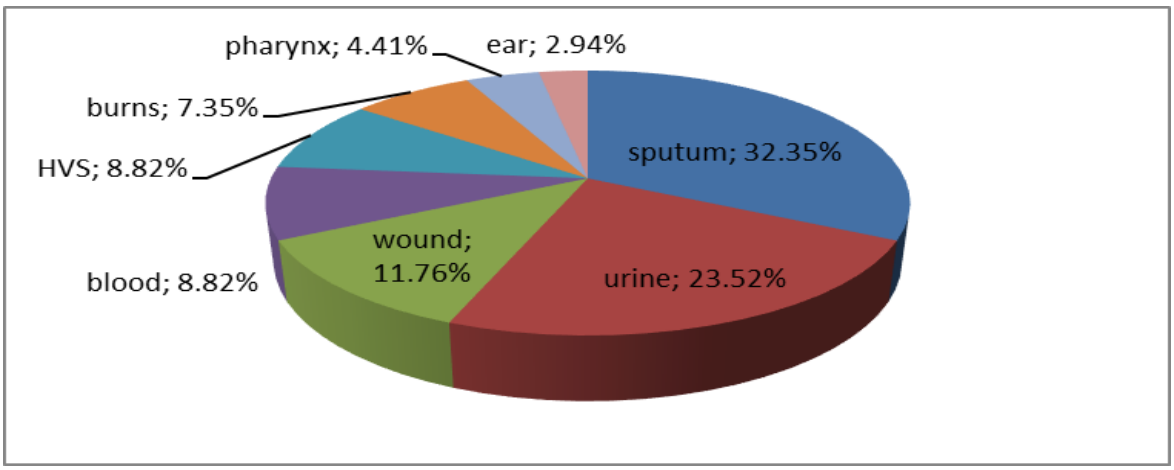


Figure1: Percentage of *Klebsiella pneumoniae* isolates from different clinical samples.

3.2 Antibiotic Susceptibilities: The results of antibiotic susceptibility tests showed that *K. pneumoniae* isolates were highly resistant to Carbapenem antibiotics including Imipenem (88%), and Cephems group including cefotaxime (74%), Ceftazidime (66%), Aztreonam (57%), Cefipeme (56%), Ciprofloxacin (55%), Gentamicin (51%), Meropenem (41%), and Trimethoprim/sulfamethoxazole (43%). The results also, have indicated that *K. pneumoniae* isolates were intermediate to Cefipeme (15%), meropenem (12%) and imipenem (4%), Gentamicin (9%), Ceftazidime (6%), Trimethoprim-sulfamethoxazole (3%), Ciprofloxacin and aztreonam (1) % .The results of antibiotic susceptibility tests indicated that *K. pneumoniae* isolates were highly sensitive to Sulfonamide (folate pathway) including Trimethoprim-sulfamethoxazole (55%), Levofloxacin (51%) Ciprofloxacin (44%), Gentamicin (40%), Meropenem (47%), Aztreonam (41%), Cefipeme (29%) cefotaxime and Ceftazidime (26%), as shown in table 1 below

Table1: Antimicrobial susceptibility test of *Klebsiella pneumoniae* isolate.

Antibiotics	Sensitive	Intermediate	Resistance	Total
Cefotaxime 30mg	18 (26%)	-	50 (74%)	68
Levofloxacin 5mg	35 (51%)	3(4%)	25 (37%)	68
Cefipeme 30mg	20 (29%)	10(15%)	38(56%)	68
Trimethoprim/sulfamethoxazole 25 mg	37(55%)	2(3%)	29 (43%)	68
Ceftazidime 30mg	18(26%)	5(6%)	45(66%)	68
Meropeneme 10mg	32(47%)	8(12%)	28 (41%)	68
Imipenem 10mg	5 (7%)	3(4%)	60(88%)	68
Gentamicin 10mg	27 (40%)	6(9%)	35 (51%)	68
Ciprofloxacin 5mg	30 (44%)	1(1%)	37(55%)	68
Aztreonam 30 mg	28 (41%)	1(1%)	39(57%)	68

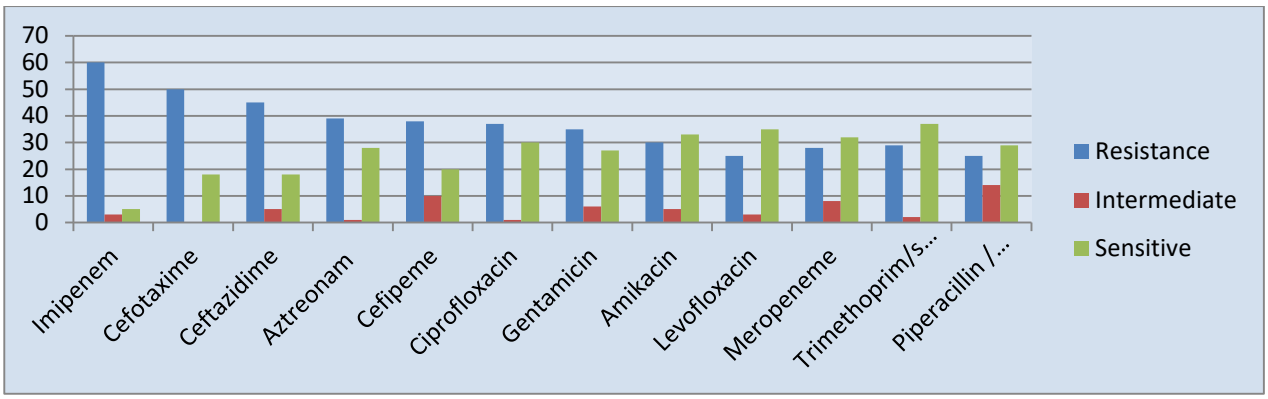


Figure 2: Antimicrobial susceptibility test of *Klebsiella pneumoniae* isolates.

Among sixty-eight *K. pneumoniae* isolates 40/68 (59%) were diagnosed as multi-drug resistant (MDR) *K. pneumoniae*, 28 /68(41%) non MDR.In this study *K. pneumoniae* isolates as MDR were resisted to 3 antibiotics up to 10 antibiotics for 7 classes, its resistant to 7 classes were the highest present with 20/40 also considered extensively drug-resistant XDR 50% followed by 4, 6 classes MDR 7/40 (15%), 3 classes MDR 4/40(10%), and 5 classes MDR 2/40 (5%) as table2 below.

Table: Resistance patterns among *Klebsiella pneumoniae* isolates.

Classes	MDR Phenotype	No.	%
3	Cephems /Carbapenem /Fluoroquinolone	1	10%
	Cephems /Carbapenem / beta lactam	2	
	Cephems / beta lactam / Sulfonamide	1	
4	Monobactam / Cephems / beta lactam / Fluoroquinolone	1	15%
	Monobactam / Carbapenem/ Aminoglycoside /Fluoroquinolone	1	
	Monobactam /Carbapenem/ Beta lactam / Cephems	5	
5	Beta lactam/Monobactam/Carbapenem/ Sulfonamide /Fluoroquinolone	1	5%
	Beta lactam / Sulfonamide / Monobactam / Cephems / Fluoroquinolone	1	
6	Cephems / Monobactam / Carbapenem / Fluoroquinolone / Aminoglycoside/ Beta lactam	4	20%
	Monobactam /Carbapenem/ Beta lactam / Fluoroquinolo Aminoglycoside / Sulfonamide	1	
	Cephems /Carbapenem/ Beta lactam / Fluoroquinolo Aminoglycoside / Sulfonamide	2	
7	Monobactam /Carbapenem/ Beta lactam / Fluoroquinolo Aminoglycoside / Sulfonamide / Cephems	20	50%
Total		40	

The current investigation found that all *K. pneumoniae* isolates were multidrug resistant to at least one antibiotic in three or more of the 7 antimicrobial classes tested in this study. Multi-resistant isolates were categorized as MDR, perhaps XDR, or

possibly PDR based on this criterio[21]. The reason may be attributed to this: efflux pump systems and enzymatic degradation are important in the spread of multidrug-resistant *K. pneumonia* [22].

3.4 Genotypic Detection of Carbapenemases enzymes: The results showed that 18/40 (47.5%) of the isolates have bla_{OXA48} gene and 1/40 isolate have bla_{KPC} gene, while 21/40 (52.5%) of the isolates did not have both.

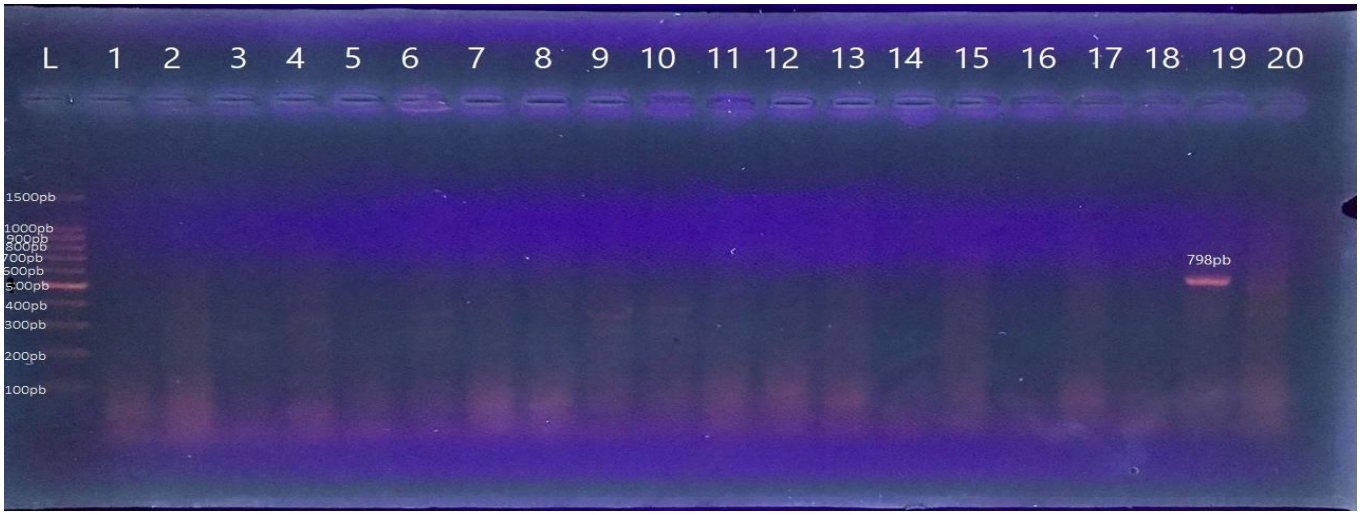
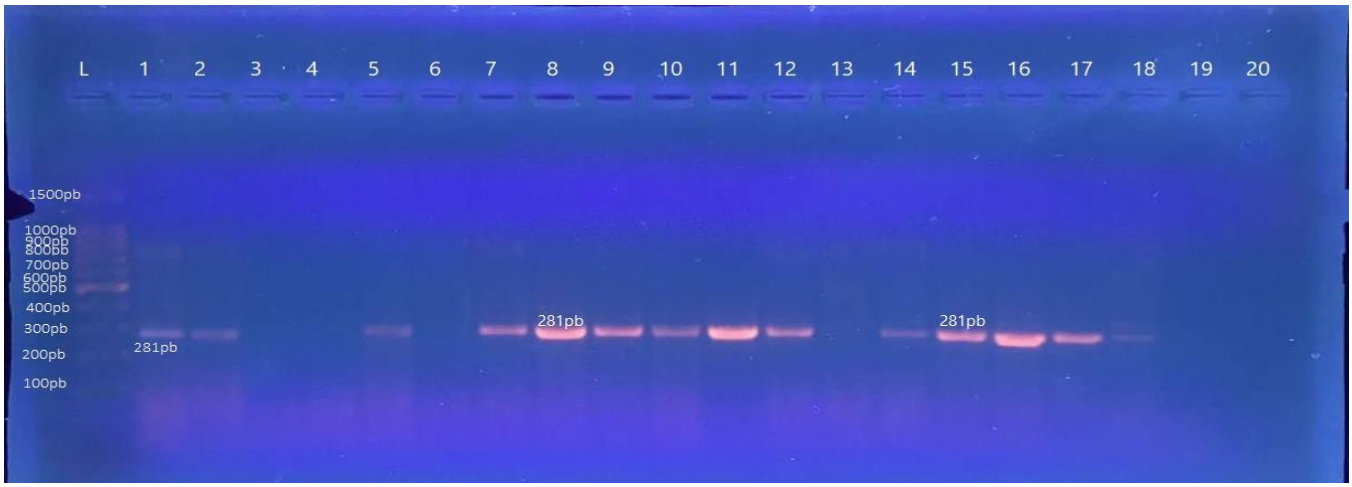


Figure2: Agarose gel (1.5% in TBE) electrophoresis for *K. pneumoniae* bla_{KPC} amplicon (798bp) primer for carbapenem-resistant *K. pneumoniae*, L is 100bp DNA ladder, 1/40(2.5%) lane 19 isolate positive bla_{KPC} genes, at Voltage 75 volts for 90 min.



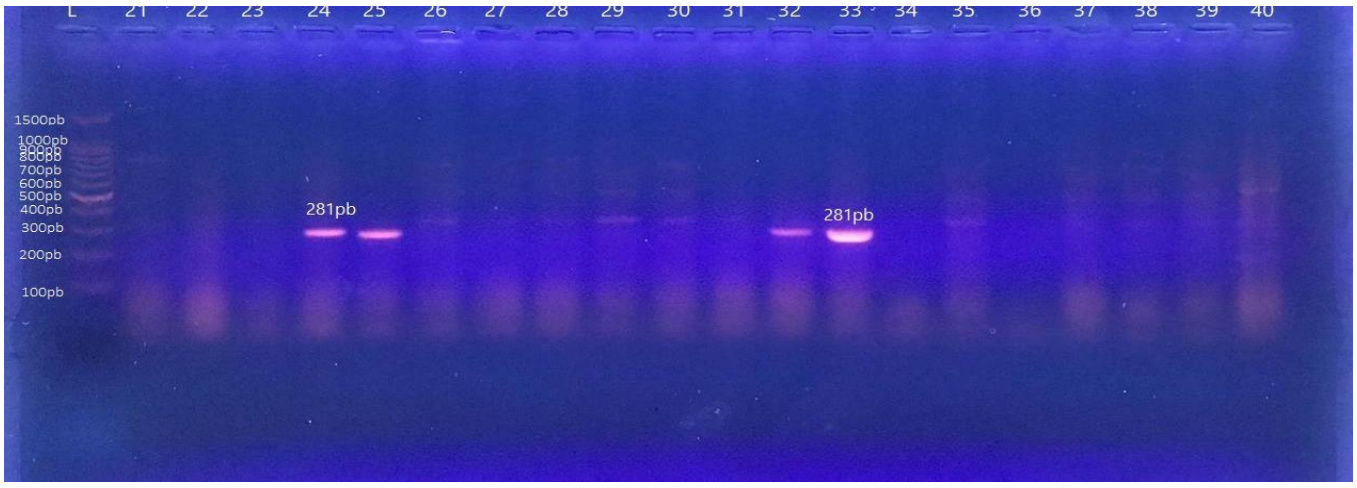


Figure 3: Agarose gel (1.5% in TBE) electrophoresis of amplified product of 281bp *bla$_{OXA-48}$* gene for *K. pneumoniae* isolates. For 90 min at 75 volts. Lane L is 100bp DNA ladder; 18/40(45%) Lanes 1, 2, 5, 7, 8, 9, 10,11,12,14,15,16,17, 18, 24, 25, 32 and 33 positive *bla$_{OXA-48}$* gene**.**

In this study, found that the prevalence of KPC-producing *K. pneumoniae* was relatively low compared to OXA-48 gene. *K. pneumoniae* isolates expressing KPC-type carbapenemases are among the most troublesome multiple antibiotic-resistant infections to emerge in recent years, attributable to their XDR phenotypes and propensity for rapid diffusion in hospitals, with a significant effect on mortality[23]. Klebsiella species are the major source of mortality and morbidity due to endemic, epidemic, and nosocomial diseases[24]. Based antimicrobial susceptibility assay, PCR and carbapenemase gene typing, substantial prevalence of highly virulent MDR *K. pneumoniae* isolates were present in clinical specimens. Due to the multidrug resistance produced by the emergence of drug-inactivating enzymes, particularly beta-lactamases. The carbapenemase enzyme is increasingly being identified in bacteria that cause nosocomial infections[16].

CONCLUSION

We have shown that increasing trends of *K. pneumoniae* isolates from different clinical samples (blood, sputum, urine, wounds, burns, higher vagina swab, pharynx swab and ear swab) from patients in separate hospitals in Karbala. The antibiotic susceptibility indicated that *K. pneumoniae* isolates were resistant to Cefotaxime, Levofloxacin, Cefipeme, Trimethoprim/sulfamethoxazole, Ceftazidime Meropeneme, Imipenem, Gentamicin, Ciprofloxacin, Aztreonam. It was diagnosed as multi-drug resistant (MDR) *K. pneumoniae.* This study found that *blaKPC* was the most common carbapenemase gene that found in1/40 (2.5%) isolates followed by *blaOXA-48* 18/40(45%) isolates. As a result, the *K. pneumoniae* strains that produce OXA-48 and KPC are an emerging threat in this medical center and should be targeted for early identification and stringent control of infections brought on by *K. pneumoniae*.

ACKNOWLEDGMENT

Gratitude to everyone who helped me conduct the current study.

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