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Original Research Article

Occurrence of *Bla*$_{VIM}$ in carbapenem resistant isolates of *Klebsiella pneumoniae* in a tertiary care hospital

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Abstract

Background: *Klebsiella pneumoniae*, causes a wide range of infections in humans such as pneumonia, soft tissue infections, septicaemia, and urinary tract infections. The rise of multidrug resistant *Klebsiella pneumoniae* has alarming implications for public health, drastically limiting the treatment options for severe infections caused by this bacterium. *Bla*$_{VIM}$ is a clinically important subgroup of Class B metallo beta-lactamases, posing a significant challenge to antibiotic therapy.
Aim and Objective: This study aimed to investigate the occurrence of *bla*$_{VIM}$ gene in *K. pneumoniae* isolates, and its co-existence with other carbapenemase genes.
Materials and Methods: The study analysed a collection of 200 unique clinically relevant *K. pneumoniae* isolates recovered from diverse clinical samples over a 12 month period. Antibiotic susceptibility testing was conducted using the disc diffusion technique, adhering to CLSI guidelines, to assess the effectiveness of diverse antimicrobial classes against the isolates. Polymerase chain reaction (PCR) assays were employed to identify the presence of the *bla*$_{VIM}$ gene in the isolated strains.
Results: Of the 200 isolates, 50 (25%) were resistant to meropenem by disc diffusion method. *Bla*$_{VIM}$ was detected in 9 (18%) isolates by PCR. They were isolated from urine (n=7), exudative specimen (n=1) and respiratory (n=1).Co-existence of other carbapenemase genes such as *bla*$_{IMP}$, *bla*$_{NDM}$, *bla* $_{OXA-48}$ and *bla*$_{KPC}$ were not detected alongside the *bla*$_{VIM}$ in this study.
Conclusion: Detection of the resistance mechanism by molecular methods such as PCR will help to prevent therapeutic failure and the spread of multidrug resistant *Klebsiella pneumoniae*.

Keywords: *Klebsiella pneumoniae*, Antimicrobial susceptibility, Metallo beta lactamases, Bla$_{VIM}$.

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1. Introduction

K.pneumoniae (K.pneumoniae), is a versatile pathogen responsible for a broad spectrum of infections, encompassing life threatening septicemia, respiratory pneumonia, suppurative infections and urinary tract infections.[1] Carbapenems (imipenem, meropenem, ertapenem, and doripenem) represent the final therapeutic resort for combating severe infections triggered by multidrug resistant bacteria, owing to their expansive antimicrobial spectrum and proven efficacy against recalcitrant strains. A worldwide surge in carbapenem resistance in *Enterobacteriaceae* has been documented, sparking international concern.[2] Carbapenemases, are enzymes that hydrolyze beta-lactam antibiotics, including carbapenems, thereby conferring resistance to these drugs.[3] In *Enterobacteriaceae*, the carbapenem hydrolysing beta-lactamases are the class A carbapenemases (eg. KPC), class B carbapenemases (eg. IMP, VIM and NDM) and class D carbapenemases (eg.OXA-48 and its variants).[2,4]

The class B metallo beta lactamases (MBL) family encompasses several notable enzymes, including Verona integron encoded metallo beta lactamases (VIM), Imipenemase (IMP) and New Delhi metallo beta lactamase (NDM), which confer resistance to a broad range of beta-

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lactam antibiotics.[5] Class B metallo beta- lactamases possess abroad hydrolytic spectrum, enabling them to breakdown virtually all beta lactam antibiotics, with aztreonam being the notable exception. They are uniquely inhibited by chelating agents such as EDTA, whereas they remain unaffected by beta-lactamase inhibitors like clavulanic acid. A significant proportion of MBL producers are *K.pneumoniae* isolates, which often exhibit multidrug resistant profiles.[6] The first reported detection of the Class B metallo beta lactamase VIM occurred in 1996, in a strain of *Pseudomonas aeruginosa*.[7] Previous studies have documented sporadic cases, outbreaks, and endemic situations involving VIM producing organisms.[5]

Understanding the antimicrobial susceptibility patterns and the presence of metallo-beta lactamase producing strains is crucial or guiding effective treatment strategies against infections caused by these resistant organisms. This research aimed to investigate the presence of *Bla*$_{VIM}$ genes in *K. pneumoniae* clinical isolates obtained from a tertiary care hospital setting and its co-existence with other carbapenemase genes.

2. Materials and Methods

2.1. Bacterial isolates

This study analysed a collection of 200 non-duplicate *K.pneumoniae* isolates gathered over a period of one year. The *K.pneumoniae* isolates originated from various clinical specimens including urine (n=74), respiratory secretions (n=73) exudative specimens (n=50), and blood samples (n=3).

2.2. Inclusion criteria

K.pneumoniae isolated from the various clinical samples of hospitalised and non-hospitalised patients were collected and used for the current study.

2.3. Exclusion criteria

Isolates deemed as colonizers.

Repetitive isolates from the same source

2.4. Antimicrobial susceptibility test

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion technique for cefotaxime (30 µg), ceftriaxone (30µg), ciprofloxacin(5µg), amikacin(30µg), piperacillin/tazobactam (100µg/10µg) and meropenem(10µg) (HiMedia laboratories, Mumbai, Maharashtra, India) as per the Clinical and Laboratory Standards Institute guidelines 2022. [8] ATCC strain of *Escherichia coli* 25922 was used as the quality control.

2.5. Detection of beta-lactamase-encoding genes

bla$_{VIM}$ gene by polymerase chain reaction

Template DNA was extracted from the isolates by boiling method.[9] All the carbapenem resistant isolates underwent PCR testing for the *bla*$_{VIM}$ gene, utilizing the previously mentioned primers. [10]The primers were VIM forward - GATGGTGTTTGGTCGCATA and VIM reverse – CGAATGCGCAGCACCAG. Amplicon size of VIM gene was 390 bp.

PCR was performed with a final volume of 25 µl. Each reaction contained 10 pmol of each primer (Sigma-Aldrich, India),10mM of dNTP mixture (Takara, India), 5U Taq polymerase (Takara, India) in 2.5µl of 10X Taq polymerase buffer (Mg2+plus). A volume of 2 µl of template DNA was added to 23 µl of the master mixture. Negative controls were PCR mixture with water (instead of template DNA) and were included in every PCR run. Previously, characterized strains were used as positive controls.

Amplification reactions were performed under the following conditions: initial denaturation 10 min at 94°C, followed by 36 cycles of amplification for 30 s at 94°C, annealing at 52°C for 40 s, and 50 s at 72°C with final extension for 5 min at 72°C. DNA fragments were analysed by electrophoresis in a 2% agarose gel containing ethidium bromide at 100 V in IX TBE buffer.

2.6. Other carbapenemase genes

Some of the other commonly occurring carbapenemase genes were also looked for employing PCR. They were *bla*$_{IMP}$, *bla*$_{NDM}$, *bla* $_{OXA-48}$ and *bla*$_{KPC}$. They were also tested using primers described previously.[10]

3. Results

Among the 200 *K.pneumoniae* isolates, 50 (25%) exhibited resistance to meropenem by disc diffusion method. The susceptibility patterns of the isolates to other classes of antimicrobial agent were as follows: amikacin (69%), piperacillin/tazobactam (67.5%), ciprofloxacin (59%), and cefotaxime (53.5%).

Among the 50 carbapenem resistant *Klebsiella pneumoniae* isolates, *Bla*$_{VIM}$ was detected in 9(18%) isolates. The entire VIM gene producing *K.pneumoniae* was isolated from the clinical samples of inpatients. Gel picture of the amplified gene was showed in **Figure 1**. In **Figure 1** lane 1, lane 2 shows positive control and test strain of VIM gene (390bp), lane 3 negative control and lane 4 shows 100bp ladder. The source of the isolates were urine (n=7), exudative specimen (n=1) and Respiratory (n=1). Notably, the other carbapenemases including *bla*$_{IMP}$, *bla* $_{KPC}$, *bla*$_{NDM}$ and *bla*$_{OXA\ 48}$ were not detected along with these *Bla*$_{VIM}$ harbouring *K.pneumoniae* isolates.

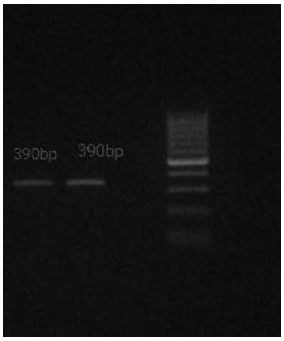

Figure 1: Gel electrophoresis of PCR products targeting the VIM gene (390 bp). Lane 1: Positive control; Lane 2: Test strain; Lane 3: Negative control; Lane 4: 100 bp DNA ladder.

4. Discussion

K.pneumoniae is a versatile opportunistic pathogen that can trigger a diverse array of infections, encompassing both community onset and hospital acquired infections.[11] Carbapenems represent a critical last line of defense in the antimicrobial arsenal, reserved for combating severe, complicated infections caused by multidrug-resistant gram negative pathogens. In the past decade, a disturbing trend has emerged: a significant and alarming rise in carbapenem resistance among *K.pneumoniae* isolates.[12] The global rise of antibiotic resistant *Klebsiella pneuoniae* strains has sparked alarm, as isolates producing extended spectrum beta lactamases and carbapenemases are being reported with escalating frequency, posing a significant threat to public health.[11]

Class B metallo beta-lactamases possess a remarkably broad catalytic capacity, enabling them to hydrolyze a wide range of beta-lactam antibiotics, including carbapenems, with the notable exception of monobactams.[2] Class B metallo beta-lactamases are not inhibited by mechanism-based inhibitors such as clavulanate, sulbactam, tazobactam, whereas they are inactivated by metal chelators such as EDTA (ethylene diamine tetraacetic acid). Class B carbapenemases mostly identified in *Enterobacteriaceae* includes VIM, IMP and NDM. A total of 41 distinct variants of VIM type carbapenemase have been detected in *Enterobacteriaceae,* highlighting the extensive diversity within this enzyme family.[3] Following its first detection in 1996, VIM gene has been reported globally.Worldwide, VIM-2 is the most frequently reported metallo beta-lactamase, underscoring its widespread distribution and prevalence.[13]

In this study, 25% of the isolates were resistant to meropenem by disc diffusion method. Among the 50 carbapenem resistant isolates18% (9/50) carried bla_{VIM} gene. It was detected highest in urinary isolates (77%). In Vellore, India it has been observed that of the 134 isolates of *K.pneumoniae*, 10 harbored $bla_{VIM.}$ They also identified the co-existence of other carbapenemase genes including NDM and OXA-48 along with VIM.[14] Elsewhere, among 21 uropathogenic *K.pneumoniae*, 2 carry VIM gene.[15] A previous study reported the presence of VIM gene in *K.pneumoniae* was 1%.[16] According to Mohanty *et al*., the VIM gene was detected in 8.8% of the isolates.[17] In India, a high prevalence of the blaVIM gene has been reported, with up to 18% of the *K.pneumoniae* isolates harbouring this gene.[18]

VIM producing *K.pneumoniae* have reported more in Asia and Europe, whereas rarely from America and Africa.[2] In Greece, bla_{VIM} detected in 17 *K.pneumoniae* isolates.[19] In Egypt, it has been observed that, of the eighty carbapenem resistant *K.pneumoniae* isolates, 27.5% isolates carry VIM gene.[20] This gene has been encountered in carbapenem resistant *K.pneumoniae* in Iran (26.7%).[21] A study from Nepal in 2021 reported, VIM gene was 3.33% in *K.pneumoniae* isolates.[22] The VIM gene exhibits a higher prevalence in Greece, yet its presence has been documented in sporadic cases and outbreaks globally, underscoring its widespread distribution.[23] In Venezuela, 19 carbapenem resistant isolates carried VIM gene along with KPC gene.[24] In Spain, Italy, and Hungary, VIM is the most common metallo-beta-lactamase found among carbapenemase producing *Enterobacteriaceae*.[25] Greece is recognized as a European hotspot for the endemic presence of VIM-1 producing *Enterobacteriaceae*, highlighting a significant regional concern. Numerous Greek studies have documented the widespread dissemination of VIM-1 producing *Klebsiella pneumoniae* nationwide, while additionally, this enzyme has been detected in various other bacterial species, underscoring its expanding presence.[13] A recent study in Iraq reported, of the 27 carbapenem resistant *K.pneumoniae* isolates, bla_{VIM} was detected in 11.1% of the isolates.[26] Mushi *et.al*, from Tanzania, reported among 68 isolates of *K.pneumoniae*, 11 harboured VIM gene.[27] A study from Lahore, Pakistan reported, of the 19 carbapenem resistant *K.pneumoniae* isolates four harboured bla_{VIM} genes.[28]

In this study, co-existence of other carbapenemase genes with bla_{VIM} was not detected. A study from South Korea reported co-occurrence of KPC along with VIM gene.[29] Flores *et al*. detected co-carriage of other carbapenemase genes with VIM gene in *Klebsiella pneumoniae* isolates.[30] A recent study from Greece, *K.pneumoniae* strain co-producing NDM-1 and VIM-1, isolated from a blood culture of the patient.[31] Bayraktar *et al*. reported co-occurrence of OXA-48 and VIM in one isolate of *K.pneumoniae*.[32] Mohammed *et al.* noted the co-existence of bla_{NDM} with bla_{VIM} in their study isolates.[33]

Ceftazidime-avibactam demonstrates efficacy against OXA-48- and KPC-producing *Enterobacteriaceae*. However, it lacks activity against VIM- or NDM producing *Enterobacteriaceae,* which restricts treatment options for infections caused by these resistant organisms.[34] A novel therapeutic combination, pairing ceftazidime/avibactam with aztreonam, has shown promising in vitro results, demonstrating substantial efficacy against *Klebsiella pneuoniae* strains that produce metallo beta-lactamases. This

combination therapy successfully counters resistance conferred by both metallo beta lactamases and serine beta lactamases, providing a potentially effective treatment strategy for combating highly resistant carbapenem resistant *Klebsiella pneumoniae* infections. Further in vivo investigations are necessary to corroborate these results and assess the translational potential of this combination therapy in clinical settings.[35] A prompt detection of carbapenemase producing organisms, particularly in healthcare facilities, can play a crucial role in curbing their dissemination. The effective control of carbapenemase producing organisms on the swift deployment of advanced diagnostic tools, coupled with the rigorous enforcement of infection prevention and control protocols.[13]

5. Conclusion

Occurrence of bla_{VIM} in this study among carbapenem resistant *Klebsiella pneumoniae* was 18% as by PCR. Carbapenems serve as the ultimate therapeutic recourse in combating severe and multidrug resistant *K.pneumoniae* infections, representing a stronghold against these formidable pathogens. Detection of carbapenemase genes is essential, because they can easily be transmitted to other susceptible gram negative bacterial isolates in the hospital settings. Monitoring trend of antimicrobial resistance among *K.pneumoniae* is necessary for formulating antimicrobial stewardship programmes in healthcare settings.

6. Ethical Approval

This study was approved by ethical approval committee of the institute with ref. no. AAMC/IEC/2023-2024/5.

7. Source of Funding

None.

8. Conflict of Interest

None.

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