Detection of Carbapenemase Genes Among Multi-Drug Resistant *Klebsiella Pneumoniae* from Clinical Specimens in Ibadan

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Abstract- ***Carbapenem-resistant Klebsiella pneumoniae (CRKP) has become a serious public health concern as it can resist last-resort antibiotics, making treatment more challenging. This research examines the genetic characteristic of multidrug-resistant (MDR) K. pneumoniae obtained from clinical specimens in Ibadan tertiary facility clinics, particularly detecting carbapenem resistance genes. 156 clinical specimens, including sputum, wound swabs, and urine, were collected from selected healthcare facilities. Bacterial isolation and identification were performed using standard microbiological methods and antimicrobial susceptibility testing to assess resistance patterns. Molecular analysis was conducted using polymerase chain reaction (PCR) to detect genes responsible for carbapenem resistance, such as blaKPC, blaNDM, and blaVIM. Of the 156 clinical specimens examined, 52(33.8%) were positive for Klebsiella pneumoniae with the most significant proportion obtained from urine, 28(53.8%). The antimicrobial susceptibility testing revealed multi-drug resistance, with imipenem (98.1%), cefotaxime (90.4%), and cefuroxime (92.3%) among the most affected antibiotics. However, meropenem (69.2%) showed the highest susceptibility, suggesting potential differences in carbapenemase gene spread and expression. The average Multiple Antibiotic Resistance (MAR) index (0.85) obtained from AST assay further suggests strong antibiotic selection pressure among hospitals in this region, identifying the genetic factors driving carbapenem resistance. Molecular analysis via PCR assays confirmed the presence of carbapenemase genes, with bla_NDM (47%), bla_VIM, and bla_KPC identified in several isolates. The detection of these genes highlights the growing threat of carbapenem-resistant strains and suggests horizontal gene transfer as a potential mechanism for resistance spread. The widespread use and misuse of imipenem and meropenem may have contributed to the selection pressure driving this resistance. This study, however, emphasizes the pressing need for stronger antimicrobial stewardship, surveillance programs, and alternative treatment options to curb the spread of carbapenem-resistant bacteria in both clinical and public health environments.***

Indexed Terms- ***Resistance, Klebsiella pneumoniae, Carbapenemase, resistance genes(carbapenemase), Ibadan***

I. INTRODUCTION

Klebsiella pneumoniae is a significant bacterial species within the Enterobacteriaceae family. This Gram-negative bacterium is naturally found on human skin and in the mouth and intestines, contributing to the normal microbial community (Martin and Bachman, 2018). Klebsiella pneumoniae frequently exists in certain body areas, where it sustains itself, yet it exhibits well-known behavior as an opportunistic infection agent. The risks of infection associated with Klebsiella pneumoniae emerge when there is a weakness in the immune system or a disruption of natural body defenses(CDC, 2013). . The pathogenicity of Klebsiella pneumoniae infections among hospitalized patients with

compromised immune systems leads to infections such as UTIs, septicemia, skin wounds, liver sores, and brain inflammation (Effah et al.,2020). Treating these infections becomes increasingly challenging because the bacteria can quickly develop resistance to multiple antibiotics. Klebsiella pneumoniae is most commonly implicated in CRE infections. The rapid increase in carbapenem-resistant Klebsiella pneumoniae is particularly concerning because carbapenems are typically regarded as the last option for treating serious bacterial infections. The ability of Klebsiella pneumoniae to withstand these strong antibiotics severely limits treatment options, posing a critical challenge to public health systems globally. K. pneumoniae is a major contributor to nosocomial infections, as it can persist in medical facilities, survive on surfaces, and colonize the skin, respiratory system, and intestines of individuals (Ashurst and Dawson, 2023). Transmission can easily occur through contact with medical staff (Pessoa-Silva et al., 2007). Consequently, a frequent source of outbreaks in newborn intensive care units (Fabbri et al., 2013).

Antimicrobials have long been employed to combat Klebsiella pneumoniae infections. However, these infections often prove highly resistant to treatment efforts. Persistent antibiotic treatment of Klebsiella pneumoniae strains with various drugs has created conditions where multidrug-resistant (MDR) strains naturally proliferate. These MDR strains develop resistance toward multiple antibiotic groups which include both broad-range beta-lactam drugs, together with two key antibiotic classes, aminoglycosides and fluoroquinolones. The dangerous aspect of these bacterial strains is their ability to overcome carbapenem antibiotics (Peter et al., 2018. The rising resistance patterns demonstrate the immediate need to develop novel therapeutic methods and improved antibiotic management practices to control and lower Klebsiella pneumoniae infection impacts. The isolation of Klebsiella pneumoniae strains resistant to multiple antibiotics increases due to different variables; The bacterium expresses essential efflux pump channels inside cells that forcefully push out numerous antimicrobial compounds through the cellular membrane thus lowering their therapeutic potential. Excessive and uncontrolled antibiotic use creates an environment that allows resistant strains to multiply because it encourages their selection. The critical nature of these isolates includes their ability to create novel beta-lactamase enzymes with enhanced hydrolytic functions. Three types of beta-lactamase enzymes, including ESBLs and AmpC variants and carbapenem-hydrolyzing enzymes, specifically carbapenemases, function to break down significant antimicrobials of all beta-lactam antibiotic classes, including cephalosporins and carbapenems, which are considered the most potent antibiotic drugs available variants (Thomson,2010). Klebsiella pneumoniae beta-lactamases lower the effectiveness of essential antibiotics, thus creating a significant threat for treating this infection and emphasizing the necessity for alternative treatment approaches and better antibiotic practices.

Several studies, including Akinyemi et al. (2021) and Raji et al. (2013), have documented Klebsiella pneumoniae drug resistance. However, there is a paucity of molecular information about the prevalence of MDR Klebsiella pneumoniae in Ibadan and throughout southwest Nigeria. Specimens will be collected from different locations before evaluating drug-resistant Klebsiella pneumoniae using phenotypic and molecular techniques. Klebsiella pneumoniae is one of the numerous opportunistic pathogens that lead to various infections, while carbapenem-resistant Klebsiella pneumoniae is recognized as a significant worldwide health concern. This study focused on evaluating the prevalence and investigation of CRKP, a strain resistant to carbapenems, while detecting carbapenem-resistant genes from clinical specimens in Ibadan, Nigeria.

II. METHODS

- Study area and design

A descriptive cross-sectional study design was used for this study. Specimens were obtained from both male and female patients of different ages between July and December 2024 among in-patients attending University College Hospital Ibadan, Adeoyo Maternity Hospital Ibadan, Adeoyo State Hopsital Ibadan, and University of Ibadan Clinic (Jaja). Ethical approval for this study was obtained from the research and ethics committee, Ministry of Health, Oyo State. Patient confidentiality was strictly

maintained while ensuring the disposal of used specimens via autoclaving.

- Specimen collection and culturing

A total of 156 clinical specimens were collected in a sterile disposable container and taken to the Pharmaceutical Microbiology Laboratory, University of Ibadan, for processing and examination.

The clinical 156 specimens (73 urine, 67 sputum and 16 wound swab); sputum and wound swab were cultured on MacConkey agar while Urine specimens were cultured on cysteine Lactose electrolyte deficient agar(CLED) and incubated at 37°C for 24 hours. Morphological appearance of pink colony with string upto 3cm long were noted for suspected Klebsiella pneumoniae Gram stain and biochemical test(Lactose fermentation test, catalase test, citrate test, oxidase test, urease test, indole test) was further conducted as described by Vandepitte et al, (2003), in the identification of Klebsiella pneumoniae

- Antimicrobial Sensitivity Testing

Antimicrobial susceptibility testing was carried out on the isolates using the disc diffusion (Kirby–Bauer) method on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2024). The antibiotic discs used were purchased from Celtech Diagnostic Limited, Belgium, and were impregnated with the following antibiotics: Penicillins (amoxicillin 30µg, ampiclox 10 µg), Cephalosporins (cefotaxime 25 µg, cefixime 5 µg, cefuroxime 30 µg, ceftriaxone 45 µg), Aminoglycoside (gentamicin 10 µg), Carbapenems (Meropenem 10µg, imipenem 10 µg), and Fluoroquinolones (levofloxacin 5 µg, ofloxacin 5 µg), Quinolones (Nalidixic Acid 30µg), Nitrofurans (Nitrofurantoin 300µg). Colonies from a 24-hour bacterial culture were suspended in normal saline, and the turbidity of the suspension was adjusted to match the 0.5 McFarland standard.The colony was evenly seeded onto the Mueller-Hinton agar plates using sterile swabs to provide proper distribution of the bacteria. The antibiotic discs were then placed on the inoculated agar using sterile forceps with caution and firmly. The plates were then turned over and incubated at 37°C for 18 hours (CLSI, 2024). After incubation, isolates with zone diameters <19 mm to meropenem and Imipenem (10µg) as stated in the CLSI standard guidelines for detecting resistance were classified as carbapenem resistant.

- PCR amplification of resistance genes (VIM, KPC, NDM)

Molecular investigations of carbapenemase genes in the selected isolates was done by simple PCR on the extracted DNA using carbapenemase-coding region-specific primers sequence (Table 3.1.). The reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR green buffer (2.5), MgCl2 (0.75), 10pM DNTP (0.25), 10pM of each forward and backward primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification PCR was carried out in a GeneAmp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

Table 1. Primers used in gene amplification

Gene	Primer	Primer sequence	Fragment size(bp)	Ref.
blakpc	Forward Reverse	TGTTGCTGAAGGAGTTGGGC ACGACGGCATAGTCATTTGC	498	Mlynarcik *et al*
blavim	Forward Reverse	CGCGGAGATTGARAAGCAAA CGCAGCACCRGGATAGAARA	500	
blandm	Forward Reverse	CGCAGCACCRGGATAGAARA AAATGGAAACTGGCGACC	634	
Kelbp	Forward Reverse	GGAGGTATCAGAAGTGCGAATG CACACCTCAGCCTTGATTATCC	270	

Statistical Analysis

Data generated from the study was analyzed using SPSS version 26 statistical software.

III. RESULTS

A total of 156 clinical specimens, including urine, wound swabs, and sputum, were collected between July 2024 and November 2024 from four selected healthcare centers in Ibadan Metropolis, Oyo State as shown in table 1. Among the isolates obtained, the highest incidence of Klebsiella pneumoniae was found in urine samples, accounting for 28 isolates (53.8%), followed by sputum with 21 isolates (40.4%), and wound swabs with 3 isolates (5.8%), as presented in Table 2, with a statistically significant p-value of 0.001.

Among the 52 Klebsiella isolates obtained, 28 (54%) were from male patients and 24 (46%) from females, with a p-value of 0.58. Additionally, 28 (53.8%) of the Klebsiella pneumoniae isolates were recovered from patients aged between 0 and 39 years, while the remaining 24 (46.3%) were from patients aged 40 years and above, with a p-value of 0.579, as detailed in Table 4.3.

Antimicrobial susceptibility testing was conducted on 52 Klebsiella pneumoniae isolates using 13 antibiotics. The highest resistance rates were observed against amoxicillin (100%), imipenem (98.1%), cefuroxime (92.3%), cefotaxime (90.4%), nitrofurantoin (90.4%), and nalidixic acid (86.5%). Resistance to fluoroquinolones, such as levofloxacin (84.6%), and aminoglycosides such as gentamicin (82.7%), was also significant. Despite the widespread resistance, some isolates retained susceptibility to a few antibiotics. Meropenem exhibited the highest activity level, with 69.2% of isolates being susceptible, followed by ceftriaxone (17.3%) and ofloxacin (17.3%). However, minimal susceptibility was recorded for levofloxacin (1.9%) and imipenem (1.9%). Most isolates had a MAR index ≥ 0.85, suggesting that they originated from high-risk environments with significant antibiotic pressure, such as hospital settings and intensive care units (ICUs). The highest recorded MAR index was 0.92, indicating resistance to nearly all tested antibiotics.

The 15 isolates that exhibited resistance to both meropenem and Imipenem were further subjected to detection of ¬blaKPC, blaNDM, and blaVIM resistance genes using Polymerase chain reaction. The reaction confirmed the presence of blaNDM in four of the isolates, blaVIM in seven of the isolates, and blaKPC in three of the isolates as shown in figure 4 and 5.

Table 1. Distribtion of Klebsiella pneumoniae obtained from clinical specimens across study sites.

Isolates Codes	*K. Pneumoniae*	Total
Uch	18(12.0%)	53(34.0%)
Amh	31(19.8%)	91(58.3%)
Ash	0(0.0%)	1(0.7%)
Jc	3(2.0%)	11(7.0%)
Total	52(33.8%)	156(100.0%)

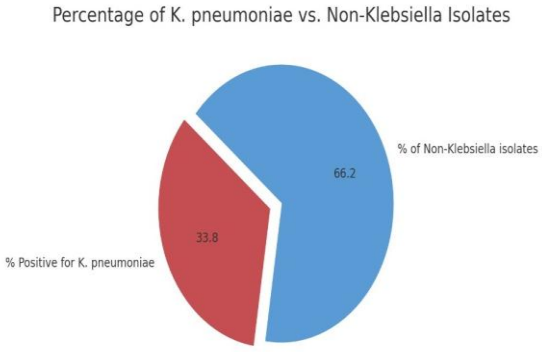

Figure 1 Prevalence of Klebsiella pneumoniae isolated in some clinical specimens in Nigeria, Nigeria

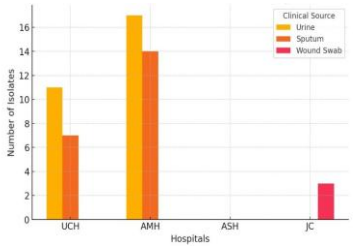

Figure 2 Distribution of Klebsiella pneumoniae from different clinical specimens in Ibadan, Nigeria

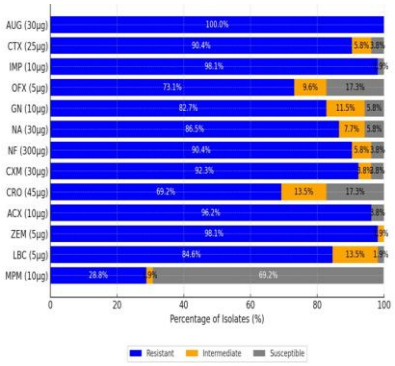

Figure 3. Antibiotic susceptibility pattern of Klebsiella pneunomiae isolates from Ibadan, Nigeria. (N=52).

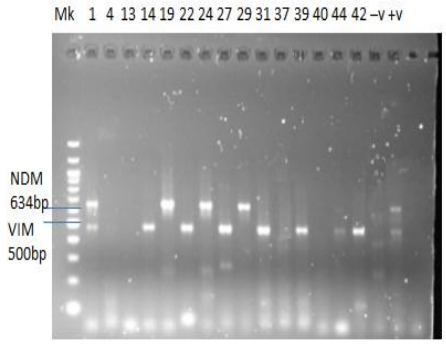

Figure 4. Gel electrophoresis of the PCR amplicons showing bla_NDM and bla_VIM genes from the representative CRKP isolates

KEYS: 1= U56, 4=U27, 13=S48, 14=S51, 19=U38, 22= U69, 24=U67, 27=U39, 29=WS7, 31= S53, 37=U15, 39=U13, 40=U12, 44=U37, 42=S43, +ve=positive control, M= DNA ladder band size

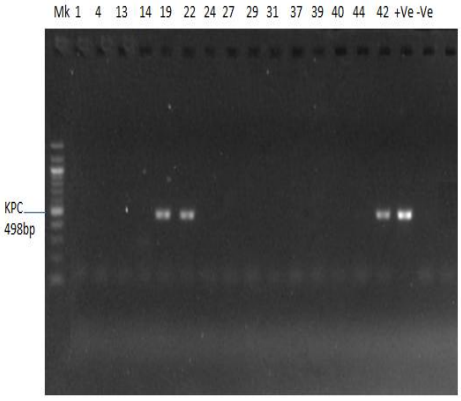

Figure 5.Gel electrophoresis of the PCR amplicons showing bla_KPC gene from the representative CRKP isolates

KEYS: 1= U56, 4=U27, 13=S48, 14=S51, 19=U38, 22= U69, 24=U67, 27=U39, 29=WS7, 31= S53, 37=U15, 39=U13, 40=U12, 44=U37, 42=S43, +ve=positive control, M= DNA ladder band size

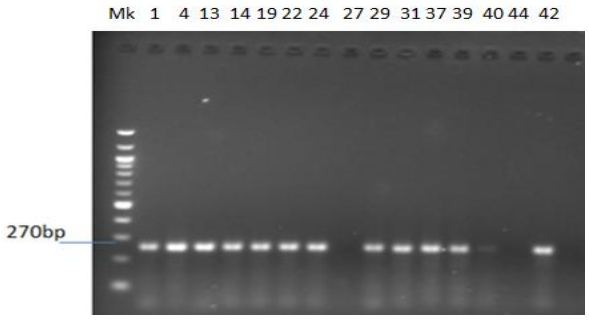

Figure 6. Gel electrophoresis of the PCR amplicons showing 16SrRNA from the representative CRKP isolates

KEYS: 1= U56, 4=U27, 13=S48, 14=S51, 19=U38, 22= U69, 24=U67, 27=U39, 29=WS7, 31= S53, 37=U15, 39=U13, 40=U12, 44=U37, 42=S43, +ve=positive control, M= DNA ladder band size

IV. DISCUSSION

The rising occurrence of multi-drug resistant (MDR) Klebsiella pneumoniae in clinical specimens is a global public health concern. This organism demonstrates significant resistance to available antibiotics, particularly carbapenems, which are considered the last line of defense. Infections caused by carbapenem-resistant Klebsiella pneumoniae (CRKP) are associated with high morbidity and mortality rates (Li et al., 2023). In this study, a significant number of K. pneumoniae isolates were obtained from clinical samples, reflecting the widespread occurrence of this Uropathogen in hospital settings. An overall prevalence of Klebsiella pneumoniae of 33.8% was observed among the clinical specimens collected across the four study sites of the metropolis. This is consistent with the 50% prevalence reported in Uganda (Lubega and Joel, 2017), which highlights K. pneumoniae as a major uropathogen and wound-infecting agent, and similar to the 20% reported in a cross-sectional study in Ethiopia (Minichil et al., 2024). The prevalence of 12.5% obtained from a study in Nassarawa (Ayeni et al., 2024) reports a lower occurrence among Urinary tract infection patients

The incidence of Klebsiella pneumoniae infection differs by geographical region (Ko et al., 2002; Shamanna et al., 2024). The ability of K. pneumoniae to persist in hospital environments, colonize medical devices, and exhibit high transmission rates among patients contributes to its clinical significance (Vock et al., 2019). Elements including unprotected sexual intercourse, peer group influence, and low socio-economic status among Nigerian genders could have contributed to the high prevalence obtained from this study (Hooton, 2000).

Contrary to study in Ethiopia (Worku et al, 2024) which reported the highest prevalence of Klebsiella pneumoniae in wound The highest incidence of isolates obtained in this study was from urine specimens 28 (53.8%), followed by wound swabs 3(5.8%) and sputum 21(40.4 %) with a P-value of 0.001. This finding is consistent with the previous study conducted by Olowo-okere et al. (2020). Echendu et al, (2024) also reported a prevailence in urine from a cross sectional study on the prevalence of Klebsiella pneumoniae among pregnant women in the south easten part of Nigeria. In a fairly separate study from this research Okoch et al (2015) reported the highest prevalence of Klebsiella pneumoniae in pus swab. The urine sample, which is the primary source of K. pneumoniae in this study, indicates urinary tract infections and represents nearly 45% of healthcare-associated infections globally (Vidal-Cortés, 2022)

The results of antimicrobial susceptibility testing revealed extensive resistance to multiple antibiotics across different classes. The phenotypic resistance rate of the isolates was highest to amoxicillin/clavulanate (100%), followed by imipenem (98.1%), followed by cefotaxime (90.4%), and cefuroxime (92.3%), High resistance was also observed for nalidixic acid (86.5%), levofloxacin (84.6%), and gentamicin (82.7%). Least resistance and susceptibility was reported in meropenem with 69.2% of isolates being susceptible, followed by ceftriaxone (17.3%) and ofloxacin (17.3%). This is fairly similar to a study by Worku et al., 2024, where 79% meropenem and 85.7% Imipenem susceptibility was reported. The high resistance to Imipenem obtained in this study raises concerns expressed by a laboratory attendant, who noted that Imipenem is often prescribed for many inpatients at the Adeoyo Maternity Clinic in Ibadan.

Unlike a related study in the northern part of Nigeria (Nkup et al.,2022), where similar genes in this study were screened among patients attending a tertiary Clinic in the capital city of Jos, Nkup et al. reported that none of the resistance genes were identified. Findings in this study indicated that there was a significant proportion of the isolates carrying the bla_NDM gene Seven(47%), which is indicative of the presence of New Delhi Metallo-β-lactamase-producing Klebsiella pneumoniae among the clinical specimens being examined. The bla_VIM gene was also identified in several isolates, indicating the presence of Verona Integron-encoded Metallo-β-lactamase, another carbapenemase enzyme associated with multidrug resistance. In contrast, fewer isolates tested positive for bla_KPC. This is in contrast to a study reported by Mohammed et al.,whose research revealed KPC as a dominant resistance gene in a relative study conducted in Maiduguri, Nigeria.

The presence of bla_VIM, bla_KPC, and bla_NDM in this study could probably be a result of frequent use of the Imipenem or meropenem for treatment of Klebsiella-related infection in Ibadan

CONCLUSION

The emergence and spread of carbapenemase-resistant Klebsiella pneumoniae as revealed in this study pose a significant threat to global public health. The high phenotypic antibiotic resistance observed suggests the possible presence of additional resistance genes that were not investigated. Therefore, continuous surveillance of carbapenem-resistant K. pneumoniae, with a focus on screening for other antibiotic resistance genes, is strongly recommended.

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