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# Molecular detection of carbapenemase-producing *Klebsiella* spp from clinical samples in Ibadan, Nigeria

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## Abstract:

**Background:** *Klebsiella* species are significant pathogens responsible for various healthcare-associated infections. Despite the existence of several mechanisms of resistance against high priority antibiotics such as carbapenems, the production of carbapenemases remains the most important mechanism of resistance among *Klebsiella* spp. The aim of this study was to determine the prevalence of carbapenemase-production among *Klebsiella* spp isolated from clinical specimens at the University College Hospital (UCH) Ibadan, Nigeria.
**Methodology:** A total of 120 clinical bacterial isolates were collected from the routine laboratories in the Department of Medical Microbiology and Parasitology, UCH, Ibadan between January and August 2023. These isolates were re-identified using conventional microbiological methods, including Gram staining, and biochemical identification tests. Eighty viable isolates were screened for carbapenem resistance using imipenem, following CLSI guidelines. Carbapenemase production was confirmed by the modified carbapenem inactivation (mCIM) and EDTA carbapenem inactivation (eCIM) methods. Conventional multiplex polymerase chain reaction (PCR) was used to amplify and detect carbapenemase resistance genes ($bla_{NDM-1}$, $bla_{VIM}$, $bla_{KPC}$, $bla_{OXA-48}$, and $bla_{IMP}$) in the isolates, with the amplified products resolved by agarose gel electrophoresis. Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0.
**Results:** Ninety-five of the 120 isolates were biochemically identified as *Klebsiella* spp while only 80 remained viable. Twenty-three (28.8%) biochemically confirmed isolates were resistant to imipenem (carbapenem resistant *Klebsiella*), and 20 (25.0%) were confirmed as *Klebsiella* spp by PCR, of which 11 (55.0%) were resistant to all tested antibiotics. Carbapenemase production was detected in 5 (6.3%) isolates using mCIM and eCIM. PCR identified $bla_{NDM-1}$ gene in 3 (3.8%) of the isolates, while $bla_{VIM}$, $bla_{KPC}$, $bla_{OXA-48}$, and $bla_{IMP}$ genes were not detected in any isolate.
**Conclusion**: The detection of $bla_{NDM-1}$ and high resistance levels among isolates is of great concern. The emergence of carbapenem-resistant *Klebsiella*, especially with $bla_{NDM-1}$ gene, threatens the effectiveness of 'last-resort' antibiotics. There is a need for antimicrobial resistance surveillance and antimicrobial stewardship to stem the tide of antimicrobial resistance in healthcare settings.

**Keywords**: *Klebsiella*, carbapenemase, antimicrobial resistance, Ibadan, Nigeria.

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# Détection moléculaire de *Klebsiella* spp productrices de carbapénémases dans des échantillons cliniques à Ibadan, au Nigéria

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## Résumé:

**Contexte:** Les espèces de *Klebsiella* sont des agents pathogènes importants responsables de diverses infections associées aux soins. Malgré l'existence de plusieurs mécanismes de résistance aux antibiotiques prioritaires tels que les carbapénèmes, la production de carbapénémases demeure le principal mécanisme de résistance chez les espèces de *Klebsiella*. L'objectif de cette étude était de déterminer la prévalence de la production de carbapénémases chez les espèces de *Klebsiella* isolées à partir d'échantillons cliniques à Hôpital Universitaire (UCH) d'Ibadan, au Nigéria.
**Méthodologie:** Au total, 120 isolats bactériens cliniques ont été collectés dans les laboratoires de routine du Département de microbiologie médicale et de parasitologie de l'UCH d'Ibadan entre janvier et août 2023. Ces isolats ont été réidentifiés à l'aide de méthodes microbiologiques conventionnelles, notamment la coloration de Gram et des tests d'identification biochimique. Quatre-vingts isolats viables ont été testés pour leur résistance aux carbapénèmes à l'imipénème, conformément aux directives du CLSI. La production de carbapénémases a été confirmée par les méthodes d'inactivation modifiée des carbapénèmes (mCIM) et d'inactivation des carbapénèmes par EDTA (eCIM). Français La réaction en chaîne par polymérase multiplex (PCR) conventionnelle a été utilisée pour amplifier et détecter les gènes de résistance aux carbapénémases (*bla*$_{NDM-1}$, *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{OXA-48}$ et *bla*$_{IMP}$) dans les isolats, les produits amplifiés étant résolus par électrophorèse sur gel d'agarose. Les données ont été saisies et analysées à l'aide de la version 26.0 de Statistical Package for the Social Sciences (SPSS).
**Résultats:** Quatre-vingt-quinze des 120 isolats ont été identifiés biochimiquement comme Klebsiella spp tandis que seulement 80 sont restés viables. Vingt-trois (28,8%) isolats confirmés biochimiquement étaient résistants à l'imipénème (*Klebsiella* résistant aux carbapénèmes) et 20 (25,0%) ont été confirmés comme *Klebsiella* spp par PCR, dont 11 (55,0%) étaient résistants à tous les antibiotiques testés. La production de carbapénémase a été détectée dans 5 isolats (6,3%) à l'aide des techniques mCIM et eCIM. La PCR a identifié le gène *bla*$_{NDM-1}$ dans 3 isolats (3,8%), tandis que les gènes *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{OXA-48}$ et *bla*$_{IMP}$ n'ont été détectés dans aucun isolat.
**Conclusion:** La détection de blaNDM-1 et de niveaux élevés de résistance parmi les isolats est très préoccupante. L'émergence de *Klebsiella* résistantes aux carbapénèmes, en particulier celles porteuses du gène *bla*$_{NDM-1}$, menace l'efficacité des antibiotiques de «dernier recours». Il est nécessaire de surveiller la résistance aux antimicrobiens et d'en assurer la gestion afin d'enrayer la vague de résistance aux antimicrobiens dans les établissements de santé.


**Mots-clés:** *Klebsiella*, carbapénémase, résistance aux antimicrobiens, Ibadan, Nigéria.

## Introduction:

*Klebsiella* species are significant pathogens responsible for various healthcare-associated infections (1,2). Their ability to form biofilms facilitates persistence and rapid spread in hospital environments, posing a serious threat to public health due to frequent resistance to critically important antibiotics, especially carbapenems (3-5). The rising prevalence of multidrug-resistant (MDR) bacteria (resistance to at least one member in three or more classes of antibiotics), including carbapenem-resistant *Klebsiella* strains, is associated with increased morbidity, mortality (approaching 60%), and healthcare costs (6-9).

In *Klebsiella* spp, carbapenem resistance, primarily due to carbapenemase production, is the most significant β-lactam resistance mechanism. *Klebsiella pneumoniae* harbors several types of carbapenemases, including KPCs (Class A), NDM (Class B), and OXA-type (Class D) (10-12). Non-carbapenemase mechanisms such as loss of outer membrane proteins and efflux pump production, also contribute to carbapenem resistance (13). Despite these mechanisms, carbapenemase production remains the most important resistance mechanism.

The modified carbapenem inactivation method helps distinguish between these detection mechanisms but other standardized phenotypic tests for carbapenemase detection include multi-disk mechanism testing and combined disk synergy test (14). Molecular detection methods such as PCR are favoured due to the low turnaround time, high accuracy, and specificity. The spread of carbapenemase genes among Enterobacteriaceae, coupled with inadequate surveillance and infection control measures, exacerbates the problem. Detection and characterization of carbapenemase-producing *Klebsiella* strains are essential for effective management and infection control.

In Nigeria, lack of adequate surveillance and monitoring of carbapenem-resistant Enterobacteriaceae increases the risk of further dissemination of antibiotic-resistant genes (15-18). Continuous detection and characterization of carbapenemases are crucial for understanding their epidemiology and preventing their spread. Therefore, this study aimed to detect carbapenemase-producing strains of *Klebsiella* spp isolated from clinical samples in University College Hospital, Ibadan, southwest Nigeria.

## Materials and method:

**Study site:**

This laboratory-based study was conducted in the Department of Medical Microbiology and Parasitology of the University College Hospital (UCH) in Ibadan, Nigeria between January and August 2023.

**Clinical bacterial isolates:**

Presumptively identified *Klebsiella* spp isolates were collected from the Department of Medical Microbiology and Parasitology laboratory. These isolates were recovered from sam-

ples collected from both in-patients and out-patients across 31 different wards and various clinics in UCH and a few other hospitals in Ibadan (Oluyoro Catholic Hospital and Adeoyo Maternity Teaching Hospital, Yemetu).

**Ethical approval:**

Approval was obtained from the Ethics and Research committee of University College Hospital and University of Ibadan, Nigeria (UI/EC/22/0403).

**Bacterial isolates and preliminary analyses:**

Presumptively identified non-duplicate *Klebsiella* isolates from clinical samples on the routine bench were re-characterized using standard microbiological techniques (19). Isolates were subcultured onto MacConkey agar (Oxoid, UK) and incubated under aerobic conditions at 37ºC for 24 hours. Re-characteri zation was carried out by Gram stain reaction, urease test, citrate utilization, indole production, and motility assessment, using standard methods (19).

**Antibiotic susceptibility testing (AST):**

Antibiotic susceptibility testing (AST) for each isolate was done using the modified Kirby-Bauer disk diffusion test on Mueller Hinton agar (Oxoid, UK) according to the Clinical and Laboratory Standards Institute guidelines (20). The antimicrobial discs used included imipenem (IPM, 10µg), ceftazidime (CAZ, 30 µg), levofloxacin (LEV, 5µg), piperacillin (PRL, 30µg), gentamicin (GM, 120µg), cefoxitin (FOX, 30µg), amoxicillin-clavulanate (AUG, 30 µg) and ciprofloxacin (CIP, 5µg). *Escherichia coli* (ATCC 25922) was used as quality control strain to validate media and disc potency.

**Phenotypic detection of carbapenemases:**

Imipenem-resistant isolates were tested for carbapenemase production using the Modified Carbapenem Inactivation method (m CIM) and EDTA Inactivation Method (eCIM). Isolates were subcultured from Nutrient agar slants onto MacConkey agar and incubated overnight. Overnight cultures of both test isolates and quality control strains were inoculated into 2mL of Typtic Soy Broth (TSB) for mCIM and 2mL of TSB containing 20µL of 0.5M EDTA for eCIM. After homogenization, a meropenem disk was added to each tube, followed by a 4-hour incubation at 32±2˚C. Discs were then placed on *E. coli* ATCC 25922-inoculated Mueller Hinton agar (MHA) plates and incubated at 37˚C for 24 hours.

A zone of inhibition with a diameter of 6-15 mm indicated a positive mCIM. An increase of ≥5mm in the zone size for eCIM compared to mCIM suggested metallo-carbapenemase production. Control strains used were *Klebsiella pneumoniae* ATCC® BAA-1705™ (KPC positive), *Klebsiella pneumoniae* ATCC® BAA-1706™ (carbapenemase negative), and *Klebsiella pneumoniae* ATCC® BAA-2146™ (NDM positive).

**Molecular detection of *Klebsiella* isolates:**

All molecular analyses were carried out at the molecular unit of the Biorepository and Clinical Virology Laboratory (BCVL), College of Medicine, University of Ibadan.

***DNA extraction:***

Freshly cultured carbapenem resistant *Klebsiella* isolates were sub-cultured into Tryptic Soy broth (TSB) and stored at -20$^0$C for molecular studies. Stored isolates were recovered by centrifugation. The supernatant was discarded, and the pellet composed of the bacterial cells were subjected to DNA extraction process as described by Trindade et al., (21). The broth culture was centrifuged (10,000rpm for 5 minutes) to collect the cell pellet. Five hundred microliters of Tris-EDTA buffer were added, followed by 15µL of Sodium Dodecyl Sulphate (SDS) and 3µL of proteinase K. The mixture was vortexed thoroughly to facilitate cell lysis and incubated at 37˚C for 1 hour.

After incubation, 100µL of 5M NaCl and 80µL of 10% CTAB solution in 0.7M NaCl were added and vortexed, followed by a 10 minutes' incubation at 65˚C on the heating block. Phenol-Chloroform-Isoamylalcohol (PCI, 25:24:1) was thereafter added to separate DNA from proteins and other cellular debris. After centrifugation (10,000rpm for 10 mins), the DNA-containing supernatant was precipitated with 400µL of isopropanol (8˚C for 1 hr) and further purified with 500µL 70% ethanol. The DNA pellets were re-suspended with 50µL of nuclease-free PCR graded water and stored at -80˚C for further analyses.

***Confirmatory identification of Klebsiella isolates by PCR:***

The identity of carbapenem-resistant *Klebsiella* isolates (n=23) was confirmed by conventional PCR assay and products were resolved using agarose gel electrophoresis. The set of primers and reaction protocols applied are highlighted in Table 1.

Table 1: Primer sequences for targeted Klebsiella species, with corresponding amplicon sizes and PCR cycling parameters

| Target strain | Primer sequence (5' → 3') | Amplicon size (bp) | PCR cycling condition |
|---|---|---|---|
| ***Klebsiella* genus** | F:GAGCAAGCGGACCTCATAAA<br>R:CGGTTACCTTGTTACGACTTCA | 241 | Initial denaturation of 5 min at 94$^0$C followed by 35 cycles, denaturation at 95$^0$C for 30 sec, annealing at 49$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 10 min |
| ***Klebsiella pneumoniae*** | F: GGAGGTATCAGAAGTGCGAATG<br>R: CACACCTCAGCCTTGATTATCC | 300 | Initial denaturation of 5 min at 94$^0$C followed by 35 cycles, denaturation at 95$^0$C for 30 sec, annealing at 50$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 10 min |

Table 2: Primer sequences for detection of selected carbapenem resistance genes

| Target gene | Primer sequence (5' → 3') | Amplicon size (bp) | PCR cycling condition |
|---|---|---|---|
| *bla*$_{NDM-1}$ | F: GGTTTGGCGATCTGGTTTTC<br>R: CGGAATGGCTCATCACGATC | 624 | Initial denaturation of 5 min at 94$^0$C followed by 30 cycles, denaturation at 95$^0$C for 30 sec, annealing at 50$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 5 min |
| *bla*$_{OXA-48-like}$ | F: TTCTGTTGTTTGGGTTTCGC<br>R: ACGCAGGAATTGAATTTGTTC | 190 | Initial denaturation of 5 min at 94$^0$C followed by 30 cycles, denaturation at 95$^0$C for 30 sec, annealing at 47$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 5 min |
| *bla*$_{KPC}$ | F: CATTCAAGGGCTTTCTTGCTGC<br>R: ACGACGGCATAGTCATTTGC | 498 | Initial denaturation of 5 min at 94$^0$C followed by 30 cycles, denaturation at 95$^0$C for 30 sec, annealing at 47$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 5 min |
| *bla*$_{VIM}$ | F: GGTGTTTGGTCGCATATCGCAA<br>R: ATTCAGCCAGATCGGCATCGGC | 502 | Initial denaturation of 5 min at 94$^0$C followed by 30 cycles, denaturation at 95$^0$C for 30 sec, annealing at 50$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 5 min |
| *bla*$_{IMP}$ | F: TCGTTTGAAGAAGTTAACG<br>R: ATGTAAGTTTCAAGAGTGATGC | 568 | Initial denaturation of 5 min at 94$^0$C followed by 30 cycles, denaturation at 95$^0$C for 30 sec, annealing at 47$^0$C for 40 sec, extension at 72$^0$C for 40 sec, and final extension at 72$^0$C for 5 min |

***Detection of carbapenemase genes by multiplex PCR:***

*Klebsiella* spp isolates that exhibited phenotypic resistance to imipenem (n=23) were further screened for relevant carbapenemase-coding genes using conventional multiplex PCR assay and PCR products were viewed using Agarose Gel Electrophoresis (AGE). The set of primers and reaction protocols applied are highlighted in Table 2.

**Statistical analysis:**

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. Descriptive statistics such as frequencies and percentages were used to summarize the distribution of carbapenemase-producing isolates and their antimicrobial susceptibility patterns.

## Results:

**Frequency distribution of *Klebsiella* isolates across clinical samples and hospital wards:**

Of the 120 presumptive *Klebsiella* spp tested, 95 (80.0%) were biochemically confirmed as *Klebsiella* spp while only 80 remained viable after subsequent subculturing. The pathogen was predominantly recovered from urine specimen (n=30, 37.5%).

Majority of the confirmed isolates were recovered from the medical (n=28, 35.0%) and surgical (n=28, 35.0%) wards in UCH while 17 (21.3%) were from outside facilities (Oluyoro Catholic hospital and Adeoyo Maternity Teaching hospital Ibadan) (Table 3 and 4).

Table 3: Distribution of *Klebsiella* spp across clinical samples

| Clinical sample | Frequency (%) | | | |
|---|---|---|---|---|
| | ***Klebsiella* spp*** | **Culture positive *Klebsiella* spp** | **CRKP** | **CpCRKP** |
| Urine | 42 (35.0) | 30 (37.5) | 12 (40.0) | 5 (16.7) |
| Wound **s**wab | 22 (18.3) | 14 (17.5) | 2 (14.3) | 0 |
| Eye **s**wab | 3 (2.5) | 2 (2.5) | 0 | 0 |
| Pus | 4 (3.3) | 1 (1.3) | 1 (100.0) | 0 |
| Wound **a**spirate | 6 (5.0) | 2 (2.5) | 0 | 0 |
| Sputum | 15 (12.5) | 12 (15.0) | 3 (25.0) | 0 |
| High **v**aginal **s**wab | 6 (5.0) | 5 (6.3) | 1 (20.0) | 0 |
| Blood **c**ulture | 6 (5.0) | 6 (7.5) | 2 (33.3) | 0 |
| Tracheal **a**spirate | 1 (0.8) | 1 (1.3) | 1 (100.0) | 0 |
| Wound **b**iopsy | 5 (4.2) | 1 (1.3) | 0 | 0 |
| Throat **s**wab | 3 (2.5) | 1 (1.3) | 0 | 0 |
| Stool | 3 (2.5) | 3 (3.8) | 1 (33.3) | 0 |
| Ear **s**wab | 4 (3.3) | 2 (2.5) | 0 | 0 |
| Total | **120 (100.0)** | **80 (100.0)** | **23 (28.8)** | **5 (6.3)** |

*Presumptive isolates; CRKP - Carbapenem-resistant *Klebsiella* spp; CpCRKP-Carbapenemase-producing Carbapenem-resistant *Klebsiella* spp

Table 4: Distribution of *Klebsiella* isolates across different wards in UCH Ibadan and outside facility

| Ward | Frequency (%) | | | |
|---|---|---|---|---|
| | ***Klebsiella* spp.*** | **Culture positive *Klebsiella* spp** | **CRKP** | **CpCRKP** |
| Medical wards | 38 (31.7) | 28 (35.0) | 5 (17.9) | 2 (7.1) |
| Surgical wards | 50 (41.7) | 28 (35.0) | 6 (21.4) | 2 (7.1) |
| Emergency | 10 (8.3) | 7 (8.8) | 1 (14.3) | 0 |
| Outside UCH | 22 (18.3) | 17 (21.3) | 11 (64.7) | 1 (5.9) |
| Total | **120 (100.0)** | **80 (100.0)** | **23 (28.8)** | **5 (6.3)** |

*Presumptive isolates; CRKP - Carbapenem-resistant *Klebsiella* spp; CpCRKP-Carbapenemase-producing Carbapenem-resistant *Klebsiella* spp

**Antibiotic susceptibility test results:**

Of 80 viable isolates following sub-culturing, 40 (50.0%) were resistant to ceftazidime, 62 (77.5%) to cefoxitin, 36 (45.0%) to levofloxacin, 49 (61.3%) to ciprofloxacin, 30 (37.5%) to gentamicin, and 23 (28.8%) to imipenem (Fig 1).

All 80 isolates were resistant to both piperacillin and amoxicillin-clavulanate while 50 (62.5%) expressed multidrug resistance phenotype, with 19 different antimicrobial resistant patterns identified, CAZ/FOX/LEV/CIP/PRL/AUG/GM/IPM being the most predominant (n=11, 22.0%) resistance pattern (Table 5).

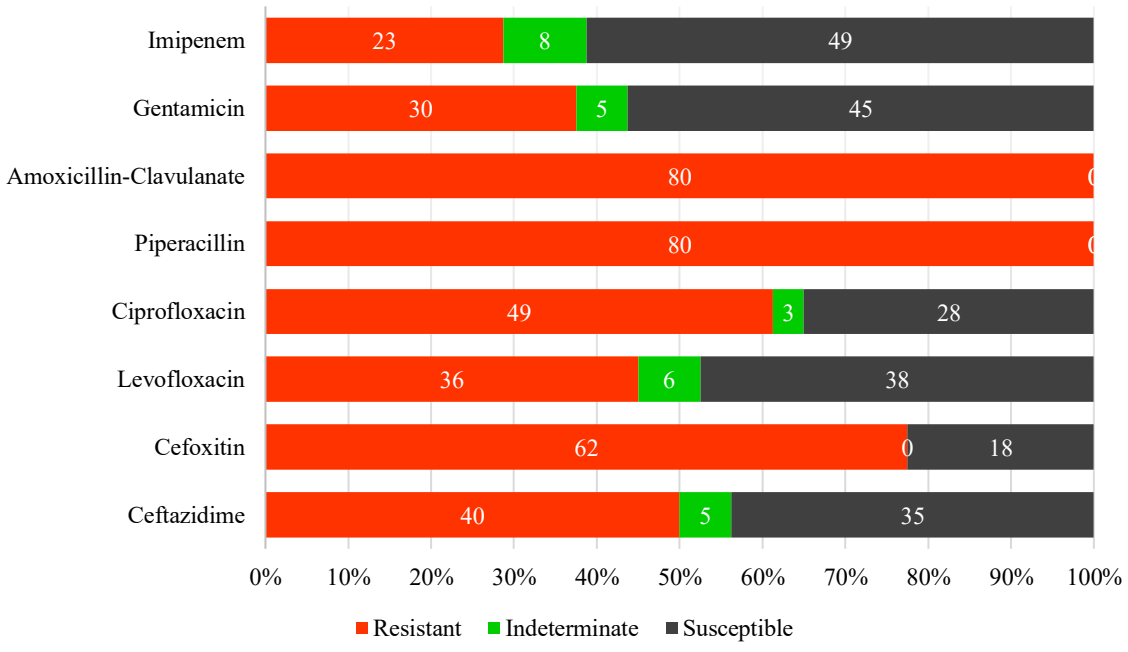


Fig 1: Antibiotic sensitivity pattern of *Klebsiella* isolates

Table 5: Multi-drug-resistant patterns of *Klebsiella* isolates

| S/N | MDR patterns | No of isolates (%) | MDR pattern |
|---|---|---|---|
| 1 | CAZ/FOX/LEV/CIP/PRL/AUG/GM/IPM | 11 (22.0) | MDR |
| 2 | CAZ/FOX/LEV/CIP/PRL/AUG/GM | 6 (12.0) | MDR |
| 3 | CAZ/FOX/LEV/CIP/PRL/AUG/IPM | 3 (6.0) | MDR |
| 4 | CAZ/FOX/CIP/PRL/AUG/GM/IPM | 3 (6.0) | MDR |
| 5 | CAZ/FOX/CIP/PRL/AUG/IPM | 3 (6.0) | MDR |
| 6 | CAZ/FOX/LEV/CIP/PRL/AUG | 5 (10.0) | MDR |
| 7 | CAZ/LEV/CIP/PRL/AUG/GM | 1 (2.0) | MDR |
| 8 | CAZ/FOX/PRL/AUG/GM/IPM | 1 (2.0) | MDR |
| 9 | CAZ/FOX/PRL/AUG/GM | 1 (2.0) | MDR |
| 10 | CAZ/CIP/PRL/AUG/GM | 1 (2.0) | MDR |
| 11 | CAZ/PRL/AUG/GM | 1 (2.0) | MDR |
| 12 | CAZ/CIP/PRL/AUG | 1 (2.0) | MDR |
| 13 | FOX/LEV/CIP/PRL/AUG/GM | 2 (4.0) | MDR |
| 14 | FOX/CIP/PRL/AUG/GM/IPM | 1 (2.0) | MDR |
| 15 | FOX/CIP/PRL/AUG | 2 (4.0) | MDR |
| 16 | FOX/LEV/CIP/PRL/AUG | 5 (10.0) | MDR |
| 17 | LEV/CIP/PRL/AUG | 1 (2.0) | MDR |
| 18 | LEV/CIP/PRL/AUG/GM | 1 (2.0) | MDR |
| 19 | LEV/PRL/AUG/GM | 1 (2.0) | MDR |
| | **Total** | **50 (100.0)** | **MDR** |

Ceftazidime –CAZ; Cefoxitin –FOX; Levofloxacin –LEV; Ciprofloxacin –CIP; Piperacillin –PRL; Amoxicillin-clavulanate -AUG; GM –Gentamicin; IPM- Imipenem.

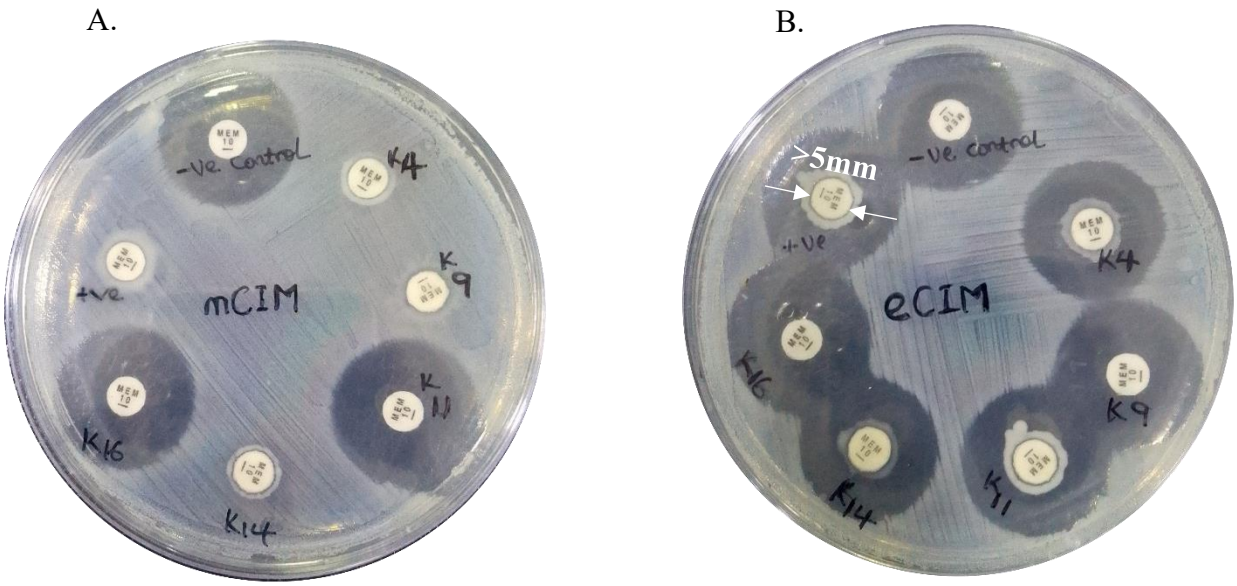


Fig 2: Phenotypic Test result for carbapenemase production. A. Modified Carbapenem Inactivation Method results: K11 and K16 -Negative isolates, K4, K9, & K14 -Positive isolates. B. EDTA- Carbapenem Inactivation Method results: K4, K9, & K14 -Positive isolates.

**Phenotypic detection of carbapenemase production:**

In this study, 5 (6.3%) of the 80 isolates were phenotypically identified as carbapenemase producers, representing 25.0% of the carbapenem-resistant strains confirmed by both mCIM and eCIM methods. Fig 2 below shows the result of mCIM and eCIM simultaneously carried out on carbapenem-resistant isolates.

**Molecular confirmation of carbapenem-resistant *Klebsiella* spp:**

Twenty-three (28.8%) biochemically confirmed *Klebsiella* spp isolates were resistant to imipenem, 20 (25.0%) were confirmed as *Klebsiellae* using PCR (Fig 3) and 17 (21.3%) were identified as *Klebsiella pneumoniae* with the aid of specific primers (Fig 4).

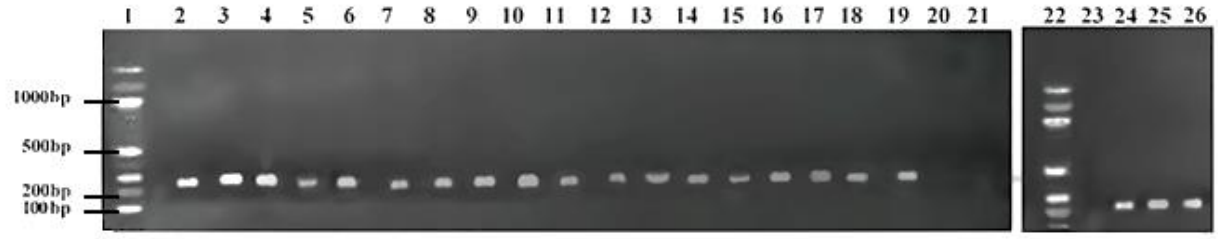


Fig 3: PCR products of the amplification of *Klebsiella* spp.-specific gene. Lane 1 & 22: 100.0 bp molecular weight marker; Lane 2: positive control; Lane 3-19, & 24-26: positive isolates; Lanes 20, 21 & 23: negative isolates.

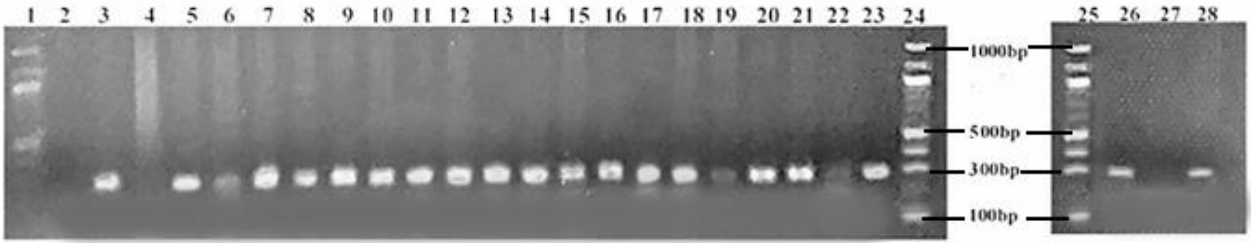


Fig 4: PCR products of the amplification of *Klebsiella pneumoniae*-specific gene. Lane 1, 24 & 25: 100.0 bp molecular weight marker; Lane 2: negative control; Lane 3: positive control; Lanes 4, 22 & 27: negative isolates; Lanes 5-19, 26 & 28: positive isolates. Lanes 20/21 (repeated), 22 & 23: *Klebsiella* spp. negative isolates.

**Molecular detection of the relevant carbapenem resistance genes:**

The PCR amplification products for the $bla_{\text{NDM-1}}$ gene multiplexed with $bla_{\text{VIM}}$, $bla_{\text{KPC}}$ with $bla_{\text{OXA-48}}$, and $bla_{\text{IMP}}$ genes are shown in Figs 5 and 6. Of the 5 isolates positive for phenotypic carbapenemase detection, 3 harbored the $bla_{\text{NDM-1}}$ gene, while none expressed other resistance genes.

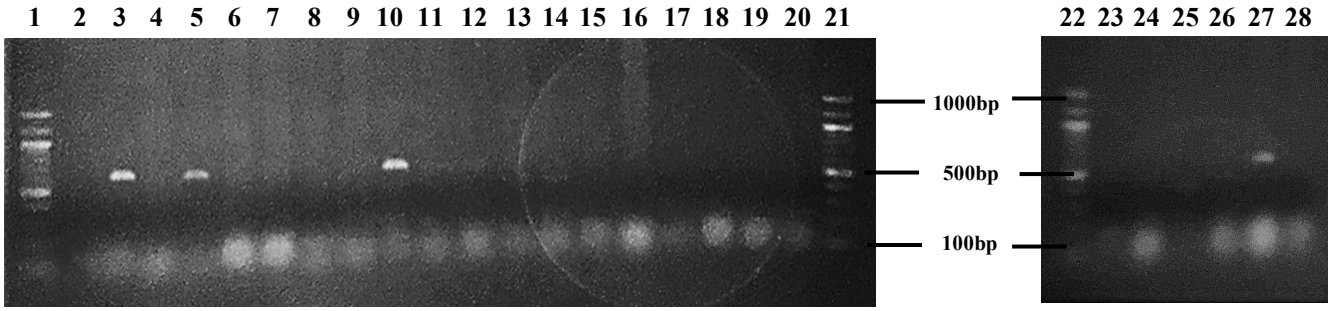


Fig 5: PCR products of the amplification of $bla_{\text{NDM-1}}$ multiplexed with $bla_{\text{VIM}}$, and $bla_{\text{KPC}}$ genes. Lane 1, 21 & 22: 100.0 bp molecular weight marker; Lane 2: negative control; Lane 3: NDM positive control; Lanes 4, 6-9, 11-20, 23-26, & 28: negative isolates; Lanes 5, 10 & 27: positive isolates.

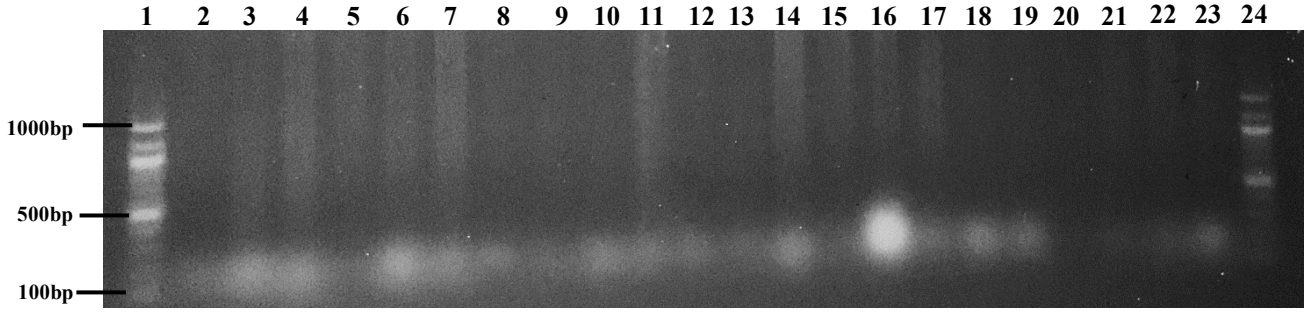


Fig 6: PCR products of the amplification of $bla_{\text{OXA-48}}$ and $bla_{\text{IMP}}$ genes. Lane 1, & 24: 100.0 bp molecular weight marker; Lane 2: negative control; Lanes 3-23: negative isolates.

## Discussion:

The emergence of antibiotic resistance in Gram-negative bacteria is a significant threat to public health (22). *Klebsiella* spp is associated with infections affecting the lungs, urinary tract, gastrointestinal tract, bloodstream, wound or surgical site and other body sites (23). These bacteria have demonstrated notable resistance to several antibiotics, including carbapenems, the 'last-resort' drug for multidrug-resistant infections. The spread of carbapenemases has worsened this problem, with continuous dissemination across clinical settings, communities and the environment (24).

This study investigated the carbapenemase-producing *Klebsiella* isolates in clinical samples, providing a comprehensive analysis of AMR patterns, MDR, and the mechanisms underlying carbapenem resistance. From this study, *Klebsiella* spp were mostly isolated from urine samples (37.5%), wound swabs (17.5%) and sputum (15.0%). Previous studies show similar distributions with 35.4% in urine, 26.2% in wound swabs, and 13.4% in sputum as reported by Mukail et al., (25), and 45.8% in urine, 29.2% in sputum, and 12.5% in wound swab as reported by Jeremiah et al., (26). The lower rates in our study may be due to differences in sample size. The predominance of isolates from urine samples highlights the urinary tract as a significant reservoir of the pathogen (27,28). Wound swabs and sputum samples contributed to the spectrum, aligning with findings that emphasize the organisms' role in wound and respiratory infections (29).

*Klebsiella* infections were widespread in different clinical wards, with the highest frequency in both surgical (35.0%) and medical (35.0%) wards. Surgical site infections (SSIs) are common healthcare-associated infections, often caused by bacteria from patients' normal flora introduced during surgery (30-32). Immunocompromised individuals are particularly at risk of developing SSIs (33). Other studies carried out in Russia and Romania have reported *Klebsiella* spp to be the predominant cause of infections in surgical patients (34, 35). In the Romania study, *Klebsiella* infections in hospital wards showed a prevalence range of 5.25% to 19.49% in surgical wards, and 5.15% to 17.36% in medical wards. In our study, similarly high *Klebsiella* prevalence was observed in the medical wards (35.0%), reflecting *Klebsiella* versatile clinical presentations from pneumonia to sepsis. Interestingly, the high frequency of isolation of *Klebsiella* spp from out-patient samples in other hospitals (21.3%) indicates a potential community source.

In this study, antibiotic resistance among *Klebsiella* isolates is quite alarming, with 100.0% resistance to piperacillin and amoxicillin/clavulanate. Similar observations have been reported in other study (36), suggesting a consistent trend of high-level resistance of *Klebsiella* spp to piperacillin; although a lower resistance rate of 54.2% against amoxicillin-clavulanate was observed. A similar observation of 50.0% resistance rate was reported by Mukail and colleagues in Kaduna, northern Nigeria (25). The isolates also exhibited considerable resistance to cefoxitin (77.5%), comparable to an Ethiopian study where 79.3% resistance was reported (37). Resistance to gentamicin, ceftazidime, levofloxacin, and ciprofloxacin was also high, reflecting limited treatment options for infected patients.

The observation of carbapenem resistance among *Klebsiella* isolates in this study highlights a concerning trend with significant clinical implications. Twenty (25.0%) of the 80 PCR-confirmed *Klebsiella* isolates were resistant to imipenem. Other studies have reported lower resistance rates of 4.2%, 10.9% and 21.9% respectively (25,36,38). The *invitro* efficacy of imipenem in our study is however lower compared to previous studies in Nigeria and India (39,40). Differences in antibiotic use and healthcare practices may likely be responsible for these variations.

Reports of carbapenemase production in *Klebsiella* spp which hydrolyze carbapenems and render them ineffective, have increased globally. Our study found a 6.3% prevalence of carbapenemase production, similar to the study of Jeremiah et al., (26) who reported a 6.2% phenotypic expression of carbapenemase in *Klebsiella pneumoniae*. Marked variation in prevalence has also been observed, such as the Kaduna study with prevalence as high as 24.0% (25). Alternative resistance mechanisms, such as efflux pumps or cell wall modifications, may also be involved (26,41)

Molecular detection of carbapenem resistance genes helps to better understand the genetic basis underlying antibiotic resistance. In this study, NDM-1 gene was found in 60.0% (3 of 5) of isolates that produced carbapenemase. The gene coding for NDM-1 is usually found on distinct large plasmids, facilitating its high frequency transfer to susceptible bacteria. These plasmids are implicated in conferring resistance to nearly all antibiotics (42). This can explain why all the isolates harboring the NDM-1 gene in this study were resistant to all antibiotics used. The observed prevalence of NDM-1 (3.8%) in this study is comparable to the findings by Mushi *et al*. (43) and Okoche et al., (44) who reported 3.1% and 2.6% prevalence rates respectively.

The non-detection of other genes tested in this study, however positive Phenotypically, could be explained by the potential occurrence of false negative PCR results associated with mutations that could affect the anne-

aling of primers (45). The non-detection of other resistance genes may also suggest a specific regional prevalence or a unique genetic profile of the studied *Klebsiella* population. This observation is comparable to the findings in a recent study conducted by Onyeji *et al*. (46), who reported the non-detection of carbapenemase genes in 46.0% of the isolates resistant to carbapenems. Likewise, in a study carried out in Iran by Moradi et al., (47), a discrepant result was observed with 15.7% of isolates phenotypically testing positive by modified Hodge Test, while PCR was negative.

Our study is limited by a number of factors. The exact prevalence of carbapenem resistance across individual species of *Klebsiella* was not estimated. Also, other mechanisms responsible for resistance in PCR negative isolates were not investigated. The omission of these investigations was primarily due to limitations in funding, laboratory resources, and time. This could have provided insights on uncommon or emerging carbapenemases and non-carbapenemase-controlled mechanism of resistance in *Klebsiella* spp.

## Conclusion:

The detection of $bla_{NDM-1}$ and high antimicrobial resistance rates among clinical *Klebsiella* isolates is alarming in this study. The emergence of carbapenem-resistant *Klebsiella*, especially with the $bla_{NDM-1}$ gene, threatens the effectiveness of last-resort antibiotics. The recovery of both multidrug-resistant and carbapenem-resistant strains from various hospital wards and the community calls for a need to ensure implementation of infection prevention and control policy in the hospital. Additionally, it also justifies the need for antimicrobial stewardship.

## Contributions of authors:

SAA was involved in the study conceptualization, design, methodological activities, and initial draft production; KAO was involved in the study conceptualization, design, initial draft production and supervision; AI was involved in data collection and methodological activities; AVO designed all primers used; MOB and OAB were involved in the critical review of the initial draft; All authors were involved in findings interpretation, critical review and approval of the final draft.

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## Conflict of interest

Authors declare no conflict of interest.

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