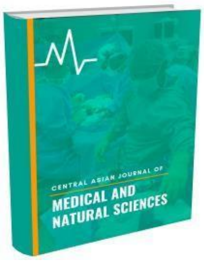

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Molecular Detection of Metallo-β-Lactamase (MBL) and Other Carbapenemase Genes and Their Resistance Patterns in *Klebsiella pneumoniae* Isolates from Hospitals in Diwaniyah, Iraq

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Abstract: *Klebsiella pneumoniae* is an ubiquitous opportunistic pathogen, responsible for infections in hospital and non-hospital settings. It is becoming increasingly well-known for its capacity to grow resistant to so many drugs, including carbapenems. The aim of the present study was to detect MBL and other carbapenemase genes, to further understand the variation in susceptibilities between *K. pneumoniae* strains obtained from hospitals in the Diwaniyah Governorate, Iraq.40 medical samples were taken between November 2025 and January 2026 from burns, wounds and urine. Of them, 20 represented unique *K. pneumoniae* tests. We used a PCR approach to detect the presence of the carbapenemase genes (VIM, NDM, KPC and SME) and the production of carbapenemase was determined by Modified Hodge Test (MHT). The findings showed a high degree of resistance to carbapenem antibiotics and the ability of 80% of the isolates to produce carbapenemase enzymes. Molecular analysis revealed that NDM was found in only one isolate (5%), while the VIM and SME genes were the most common (55% each), followed by KPC (50%). Multidrug resistance poses a serious threat, as evidenced by the coexistence of multiple carbapenemase genes in multiple isolates. To prevent carbapenem-resistant *K. pneumoniae* from spreading in healthcare settings, these findings highlight the critical need for ongoing molecular surveillance, stringent infection control protocols, and prudent antibiotic use.

Keywords: *Klebsiella pneumoniae,* metallo-β-lactamase, Carbapenemase Genes, Polymerase Chain Reaction and Modified Hodge Test

1. Introduction

Klebsiella pneumoniae is a member of the Enterobacteriaceae family and is one of the most important opportunistic pathogens causing both community-acquired and hospital-acquired infections. This bacterium can cause various types of infections related to human health, including meningitis, pneumonia, bacteremia, urinary tract infections, and infections at wound and burn sites. *Klebsiella pneumoniae* has developed a repeated pattern of antibiotic resistance, accompanied by an increased mortality rate, due to the widespread and random use of most antibiotic groups [1].

Recently, the dissemination of *Klebsiella pneumoniae* resistant to carbapenems has raised public health concerns. According to the Ambler classification, carbapenemase enzymes are categorized into classes A, B, and D based on their molecular properties. While classes A and D necessitate the amino acid serine at their active sites, class B utilizes zinc ions (Zn^{+2}) at its active site and is known as metallo-beta-lactamases [2].

Carbapenem resistance can arise through the acquisition of resistance genes that encode metallo-beta-lactamases (MBLs) or non-metallo carbapenemases (such as blaNDM, blaSME, blaKPC and blaVIM), alterations in outer membrane permeability (OMP), efflux pumps, or a combination of these mechanisms. Carbapenemase enzyme production is regarded as the primary resistance mechanism against carbapenem antibiotics [3].

For clinical infections caused by carbapenem-resistant *Klebsiella pneumoniae*, treatment options are very limited. The antibiotic colistin is the last resort in treating these isolates. A surveillance study in the UK clearly revealed that colistin was the only drug that remained effective against more than 90% of carbapenem-resistant Klebsiella pneumoniae isolates associated with diverse resistance patterns [4].

In particular, the presence of plasmid-mediated carbapenem-resistant *K. pneumoniae* in Diwaniyah, its antibiotic resistance pattern and spread among local hospitals are a public health concern overall. This is due to the fact that these resistance genes can be easily transferred to other bacteria [5].

2. Materials and Methods

2.1. Sample collection

The study included a variety of clinical samples from hospitalised patients of diverse ages and genders, sourced from different body sites, including wounds, burns, and urine. From November 2025 to January 2026, 40 samples were taken from different hospitals and health centers in the Diwaniyah Governorate. These included the Women's and Children's Hospital, the Diwaniyah General Teaching Hospital, and the Public Health Laboratory.

2.2 Isolation and identification

Swabs and samples were sent straight to the lab so that the case could be diagnosed based on its cultural, microscopic, and biochemical traits. They were then cultured for 24 hours at 37°C in aerobic conditions. Culture media were used to isolate bacterial species, and a variety of biochemical tests (catalase, oxidase, IMVC, and TSI) were done along with looking at the isolates' morphological and microscopic traits on the plate. The Vitek2 Compact test, along with a previous study, has shown that it is possible to accurately diagnose *Klebsiella pneumoniae* isolates.

2.3 Modified Hodge Test (MHT)

The standard bacterial isolate E. coli ATCC 25922 was used for the Hodge test. A saline solution was used to make a bacterial suspension, which was then diluted to a 1:10 ratio after its turbidity was changed to match the 0.5 McFarland standard. After that, the standardised suspension was swabbed onto a Mueller-Hinton agar plate and left there for 10 minutes to soak up the moisture. In the middle of the plate, there was a 10 μg Meropenem disc. The test isolates were streaked in a straight line from the edge of the disc to the edge of the plate, making sure that the line was at least 20 mm long. The plate was incubated at 37°C for 24 hours. After incubation, the formation of a cloverleaf-like indentation indicated a positive test result, confirming the production of metallo-β-lactamase enzymes.

Table 1. Primers used in this study, with their nucleotide sequence and amplification size.

Gene name		sequence (5'-3') (primer)	Product Size (bp)	Reference
KPC	F	ATG TCA CTG TAT CGC CGT CT	893 bp	Hatrongjit *et al.*, 2023 [6]
	R	TTT TCA GAG CCT TAC TGC CC		
NDM	F	GGT TTG GCG ATC TGG TTT TC	621 bp	

	R	CGG AAT GGC TCA TCA CGA TC		Yuan et al.,2024 [7]
VIM	F	GAT GGT GTT TGG TCG CAT A	690 bp	Sisay et al.,2025 [8]
	R	CGA ATG CGC AGC ACC AG		
SME	F	ACT TTG ATG GGA GGAT TGG C	551 bp	Lian *et al.,* 2024 [9]
	R	ACG AAT TCG AGA TCA CCA G		

2.4 Genomic DNA extraction

Nucleic acid extraction from Klebsiella pneumoniae was performed using Geneaid USA's pre-made Genomic DNA Mini Kit, following the provided instructions.

2.5 Preparation of Agarose Gel

It was prepared using the method developed by Sambrook and his group, as follows:

a. Employing a magnetic hot plate stirrer, mix 1.5 g of agarose gel into 100 ml of TBE buffer solution at a (1X) concentration for 15 minutes.
b. After the gel had cooled to 50°C, three microliters of the radioactive DNA stain ethidium bromide were incorporated into it and mixed thoroughly.
c. The acro gel was poured into the migration mold (the tray with the comb) and allowed to set for fifteen minutes at room temperature. Next, the comb was carefully removed from the gel to create and define the holes (wells) necessary for injecting the amplified samples.

2.6 PCR Master Mix Preparation

1. Prepare this mixture in PCR tubes, as instructed by the company that supplied the Wizpure PCR FDmix and as shown in Table 2 below:

Table 2. Components of the PCR master mix.

Mixture Ingredients	Size
PCR FD mix	1Tube
10mM Forward Primer	1.5 μL
10mM Reverse Primer	1.5 μL
Template DNA	5 μL
PCR water	12 μL
Total	20 μL

2. After the mixture tubes were prepared, they were placed in the Vortex machine for five seconds. The tubes were subsequently placed in the PCR thermocycler machine to perform the DNA amplification process under optimal thermal cycle conditions. 2

3. The program employs heat cycles for DNA amplification. The genes under examination were analyzed with a thermocycler PCR apparatus that was configured for this purpose.

3. Results and Discussion

3.1 Numbers and percentages of *Klebsiella pneumoniae* isolation

Bacterial isolates were obtained from 20 out of 40 clinical samples, which means the overall isolation rate was 50%. *Klebsiella pneumoniae* had the highest isolation rate from urine samples at 60%, followed by burn injury samples at 30%, and wound samples had the lowest rate at 10%. The findings are consistent with previous studies in part, while they differ from them in certain respects. As an example, our results align with those of [10], who noted that *Klebsiella pneumoniae* was one of the most frequently isolated pathogens in urinary tract infections, with isolation rates between 55% and 65%. In a similar vein, [11] identified *Klebsiella pneumoniae* as often isolated from infections in burn wounds, noting rates of about 30–35% that are akin to those seen in our samples of burn injuries. Nonetheless, the study's findings reveal a low isolation rate in wound samples that contrasts with those of earlier studies. [12] recorded isolation rates of over 40% for wound swabs, especially for *Klebsiella pneumoniae*, which was found to be significantly higher. These discrepancies may be attributed to variations in sampling methodologies, hospital infection control protocols, patient population demographics, and regional microbial epidemiology. Moreover, the variation in outcomes may be attributed to disparities in healthcare settings, antibiotic prescribing practices, and environmental factors affecting microbial colonization. Variations in the instruments and models employed for laboratory diagnosis, encompassing the sensitivity and selectivity of testing methods, may significantly elucidate these discrepancies.

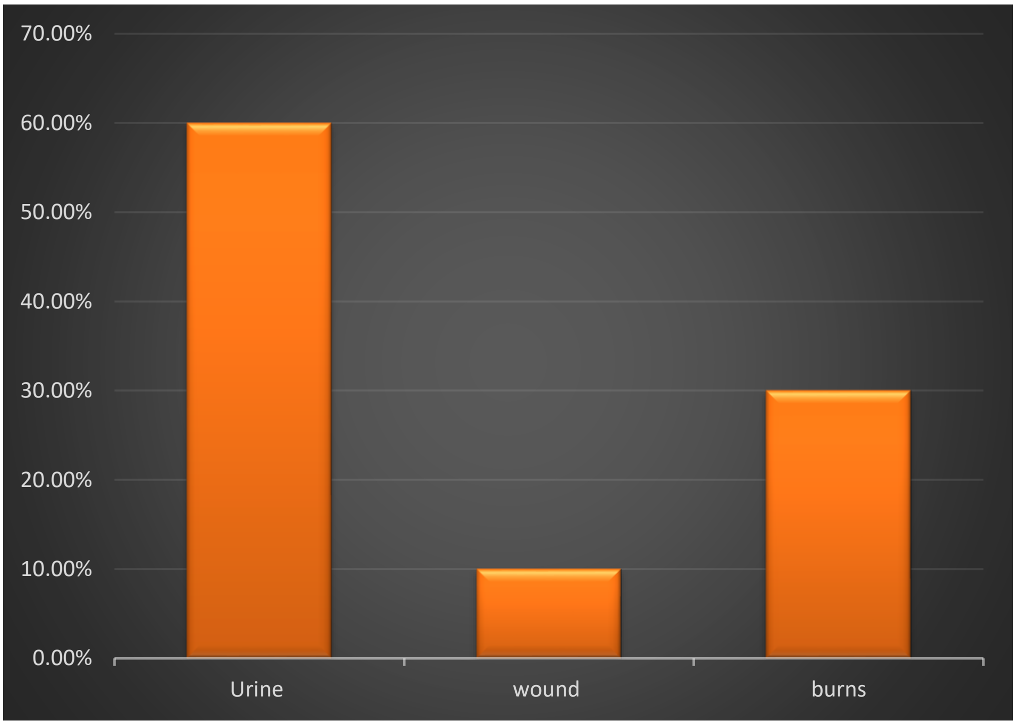

Figure 1. Distribution of *Klebsiella pneumoniae* isolates according to sample source.

The findings demonstrated that 16 of the 20 isolates (80%) had the capability of generating carbapenem enzymes and that the bacteria exhibited resistance to certain antibiotics, including imipenem and meropenem, which have a beta-lactam ring in their structure.

The Vitek device results indicated that most isolates exhibited resistance to the examined antibiotics, with a resistance rate of 100%. However, tigecycline was effective against the bacteria, as its sensitivity percentage ranged from 0.3% to 1%.

The proportion of carbapenemase production in *Klebsiella pneumoniae* isolates from various sources is illustrated in Table 3. Carbapenemase producers accounted for 90% of the isolates, while non-producers made up 10%. Out of these isolates, one was from burns, and one was from urine; the latter did not generate carbapenemases. Based on the Modified Hodge Test (MHT).

Table 3. Ability of *K. pneumoniae* isolates to produce Carbapenemase enzyme according source isolated.

Ability to produce Carbapenemase enzymes	Burn	Wound	Urine	Percentage %
Producing isolate	6	1	11	90%
Non-producing isolate	0	1	1	10%

3.2. Molecular diagnosis of *K. pneumoniae* through polymerase chain reaction

Twenty samples containing two metallo-β-lactamase (MBL) genes, NDM and VIM, and two non-metallo-β-lactamase genes, KPC and SME, were examined using polymerase chain reaction (PCR) to detect their production by *Klebsiella pneumoniae*. It was found that 11 out of 20 samples produced both the VIM and SME genes (55%), while 10 out of 20 produced the KPC gene. Only one isolate was able to produce the NDM gene. The results of amplification by PCR for these genes are displayed in Figures (2, 3, 4, and 5)

The results of this study revealed a high prevalence of carbapenemase genes among the *Klebsiella pneumoniae* isolates examined, reflecting an increasing risk of antibiotic resistance, particularly to carbapenems, which are considered last-line treatments. The KPC gene was found in 10 isolates (50%), and the VIM and SME genes were found in 11 out of 20 isolates (55%). The NDM gene was the least common, showing up in only one isolate (5%). The high prevalence of the VIM gene is an important discovery because research shows that metallo-β-lactamases, especially VIM, can break down a wide range of beta-lactam antibiotics, including carbapenems, but there aren't many good ways to treat them. Several studies have shown that the VIM gene is present in clinical K. pneumoniae isolates, especially in hospitals. The prevalence of this gene varies a lot depending on where you are and what treatment policies are in place [13],[14]. The co-detection of the SME gene with VIM in a high percentage of isolates suggests the potential for multiple resistance mechanisms to accumulate within a single isolate. Although the SME gene is less common than genes such as KPC and NDM, its presence may contribute to enhanced carbapenem resistance and further complicate the genetic profile of resistance. Some studies have indicated that carrying more than one carbapenemase gene in the same isolate reflects horizontal gene transfer via plasmids and mobile genetic elements, which increases the risk of resistance spreading within hospitals [15], [16] . The KPC gene showed a relatively high prevalence in this study (50%), consistent with numerous international studies that have confirmed KPC as one of the most prevalent carbapenemase genes in *K. pneumoniae*. This gene is closely associated with outbreaks of nosocomial infections and high rates of treatment failure due to its ability to confer widespread resistance to beta-lactam antibiotics and other antibiotics [17], [18]. The NDM gene, on the other hand, was found in only one isolate, which is a lot less common. Even though it is found all over the world, especially in South Asia and parts of the Middle East, many studies have shown that its prevalence varies greatly from region to region. In some clinical settings, the gene is very low or even absent, while other genes like KPC and VIM are much more common [19], [20]. The difference could be because of other sources of infection or because this gene doesn't spread as easily in the area where the study was done. In this study, the *Klebsiella pneumoniae* isolates analysed were predominantly resistant to carbapenems due to the VIM and KPC genes, while a minor role was played by the NDM gene. The finding of multipleresistance genes in such a great number of isolates is a bad clinical indicator since it directly contributes to the failure of therapy and morbidity/mortality figures. These findings also demonstrate the importance of improve molecular surveillance programs for antibiotic resistance, encourage the smart use of antibiotics, and strengthen infection control measures in healthcare settings.

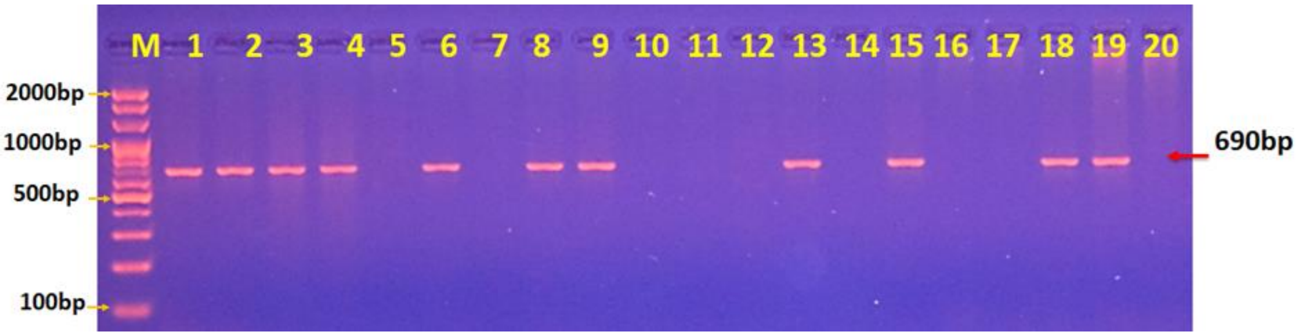

Figure 2. PCR product of the VIM gene (690 bp). Line (M): DNA ladder 2000 base-pairs. Lines (1,2,3,4,6,8,9,13,15,18 and 19): Positive result of the VIM gene.

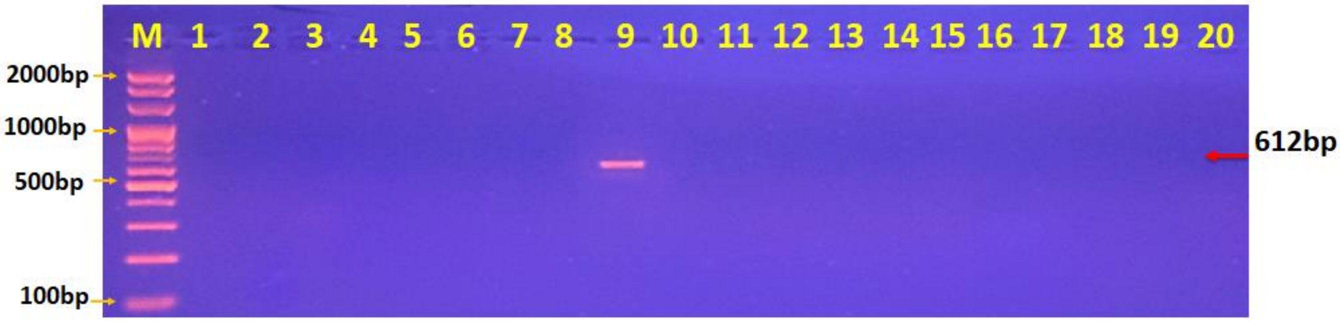

Figure 3. PCR product of the NDM gene (612 bp). Line (M): DNA ladder 2000 base-pairs. Lines (9): Positive result of the NDM gene.

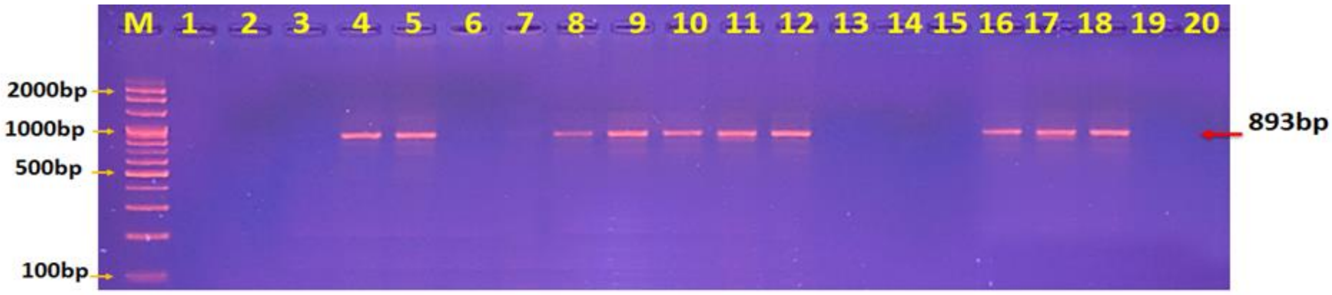

Figure 4. PCR product of the KPC gene (893bp). Line (M): DNA ladder 2000 base-pairs. Lines (4,5,8,9,10,11,12,16,17 and 18): Positive result of the KPC gene.

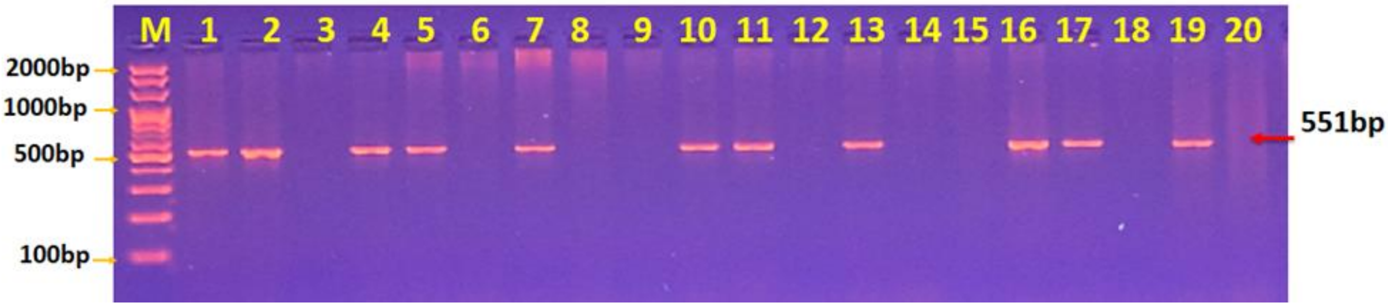

Figure 5. PCR product of the SME gene (551bp). Line (M): DNA ladder 2000 base-pairs. Lines (1,2,4,5,7,10,11,13,16,17 and 19): Positive result of the SME gene.

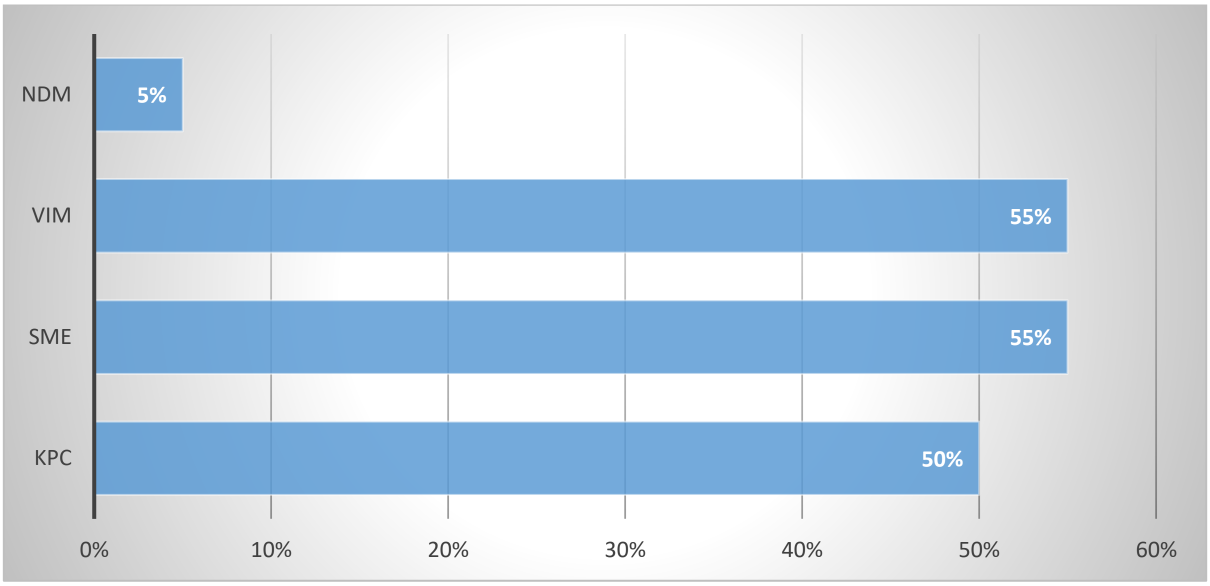

Figure 6. The percentage of gene prevalence in *Klebsiella pneumoniae* bacteria under study.

4. Conclusion

The present research shows that amongst clinical specimens taken from hospitals in the Diwaniyah province, carbapenem-resistant *Klebsiella pneumoniae* is very common. A majority of isolates produced carbapenemase, and molecular examination confirmed that significant resistance genes, such as VIM, SME, and KPC, were widely present. Multiple carbapenemase genes found in the same isolates indicate that resistance determinants are actively propagating, most likely by plasmids and other mobile genetic elements. This situation makes therapy much less likely to work, and it also makes it more likely that therapy will fail and that outbreaks will happen in the facility. The study shows that regular biological testing of antibiotic resistance is necessary to stop the spread of carbapenem-resistant *K. pneumoniae* and keep last-line antimicrobial drugs effective. It also calls for stronger prevention and treatment measures and the careful use of antimicrobial drugs.

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