

## Isolation and Characterization of Carbapenemase-Producing *Klebsiella* spp from Fomites in Madonna University, Elele

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### Abstract

Carbapenemase-producing *Klebsiella* spp. (CPK) constitutes a serious public health hazard owing to their multidrug-resistant characteristics, limiting treatment choices, and worsening healthcare difficulties. This work is intended to identify and characterize CPK from fomites at Madonna University, Elele, Nigeria. One hundred (100) samples were collected aseptically from four locations: the girls' hostel, the boys' hostel, the hospital area, and the church area at Madonna University, Elele Campus. Using nutritional broth and Eosin Methylene Blue agar, *Klebsiella* spp. were identified based on colonial appearance and biochemical properties. Twelve antibiotics were used for an antibiotic susceptibility test conducted on Mueller-Hinton Agar, utilising the disc diffusion method. Results showed that the prevalence of *Klebsiella* spp. was 35%, distributed uniformly throughout the studied locales. Alarming, all isolates indicated Multidrug resistance (MDR), including 100% resistance to Amoxicillin/Clavulanate, Nalidixic Acid, and Nitrofurantoin. Imipenem demonstrated the greatest susceptibility rate at 22.9%. Carbapenemase production was found in 11.4% of isolates, limited to the girls' hostel and hospital regions. Statistical analysis revealed no significant difference in the occurrence of *Klebsiella* sp. across sites ( $p = 0.3175$ ), suggesting broad environmental contamination. The results underline the critical need for antimicrobial stewardship and better infection control procedures. Additionally, the research reveals the environmental reservoirs of CPK, underlining the crucial function of fomites in pathogen spread across hospital and community settings. This work adds significant data to assist treatments aimed at reducing the development of Multi Drug Resistant (MDR) *Klebsiella* spp. It illustrates the necessity of focused policies for antibiotic usage and hygiene management in institutional contexts.

**Keywords:** Antimicrobial resistance, Carbapenemase, Environmental reservoirs, Fomites, Healthcare-associated infections, Madonna University, Multidrug resistance

### Introduction

A rise of microbes resistant to many antibiotics, including carbapenem, has been seen in recent years. This resistance is due to the production of enzymes that can break down and alter the activity of the antibiotic hence rendering them ineffective, and this problem of antimicrobial resistance has been of major concern to the healthcare system [1].

Gram-negative, rod-shaped, and non-motile, *Klebsiella* spp. is a member of the Enterobacteriaceae family and is deadly because of its resistance to the environment, disinfectants, and several medications. In addition to endotoxin and capsular and somatic antigens, it has a complex antigenic structure, and some strains are capable of producing exotoxins. Nonetheless, *Klebsiella* species showed the capacity to acquire genetic components and mutations that give resistance to antibiotics, ultimately resulting in the creation of convergent clones known as multidrug-resistant isolates [2-3]. There are several species of *Klebsiella*, which are: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella terrigena* and *Klebsiella planticola*, etc.

Carbapenemase-producing *Klebsiella* (CPK) bacteria are a significant public health concern due to their high resistance to antibiotics, including carbapenem, which is often considered the last line of defense against multidrug-resistant infections. In hospital settings, these bacteria cause a variety of illnesses that raise morbidity, death, and medical expenses. One of the major challenges associated with CPK infections is their ability to survive and spread in the environment, including on inanimate objects known as fomites.

Fomites are surfaces or objects that can carry infectious microorganisms, including bacteria, viruses, and fungi. In healthcare settings such as hospitals and clinics, fomites play a crucial role in transmitting infections, as they can become contaminated with pathogenic

bacteria and serve as reservoirs for the spread of diseases. Fomites can potentially harbour and disseminate these antibiotic-resistant bacteria, posing a significant threat to patients, healthcare workers, and visitors.

Madonna University Elele is not immune to the challenges posed by these infections. Understanding the presence and characteristics of CPK on fomites within Madonna University healthcare facilities is essential for several reasons. Identifying fomites associated with Carbapenemase-producing *Klebsiella spp* can help the institution implement targeted infection control measures to prevent the transmission of these bacteria among patients and staff members.

Knowledge about the prevalence and types of antibiotic resistance mechanisms, such as Carbapenemase, in Carbapenemase-producing *Klebsiella spp* isolates from fomites can guide antibiotic stewardship programs, ensuring appropriate use of antibiotics and minimising the development of further resistance. By studying Carbapenemase-producing *Klebsiella spp* on fomites, researchers can assess the potential public health impact of these bacteria in Madonna University and the surrounding community, leading to informed decisions on healthcare policies and interventions.

Therefore, conducting a study on the isolation and characterization of Carbapenemase-producing *Klebsiella spp* from fomites at Madonna University is vital for enhancing the knowledge base, improving infection control practices, and ultimately safeguarding the health and well-being of the patients and healthcare workers within the institution. This study aims to isolate and characterize Carbapenemase-producing *Klebsiella spp* from fomites obtained at different locations in Madonna University, Elele.

## Materials and Methods

### Study Area

The study was carried out at Madonna University, Elele Campus, located in Rivers State, Nigeria, between September 2023 and October 2023. The Elele campus is situated in Ikwerre Local Government Area of Rivers State, in the south-south geopolitical zone of Nigeria. It lies approximately at the geographic coordinates 5°59'34" N latitude and 6°47'26" E longitude, along the Port Harcourt–Owerri expressway, making it easily accessible from neighbouring states such as Imo, Abia, and Bayelsa. The area experiences a tropical rainforest climate characterized by high humidity, a long rainy season (March to October), and an average annual temperature of about 26–28°C.

### Collection of samples

A total of 100 samples were collected aseptically with a swab stick from fomites, found at 4 different locations in Madonna University (girls' hostel, boys' hostel, hospital area of the university, church area). The samples obtained were taken quickly to the microbiology laboratory and were subjected to bacterial culturing and further laboratory analyses were conducted.

### Isolation and Identification of *Klebsiella* species

Nutrient broths (ReadyMED RDM-NB001 dehydrated cell culture media (Chaitanya Agro Biotech Pvt Makapur, India) were prepared and the samples were inoculated into each test tube containing the already prepared nutrient broth, and incubated at 37°C for 24 hours. The samples were subcultured Eosin methylene blue agar (TM MEDIA, Titan biotech LTD, Rajasthan, India) plate and incubated at 37°C for 24 hours after incubation. The isolates' morphological and biochemical characteristics were compared to Bergey's Determinative Bacteriology Manual (1994). The *Klebsiella* growth was distinguished by pinkish-purple mucoid colonies. The isolates were then purified and stored in agar slants. [4]. The isolates were taken for biochemical tests for phenotypic concentration. They include Gram staining (negative); Indole test (Negative); Catalase test (Positive); and Oxidase test (Negative)

### Antibiotic Sensitivity Assay

The assay for antibiotic sensitivity was done on a freshly prepared/sterilised Muller-Hinton Agar (MHA) plate. 0.5McFarland standard of the identified isolates was prepared and the bacterial suspension was then spread on the MHA plates with a swab stick and twelve gram-negative sensitivity discs (Celtec Diagnostics); Amoxicillin clavulanate (30ug), Cefotaxime (25ug), imipenem (10/10ug), Ofloxacin (5ug), Gentamicin (10ug), Nalidixic Acid (30ug), Ampiclox (10ug), Cefexime (5ug), Levofloxacin (5ug) Nitrofurantoin (300ug), Cefuroxime (30ug), Ceftriaxone Sulbactam (45ug) were placed at the center of the surface of the inoculated MHA plate using forceps and the plates were incubated overnight at 37°C. The inhibition zone around each disc was measured and recorded against each antibiotic the next day after growth. All experiments were carried out under aseptic conditions [5].

### Carbapenemase production

The indicator organism, *Klebsiella spp.*, susceptible to carbapenem, was produced in a nutrient broth using a 0.5 McFarland of standard suspension. A lawn of 300 microlitres of 0.5 McFarland suspension of *Klebsiella* species was applied on the Mueller-Hinton agar plate. Background lawn streaking was followed by the placement of a 10 µg meropenem disc (Lioflchem, Italy) in the centre of the agar plate. Using a sterile loop to aid, the meropenem disc was near the centre of the plate, and the test isolates were streaked towards the edge. After a 24-hour incubation period, the growth was measured [6].

### Data analysis

A two-tailed P value of less than 0.05 was deemed statistically significant. All statistical analyses were carried out using Graphpad Prism 5, and the findings were compared using the Student's t-test to examine the statistical difference in the incidence of the isolates in the locations.

## Results

The study was conducted to assess the distribution of environmental samples collected from various locations within the study area. The samples were obtained from key sites with differing population densities and activities, including residential hostels, religious areas, and healthcare settings. The breakdown of the sample collection is presented in the Table 1.

**Table 1: Sample collection**

| Study Area    | Sample collected |
|---------------|------------------|
| Girls Hostel  | 10               |
| Boys Hostel   | 11               |
| Church Area   | 10               |
| Hospital Area | 4                |
| Total         | 35               |

The prevalence of *Klebsiella* isolates across various sampling locations was analyzed to determine their distribution and relative frequency. The result in Table 2 presents the number of *Klebsiella* isolates identified in comparison to non-*Klebsiella* isolates from samples collected in girls' hostels, boys' hostels, church areas, and hospital areas. The data highlights the percentage prevalence of *Klebsiella* isolates across these locations. The statistical evaluation shows no statistical difference between the *Klebsiella* and the non-*Klebsiella* isolates present ( $p=0.3175$ ).

**Table 2: Prevalence of the *Klebsiella* Isolates**

| Isolates                               | Girls Hostel | Boys Hostel | Church area | Hospital area | Prevalence (%) |
|----------------------------------------|--------------|-------------|-------------|---------------|----------------|
| No. of <i>Klebsiella</i> Isolates      | 10           | 11          | 10          | 4             | 35             |
| No. of Non- <i>Klebsiella</i> Isolates | 20           | 19          | 15          | 11            | 65             |
| Total                                  | 30           | 30          | 25          | 15            | 100            |

The result presented in Table 3 summarizes the antimicrobial susceptibility profiles of *Klebsiella spp* isolates (N=35) against a range of antibiotics from different classes. The table presents the number and percentage of isolates categorized as susceptible, intermediate, or resistant to each antibiotic tested. All isolates (100%) were resistant to Amoxicillin/Clavulanate (AUG) and Nalidixic Acid (NA), while Ceftriaxone Sulbactam (CRO) showed moderate resistance (80%) with a small percentage of susceptible isolates (11.4%). Similarly, Cefotaxime (CTX), Cefexime (ZEM), and Cefuroxime (CXM) had very high resistance rates (94.3%–97.1%). Imipenem/Cilastatin (IMP) exhibited the best efficacy among all antibiotics, with 22.9% of isolates classified as susceptible. However, 65.7% of isolates were still resistant, suggesting emerging resistance to carbapenems. Levofloxacin (LBC) showed a relatively high susceptibility rate (48.6%), while Ofloxacin (OFX) had a susceptibility rate of 37.1%. Gentamycin (GN) demonstrated low efficacy, with only 2.9% of isolates being susceptible and 94.3% being resistant. All isolates were highly resistant to Nitrofurantoin (NF), with 97.1% resistance. Resistance was similarly high (94.3%), with only 5.7% susceptibility observed.

As seen on Figure 1, the bar chart compares the number of carbapenem-susceptible and carbapenem-resistant isolates obtained from four different sampling areas: Girls Hostel, Boys Hostel, Church area, and Hospital area. For carbapenem-susceptible isolates, the Girls Hostel recorded the highest number (about 6), followed by the Church area (4), Boys Hostel (3), and Hospital area (around 3). For carbapenem-resistant isolates, the Boys Hostel had the highest count (8), followed by the Hospital area (7), Church area (6), and Girls Hostel (3). Overall, the chart shows a higher prevalence of carbapenem-resistant isolates in the Boys Hostel and Hospital area compared to other locations.

**Table 3: Antimicrobial susceptibility to selected isolates (N=35)**

| S/N | Antibiotics                   | Class of antibiotics used                                    | Number of susceptible isolates (%) | Number of intermediate isolates (%) | Number of resistant isolates (%) |
|-----|-------------------------------|--------------------------------------------------------------|------------------------------------|-------------------------------------|----------------------------------|
| 1.  | Amoxicillin/Clavulanate (AUG) | Penicillin class with a beta-lactamase inhibitor             | 0(0)                               | 0(0)                                | 35(100)                          |
| 2.  | Ceftriaxone Sulbactam (CRO)   | 3rd-generation cephalosporin with a beta-lactamase inhibitor | 4(11.4)                            | 3(8.6)                              | 28(80)                           |
| 3.  | Cefotaxime (CTX)              | 3rd-generation cephalosporin                                 | 1(2.9)                             | 1(2.9)                              | 33(94.3)                         |
| 4.  | Imipenem/Cilastatin (IMP)     | Carbapenem with a renal dehydropeptidase inhibitor           | 8(22.9)                            | 5(14.3)                             | 22(62.8)                         |
| 5.  | Ofloxacin (OFX)               | Fluoroquinolone                                              | 13(37.1)                           | 6(17.1)                             | 16(45.7)                         |
| 6.  | Gentamycin (GN)               | Aminoglycoside                                               | 1(2.9)                             | 1(2.9)                              | 33(94.3)                         |
| 7.  | Nalidixic acid (NA)           | first-generation quinolone                                   | 0(0)                               | 0(0)                                | 35(100)                          |
| 8.  | Ampiclox (ACX)                | Beta-lactam                                                  | 2(5.7)                             | 0(0)                                | 33(94.3)                         |
| 9.  | Cefexime (ZEM)                | 3rd-generation cephalosporin                                 | 1(2.9)                             | 1(2.9)                              | 33(94.3)                         |
| 10. | Levofloxacin (LBC)            | Fluoroquinolone                                              | 17(48.6)                           | 7(20)                               | 11(31.4)                         |
| 11. | Nitrofurantoin (NF)           | Nitrofurantoin derivative                                    | 0(0)                               | 1(2.9)                              | 34(97.1)                         |

|     |                  |                              |      |        |          |
|-----|------------------|------------------------------|------|--------|----------|
| 12. | Cefuroxime (CXM) | 2nd-generation cephalosporin | 0(0) | 1(2.9) | 34(97.1) |
|-----|------------------|------------------------------|------|--------|----------|

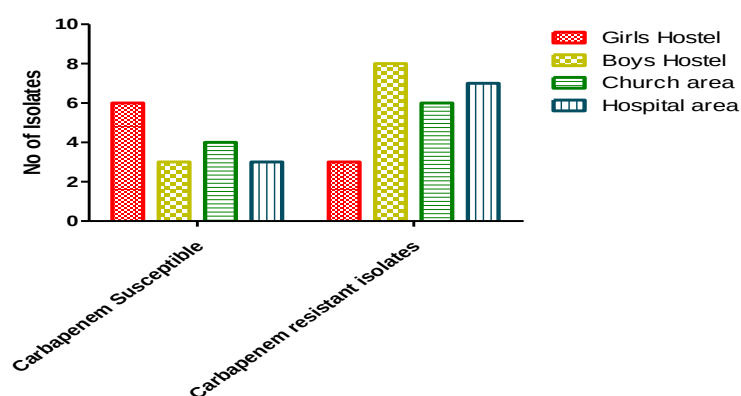


Figure 1: Carbapenem susceptibility of the isolates according to the sites of the collection

The distribution of multidrug-resistant (MDR) *Klebsiella* isolates across various sample collection sites was analyzed. Figure 2 depicts the number of multidrug-resistant and non-multidrug-resistant isolates identified in girls' hostels, boys' hostels, church areas, and hospital areas. All the isolates are multidrug resistant.

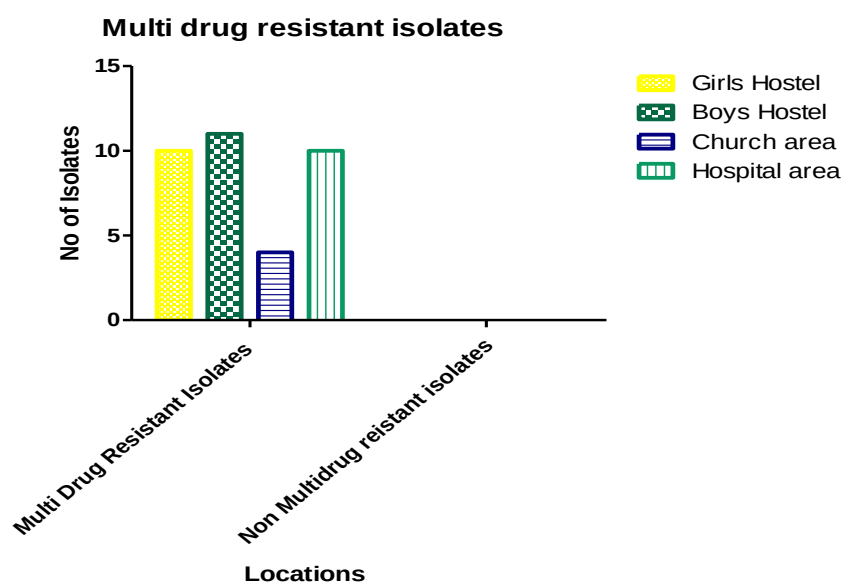


Figure 2: Multi-drug resistant isolates from different sites of collection

The production of carbapenemase, an enzyme responsible for resistance to carbapenem antibiotics, was evaluated in *Klebsiella* spp. Isolates were obtained from different sampling locations. The result on Table 4 presents the distribution of isolates and the corresponding number of carbapenemase-producing *Klebsiella* spp. identified in girls' hostels, boys' hostels, church areas, and hospital areas.

Table 4: Carbapenemase producing isolates from the various locations

| Location      | Total Isolates | Carbapenemase-Producing isolates | Prevalence (%) |
|---------------|----------------|----------------------------------|----------------|
| Girls Hostel  | 10             | 2                                | 20             |
| Boys Hostel   | 11             | 0                                | 0              |
| Church Area   | 4              | 0                                | 0              |
| Hospital Area | 10             | 2                                | 20             |
| <b>Total</b>  | <b>35</b>      | <b>4</b>                         | <b>11.43</b>   |

## Discussion

The detection and characterization of carbapenemase-producing *Klebsiella* species from environmental surfaces, also known as fomites, play a critical role in comprehending the spread and persistence of antibiotic resistance within healthcare settings [7]. The environmental sampling was conducted across four key locations within the study area: girls' hostels, boys' hostels, church areas, and hospital areas. A total of 35 samples were collected, with the highest number from boys' hostels (11 samples) and the lowest from hospital areas (4 samples). The distribution of sample collection reflects the diversity in population density and activities across these locations, which are critical factors in microbial distribution. This approach ensures a comprehensive assessment of potential reservoirs for *Klebsiella* spp. and other pathogens in varied environmental contexts.

The prevalence study revealed a total of 35 *Klebsiella* isolates across the sampled sites. Girls' hostels, boys' hostels, and church areas each contributed 10 isolates, while hospital areas accounted for 4 isolates. Non-*Klebsiella* isolates were more prevalent, making up 65% of the total isolates. Statistical analysis showed no significant difference in the prevalence of *Klebsiella* and non-*Klebsiella* isolates across the sampling locations ( $p=0.3175$ ). This finding suggests a relatively uniform distribution of *Klebsiella* isolates in the study area, irrespective of site-specific environmental conditions. The prevalence of *Klebsiella* sp in this study is 35% which is higher than the report of Aghanya et al [8] who recorded a prevalence of 2.8% in a Nigerian hospital. A lower prevalence rate was recorded in the work of Mohammed et al [9] who recorded a rate of 0.04% in an Egyptian hospital.

The antimicrobial susceptibility testing highlighted alarming resistance rates among the *Klebsiella* isolates. Resistance to commonly used antibiotics, such as Amoxicillin/Clavulanate (100%), Nitrofurantoin (100%), and Cefuroxime (100%), indicates a high prevalence of multidrug-resistant (MDR) strains. Additionally, resistance to other critical antibiotics, including Cefotaxime (97.1%), Gentamycin (97.1%), and Cefexime (97.1%), shows the limited therapeutic options for treating infections caused by these isolates. The findings from the antimicrobial susceptibility testing highlight the critical issue of multidrug resistance (MDR) among *Klebsiella* spp. isolates in the study. The complete resistance to Amoxicillin/Clavulanate and Nalidixic Acid, as well as high resistance to other beta-lactams like Cefotaxime, Cefexime, and Cefuroxime, shows the limited efficacy of beta-lactam antibiotics against these isolates. Carbapenems, represented by Imipenem/Cilastatin, showed the best performance, with 22.9% susceptibility. However, the 65.7% resistance rate is alarming and suggests the emergence of carbapenem-resistant *Klebsiella* strains, which poses a significant challenge in clinical settings. Fluoroquinolones, such as Levofloxacin and Ofloxacin, exhibited moderate susceptibility rates compared to other classes, indicating potential as treatment options for some cases. However, resistance remains substantial. The high resistance to Gentamycin, a commonly used aminoglycoside, raises concerns about the efficacy of this class in treating infections caused by these isolates. Resistance to Nitrofurantoin, typically used for urinary tract infections, highlights the challenge of managing such infections in clinical settings. This study is consistent with the findings of Aghanya et al. [8], who reported that *K. pneumoniae* was resistant to antibiotics such as Cefuroxime susceptible to the Carbapenem class of antibiotics, with Imipenem exhibiting the highest sensitivity (73.3%). In contrast, an Egyptian study found 100% resistance to meropenem [10]. The lower rates of resistance to the carbapenems in this study may be because carbapenems are the preferred treatment for infections caused by drug-resistant Enterobacteriaceae [11], and it has been well documented that there is an increasing bacterial resistance to carbapenems. Previous studies have shown that *Klebsiella pneumoniae* strains isolated from environmental sources exhibit biochemical characteristics, virulence factors, and pathogenic potential comparable to those obtained from clinical sources [12]. In contrast to environmental *K. pneumoniae*, clinical *K. pneumoniae* had been shown to have a much higher level of antibiotic resistance [13]. The report of this work is dissimilar to the report of Osuala et al. [14] who recorded 85% of *E. coli* isolate (Enterobacteriaceae) resistant to imipenem.

Carbapenem resistance, evaluated through susceptibility to Imipenem/Cilastatin, was observed in 65.7% of isolates, highlighting the emergence of carbapenem-resistant strains. Figure 1 illustrates the site-specific variation in carbapenem susceptibility, with hospital areas showing a notable proportion of resistant isolates. The findings emphasize the need for stringent antimicrobial stewardship and infection control measures. The study revealed that all *Klebsiella* isolates were multidrug-resistant (MDR), as depicted in Figure 2. The distribution of MDR isolates across the study locations was consistent, with significant numbers identified in girls' hostels, boys' hostels, and hospital areas. This widespread resistance further complicates treatment options and underscores the potential for dissemination of MDR strains in both community and healthcare settings. The prevalence of MDR isolates necessitates immediate public health interventions to curb the spread of resistant pathogens.

Carbapenemase production, a key mechanism of resistance to carbapenem antibiotics, was evaluated among the *Klebsiella* isolates. Out of the total 35 isolates, 4 were identified as carbapenemase producers (11.4%). These were isolated exclusively from girls' hostels and hospital areas, with each site contributing two carbapenemase-producing isolates. The absence of carbapenemase producers in boys' hostels and church areas suggests possible site-specific factors influencing the dissemination of these resistant strains. This work is in agreement with the report of Aghanya et al [8] who isolated carbapenemase-producing *K. pneumoniae* but with a higher prevalence rate of 53% from fomites in a hospital setting in Nigeria. The northern region of Brazil had a higher prevalence rate, with 25/25 (100%) of the *K. pneumoniae* isolates confirmed to be carbapenemase producers [15]. However, a Chinese study found that 4/153 (2.6%) of the clinical isolates of *K. pneumoniae* were carbapenemase-producing, and in Kano, Nigeria, a low prevalence of carbapenemase-producing *K. pneumoniae* was also found (6/73 (8.2%)) [16]. These inconsistent observations were brought to light by Oduyebo et al. [17], who noted that carbapenemase production among the Enterobacteriaceae has been widely reported, with prevalence ranges between 2.8% and 53.6%.

## Conclusion

This study highlights the critical role of environmental reservoirs in the dissemination of carbapenem-resistant and multidrug-resistant *Klebsiella* spp within community and healthcare settings. The detection of carbapenemase-producing isolates from high-contact areas, such as girls' hostels and hospital environments, highlights the potential for localized transmission of resistant strains. The observed resistance to multiple antibiotic classes, including beta-lactams, aminoglycosides, and fluoroquinolones, reinforces the urgency of implementing antimicrobial stewardship programs and stringent infection control measures. The prevalence of carbapenemase producers (11.4%) and their restriction to specific sites suggests that environmental factors and human activity patterns may influence

the persistence and spread of these strains. Notably, the resistance rates for carbapenems, though relatively lower than in some international studies, signal the emerging threat of carbapenem-resistant *Klebsiella* spp., with significant implications for treatment efficacy and public health. The findings align with global and regional reports on multidrug resistance trends, emphasizing the necessity for continuous surveillance and targeted interventions to curb the spread of antibiotic-resistant pathogens. Furthermore, the high resistance rates observed in this study call for urgent investment in diagnostic capacity, affordable access to effective antibiotics, and the development of alternative treatment strategies to manage infections caused by multidrug-resistant and carbapenem-resistant *Klebsiella* spp.

#### Ethical approval

Not applicable

#### Conflict of interest

No conflict of interest was recorded

#### Authors' Contributions

O.O.J.: Conceptualization, Supervision, editing; S.Y.F.: Conceptualization, editing; C.E.C.: Supervision; V.E.O.: Writing and Editing; V.I.E.: Writing and Editing; C.N.O.: Writing and Editing; O.A.N.: Writing and Editing

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