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Low Prevalence of Carbapenemase Gene NDM-1 in Uropathogenic Klebsiella pneumoniae and Escherichia coli: A Molecular Surveillance Study

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ABSTRACT

Introduction: The New Delhi Metallo-β-lactamase-1 (NDM-1) gene has emerged as a major global health concern because of its ability to confer resistance to β-lactam antibiotics, particularly carbapenems. These antibiotics are often considered last-line agents, and resistance severely limits treatment options. NDM-1 is frequently associated with urinary tract infections (UTIs) caused by multidrug-resistant Gram-negative bacteria, and its spread poses a serious challenge to patient care. Continuous surveillance is therefore essential to track its prevalence and guide effective treatment and prevention strategies.

Aim: This study set out to determine the prevalence of the NDM-1 gene in clinical isolates obtained from patients with UTIs in Onitsha Metropolis.

Methods: Thirty clinical isolates from patients diagnosed with UTIs were analyzed. Genomic DNA was extracted from each isolate, and the presence of the NDM-1 gene was assessed using Polymerase Chain Reaction (PCR). Amplified products were separated on agarose gel electrophoresis and visualized with ethidium bromide staining to confirm gene detection.

Results: Of the thirty isolates examined, only one tested positive for the NDM-1 gene. This finding corresponds to a prevalence rate of 3.33 percent among the studied samples. Although the prevalence was low, the detection of NDM-1 in even a single isolate is significant, given the potential for plasmid-mediated dissemination across bacterial populations.

Conclusion: The study demonstrates a low prevalence of NDM-1 among UTI isolates in Onitsha. However, the presence of the gene underscores the importance of ongoing surveillance, strict infection control measures, and antimicrobial stewardship to prevent its wider spread. Larger studies are recommended to provide a more comprehensive understanding of the distribution and clinical impact of NDM-1 in Nigeria and similar settings.

KEYWORDS

NDM 1, urinary tract infections, antimicrobial resistance, PCR, Onitsha

INTRODUCTION

Antimicrobial resistance (AMR) remains one of the most urgent threats to global public health, with the World Health Organization (WHO) warning that it jeopardises the effective treatment of infections and undermines decades of medical progress (WHO, 2025). Among the most concerning forms of resistance is the rapid emergence and dissemination of carbapenemase-producing Gram-negative bacteria. Carbapenems are often regarded as last-line agents for severe infections, and resistance to them significantly limits therapeutic options. Of particular concern is the New Delhi metallo-β-lactamase-1 (NDM-1), an enzyme capable of hydrolysing a broad range of β-lactam antibiotics, including carbapenems, thereby conferring high-level resistance (Alvisi et al., 2025; Ramesh et al., 2025).

Since its first identification in 2008 in a patient returning from India, NDM-1 has spread across continents, carried predominantly by Enterobacteriaceae such as *Klebsiella pneumoniae* and *Escherichia coli* (Zhao et al., 2023). These organisms are major causes of urinary tract infections (UTIs), bloodstream infections, and hospital-acquired pneumonia. In low- and middle-income countries (LMICs), the burden is particularly acute due to diagnostic limitations, widespread overuse of antimicrobials, and weak infection-control systems, which together create favourable conditions for the propagation of resistance (Laxminarayan et al., 2016).

Globally, carbapenem resistance among Enterobacteriaceae has been widely documented in Asia, the Middle East, and parts of Europe. South Asia remains a hotspot, with India, Pakistan, and Bangladesh reporting high prevalence rates of NDM-positive isolates (Joshi et al., 2023). In the Middle East, recent studies have shown worrying trends: Izhari et al. (2025) reported *blaNDM* in over 40% of *K. pneumoniae* isolates in Saudi Arabia, while Abbasi and Ghaznavi-Rad (2023) documented detection rates exceeding 60% among carbapenem-resistant isolates in Iran. These figures highlight the global expansion of NDM-positive organisms and underscore the importance of early detection in regions where prevalence remains comparatively low.

In Africa, however, data remain limited. Nigeria, the most populous country on the continent, faces significant challenges in AMR surveillance. Molecular detection of carbapenemase genes such as NDM-1 is not routinely integrated into clinical microbiology workflows, and surveillance capacity is inconsistent across institutions (Ibadin et al., 2023). This gap means that the true burden of carbapenemase-producing organisms may be underestimated, hindering effective antimicrobial stewardship and policy development. Local evidence is therefore essential to understand circulating resistance mechanisms and to inform both clinical practice and public-health interventions.

Onitsha, a major commercial hub in Southeastern Nigeria, presents a unique setting for such investigations. The city's dense population, high patient turnover, and extensive trade networks increase the risk of rapid dissemination of resistant pathogens. Informal healthcare practices and easy access to antibiotics without prescription further contribute to the selection and spread of multidrug-resistant organisms (Kariuki et al., 2022; James et al., 2025; Khadija et al., 2025). Yet, molecular studies targeting carbapenemase genes in this environment remain sparse, leaving a critical gap in knowledge.

Against this backdrop, the present study investigates the presence of the NDM-1 gene in *K. pneumoniae* and *E. coli* isolates recovered from patients with UTIs in Onitsha Metropolis. By providing baseline molecular data, this study contributes to the growing body of evidence needed to guide clinical decision-making, strengthen laboratory detection capacities, and support public-health interventions aimed at reducing the dissemination of carbapenem-resistant pathogens. The findings are expected to inform antimicrobial stewardship programmes and highlight the urgent need for routine molecular surveillance in Nigerian healthcare facilities.

MATERIALS AND METHODS

Study Design and Setting

This laboratory-based cross-sectional study investigated the presence of the NDM-1 gene in previously confirmed uropathogenic *Klebsiella pneumoniae* and *Escherichia coli* isolates. The isolates were obtained from patients diagnosed with urinary tract infections (UTIs) at two major healthcare facilities in Onitsha Metropolis, Southeastern Nigeria:

1. Federal Medical Centre (FMC) Onitsha, and
2. St. Charles Borromeo Specialist Hospital, Onitsha.

Both facilities serve large and diverse patient populations, providing a representative setting for examining antimicrobial resistance patterns in the region.

Sample Collection and Bacterial Identification

A total of 271 urine samples were originally processed at the participating hospitals as part of routine clinical investigations for suspected UTIs. Standard microbiological procedures, including culture on differential media, Gram staining, and biochemical testing, were used to identify uropathogenic isolates. From these, 30 pure isolates (comprising *K. pneumoniae* and *E. coli*) were confirmed phenotypically to possess metallo-β-lactamase activity and were selected for molecular analysis.

DNA Extraction

Genomic DNA had been previously extracted and stored. To ensure viability, each stored DNA sample was re-processed using a simple boiling lysis method.

1. Approximately 200 µL of each stored sample was transferred into a sterile microcentrifuge tube.
2. Samples were vortexed and heated at 95°C for 10 minutes to lyse cells and release DNA.
3. The lysates were centrifuged at 12,000 rpm for 1 minute, and the supernatant containing crude DNA was transferred into fresh tubes for PCR. The presence of genomic DNA was verified via agarose gel electrophoresis.

PCR Amplification for NDM-1 Detection

Polymerase chain reaction (PCR) was performed using NDM-1–specific primers:

- Forward: GGTGCATGCCCGGTGAAATC
- Reverse: ATGCTGGCCTTGGGGAACG

Each reaction mixture (25 µL total volume) included:

- 12.5 µL OneTaq Quick-Load 2× Master Mix (New England Biolabs),
- 0.5 µL each of forward and reverse primers,
- 6.5 µL nuclease-free water,
- 5 µL DNA template.

Thermocycling conditions were as follows:

- Initial denaturation: 94°C for 5 min

- 35 cycles of:
    - Denaturation at 94°C for 30 s,
    - Annealing at 55°C for 30 s,
    - Extension at 72°C for 60 s
- Final extension: 72°C for 5 min

Amplification was carried out using an Eppendorf Nexus Gradient Mastercycler.

Gel Electrophoresis and Visualisation

PCR products were resolved on a 1.5% agarose gel prepared in 1× TBE buffer. Ethidium bromide (5 µL per 100 mL) was incorporated for nucleic acid staining.

- A 100 bp DNA ladder was used as a molecular size marker.
- Electrophoresis was conducted at 120 V for 40 minutes.
- Bands were visualised using a VILBER gel documentation system. A band size of approximately 660 bp was interpreted as positive for the NDM-1 gene.

Data Analysis

Results were compiled manually and summarised using simple descriptive statistics. The prevalence of NDM-1 among the isolates was calculated as a percentage of the total number of samples analysed.

RESULTS

A total of eighteen stored samples that had previously been phenotypically confirmed to harbor Metallo-Beta-lactamase genes were selected for this study. DNA was extracted from each isolate, and the presence of the MBL gene NDM-1 was investigated through amplification using the polymerase chain reaction. The amplified products were then demonstrated using gel electrophoresis and visualized with a gel documentation system, allowing clear confirmation of the outcomes.

Genomic DNA Extraction

To establish the viability of the isolates and to ensure that DNA had been successfully extracted, the boiling method was employed. Portions of the DNA obtained from each isolate were subjected to agarose gel electrophoresis. The gels revealed distinct DNA bands across all samples, confirming that extraction was successful and that the isolates were suitable for downstream molecular analysis. This step provided confidence that the material used in subsequent assays was intact and reliable.

Detection of NDM-1 using PCR Amplification

PCR amplification targeting the NDM-1 gene was carried out, and the resulting products were separated on a 1.5% agarose gel. Visualization was achieved using ethidium bromide staining, which highlighted DNA bands under ultraviolet light. A single PCR product of approximately 660 base pairs was expected for positive samples. Figure 1 illustrates the gel image obtained, showing the amplified fragments for samples numbered 21 to 50, with a control included for comparison.

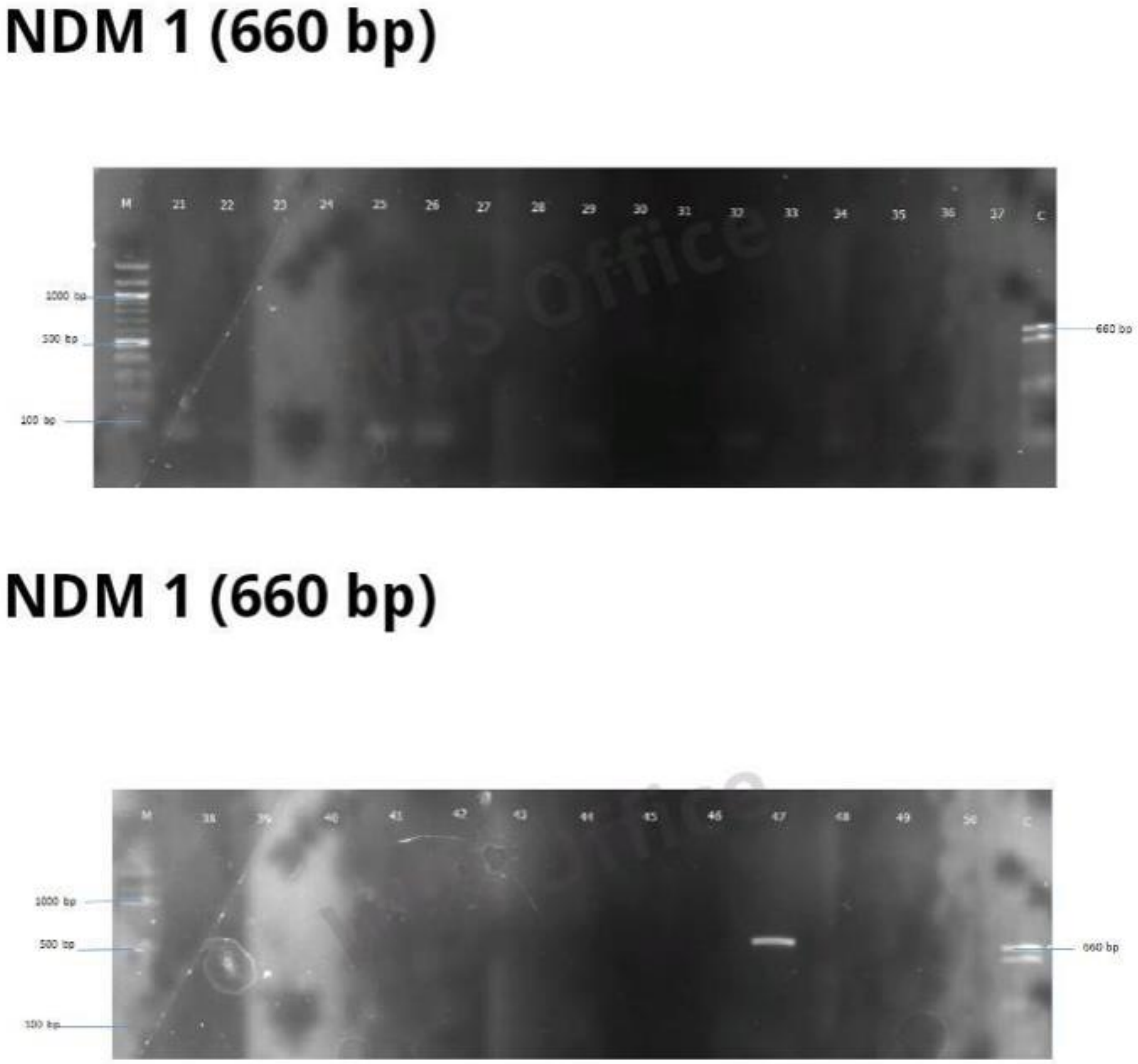

Figure 1: Gel electrophoresis image showing detection of the NDM-1 gene. A single PCR assay targeting NDM-1 produced an expected band size of approximately 660 bp. Samples 21–50 were analyzed, with "C" serving as the control. Amplification at 660 bp indicates the presence of the NDM-1 gene.

The results were carefully examined, and the number of isolates carrying the gene under study was summarized in Table 1. Most of the samples did not demonstrate amplification at the expected band size, indicating absence of the NDM-1 gene. However, one isolate, designated Ch47, showed a clear band at approximately 660 bp, consistent with the presence of NDM-1. This finding was further confirmed by comparison with the control, which validated the accuracy of the amplification process.

Table 1: Number of isolates carrying the NDM-1 gene

Samples	NDM-1	Genes present
Ch21	Absent	0
Ch22	Absent	0
Ch23	Absent	0
Ch24	Absent	0
Ch25	Absent	0
Ch26	Absent	0
Ch27	Absent	0
Ch28	Absent	0
Ch29	Absent	0
Ch30	Absent	0
Ch31	Absent	0
Ch32	Absent	0
Ch33	Absent	0
Ch34	Absent	0
Ch35	Absent	0
Ch36	Absent	0

Ch37	Absent	0
Ch38	Absent	0
Ch39	Absent	0
Ch40	Absent	0
Ch41	Absent	0
Ch42	Absent	0
Ch43	Absent	0
Ch44	Absent	0
Ch45	Absent	0
Ch46	Present	0
Ch47	Absent	1
Ch48	Absent	0
Ch49	Absent	0
Ch50	Absent	0

Summary of Findings

Table 2 presents the overall occurrence of NDM-1 among the isolates tested. Out of the eighteen samples analyzed, only one isolate carried the gene, representing 3.33% of the total. Although the prevalence was low, the detection of NDM-1 in even a single isolate is significant, given the potential for plasmid-mediated dissemination of resistance genes across bacterial populations.

Table 2: Number of samples habouring NDM-1 genes

Gene	**No of Occurrences**	**% Occurrence**
NDM-1	1	3.33%

The identification of NDM-1 in isolate Ch47 highlights the importance of continued surveillance and molecular characterization of resistant organisms. While the majority of samples did not harbor the gene, the presence of NDM-1 in one isolate underscore the risk of silent spread within clinical and community settings. This result demonstrates that even limited detection should be treated as an early warning, prompting further investigation and strengthening of laboratory capacity to monitor carbapenem resistance.

The study confirmed successful DNA extraction from all isolates, validated the use of PCR amplification for detection of NDM-1, and identified one isolate carrying the gene. The findings emphasize the need for vigilance, as the emergence of NDM-1, even at low frequency, has serious implications for antimicrobial resistance management.

DISCUSSION

This study investigated the presence of the NDM-1 carbapenemase gene in *Klebsiella pneumoniae* and *Escherichia coli* isolates associated with urinary tract infections in Onitsha, Southeastern Nigeria. The findings revealed a low prevalence of 3.33%, with only one isolate testing positive for NDM-1. Although the detection rate was low, the presence of even a single NDM-1-positive isolate carries significant clinical and epidemiological implications because of the ease with which this gene spreads across bacterial species via mobile genetic elements such as plasmids and integrons (Zhao et al., 2023; Alvisi et al., 2025).

The low frequency observed in this study contrasts with reports from several regions where NDM-1 has become more entrenched. Studies from South Asia consistently report higher prevalence rates, reflecting both the early emergence of NDM-1 in that region and ongoing transmission in healthcare and community environments. For example, Abbasi and Ghaznavi-Rad (2023) reported NDM detection rates exceeding 60% among carbapenem-resistant isolates in Iran, while Joshi et al. (2023) documented a prevalence of 17.6% in South India. In the Middle East, Izhari et al. (2025) found *blaNDM* in over 40% of *K. pneumoniae* isolates in Saudi Arabia. These higher rates highlight the global expansion of NDM-positive organisms and underscore the importance of early detection in regions where prevalence remains comparatively low.

The low detection rate in our study may reflect several contextual factors. First, geographical differences in antibiotic consumption, infection-control infrastructure, and healthcare system organisation strongly influence local resistance patterns. Nigeria's resistance landscape is heterogeneous, with some tertiary centres reporting high carbapenemase rates, while others, particularly those with limited molecular diagnostic capacity, may under-detect these genes (Ibadin et al., 2023). Second, many isolates in the present study were phenotypically positive for metallo-β-lactamase activity but negative for NDM-1, suggesting that other carbapenemase genes (e.g., VIM, IMP, OXA-48-like variants) or non-enzymatic mechanisms such as porin loss or efflux pump overactivity may be contributing to resistance. Previous Nigerian studies have reported the dominance of OXA-48-like enzymes and co-carriage of multiple genes, further supporting this explanation (James et al., 2025).

This finding aligns with evidence from elsewhere showing that NDM-1 is only one component of the broader carbapenem resistance problem. For instance, Kazi et al. (2015) in Mumbai highlighted the frequent co-existence of NDM with other carbapenemase genes, while Camargo et al. (2022) demonstrated wide genetic diversity among resistant *K. pneumoniae* isolates in Sao Paulo, including numerous resistance determinants beyond NDM. These studies emphasise the dynamic and rapidly evolving nature of carbapenem resistance globally.

Although the prevalence of NDM-1 in this study was low, the public health risk remains substantial. NDM-1-producing organisms often harbour additional resistance genes, limiting treatment options to a few relatively toxic or expensive antibiotics such as colistin or tigecycline (Logan & Weinstein, 2017). The spread of NDM-1 in settings where antibiotic use is poorly regulated, such as community pharmacies and informal health centres, could lead to rapid dissemination, particularly in high-density urban environments like Onitsha. Therefore, the detection of even a single NDM-positive isolate should be viewed as an early warning signal rather than a reassuring finding.

Another critical implication of this study is the value of incorporating molecular diagnostics into routine microbiology workflows. Phenotypic tests alone cannot reliably differentiate between different carbapenemase types, and delays in detecting highly transmissible genes like NDM-1 may contribute to silent spread in hospitals. The successful use of PCR in this study demonstrates the feasibility of molecular surveillance even in resource-limited settings, provided training, equipment, and sustained funding are available (WHO, 2025).

Overall, this study contributes important baseline data on the molecular epidemiology of carbapenem resistance in Southeastern Nigeria. Continued surveillance, larger multi-centre studies, and the integration of rapid molecular diagnostics are essential for understanding local resistance trends and preventing the establishment of NDM-1 as a dominant carbapenemase in the region.

LIMITATIONS

Several limitations should be acknowledged in interpreting the findings of this study. First, the sample size was relatively small and restricted to a single tertiary hospital in Onitsha. While the detection of NDM-1 in one isolate is epidemiologically significant, the limited scope reduces the generalisability of the results to other regions of Nigeria or West Africa. Larger, multi-centre studies are needed to provide a more comprehensive picture of carbapenemase prevalence and distribution (Ibadin et al., 2023; WHO, 2025).

Second, the study focused exclusively on *Klebsiella pneumoniae* and *Escherichia coli* isolates associated with urinary tract infections. Although these organisms are major contributors to carbapenem resistance globally, restricting analysis to two species may underestimate the broader burden of NDM-1 and related genes in other Gram-negative pathogens such as *Acinetobacter baumannii* or *Pseudomonas aeruginosa* (Logan & Weinstein, 2017).

Third, only PCR amplification targeting the NDM-1 gene was performed. Other carbapenemase genes, including *blaOXA-48-like*, *blaVIM*, *blaIMP*, and *blaKPC*, were not screened. This limits the ability to fully characterise the molecular mechanisms underlying carbapenem resistance in the study population. Previous Nigerian studies have shown that OXA-48-like enzymes are common and often co-exist with NDM-1, highlighting the need for multiplex PCR or whole-genome sequencing to capture the full resistance landscape (James et al., 2025; Izhari et al., 2025).

Fourth, phenotypic assays were not systematically compared with molecular results. While PCR provides high specificity, combining molecular and phenotypic methods such as the modified carbapenem inactivation method (mCIM) or EDTA synergy tests would strengthen diagnostic accuracy and help identify discrepancies between genotype and phenotype (CDC, 2023; CLSI, 2024).

Finally, resource constraints limited the inclusion of advanced molecular epidemiology techniques such as plasmid typing or sequencing. These approaches would have provided insights into the genetic context of NDM-1, including its association with mobile elements and potential for horizontal transfer. Such data are critical for understanding transmission dynamics and informing infection-control strategies (Koppenhöfer, Tomasch & Lang et al., 2022; Abbasi & Ghaznavi-Rad, 2023).

Despite these limitations, the study provides valuable baseline data on carbapenem resistance in Southeastern Nigeria. It highlights the feasibility of molecular surveillance in resource-limited settings and underscores the urgent need for expanded diagnostic capacity, larger epidemiological studies, and integrated stewardship programmes.

FUTURE DIRECTIONS

The detection of NDM-1 in urinary isolates from Southeastern Nigeria, even at low prevalence, highlights the need for sustained research and surveillance to prevent its establishment as a dominant carbapenemase in the region. Several future directions emerge from this study.

First, expanded multicentre surveillance is essential. Larger studies across multiple hospitals and regions in Nigeria would provide a clearer picture of the prevalence and distribution of NDM-1 and other carbapenemase genes. Integration into national and regional antimicrobial resistance (AMR) surveillance networks, such as the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS), would enable standardised reporting and facilitate international comparisons (WHO, 2025).

Second, molecular epidemiology and genomic studies should be prioritised. Whole-genome sequencing (WGS) and plasmid typing can reveal the genetic context of NDM-1, including its association with mobile elements and co-carriage of other resistance determinants. Such approaches have been instrumental in tracking the global spread of NDM-1 and identifying clonal lineages of *K. pneumoniae* and *E. coli* that act as high-risk reservoirs (Koppenhöfer, Tomasch & Lang et al., 2022; Zhao et al., 2023). Applying WGS in Nigerian laboratories would provide insights into transmission dynamics and guide infection-control strategies.

Third, diagnostic innovation is needed. Resource-limited settings require cost-effective, rapid molecular assays capable of detecting multiple carbapenemase genes simultaneously. Multiplex PCR, loop-mediated isothermal amplification (LAMP), and portable sequencing platforms such as Oxford Nanopore have shown promise in LMIC contexts (Abbasi & Ghaznavi-Rad, 2023). Evaluating and implementing these technologies in Nigerian hospitals could improve early detection and reduce reliance on phenotypic assays alone.

Fourth, clinical and stewardship research should examine the impact of NDM-1 on treatment outcomes. Studies assessing therapeutic efficacy, mortality, and cost implications of infections caused by NDM-positive organisms would provide evidence for optimising empiric therapy and stewardship interventions. Research into the use of newer agents such as ceftazidime-avibactam, meropenem-vaborbactam, and cefiderocol in Nigerian contexts is particularly urgent, given their limited availability and high cost (Logan & Weinstein, 2017; Khan et al., 2022).

Finally, community-level studies are critical. Informal healthcare practices and unregulated antibiotic sales in Nigeria contribute to AMR dissemination. Investigating the role of community pharmacies, self-medication, and environmental reservoirs in the spread of carbapenemase genes would broaden understanding beyond hospital settings (James et al., 2025).

Pursuing these directions enables researchers and policymakers to build a comprehensive response to carbapenem resistance in Nigeria. Early investment in surveillance, diagnostics, and stewardship will be vital to prevent NDM-1 from becoming entrenched and to safeguard therapeutic options for common infections.

RECOMMENDATIONS

Building on the findings of this study, several coordinated actions are urgently required to mitigate the threat posed by NDM-1 and related carbapenemases in Southeastern Nigeria. First, molecular surveillance and confirmatory testing should be scaled up, with routine screening for carbapenemase genes using validated PCR or rapid molecular platforms, complemented by phenotypic assays to ensure accurate classification of resistance mechanisms. Second, antimicrobial stewardship must be strengthened at the facility level, incorporating interventions such as prospective audit and feedback, formulary restrictions on carbapenems and polymyxins, optimized pathways for urinary tract infections, and diagnostics-driven prescribing with timely de-escalation. Infection prevention and control practices should also be enhanced in high-risk units, with reinforced hand hygiene, contact precautions, cohorting, environmental cleaning, and careful device management, supported by continuous training and monitoring.

Beyond hospital walls, access to and regulation of antibiotics must be improved. Collaboration with regulatory authorities and community pharmacies is needed to curb over-the-counter sales of last-line agents, promote point-of-care guidance for common infections, and expand public awareness campaigns on antimicrobial resistance. At the systems level, participation in national and regional surveillance initiatives such as GLASS should be prioritized, ensuring standardized data capture, timely reporting, and integration of clinical, microbiology, and pharmacy datasets to support real-time stewardship decisions. Finally, research should be advanced through multicentre studies that map mechanism diversity and plasmid epidemiology, evaluate cost-effective rapid tests suitable for low- and middle-income countries, and assess the impact of combined stewardship and IPC bundles on transmission and outcomes.

These measures can transform an early warning into effective prevention, safeguarding therapeutic options for urinary tract infections and other conditions in Southeastern Nigeria.

CONCLUSIONS

The detection of NDM-1 in a urinary isolate of *Klebsiella pneumoniae* or *Escherichia coli* in Onitsha represents an emerging public health threat that demands urgent attention. Although the prevalence is currently low, the clinical and epidemiological implications are significant. NDM-1 is often plasmid-borne, frequently coexists with other resistance determinants, and can spread across species and settings with minimal selective pressure. In densely populated urban environments, where antibiotics are easily accessible and infection prevention and control capacity is uneven, even a small foothold can expand rapidly. This threatens empiric therapy, increases morbidity, prolongs hospital stays, and drives up healthcare costs. Nigeria's heterogeneous resistance landscape and the absence of routine molecular surveillance further heighten the risk of underestimation and delayed response. Integrating rapid genotypic detection into standard workflows is therefore essential. Ultimately, early and sustained investment in laboratory capacity and antimicrobial stewardship offers the most cost-effective strategy to contain NDM-1 and related carbapenemases before they become entrenched.

AUTHOR CONTRIBUTION

All authors contributed meaningfully to the development of this study and the preparation of the manuscript. The study was conceptualised and designed by C.M.O. and M.N.I., who also supervised the project. Data collection, laboratory procedures, data cleaning, management and analysis were carried out by C.M.O. and M.N.I., with all

co-authors providing additional analytical input and reviewing decisions to ensure accuracy and consistency. C.M.O., C.E.E. and K.O.O. led the drafting of the manuscript, including the introduction, methods, results and discussion. All co-authors contributed to the interpretation of findings, offered substantive intellectual input, provided critical revisions and strengthened the clarity and coherence of the final text. All authors reviewed the full manuscript, approved the final version and agreed to be accountable for the integrity of the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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