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Detection of blaVIM-M and blBbKPC genes in *Streptococcus pneumoniae* isolated from sputum samples in Iraqi patients

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Abstract

Streptococcus pneumoniae (pneumococcus) is one of the leading causes of community-acquired pneumonia, as well as a major cause of morbidity and mortality around the globe. Of these carbapenemase genes, blaVIM and blaKPC are of great clinical significance and have been frequently described in various Gram-negative pathogens, whereas little work has been done on pneumococcal isolates. The aim of the present study was to detect the presence of blaVIM and blaKPC genes in S. pneumoniae isolated from sputum samples of Iraqi patients suffering pneumonia and confirm the association of these genes with antimicrobial resistance patterns. This cross-sectional study was conducted in Al-Sadr Medical City Hospital, Najaf, Iraq, extending from June 2024 to January 2025. We included 96 patients with clinical diagnosis of pneumonia. Sputum specimens were collected in sterile containers and then cultured according to standard microbiological procedures. Identification of S. pneumoniae was based on the basis of colony morphology, gram staining and biochemical tests. The antimicrobial susceptibility testing against ceftriaxone, ciprofloxacin, gentamicin, imipenem, and meropenem was performed by the Kirby–Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Conventional polymerase chain reaction (PCR) was used for the molecular detection of blaVIM and blaKPC genes. Antimicrobial resistance rates to the tested antibiotics were variable, but they were highest for gentamicin and ciprofloxacin, and the carbapenems retained relatively higher activity. Molecular analysis indicated that blaVIM and blaKPC genes were found in 11.5% and 7.3% of the isolates, respectively. The presence of both genes was significantly associated with resistance to imipenem and meropenem ($p<0.001$). On the other hand, the non-β-lactam resistance showed no significant correlation with the carbapenemase gene carriage. These findings highlight the need for ongoing molecular surveillance, rational use of antibiotics and improved infection control measures to contain the spread of carbapenem resistance from one respiratory pathogen to another..

Keywords: blaVIM and blaKPC, Streptococcus pneumoniae, pneumonia, Carbapenemase

الملخص

تُعد بكتيريا **Streptococcus pneumoniae** (المكورات الرئوية) من الأسباب الرئيسية لذات الرئة المكتسبة من المجتمع، كما تمثل سببًا مهمًا للمراضة والوفيات على مستوى العالم. ومن بين جينات إنزيمات الكاربابينيميز، يُعد الجينان **blaVIM** و **blaKPC** من الجينات ذات الأهمية السريرية الكبيرة، إذ تم الإبلاغ عنهما بشكل متكرر في العديد من الممرضات سالبة الغرام، في حين أُجريت دراسات محدودة حول وجودهما في عزلات المكورات الرئوية. هدفت الدراسة الحالية إلى الكشف عن وجود جيني **blaVIM** و**blaKPC** في بكتيريا المكورات الرئوية المعزولة من عينات القشع لمرضى عراقيين يعانون من ذات الرئة، وكذلك تأكيد ارتباط هذه الجينات بأنماط المقاومة للمضادات الحيوية.

أُجريت هذه الدراسة المقطعية في مستشفى مدينة الصدر الطبية في النجف، العراق، خلال الفترة من حزيران 2024 إلى كانون الثاني 2025، وشملت 96 مريضًا مشخصين سريريًا بذات الرئة. جُمعت عينات القشع في حاويات معقمة، ثم زُرعت وفق الإجراءات الميكروبيولوجية القياسية. تم تشخيص بكتيريا المكورات الرئوية اعتمادًا على شكل المستعمرات، وصبغة غرام، والاختبارات الكيميائية الحيوية. أُجري اختبار الحساسية للمضادات الحيوية ضد كل من السيفترياكسون، والسيبروفلوكساسين، والجنتاميسين، والإيميبينيم، والميروبينيم باستخدام طريقة الانتشار بالأقراص (كيربي–باور) وفق إرشادات معهد المعايير السريرية والمخبرية **(CLSI).** كما استُخدم تفاعل البلمرة المتسلسل التقليدي **(PCR)** للكشف الجزيئي عن جيني **blaVIM** و**blaKPC.**

أظهرت معدلات المقاومة للمضادات الحيوية المفحوصة تباينًا ملحوظًا، وكانت الأعلى تجاه الجنتاميسين والسيبروفلوكساسين، في حين احتفظت مجموعة الكاربابينيمات بنشاط نسبي أعلى. وأوضح التحليل الجزيئي أن جيني **blaVIM** و **blaKPC** وُجدا بنسبة 11.5% و7.3% من العزلات على التوالي. وكان وجود كلا الجينين مرتبطًا ارتباطًا معنويًا بمقاومة الإيميبينيم والميروبينيم **($p<0.001$)**. في المقابل، لم تُظهر المقاومة للمضادات غير التابعة لمجموعة البيتا-لاكتام ارتباطًا معنويًا مع حمل جينات الكاربابينيميز. تؤكد هذه النتائج على ضرورة الاستمرار في المراقبة الجزيئية، والترشيد في استخدام المضادات الحيوية، وتعزيز إجراءات مكافحة العدوى للحد من انتشار مقاومة الكاربابينيمات بين مسببات الأمراض التنفسية..

الكلمات المفتاحية: blaVIM و blaKPC، **المكورات الرئوية، الالتهاب الرئوي، إنزيمات الكاربابينيم**

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Introduction

Streptococcus pneumoniae is the major cause of community acquired pneumonia, meningitis, bacteremia and other invasive infections worldwide with those occurring particularly in young children and elderly or immuno-compromised adults (Dion and Ashurst, 2025). In addition to high morbidity and mortality, *S. pneumoniae* is now a threat due to the emergence of resistance to antimicrobials. *S. pneumoniae* resistance has generally been associated with changes in penicillin-binding proteins (PBPs) reducing susceptibility to β-lactams and macrolide resistance due to erm(B) or mef(A) genes. These inherent and acquired mechanisms have resulted in an extensive decline of first-line antibiotics (Mohanty et al., 2023). In contrast to these established patterns, there has also been limited documentation of the possible appearance of plasmid-encoded carbapenemase-encoding genes (e.g., blaVIM and blaKPC) in S. pneumoniae strains, which would constitute a new and disconcerting potential expansion in the spectrum of drug resistance means should this occur (Göpel et al., 2025).

Carbapenems are one of the last treatment options for severe infections from multidrug-resistant (MDR) Gram-negative strains. Carbapenemase genes (blaKPC – *Klebsiella pneumoniae* carbapenemase) are the most significant carbapenem resistance mechanisms encountered amongst members of the Enterobacteriaceae and non-fermenting bacteria, e.g., *Klebsiella pneumoniae* and *P. aeruginosa* (Yu et al., 2019). These genes are responsible for enzymes that can hydrolyze almost all β-lactam drugs, greatly constraining the choice of treatments. For instance, surveillance studies of global resistance patterns also reveal that blaKPC is one of the most steadily emerging carbapenemase genes in clinical strains from Gram-negative species and it usually appears combined with other resistance determinants (Ghasemnejad et al., 2019). The blaVIM gene, although infrequent, is most often seen in association with other MBL genes in Gram-negative isolates. The spread of these plasmid-mediated carbapenemases is driven by the horizontal gene transfer between different species and typically mediated by mobile genetic elements including transposons or integrons (Tato et al., 2010).

Although *S. pneumoniae* is a Gram-positive species, and carbapenemase genes have traditionally been associated with Gram-negative bacteria, there is now 16 evidence to support the possible horizontal transfer of resistance determinants between enterococci and other genera (Sun et al., 2023). This has not been extensively demonstrated for *S. pneumoniae*, yet this possibility deserves consideration, especially in areas with high antibiotic pressure and prevalent circulation of resistant strains. Transfer of genes from Gram-negative to Gram-positive bacteria has been also described elsewhere, for instance, plasmid-mediated vancomycin resistant gene (vanA) transferred from *Enterococci* to *Staphylococcus aureus* and resulted in the emergence of vancomycin resistant *S. aureus* (VRSA). These observations highlight the possibility of unanticipated gene transfers when bacteria are placed under selective pressure. Thus, studying the prevalence of blaKPC and blaVIM in *S. pneumoniae* from clinical samples fills a knowledge gap that may identify new resistance patterns with impact on patient care (Cillóniz et al., 2018).

The importance of carbapenemase genes is that they may decrease the susceptibility to life-saving antibiotics. Detection of the blaKPC in Enterobacteriaceae including *K. pneumoniae* has been correlated with high resistance to carbapenem, failure of therapy and mortality

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among hospitalized patients (Yu et al., 2019). In a study from southern Saudi Arabia, blaVIM was absent but blaKPC was detected in sputum and blood isolates of MDREs *K. pneumoniae*, indicating that the presence of carbapenemase genes may further complicate treatment of respiratory infections. Another frequent observation in clinical isolates is the co-occurrence of blaKPC and blaVIM a characteristic associated with VRE strains that are part of epidemic clones clonal, and the MGEs. These data reinforce the importance of careful monitoring when evaluating resistance genes outside classical species limits (Paul et al., 2015).

The burden of healthcare cost due to carbapenem resistance is significant, especially in low- and middle-income countries where access to newer or alternative antibiotics and infection control resources may be lacking, hence promoting the spread of MDR organisms. Previous molecular surveillance in Iraq found a high prevalence of carbapenem-resistant Gram-negative organisms in clinical isolates, including broad distribution of the following carbapenemase genes: blaKPC, blaNDM, and blaOXA present in *K. pneumoniae* and other pathogens (Salih et al., 2022). These are environments in which the selective pressure for resistance increases, and it is within such sorts of reservoirs that plasmid mediated genes could potentially be picked up by other species through these mobile elements. Validating whether such gene exchange has taken place in *S. pneumoniae* would be vital for understanding the resistance trends that are emerging and to help with antibiotic stewardship initiatives.

This study was designed to investigate the presence of blaVIM and blaKPC genes in *S. pneumoniae* isolates recovered from sputum specimens of Iraqi patients suffering from respiratory tract infections. This study does not only add to the current knowledge of molecular epidemiology of resistance in an essential human pathogen, but suggests new possible gene acquisition in Gram-positive bacteria. The detection of these genes would have clinical implications for *S. pneumoniae* that include patient management, infection control and prescription policy in areas where MDR bacteria are endemic.

Patients and Methods

Study Design and Setting

This is a cross-sectional study was carried out in Al-Sadr Medical City Hospital, Najaf city, Iraq from June of 2024 till the beginning of January 2025. We included a total of 96 patients who presented to the hospital with pneumonia and were diagnosed solely based on clinical presentation, chest radiological examination and laboratory tests. Inpatients aged between 18 and 75 years, admitted to respiratory or internal medicine wards within the study period composed study population (both men and women). The study only included patients with microbiological diagnosis of *Streptococcus pneumoniae* from sputum. Patients who had received systemic antibiotics during the 72-h preceding collection of the specimens and patients suffering from underlying chronic illnesses, such as diabetes mellitus, chronic renal disease (CRD), malignancy or immunocompromised disorders, were excluded in order to control any confounding effect on bacterial yield and resistance patterns.

Sample collection and bacterial isolation

The expectorated sputum samples were obtained aseptically from all patients using sterile wide-mouth containers before commencing any treatment as per standard

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guidelines for collection of sputum, early in the morning. Samples of good quality (low epithelial cells and a high polymorphonuclear lecukocyte count under microscopic examination) were prepared immediately at the bacteriology laboratory in Al-Sadr Medical City.

Sputum samples were cultured on blood agar (5% sheep blood) and chocolate agar plates (Oxoid Ltd., UK) at 37 °C in 5% CO_2 from between 24 to 48 hours. Alpha-hemolytic, Gram-positive and lancet – shaped diplococci which showed optochin sensitivity and undergoing the bile solubility test were presumed *S. pneumoniae* colonies. The frozen culture was stored at -80 °C for further molecular studies. The reference strain used for quality control was *S. pneumoniae* ATCC® 49619™.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar enriched with 5% sheep blood, which complied with guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). The tested antibiotics belonged to different antimicrobial classes frequently used for respiratory infections:

1. Penicillin (10 units)
2. Ceftriaxone (30 µg)
3. Erythromycin (15 µg)
4. Levofloxacin (5 µg)
5. Imipenem (10 µg)
6. Meropenem (10 µg)

Inhibition zones were recorded and interpreted as susceptible, intermediate or resistant using CLSI breakpoints after an overnight incubation at 37 °C with 5% CO_2. Multi-drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012). Quality control: *S. pneumoniae* ATCC® 49619™ was incorporated for quality control.

DNA extraction

All confirmed *S. pneumoniae* isolates were subjected to DNA extraction using GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's guidance. The concentration and purity of DNA were measured using spectrophotometry, and the extracted DNA was preserved at –20 °C until additional molecular analysis.

Molecular Detection Of Blavim And Blakpc Genes

Conventional PCR method and previously published primers were used for the detection of carbapenemase genes (blaVIM and blaKPC) on all confirmed S. pneumonia isolates. The PCR reaction system was performed in a final volume of 25 µL, comprising 12.5 µL 2× PCR Master Mix (Promega, USA), 1 µL (10 pmol/µL) of each the forward and reverse primers, 3 µL template DNA and nuclease-free water up to the total volume. PCR amplification was carried out in a thermal cycler (Eppendorf Mastercycler, Germany), as follows:

1. 5 min at 94 °C for denaturation.
2. 35 cycles of:
3. Conditioned at 94°C for 30 s
4. Annealing at 55 °C for 40 s
5. Extension at 72 °C for 45 s
6. Extension Final a 72 °C for 7 min

PCR products were separated on the 1.5% agarose gels containing ethidium bromide (0.5 µg/mL), and visualised under UV light using gel documentation system (Bio-Rad, USA). Target gene presence (390 bp and 798 bp) was established by a positive identification of

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amplicon size. Positive controls were performed using non-type strains known for their ability to produce carbapenemase-mediated blaVIM and blaKPC, and nuclease free water was included as a negative control. The primers' sequences, annealing temperatures and expected amplicon sizes for the molecular detection of blaVIM and blaKPC carbapenemase genes are displayed in Table 1.

Ethical considerations

The study proposal was consented from the Ethical Committee of University of Kufa / Al-Sadr Medical City Hospital. All patients signed written consent forms (informed consent) to participate and information collected from them was kept confidential.

Table1: List of primers applied for amplification of bla_{VIM} and bla_{KPC} genes

Gene	Amplicon size (bp)	Annealing temp (°C)	Primer sequence (5′–3′)
bla_{VIM}	390 bp	55	F: GTTTGGTCGCATATCGCAAC R: AATGCGCAGCACCAGGATAG
bla_{KPC}	798 bp	55	F: ATGTCACTGTATCGCCGTCT R: TTTTCAGAGCCTTACTGCCC

Results

Table 2 shows diversity in resistance of *Streptococcus pneumoniae* isolates to commonly used antibiotics. Relatively higher rates of resistance were seen with gentamicin and ciprofloxacin, indicating loss of efficacy of these agents in the management of pneumococcal pneumonia. On the other hand, carbapenems (i.e. imipenem and meropenem) and ceftriaxone still exhibited reasonable susceptibility but the prevalence of non-susceptible isolates is a cause for concern. These observations highlight the need for ongoing antimicrobial susceptibility testing and justify molecular detection of carbapenemase genes as an early indicator of new patterns of resistance (Table 2).

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Table 2. Rates of antibiotic resistance reported in the *Streptococcus pneumoniae* isolates

Groups	Resistant Isolates No. (%)	Susceptible Isolates No. (%)
Ceftriaxone	28 (29.2%)	68 (70.8%)
Meropenem	14 (14.6%)	82 (85.4%)
Ciprofloxacin	36 (37.5%)	60 (62.5%)
Gentamicin	42 (43.8%)	54 (56.2%)
Imipenem	12 (12.5%)	84 (87.5%)

As shown in Table 3, very few *S. pneumoniae* isolates were found to harbor carbapenemase (blaKPC was less common than blaVIM). While the prevalence was low overall, the identification of these genes in a traditionally Gram +ve pathogen raised concerns on possible trend towards resistance or transfer events. These results illustrate the need for ongoing molecular surveillance to identify the dissemination of clinically relevant resistance determinants (Table 3).

Table 3. Frequency and percentage of bla_{VIM} and bla_{KPC} genes in *Streptococcus pneumoniae* isolates

Groups	Positive No. (%)	Negative No. (%)
bla_{VIM}	**11 (11.5%)**	**85 (88.5%)**
bla_{KPC}	**7 (7.3%)**	**89 (92.7%)**

As revealed from Table 4, carbapenem resistance (imipenem and meropenem) was significantly correlated with blaVIM and blaKPC genes in the isolates tested suggesting a relatively high genetic component of carbapenem resistance among the *Streptococcus pneumoniae* isolates. Resistance to ciprofloxacin and gentamicin, however was not significantly associated with these genes, indicating that non-β-lactam resistance may be mediated through different mechanisms. The strong correlation of CTX could be due to global β-lactam resistance patterns or co-selection of resistance genes. In general, these data favour the involvement of blaVIM and blaKPC as relevant molecular markers associated with low susceptibility to the carbapenems.

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Table 4. Association between antibiotic resistance and Presence of bla_{VIM} and bla_{KPC} genes in *Streptococcus pneumoniae* isolates

Groups	bla_{VIM} Chi Square (P value)	bla_{KPC} Chi Square (P value)
Ceftriaxone	6.12 (0.013)*	4.89 (0.027)*
Meropenem	12.84 (0.0003)**	10.26 (0.0013)**
Ciprofloxacin	2.41 (0.12) NS	1.96 (0.16) NS
Gentamicin	3.02 (0.08) NS	2.67 (0.10) NS
Imipenem	14.57 (0.0001)**	11.33 (0.0008)**

*** Significant at P<0.05 ; ** High significant at P<0.001**

Discussion

The carbapenemase genes blaVIM and blaKPC on a Plasmid of *Streptococcus pneumoniae* were recovered from pneumonia patients. Although *S. pneumoniae* is considered susceptible to carbapenems, including imipenem and meropenem, the existence of carbapenemase gene(s) implies that the existing model of relative carbapenem resistance among Gram-positive species may be changing. Conclusion: Carbapenem antibiotics are physicians' options of last resort β-lactam antibiotics against certain types of severe infections caused by Gram-positive and Gram-negative pathogens as they have the broadest spectrum of activity of all β-lactams and hence they are highly effective antibiotics; however, due to their widespread and inappropriate use, resistance mechanisms to carba-nems have quickly developed and disseminated globally (Aurilio et al., 2022).

Antimicrobial resistance in *S. pneumoniae*, particularly for β-lactams, macrolides, and fluoroquinolones has been extensively documented and presents a major clinical problem. Global antimicrobial resistance surveillance data also reported that *S. pneumoniae* has developed resistance to multiple antibiotics, complicating the management of pneumonia and increasing the risk of treatment failure. Carbapenem resistance seems to be more infrequent in S. pneumoniae than in Gram-negative organisms, but case reports and small 244 series describe infections with carbapenem-resistant pneumococci frequently in the setting of multidrug 245 resistance and treatment challenge (Doi et al, 2014).

Broad-spectrum β-lactamases, in particular, carbapenemase genes including blaVIM and blaKPC, are recognized as key resistance determinants in Gram-negative bacteria including *Klebsiella pneumoniae* and other Enterobacteriaceae. The blaVIM and blaKPC have been found at high frequencies in clinical

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populations of *K. pneumoniae*; imports as high as 32.8% for blaVIM, and 35.9% of carbapenemase-producing isolates being blaKPC from Vietnam. In West Africa, a meta-analysis additionally described widescale circulation of the carbapenemase genes blaKPC and blaVIM among Gram-negative pathogens at varying prevalence (Ugbo et al., 2025).

The comparatively lower prevalence of blaVIM and blaKPC in *S. pneumoniae* vs Gram-negative bacteria may be a reflection of biological difference among organisms. While some Gram-negative pathogens have an outer membrane, preventing the uptake and horizontal transfer of large plasmids carrying the genes for a carbapenemase, Gram-positive organisms have the distinct cell wall structure as well as lack of outer membrane common to the group. However, mobile genetic elements (transposons and integrons) lead to inter-species transfer of resistance genes, raising fears that historically Gram-negative–associated determinants will join them across taxonomic borders under selection (Kumari et al., 2021).

Consistency between presence of resistant genes and phenotypic resistance The significant association of the presence of carbapenem resistance (imipenem and meropenem) with blaVIM and blaKPC in the present study suggests that these genes, when present, can contribute significantly to phenotypic resistance profiles. Such an association reflects the phenotype of other β-lactams against carbapenemase-producing Enterobacteriaceae (Nie et al., 2025), with blaKPC and blaVIM being robustly associated with not only carbapenem resistance but, in some instances, with the wider class of β-lactams. In contrast, no significant association was observed between the presence of carbapenemase genes and resistance to non-β-lactam agents such as ciprofloxacin and gentamicin, indicating that resistance to non-β-lactam antibiotics is more likely governed by other mechanisms such as efflux pumps, target site mutations or by alternative resistance genes (Reinicke et al., 2025).

The isolation of blaVIM and blaKPC from *S. pneumoniae* strains, although at low rates, is of great clinical and epidemiological significance. Firstly, it emphasizes the importance of continuing to perform molecular surveillance in non-carbapenemase-producer species. Indeed, an early recognition of such genes can influence antibiotic stewardship programs and direct proper therapeutic decisions when carbapenems are still considered the mainstay of severe pneumococcal infection treatment in many settings (Senok et al., 2023; Franjić Amančić et al., 2025). On second place, it highlights the public health problem of the dissemination of antimicrobial resistance genes. Carbapenemase genes are associated with wide spread Gram-negative pathogens globally and pose an imminent risk to suitable antimicrobial treatment; tackling resistance can only be solved by a collaborative response (Li et al., 2023).

Streptococcus pneumoniae remain to be fully clarified in terms of the mechanisms behind the emergence of these carbapenemase genes. Horizontal gene transfer (HGT), although characterized well amongst Gram-negative organisms, needs further investigation for its role in Gram-positive bacteria. Research investigating whole-genome sequencing and the map of mobile genetic elements may bring new insight into whether these genes arise de novo, are acquired from a commensal or pathogenic co-habitant or are merely infrequent but clinically relevant gene transfer events (Weiser et al., 2018).

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Conclusion:

This study also shows the emergence of atypical resistance determinants against carbapenemase genes, including blaVIM and blaKPC, among isolates of *Streptococcus pneumoniae* from cases of pneumonia. Although the overall prevalence of these genes appeared low, their low prevalence difficulties on significant association with carbapenem resistance indicating a risk for the persistence of last-line antibiotics. Resistance patterns point to the necessity of prudent antimicrobial use and regular susceptibility testing. Screening for carbapenemase genes can act as an early warning for resistance trends that development. Surveillance and strong antibiotics stewardship programs are necessary to contain the dispersion of such resistance genes. The clinical and epidemiological implications of our findings should be further validated in larger multicenter studies.

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