Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Genotypic and Phenotypic Characterization of NDM-5-Producing *Klebsiella pneumoniae* in Clinical Isolates

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Received: 22/02/2026
Accepted: 04/05/2026
Published: 30/06/2026

Keywords: *Klebsiella pneumonia*, Carbapenemases, Multidrug resistant (MDR)

DOI:10.62472/kjps.v17.i28.29-38

Abstract

Background

Antibiotic resistance, particularly to carbapenems, has become a major health problem in recent years, posing a significant challenge in treating hospital-acquired infections, including those caused by Klebsiella pneumoniae, a leading causative agent of these infections due to its high capacity to acquire multiple resistance mechanisms.

Objectives: The purpose of this study was to identify New Delhi Metalo β-Lactamase 5 (NDM-5) in clinical isolates of carbapenem-resistant *Klebsiella pneumoniae* obtained from patients in different hospitals at Karbala.

Methods: 158 Clinical samples were obtained at random from several hospitals in Karbala. The VITEK 2 system was used to identify and confirm *Klebsiella pneumoniae* based on staining, biochemical testing, and colony morphology. VITEK 2 was used to validate the antibiotic sensitivity test. The ABIO pure method was used to extract DNA., and the NDM gene was found using PCR.

Results: It was discovered that fifty isolates (31.6%) were *Klebsiella pneumonia.* Twenty-one (42%) of the isolates demonstrated AST resistance
to both imipenem and meropenem, according to VITEK-2. Nine samples (81.8%) contained NDM-1 and one sample (9.09%) had NDM-5, according to the results of standard sequencing.

Conclusion

This study revealed a considerable incidence of bacteria resistant to carbapenem. 42% of *Klebsiella pneumoniae* isolates from Karbala hospitals were resistant to imipenem and meropenem. More than half of these resistant bacteria had NDM genes, primarily NDM-1 and one NDM-5, indicating that treatment is challenging. The rise in NDM-5 is particularly worrisome. The results highlight the significance of good infection control, antimicrobial stewardship, and continuous monitoring. For quick identification, sophisticated diagnostic instruments like VITEK-2 were helpful. Future studies should look into the strains' genetic relatedness and possible gene transfer.

الخصائص الجينية والمظهرية ل NDM_5 الناتج من بكتريا *Klebsiella pneumonia* في عزلات سريرية

هبه مهدي محمود الهاشمي

الخلاصة

المقدمة

تعد مشكلة مقاومة المضادات الحيوية من اهم المشاكل الصحية في السنوات الأخيرة خصوصا الكاربابينيم،مما يشكل تحديا كبيرا في علاج العدوى المكتسبة من المستشفى ومنها بكتريا *Klebsiella pneumonia* التي تعد اهم العوامل المسببة لهذه العدوى نظرا لقدرتها العالية على اكتساب آليات مقاومة متعددة

الهدف

هدفت هذه الدراسة الى تحديد وجود انزيم نيو دلهي ميتالو-بيتا لاكتاماز في عزلات سريرية من بكتريا *klebsiella pneumoniae*المقاومة للكاربينيم والتي تم الحصول عليها من مرضى في مستشفيات مختلفة من مدينة كربلاء

طرق العمل

تم جمع 158 عينة سريرية بشكل عشوائي من عدة مستشفيات في كربلاء تم استخدام نظام فايتك 2 لتحديد بكتريا *klebsiella* pneumoniae اعتمادا على الصبغة والاختبارات الكيميائية الحيوية والمظهر الشكلي للمستعمرات كما تم استخدام نظام فايتك لتاكيد اختبار حساسية المضادات الحيوية. ثم استخراج الحمض النووي باستخدام طريقة ABIO pure وتم الكشف عن جين NDM باستخدام تقنية البوليميراز المتسلسل

النتائج

تبين ان خمسين عزلة (31.6%) كانت بكتريا أظهرت احدى*klebsiella pneumoniae* وعشرون عزلة (42%) مقاومة لاختبار الحساسية للمضادات الحيوية لكل من الايميبينيم والميروبينيم باستخدام نظام الفايتك 2.واظهرت نتائج التسلسل القياسي ان تسع عينات (81.8%) كانت تحتوي على جين NDM_1 بينما كانت عينة واحدة (9.09%) تحتوي على الجين الاخر 5

الاستنتاجات

كشفت هذه الدراسة عن معدل ملحوظ لانتشار البكتيريا المقاومة لمجموعة الكاربابينيم. إذ أظهرت النتائج أن 42% من عزلات Klebsiella pneumoniaeالمأخوذة من مستشفيات كربلاء كانت مقاومة لكلٍّ من الإيميبينيم والميروبينيم. كما تبيّن أن أكثر من نصف هذه العزلات المقاومة تحمل جيناتNew Delhi metallo-beta-lactamase ، وبصورة رئيسية النمط NDM-1 مع تسجيل حالة واحدة منNDM-5 ، مما يشير إلى صعوبة واضحة في خيارات العلاج. ويُعدّ الارتفاع في نمط NDM-5 أمرًا مقلقًا بشكل خاص. وتؤكد هذه النتائج أهمية تطبيق إجراءات فعّالة لمكافحة العدوى، وتعزيز برامج ترشيد استخدام المضادات الحيوية، إضافة إلى ضرورة المراقبة المستمرة. كما أثبتت التقنيات التشخيصية المتقدمة، مثل جهازVITEK 2 system ، كفاءتها في التشخيص السريع. وتوصي الدراسة بإجراء أبحاث مستقبلية لدراسة الترابط الجيني بين السلالات وإمكانية انتقال الجينات بينها

1. Introduction

The absence of sophisticated and standardized techniques makes it extremely difficult to accurately identify these diseases using traditional diagnostic techniques. An important global public health concern is the increasing reliance on antibiotics to treat illnesses brought on by multidrug-resistant (MDR) bacterial strains. The establishment, evolution, and spread of MDR phenotypes across several bacterial species have been significantly accelerated by the prolonged and careless use of antimicrobial drugs (Hassall *et al*., 2024).

The options for treating bacterial infections have been reduced by the emergence of new antimicrobial drugs and the increase in antibiotic resistance (Nathan and Cars, 2014). Using the current medications to remove nosocomial and serious community infections produced by bacteria resistant to several drugs is difficult (Cerceo *et al*., 2016). Antibiotic resistant bacteria present a problem for infection control and prevention. A recent study has shown that many harmful the majority of drugs don't work against *K. pneumoniae* isolates (Bortolaia *et al*., 2018).The NDM-1 enzyme gives bacteria resistance to several beta-lactam medications. These include the medications of the carbapenem class that are the mainstay of treatment for bacterial infections. That are resistant to antibiotics. The NDM-1 gene is one of several that encode beta-lactamase enzymes, also referred to as carbapenemases. Bacteria that produce carbapenemases are sometimes referred to as "superbugs" in the news media because of how difficult it is to treat the illnesses they cause. Typically, only polymyxin and glycylcycline are effective against these bacteria (IBRAHIM *et al*., 2022). An isolate of *Klebsiella pneumoniae* from an Indian patient in Sweden in 2018 included the first indication of NDM-1. Later, it was discovered in bacteria in Canada (Devanga Ragupathi *et al*., 2020), the United States (Abhinav and Jaiswal, 2013), the United Kingdom, India, and Japan (Yuasa). Even though this enzyme is most commonly found in Gram-negative bacteria, including *Klebsiella pneumoniae* and *Escherichia coli,* the NDM-1 gene can be horizontally transmitted between various bacterial strains (Hudson *et al*., 2014).

2. Patient and Methods

Fifty samples from clinical trials were gathered over the course of seven months, from November 2024 to May 2025, from various medical facilities in Karbala. Clinical samples used included burns, blood, urine, ear swabs, and sputum. The VITEK-2 System (Biomerieux Company) verified the diagnosis, and identification and isolation were performed using microscopic, biochemical, and macroscopic methods. Antibiotic sensitivity testing for the Carpabenem class includes the following examples: To confirm the final AST results, ipenem and meropenem (Bulik *et al*., 2010).were obtained using the diffusion disc method (Kirby-Bauer method) and the VITEK-2 compact system. Genomic DNA was extracted from the isolates' bacterial growth using the ABIOpure Extraction technique. A Quantus fluorometer was used to measure the extracted DNA concentration in order to evaluate whether the samples may be used in further procedures. The Macrogen Company provided the lyophilised primers. The final concentration of the lyophilised primer solutions was 100 pmol/µl, and they were prepared with nuclease-free water. As show in Table1. Agarose gel electrophoresis was used to confirm amplification after PCR amplification. For PCR, the extracted DNA requirements were completely dependable (Halfmann and Lindquist, 2008).

Table 1: Primer of NDM Gene

Primer Name	Sequence	Annealing Temp. (oC)	Product Size
NDM-F	5`-TTTACTAGGCCTCGCATTTG-3`	60	945 bp
NDM-R	5`-GCCCAGCTTCGCATAAA-3`		

3. Results and Discussion

3.1. Identification and Isolation

The findings revealed that 50 isolates had *K. pneumoniae.* Physiological, chemical, microscopically, and cultural features were used to make the diagnosis. The Compact System VITEK-2 subsequently verified the diagnosis. As show in Table2 and Fig.1.

Table2: *K. pneumoniae* Isolates by Type and Quantity Identified by the VITEK-2 System

Samples	Male	Female	Total
Urine	6	12	18
Burn swab	9	6	15
Ear swab	2	1	3
Blood	4	1	5
Sputum	6	3	9
Total	27	23	50

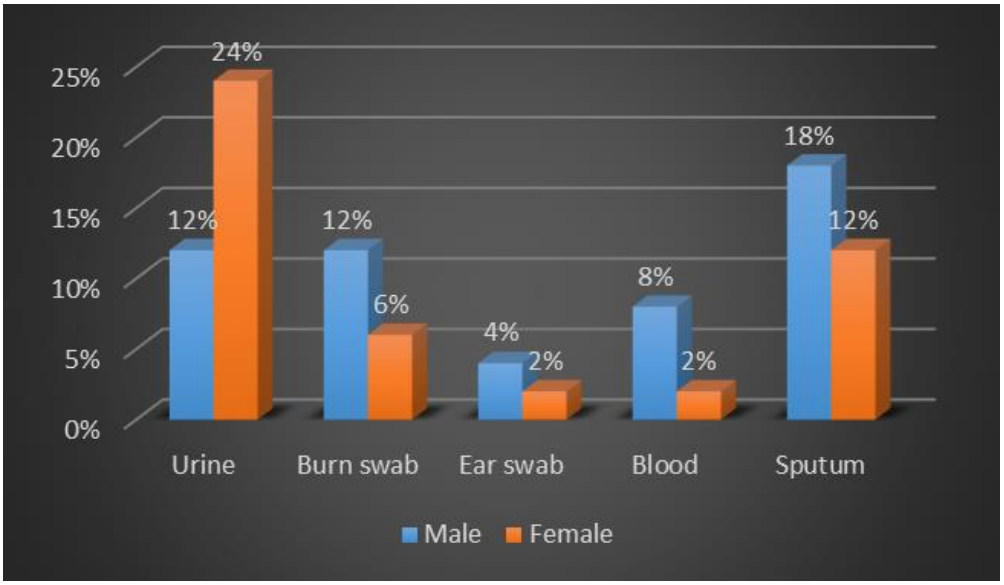

Figure1: The Percentage of Different Clinical Samples That Contained *Klebsiella Pneumoniae* Isolates

K. pneumoniae was more resistant to Imipenem 16 (32%) compared to Meropenem 20 (40%), according to the antibiotic sensitivity tests. However, 96% of patients developed imipenem and meropenem resistance, according to a recent study by (Alizadeh *et al*., 2020). This degree of resistance was consistent with another study carried out in Egypt found that 100% of patients had complete resistance to Imipenem and Meropenem (El-Kholy *et al*., 2024).

3.2. The VITEK-2 System for Testing for Antibiotic Sensitivity in *Klebsiella pneumoniae*:

Fifty strains that were recognized as *K. pneumoniae* by the Vitek 2 approach were used in this investigation. Two (4%) isolates were only resistant to imipenem, while one (2%) sample was only resistant to Meropenem, according to the AST values in VITEK-2. In contrast, 21 (42%) isolates shown resistance to both Meropenem and Ipenem. Ten isolates from fifty samples shown resistance to the carbapenem class of antibiotics in addition to eighteen other medications. The remaining isolates were resistant to at least ten drugs (i.e., XDR was extremely drug resistant). Ciprofloxacin, +Cefmetazole, +Cefixime, Minocycline, Ceftazidime + Amoxicillin/Clavulanic Acid, +Ticarcillin, +Amikacin, +Ticarcillin/Clavulanic Acid, and Trimethoprim/Sulfamethoxazole are some of these antibiotics. Of the isolates, thirty (60%) were resistant to amoxicillin/clavulanic acid, thirty (60%) to piperacillin/tazobactam, seventeen (34%) to gentamicin, and thirty-one (62%) to ticarcillin. Minocycline was 16 (32%), Ceftazidime was 44 (88%), Cefixime was 20 (40%), Ciprofloxacin was 21 (42%), and Cefmetazole was 23 (46%).23 (46%) to Imipenem, 21 (42%) to Meropenem, 34 (68%) to Ceftizoxime, 41 (82%) to Cefepime, 40 (80%) to Aztreonam, and 36 (72%). Based on resistive top, the samples' rates of resistance to Imepenem and Meropenem were as follows: Urine 6/21 (28.5%), sputum 8/21 (38.0%), ear swab 1/21 (4.76%), burn swab 5/21 (23.8%), and blood 1/21 (4.76%). According to a local study by Mohammad & Ismail (2020), 1/8 (12.5%) of ear swabs, 10/57 (17.5%) of sputum, 14/88 (15.9%) of urine, and 4/88 (4.5%) of burnt skin had antibiotic resistance in the carbapenem group. As shown in Table3, the samples' rates of Imepenem and Meropenem resistance based on resistive top were as follows: Blood 1/21 (4.76%), ear swab 1/21 (4.76%), burn swab 5/21 (23.8%), sputum 8/21 (38.0%), and urine 6/21 (28.5%). A local investigation by Mohammad & Ismail (2020) found that the carbapenem group had antibiotic resistance in 1/8 (12.5%) of ear swabs, 10/57 (17.5%) of sputum, 14/88 (15.9%) of urine, and 4/88 (4.5%) of burnt skin.

Table3: Results of Antibiotic Sensitivity Tests for The Carpabenem Families Using The VITEK-2 System and The Kirby-Bauer Method Are Contrasted.

Activity of Bacteria	Kirby-Bauer method	VITEK-2 System
Resistant to IMI	1	2
Resistant to MEM	5	0
Resistant to both	15	21
Sensitive to IMI	5	1
Sensitive to MEM	8	0
Sensitive to both	16	25
Interpretation to IMI	11	0
Interpretation to MEM	4	1
Interpretation to both	2	0

As seen fig.2, Compared to the Kirby-Bauer method (30%), the VITEK-2 System shows a higher rate of antibiotic resistance in the carbapenem group (42%). These findings were consistent with those of another study carried out in Italy by (Castellano et al., 2012). The VITEK-2 System discovered an 18.2% resistance rate to the carbapenem group of antibiotics in Pseudomonas aeruginosa, whereas the Kirby-Bauer method discovered a 15.6% resistance rate.

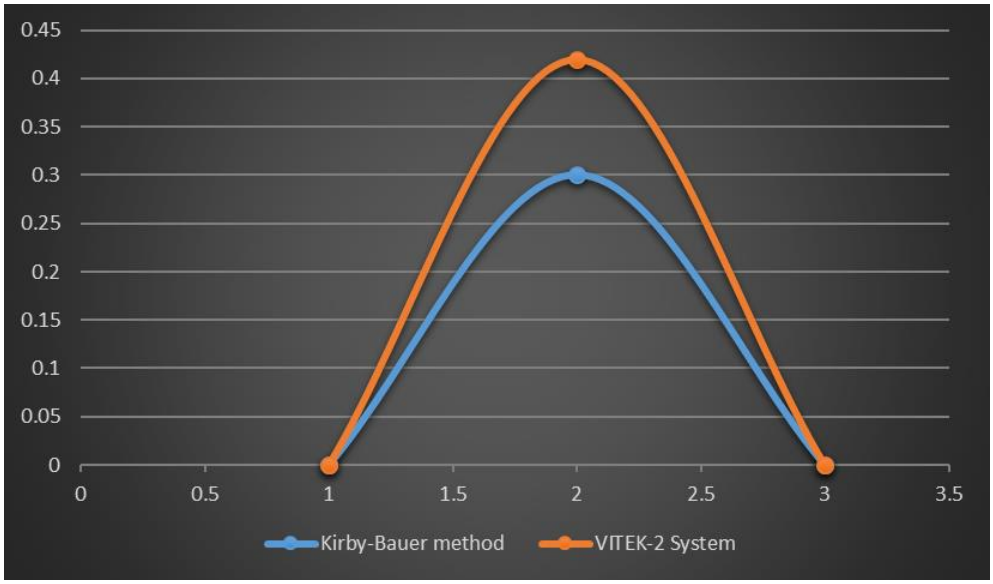

Figure2: The Kirby-Bauer methods and the VITEK-2 system's resistance in percentage

Therefore, the VITEK-2 System technique is recommended primarily due to its high specificity in detection resistance and the decrease in contamination rate. But the VITEK-2 System cuts down on time. While the Kirby-Bauer process can take up to two days, the VITEK-2 System can finish sensitivity testing in eighteen hours and diagnosis in eight after an overnight incubation time. The high cost of the VITEK-2 System is a disadvantage, but its pursuit of accurate outcomes is unaffected. Therefore, the high specificity of detection resistance and the decrease in contamination rate are the primary reasons the VITEK-2 System technique is recommended. On the other hand, the VITEK-2 System saves time. While the Kirby-Bauer process can take up to two days, the VITEK-2 System can finish sensitivity testing in eighteen hours and diagnosis in eight after an overnight incubation time. The VITEK-2 System's high cost is a disadvantage, but its pursuit of accurate outcomes is unaffected. DNA extraction and measurement from isolates of *Klebsiella pneumonia.* The genomic DNA of 21 *K. pneumoniae* isolates that showed carbapenem resistance was extracted using the ABIOpure Extraction method. DNA was taken in order to make a PCR template for amplification. The results showed that the reported DNA concentrations varied from 15 to 26 ng/µl. The quantity and quality of the recovered DNA are adequate for PCR amplification. The probability of non-specific PCR results increases with higher quantities of DNA template. The precision of amplification decreases with lower template levels. Based on the results of the gel electrophoresis analysis, the DNA samples were categorized as pure based on the presence of DNA bands.

3.2. PCR Product DNA Loading for NDM Detection

The DNA samples were analyses using gel electrophoresis, which produced DNA bands that represented pure DNA samples. As shown in Figure (3), only 11 (52.3%) of the isolates showed unique bands with the NDM gene. All isolates that formed separate bands with the NDM gene were resistant to both Meropenem and Imipenem in the Kirby-Bauer and VITEK-2 System methods, with the exception of the NO.149 isolate, which was resistant to both. The precision, accuracy, and low rate of integrity degradation of the DNA extraction and quantification processes were ensured by the bands' purity and clarity.

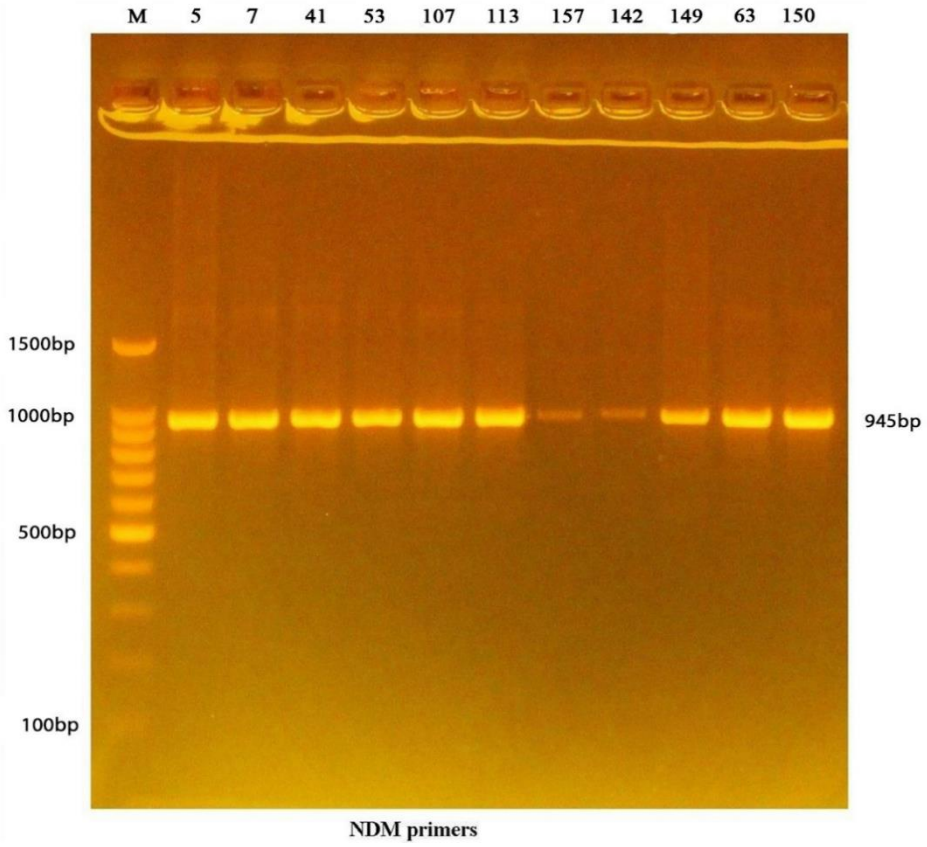

Figure3: Samples of *Klebsiella pneumoniae* with amplified NDM genes were fractionated using the Eth.Br. M: 1.5% agarose gel electrophoresis and a 100 F ladder marker were used. The 945 bp PCR results are comparable to lanes 5-150.

One of the carbapenemases that is currently most important for therapy in New Delhi, NDM-1, is the recently discovered Metallo-β lactamase. It belongs to the carbapenemase family. the Ambler B-lactamase class B, which also includes metallo-β-lactamases (MBLs). The highest similar amino acid identity, VIM-1/VIM-2, is only 32.4% similar to NDM-1 between several MBLs (Dortet et al., 2014). Septicemia, lung infections, urinary tract infections, soft tissue infections, peritonitis, and infections associated with devices are among the illnesses induced by NDM production, which were primarily detected in Enterobacteriaceae. The community's main routes of faecal transmission will be through contaminated food, water, and hands. Since other drug-resistant bacteria

have been found, it is very likely that NDM producers will colonise the gut flora prior to illness. The most commonly reported Enterobacteriaceae species that generate NDM-1 are *Escherichia coli* and *Klebsiella pneumoniae*. This carbapenemase is often found in other enter bacterial species, such as Salmonella, Providencia, Citrobacter freundii, Proteus mirabilis, Klebsiella oxytoca, and Enterobacter cloacae. *Acinetobacter* species have been shown to manufacture this carbapenemase, despite the fact that the majority of bacteria that produce NDM are members of the Enterobacteriaceae family (Bogaerts et al., 2012). in addition to the rare *Pseudomonas aeruginosa* (Flateau et al., 2012).NDM-producing bacteria have not been shown to be more hazardous than other types of bacteria. types since there are no known pathogenicity factors for plasmids. However, isolated cases of Salmonella (Cabanes *et al*., 2012).and Vibrio cholera (Walsh *et al*., 2011).two rare forms of aggressive enteric bacteria that generate NDM-1, have been documented.

Conclusion

This study revealed a considerable incidence of bacteria resistant to carbapenem. 42% of *Klebsiella pneumoniae* isolates from Karbala hospitals were resistant to imipenem and meropenem. More than half of these resistant bacteria had NDM genes, primarily NDM-1 and one NDM-5, indicating that treatment is challenging. The rise in NDM-5 is particularly worrisome. The results highlight the significance of good infection control, antimicrobial stewardship, and continuous monitoring. For quick identification, sophisticated diagnostic instruments like VITEK-2 were helpful. Future studies should look into the strains' genetic relatedness and possible gene transfer.

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