JCARM
Journal of Contemporary Academic Research and Methodologies

An Online Peer Reviewed / Refereed Journal
Volume 1 | Issue 8 | 2026
ISSN: 3139-7247 (Online)
Website: www.journal.jcarm.in

Original Research Article

Occurrence and Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* Isolated from Clinical Samples in Selected Hospitals in Akure, Ondo State, Nigeria

Fogbonja Oyindolapo Christianah[1*], Adebolu, Tinuola Tokunbo[1]

[1] Department of Microbiology, School of Life Sciences, Federal University of Technology, Akure, P. M. B. 704, Akure, Ondo State, Nigeria.

Correspondence should be addressed to: oyindolapochristianahf@futa.edu.ng

Article No: 048 | **Accepted**: 09 October 2026 | **Published**:10 October 2026

Abstract: *Klebsiella pneumoniae* is a pathogen that is of great health concern globally. This is primarily due to its rapidly increasing antimicrobial resistance (AMR) profile. *K. pneumoniae* is responsible for a wide range of life-threatening infections, including pneumonia, urinary tract infections, bloodstream infections, and sepsis, particularly among immunocompromised and hospitalized patients. The clinical management of infections caused by this organism has become increasingly difficult owing to its ability to evade the effects of multiple antimicrobial agents. This study therefore is to determine the occurrence rate of this organism in clinical samples collected from patients attending selected hospitals in the community sampled and to assess the antibiogram profile of this organism to update statistics.

A total of 120 clinical samples (urine, sputum, wound swabs, stool) were collected from patients attending selected government hospitals in Akure, Nigeria. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion assay, and the Multiple Antibiotic Resistance Index (MARI) was calculated. A total of 206 bacterial isolates were recovered from the 120 clinical samples evaluated, of which 105 (87.5%) were identified as *K. pneumoniae*. The highest incidence of *K. pneumoniae* was observed in sputum (37.4%), followed by 31.3% in urine, while the least (10.9%) was observed in wound swabs. The majority of the isolated *K. pneumoniae* were resistant to cefuroxime, ceftriaxone, cephorex, ciprofloxacin, ceftazidime, and Augmentin, with MARI values ranging from 0.2 to 1.0, confirming extensive multidrug resistance.

Keywords: *Klebsiella Pneumoniae*; Antibiogram Profile; Multiple Antibiotic Resistance Index

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https://doi.org/10.5281/zenodo.23432849

Introduction

Klebsiella species are Gram-negative bacteria that form part of the normal flora of the human gastrointestinal tract. Examples of *Klebsiella* species include *Klebsiella pneumoniae*, *K. oxytoca*, *K. veriicola*, *K. aerogenes* etc. The most common species however is *K. pneumoniae*. It is estimated that approximately one-third of the human population harbours *K. pneumoniae* asymptomatically within the gut microbiome (Wintachai *et al*., 2020). Under normal physiological conditions, these organisms function as commensals and rarely cause disease in immunocompetent individuals. However, they may act as opportunistic pathogens in vulnerable populations,

particularly in nosocomial settings. *K. pneumoniae* is of global health concern primarily due to it's rapidly increasing antimicrobial resistance (AMR) profile (Braun *et al*., 2024; Shah *et al*., 2025). This organism is responsible for a wide range of life-threatening infections, including pneumonia, urinary tract infections, bloodstream infections, and sepsis particularly among immunocompromised and hospitalised patients. The clinical management of infections caused by this organism has become increasingly difficult owing to its ability to evade the effects of multiple antimicrobial agents (Paczosa & Mecsas, 2016). The pathogen employs a diverse arsenal of resistance mechanisms to survive antibiotic pressure. These include the production of extended-spectrum β-lactamases (ESBLs), which hydrolyse a wide range of β-lactam antibiotics; the activation of efflux pump systems that expel antimicrobial agents from the bacterial cell; and porin loss or modifications, which reduce membrane permeability and limit antibiotic entry (Ding *et al*., 2025). Such mechanisms often coexist, resulting in multidrug-resistant (MDR) and, more recently, extensively drug-resistant (XDR) strains.

Recent surveillance studies have reported alarming increases in resistance rates to several classes of antibiotics, notably carbapenems, cephalosporins, and fluoroquinolones (Cireșă *et al*., 2024). Resistance to carbapenems is of particular concern, as these antibiotics are typically reserved for the treatment of severe and refractory infections. The emergence and dissemination of carbapenem-resistant *K. pneumoniae* (CR-KP) have significantly limited available therapeutic options, thereby contributing to elevated morbidity, mortality, and healthcare costs across both developed and developing countries.

Antimicrobial resistance (AMR) is the ability of microorganisms, particularly bacteria and fungi, to survive and proliferate despite exposure to antimicrobial agents. Antimicrobial resistance is a critical global health threat that severely compromise the effectiveness of antibiotics and other antimicrobial drugs in treating infections caused by bacteria, viruses, fungi, and parasites (Anyanwu & Okoye, 2017; Dadgostar, 2019).

Antimicrobial-resistant infections accounted for an estimated 1.27 million deaths globally in 2019, contributing to 4.95 million deaths overall, underscoring their significant clinical and economic burden (Murray *et al*., 2022; Islam *et al*., 2026). The global rise in antimicrobial resistance (AMR) poses a severe threat to public health, undermining decades of progress in the treatment of infectious diseases. The increasing incidence of resistance among bacterial pathogens exacerbated by the overuse of broad-spectrum antibiotics and

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inappropriate empirical therapy has significantly limited therapeutic options and increased clinical complexity (Yoshida *et al*., 2022; Islam *et al*., 2026). This trend jeopardises not only the management of routine infections but also the success of medical interventions such as chemotherapy, organ transplantation, and critical care, which depend on reliable antimicrobial prophylaxis (Teillant *et al*., 2015; Manesh & Varghese, 2021). This has made the World Health Organization to classified AMR as one of the top three public health threats of the 21st century (WHO, 2021; Gehring *et al*., 2023; Mudenda *et al*., 2024). Without intervention, AMR-related deaths could reach 10 million annually by 2050, surpassing cancer mortality (Paneri and Sevta *et al*., 2023; Nazir *et al*., 2025).

Methodology

Ethical Consideration

Ethical approval was obtained from the Health Research Ethics Committee of the Ministry of Health in Ondo State, Nigeria, with reference number (REF NO: OSHREC/28/07/2025/1067) and from the Federal University of Technology Akure Teaching Hospital, Akure, before embarking on this study.

Study Location

This research was conducted at three different hospitals in Akure viz; Federal University of Technology Akure Teaching Hospital, Arakale Comprehensive Health Centre and Police College Hospital.

All laboratory work was carried out at the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. All culturing and assays were done under aseptic environments with the aid of an appropriate laminar flow hood.

Sample size

The sample size of the clinical samples collected for this study was determined using Fisher's formula (Fisher *et al*., 1998):

$$n = \frac{Z^2 pq}{d^2}$$

n= minimum sample size, at 95% confidence level (z-score = 1.96), p = estimated prevalence of the target organism, q =1-p, d = degree of precision/ margin error (0.05). A more representative estimate of p was therefore obtained from a comparable Nigerian clinical study. Akinyemi *et al*. (2021) reported a 7.0% prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) strains among clinical *K. pneumoniae* isolates from four public hospitals in Lagos, Nigeria.

Using the literature-derived proportion (p = 0.07, q = 0.93):

$$n = \frac{(1.96)^2 \times 0.07 \times 0.93}{(0.05)^2}$$

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$$n = \frac{3.8416 \times 0.0651}{0.0025}$$

n =100

Based on this more representative prevalence estimate, the minimum required sample size for this study was determined to be 100; however, a total of 120 clinical samples were collected for this study.

Clinical samples and study population

Clinical samples (urine, sputum, stool and wound swabs) were obtained from one hundred and twenty (120) patients who sought medical attention in three hospitals, viz: Federal University of Technology Akure Teaching Hospital, Arakale Comprehensive Health Centre and Police College Hospital in Akure, Ondo State, after seeking their consent. All specimens were aseptically collected using sterile containers and swab sticks and cultured within 120 minutes of collection to maintain sample integrity.

The study was conducted from November, 2025 to July, 2026.

Isolation of bacteria from clinical samples

One 1ml of each clinical sample was pipetted into a test tube containing 9ml of sterile distilled water and serial dilution made up to $10^{-6.}$ Using pour plate techniques, 1 mL aliquots of dilution 10^{-3}, 10^{-4} and 10^{-5} were dispensed into empty Petri dishes and molten MacConkey agar medium was poured into the Petri dishes. After pouring into the Petri dishes, the plates were swirled quickly to properly mix with the medium. The plates were allowed to solidify and incubated aerobically at 37^0 C for 24hours. All distinctive colonies were counted using colony-forming units per gram (cfu/g). Suspected *Klebsiella* colonies were picked and subcultured on nutrient agar for confirmation tests (Cheesbrough, 2006).

Presumptive identification of *Klebsiella pneumoniae* isolates

Bacterial isolates were subjected to Gram's staining and standard biochemical techniques as described by Olayinka *et al*. (2014). The morphological characteristics of the colonies, such as colour, elevation, opacity and texture, were used as the first presumptive observation for the identification of bacterial isolates, as indicated by Al-Dhabaan (2019).

Molecular identification of *K. pneumoniae*

The DNA of the isolates were extracted using the QIAGEN QIAamp mini extraction kit protocol.

The extracted DNA were used in the amplification of the bacteria 16S rRNA gene using the following primers; forward: 5'- AGAGTTTGATCCTGGCT - 3' and reverse: 5'-TCGGTACCTTGTTACGACTT -3'. A PCR was carried out with a 25 µL final volume containing 12.5 µL of mastermix, 1 µL each for the 16S forward and reverse primers, 8.5 µL of PCR-grade water and 2 µL of DNA. The reaction was carried out using the following PCR conditions: initial denaturation at 94℃ for 15 minutes followed by 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 46℃ for 30 seconds and elongation at

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72℃ for 60 seconds. This was followed by a final elongation at 72℃ for 7 minutes. A known bacterial isolate was used as the positive control. The identities of the isolates were confirmed by checking the obtained sequences using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

A neighbor-joining tree was constructed for the obtained sequences using the alignment obtained between the query sequence and available sequences on the core_nt database of the NCBI.

Standardisation of bacterial isolates

Test isolates were sub-cultured onto fresh nutrient agar and incubated at 37^0C for 18-24 hours to obtain young, actively growing colonies. A loopful of the test organism were inoculated into the nutrient broth and incubated for 24 hours, and the turbidity of the suspension was adjusted to match 0.5 McFarland standard, equivalent to approximately 1.5 x10^8 colony-forming units per millilitre (CFU/mL), corresponding to an absorbance of 0.08-0.10 at a wavelength of 625 nm when measured spectrophotometrically (CLSI, 2012). Turbidity was confirmed using a spectrophotometer, with the suspension diluted with sterile water or supplemented with further bacterial growth as necessary to achieve the target absorbance range.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolated *K. pneumoniae* was evaluated using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following the standardised procedures outlined by Syal *et al*. (2017) and CLSI (2023). Multiple antibiotic resistance was indicated by the organism that showed resistance to a minimum of three classes of antibiotics.

Results

Frequency distribution of bacterial isolates from clinical samples

A total of 206 bacterial isolates were recovered from the 120 clinical samples worked on in this study (Table 1). The highest number of bacterial isolates were isolated from urine samples (78; 37.9%). This was followed by sputum (59; 28.6%), stool (42; 20.4%) and the least in the wound swabs (27; 13.1%).

Morphological and Biochemical characteristics of suspected *Klebsiella pneumoniae*

The isolates presumptively identified as *Klebsiella pneumoniae* generally exhibited the following morphological characteristics, as tabulated in Table 2, which include large colonial size, mucoid in texture, opaque, pink on MacConkey agar (lactose positive), and with a raised nature. Table 3 shows the biochemical results of representative isolates of *K. pneumoniae*. It was also observed that all strains displayed a characteristic Gram-negative enteric pattern, which is evidenced by a negative oxidase reaction and the ability to ferment glucose with the production of gas. The isolates were all able to metabolize carbohydrates, thereby utilising various sugars including monosaccharides, disaccharides, and sugar

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alcohols such as sorbitol, sucrose, and xylose. In addition, they possess a good fermentation profile as defined by their ability to utilize citrate and produce urease.

In addition, using the IMViC signature, they were positive for Voges-Proskauer while being negative for indole production, methyl red test, and motility testing. The uniformity of characteristics across multiple samples suggests a high possibility of the same phenotypic stability, which is characteristic of *K. pneumoniae.*

Table 1: Frequency distribution of bacterial isolates recovered from clinical samples

Samples	**Total Samples N**	**Bacterial isolates recovered n (%)**
Urine	50	78 (37.9)
Stool	30	42 (20.4)
Sputum	30	59 (28.6)
Wound Swab	10	27 (13.1)
Total	**120**	**206 (100.0)**

Table 2: Morphological Characteristics of Selected *Klebsiella pneumoniae*

S/n	Size	Shape	Texture	Opacity	Margin	Colour	Elevation
U1	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
U2	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
U3	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
SP65	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
SP60	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
SP81	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
SP95	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
W96	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
W97	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
W98	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised
SP71	Large	Circular	Mucoid	Opaque	Entire	Pink	Raised

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Table 3: Biochemical characteristics of suspected *Klebsiella pneumoniae*

Isolate	Shape	Citrate	Oxidase	Gas	H_2S	Lactose	Urease	Methyl Red	V. Proskauer	Catalase	Glucose	Motility	Indole	Nitrate	Sucrose	Xylose	Ribose	Fructose	Sorbitol	Mannitol	Maltose	Arabinose	Probable Organism
U1	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U2	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U3	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U4	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U5	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U6	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U7	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U8	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U9	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*

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U10	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U11	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U12	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*
U13	Rod	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	*K. pneumoniae*

Molecular Identity of Presumptive *K. pneumoniae* Isolates

Figures 1- 4 showed the results of the molecular confirmation of three (3) of isolated *K. pneumoniae* using the 16S rRNA gene. A distinct band of approximately 1,500 bp **w**as observed in all three isolates, confirming successful amplification of the bacterial 16S rRNA gene prior to sequencing. According to BLAST results (Table 4), these isolates O50, O60, and O65 were tested to be 100% identical to *Klebsiella pneumoniae* with accession numbers CP027602.1, CP129403.1, and CP129219.1, respectively.

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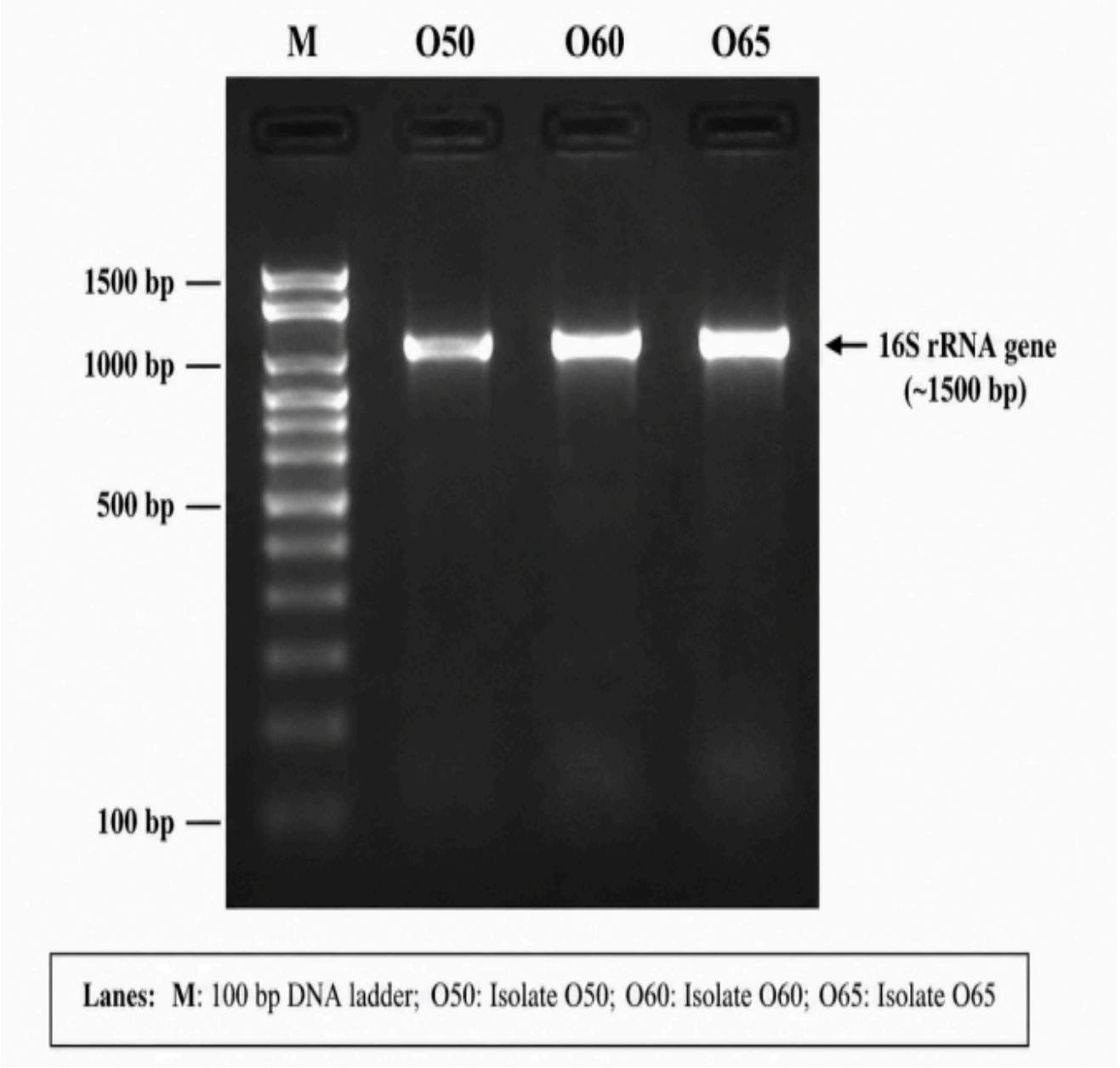


Figure 1: PCR amplification of the bacterial 16S rRNA gene (~1,500 bp). Lane M: 100 bp DNA ladder; Lane O50: *Klebsiella pneumoniae* isolate O50; Lane O60: *Klebsiella pneumoniae* isolate O60; Lane O65: *Klebsiella pneumoniae* isolate O65.

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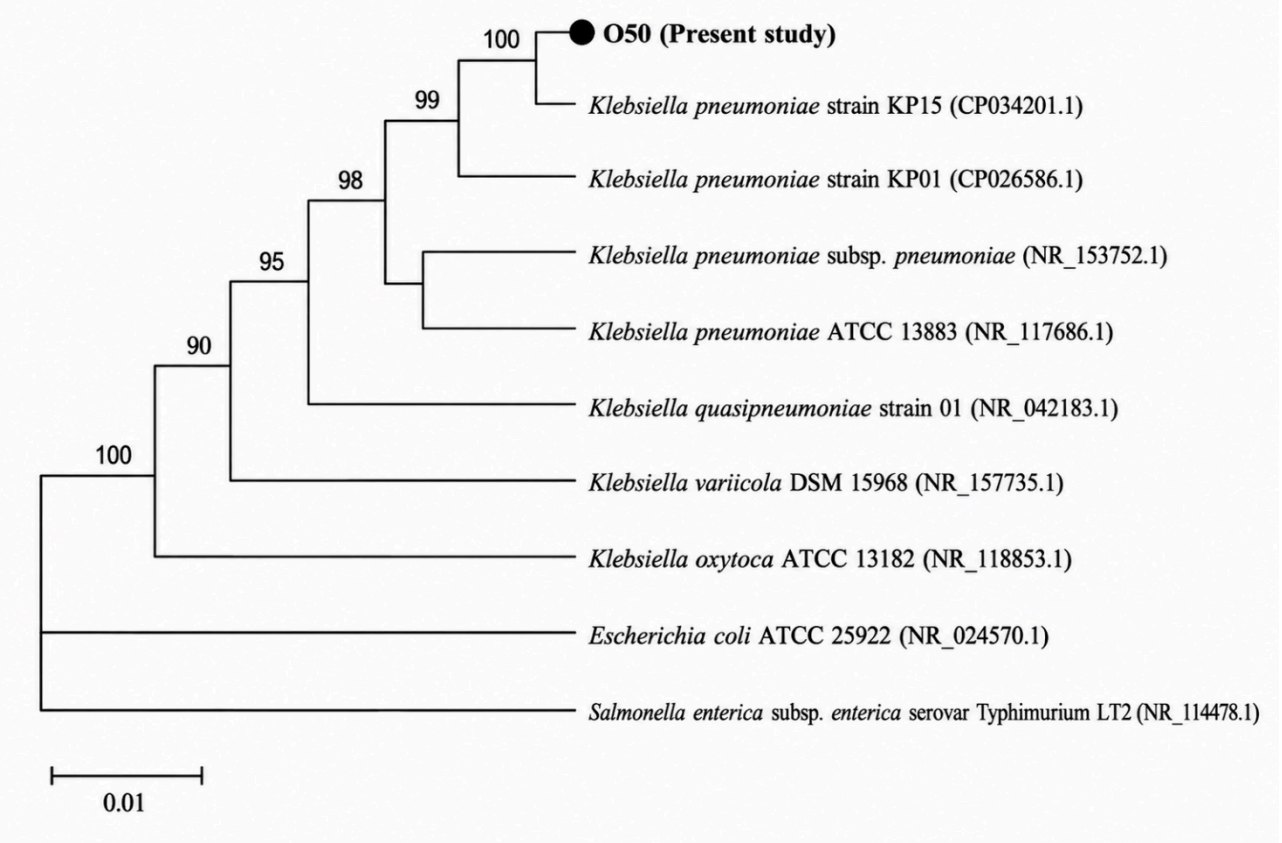


Figure 2. Neighbor-Joining phylogenetic tree based on the partial 16S rRNA gene sequence showing the evolutionary relationship between isolate **O50** and closely related *Klebsiella* species retrieved from the NCBI GenBank database. The tree was constructed using the Neighbor-Joining algorithm with **1,000 bootstrap replicates.**

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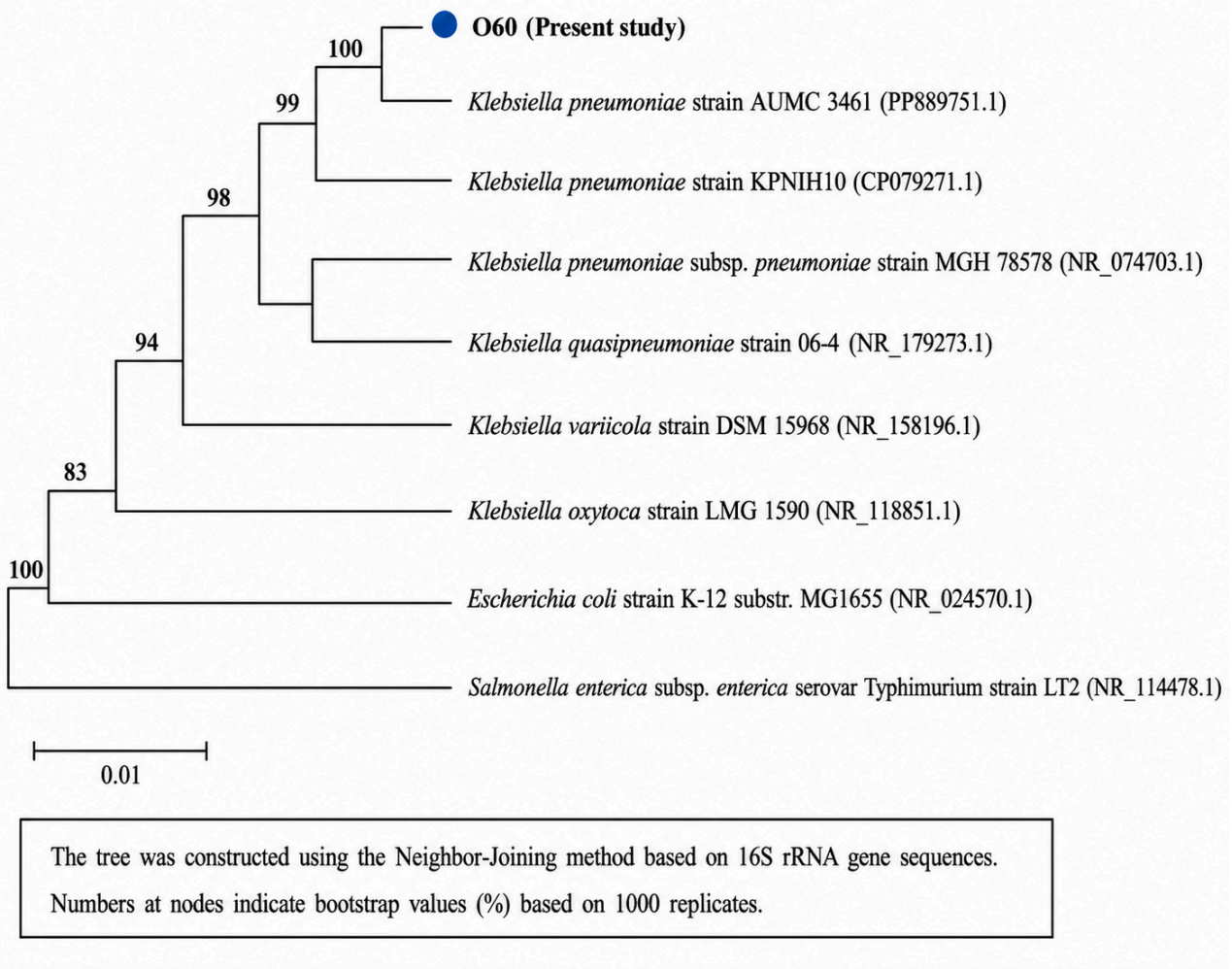


Figure 3 Neighbor-Joining phylogenetic tree based on the partial 16S rRNA gene sequence showing the phylogenetic relationship between isolate **O60** and representative *Klebsiella pneumoniae* strains obtained from the NCBI GenBank database. The tree was generated using the Neighbor-Joining method with **1,000 bootstrap replicates**. Bootstrap values are shown at the branch nodes, while *Salmonella enterica* serovar Typhimurium LT2 served as the outgroup.

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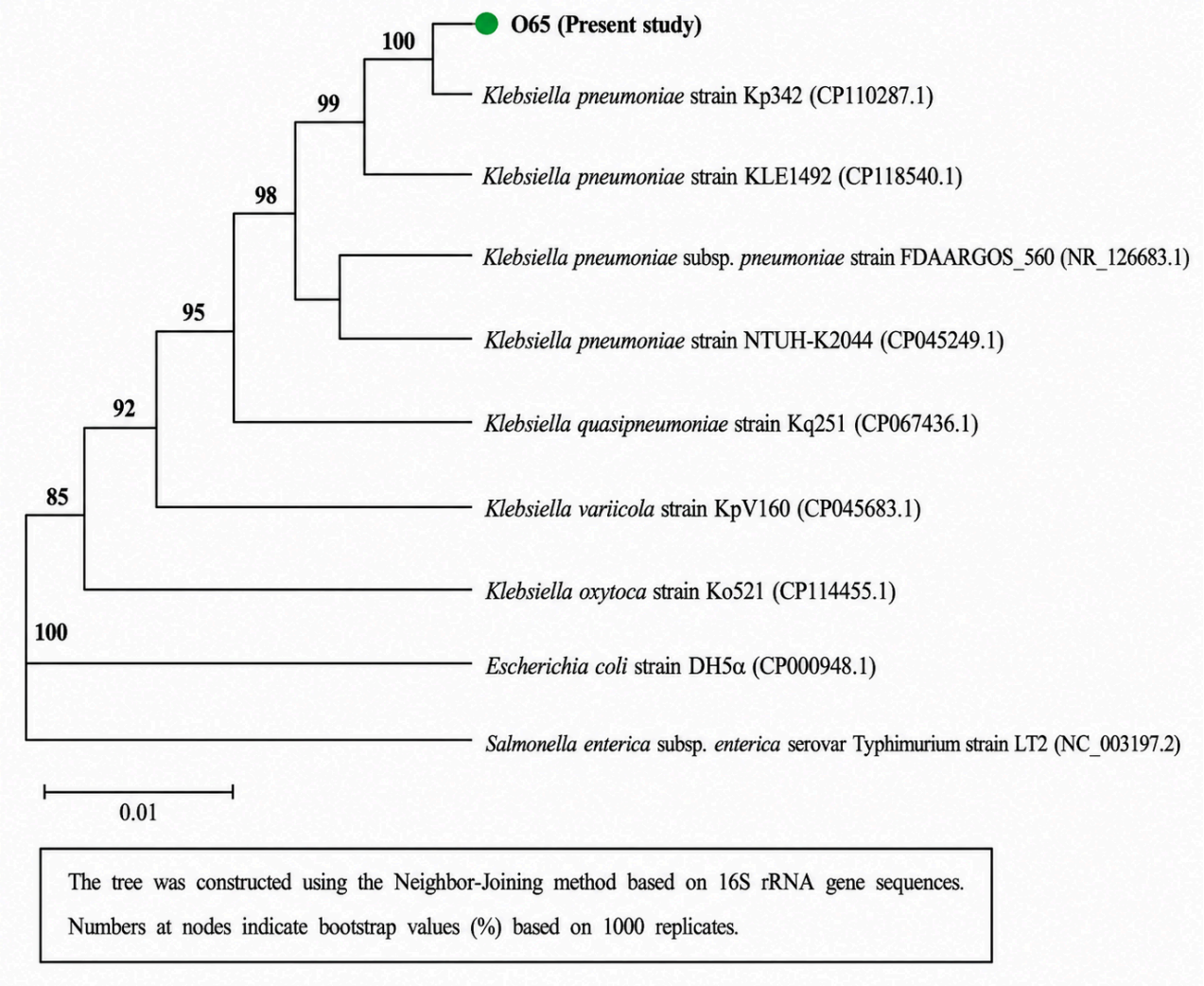


Figure 4. Neighbor-Joining phylogenetic tree based on the partial 16S rRNA gene sequence illustrating the evolutionary relationship between isolate **O65** and closely related *Klebsiella pneumoniae* reference strains retrieved from the NCBI GenBank database.

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Table 4: BLAST Prediction Identifying the Isolates

Organism	Sample ID	Sequence Length (bp)	% Identity	Accession No.	E-value	Alignment Score	Highest Query Coverage (%)
Klebsiella pneumoniae	O50	1499	100.00	CP027602.1	0.0	≥2700	100
Klebsiella pneumoniae	O60	1497	100.00	CP129403.1	0.0	≥2700	100
Klebsiella pneumoniae	O65	1498	100.00	CP129219.1	0.0	≥2700	100

Occurrence rate of *K. pneumoniae* among the clinical samples examined

Out of the 120 clinical samples analysed, 105 of the bacterial isolates recovered were identified as *K. pneumoniae,* representing 87.5% of the total samples examined (Table 5). The highest occurrence was observed from sputum samples, representing 35.24% of the suspected *K. pneumoniae*, followed by urine (31; 29.52%) and stool (27; 25.71%), while the lowest proportion of 10 (9.52%) was observed in the wound swabs.

Table 5: Percentage prevalence of *K. pneumoniae* isolates from clinical samples

Samples	Total Samples N (%)	Suspected *K. pneumoniae* (%)
Urine	50	31 (29.52)
Stool	30	27 (25.71)
Sputum	30	37 (35.24)
Wound Swab	10	10 (9.52)
Total	**120**	**105 (87.5)**

Antibiotic Susceptibility Profile of the Isolated *Klebsiella pneumoniae*

The isolates showed varying degrees of sensitivity to various classes of antibiotics as presented in Figure 5. Ofloxacin (10 mcg) emerged as the most effective agent against *Klebsiella pneumoniae* with a susceptibility rate of 79.05%, and followed by peflacine (10mcg) which inhibited 77.14% of the test *Klebsiella* isolates. Even though ofloxacin (10mcg) showed the highest cumulative efficacy percentage, peflacine (10mcg)

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demonstrated activity which was statistically superior ($P < 0.05$) against specific isolates. For example, in isolate U1, peflacine produced a zone of inhibition of 25.50 mm, SP84 with 27.50 mm and W100 with 21.00 mm whereas ofloxacin inhibited the same isolates producing 19.50 mm, 24.00 mm and 16.50 mm zone of inhibitions respectively.

With the percentage inhibition of ceporex 10mcg (34.29%) and ciprofloxacin (CPX 10mcg) (43.81%) on the tested isolates which produced inhibition zones of 9.50 mm to 18.50 mm, and 16.00 mm to 24.50 mm respectively, it is suggestive that they exhibited moderate efficacy on *Klebsiella pneumoniae*. However, augmentin (AUG 30mcg) performed poorly than every other antibiotic and they were only able to inhibit 17.14% of the total *Klebsiella* isolates tested.

Furthermore, critical resistance was observed against the cephalosporin class. The isolates showed a 100% resistant to both cefuroxime (30mcg CEF) and ceftriaxone (30mcg TRX), while ceftazidime 30mcg (CTZ) exhibited a near-total resistance of 99.95%, with only a single isolate, (SP 58) showing susceptibility, producing 22.50 m zone of inhibition. Overall, more than half of the isolates demonstrated multidrug resistance (MDR) to 60% of the total antibiotics screened.

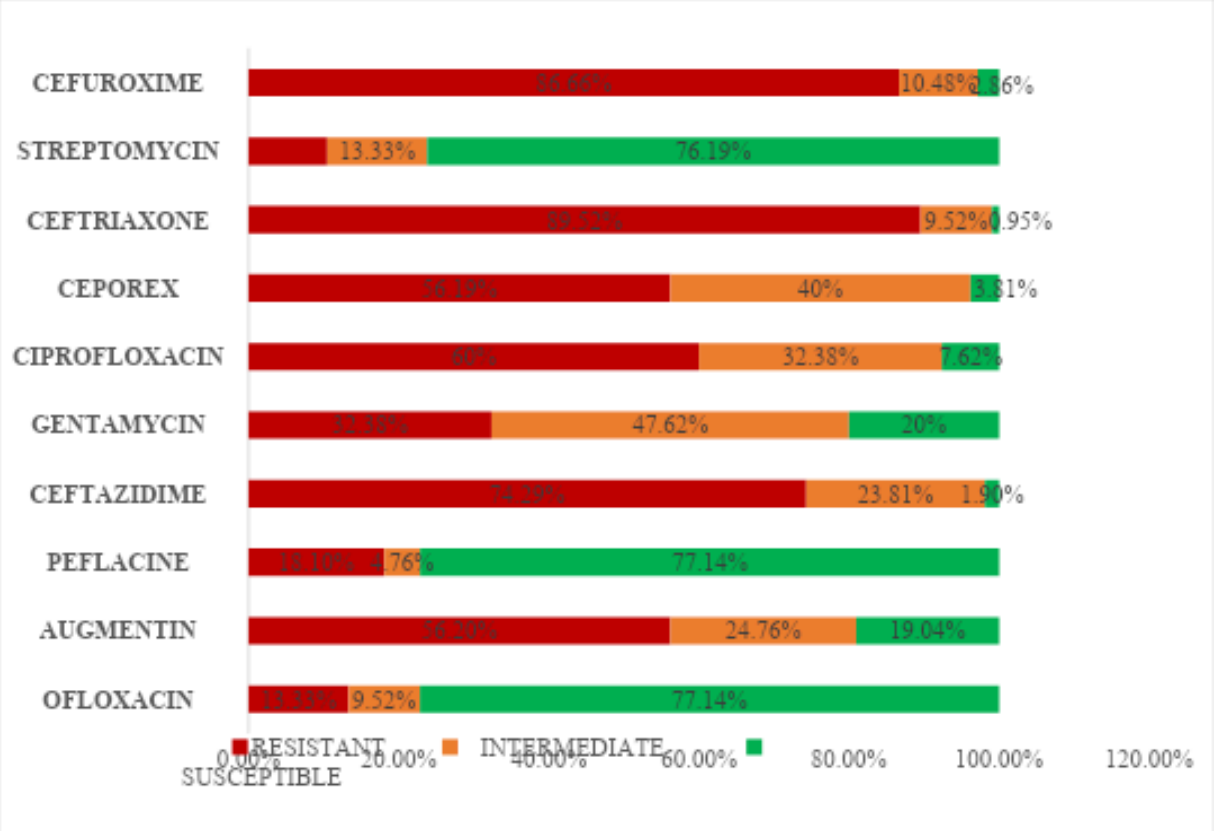


Figure 5: Antibiogram profiles of *Klebsiella pneumoniae* (n = 105) with respect to percentage resistance, susceptibility, and intermediate (CLSI, 2025)

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Multiple Antibiotic Index of the Resistant Isolated *K.pneumoniae* from Clinical Samples

Table 6 presents the Multiple Antibiotic Resistance Index (MARI) of the resistant *K. pneumoniae* isolates recovered from different clinical samples. The table shows the number of antibiotics to which each isolate was resistant (A), the total number of antibiotics tested (B = 10), and the calculated MARI values (A/B). The results revealed varying levels of multidrug resistance among the isolates, with MARI values ranging from 0.1 to 1.0. Since all isolates were tested against 10 antibiotics, the resistance index directly reflected the proportion of antibiotics resisted by each isolate.

Several isolates demonstrated extremely high resistance patterns with MARI values of 1.0, indicating resistance to all antibiotics tested. These included isolates U13, U19, ST34, and SP92. Such isolates represent highly resistant strains. Also, a high proportion of the isolates exhibited MARI values between 0.5 and 0.9, indicating resistance to 50–90% of the antibiotics tested. Examples include U3, SP86, SP89, ST48, SP94, ST50, SP87, ST35, and SP79.

Generally, the majority of the isolates had MARI values greater than 0.2, which is considered significant and indicating a relatively high prevalence of multidrug-resistant bacteria among the isolates.

Table 6: Multiple Antibiotic Resistance Index of the Resistant *K. pneumoniae* Isolates

Isolate	A	B	MARI	Isolate	A	B	MARI
U1	4	10	0.4	SP94	8	10	0.8
U2	3	10	0.4	ST34	10	10	1.0
U3	7	10	0.7	SP66	5	10	0.5
U4	4	10	0.4	ST50	7	10	0.7
U13	10	10	1.0	SP87	8	10	0.8
U19	10	10	1.0	ST35	7	10	0.7
U23	7	10	0.7	W98	4	10	0.4
U25	7	10	0.7	SP62	3	10	0.3
SP75	5	10	0.5	ST36	6	10	0.6
U26	6	10	0.6	ST46	5	10	0.5
U20	4	10	0.4	SP92	9	10	0.9
SP60	4	10	0.4	SP88	4	10	0.4
SP86	8	10	0.8	SP81	6	10	0.6
ST51	3	10	0.3	SP90	4	10	0.4
SP89	8	10	0.8	SP85	2	10	0.2
SP67	2	10	0.2	SP79	9	10	0.9

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SP71	6	10	0.6	SP73	2	10	0.2
SP72	5	10	0.5	W99	5	10	0.5
SP70	5	10	0.5	SP64	5	10	0.5
SP77	3	10	0.3	W102	5	10	0.5
W100	6	10	0.6	SP69	4	10	0.4
ST30	7	10	0.7	ST33	5	10	0.5
ST48	8	10	0.8	W105	4	10	0.4
W104	4	10	0.4	SP78	6	10	0.6
SP68	5	10	0.5	SP65	4	10	0.4

KEYS: A: Number of antibiotics indicating resistance, B: Total number of antibiotics tested, MARI: Multiple antibiotic resistance index = A/B, U: Urine, ST: Stool, SP: Sputum, W: Wound Swab.

Discussion

The present study investigated the occurrence and antibiogram profile of *K. pneumoniae* isolated from clinical samples obtained from selected hospitals in Akure, Ondo State, Nigeria. A total of 120 clinical samples comprising urine, stool, sputum and wound swabs was examined, from which 206 bacterial isolates were recovered. Of these, 105 isolates were identified as *K. pneumoniae*. The high recovery of *K. pneumoniae* in this study is of clinical importance because the organism is an established opportunistic pathogen associated with urinary tract infections, pneumonia, bloodstream infections and other healthcare-associated infections. The increasing antibiotic resistance of *K. pneumoniae* has further complicated the management of infections caused by this organism.

The distribution of *K. pneumoniae* isolates differed among specimen types, with the highest proportion recovered from sputum (37; 35.24%), followed by urine (31; 29.52%), stool (27; 25.71%) and wound swabs (10; 9.52%). The recovery of *K. pneumoniae* from respiratory, urinary, gastrointestinal and wound specimens agrees with the recognized ability of the organism to cause infections at multiple anatomical sites. Differences between the present study and other Nigerian studies may reflect differences in hospital populations, sample types, patient characteristics, infection patterns and local antibiotic-use practices.

The relatively high occurrence of *K. pneumoniae* in sputum in the present study may be related to the established role of the organism in respiratory infections, particularly among hospitalised or otherwise vulnerable patients (Giubelan *et al*., 2021). The organism possesses several characteristics that facilitate colonisation and persistence, including its prominent polysaccharide capsule and other virulence-associated traits. Therefore, occurrence data from one geographical location should be interpreted within the context of the population and clinical facilities studied.

The presumptive identification of *K. pneumoniae* was supported by characteristic colony morphology and biochemical reactions. The isolates generally produced large, circular, mucoid, opaque, pink and raised colonies on MacConkey agar, indicating lactose

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fermentation. The isolates were also Gram-negative rods, oxidase-negative, citrate-positive, and urease-positive, while showing a positive Voges-Proskauer reaction and negative indole, methyl red and motility reactions. These characteristics are compatible with the conventional phenotypic profile of K. pneumoniae. The use of both colonial and biochemical characteristics strengthened the presumptive identification of the isolates before molecular confirmation.

Molecular analysis provided further support for the identification of the organism. Three representative isolates, O50, O60 and O65, produced approximately 1,500-bp 16S rRNA amplicons and were identified by BLAST as K. pneumoniae with 100% sequence identity and 100% query coverage. The accession matches were CP027602.1, CP129403.1 and CP129219.1, respectively. These findings demonstrate agreement between the phenotypic identification and molecular identification. Molecular confirmation is valuable in studies involving *Klebsiella* because phenotypic characteristics may occasionally overlap among closely related members of the genus.

The antibiotic susceptibility results demonstrated considerable resistance among the *K. pneumoniae* isolates. However, Ofloxacin had the highest growth inhibitory rate (79.05%), followed by pefloxacin (77.14%). This suggests that these fluoroquinolones retained comparatively greater activity against the isolates than most of the other antibiotics tested. Nevertheless, the substantial proportion of isolates that were not susceptible to these agents indicates that their activity should not be interpreted as evidence of universal clinical effectiveness. Local antimicrobial susceptibility surveillance remains important because resistance patterns can vary considerably between hospitals and geographical locations.

The relatively high activity of ofloxacin and pefloxacin observed in this study however, contrasts with the considerable resistance to ciprofloxacin, for which only 43.81% of the isolates were susceptible (Wang *et al*., 2026). This variation within the fluoroquinolone group may reflect differences in antimicrobial exposure, bacterial resistance mechanisms and local prescribing practices. Fluoroquinolone resistance in *K. pneumoniae* may arise through mechanisms involving target-site alterations, reduced intracellular drug accumulation and acquisition of transferable resistance determinants.

The most striking finding in the present study was the very high level of resistance to the cephalosporins. Cefuroxime and ceftriaxone showed 100% resistance, while ceftazidime showed almost complete resistance, with only one isolate reported as susceptible. This pattern is clinically important because resistance to third-generation cephalosporins in K. pneumoniae is frequently associated with production of extended-spectrum β-lactamases (ESBLs) and other β-lactam resistance mechanisms. The high cephalosporin resistance observed in the present study is consistent with the broader antimicrobial resistance problem reported in Nigerian healthcare settings.

Augmentin demonstrated the lowest effectiveness among the antibiotics tested in the present study, with only 17.14% of isolates susceptible. The reduced activity of amoxicillin-clavulanate may reflect the extensive β-lactam resistance observed in the study population. The ability of K. pneumoniae to acquire β-lactamase-mediated resistance

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mechanisms is well established, and the coexistence of several resistance determinants can further reduce the effectiveness of β-lactam/β-lactamase inhibitor combinations.

More than half of the isolates were reported as multidrug-resistant, based on resistance to antibiotics belonging to at least three antimicrobial classes. The high frequency of multidrug resistance is concerning because it reduces the number of therapeutic options available for the management of infections. Ogefere and Idoko (2024) reported substantial multidrug resistance among clinical *K. pneumoniae* isolates in Benin City, Nigeria, with high MARI values, supporting the significance of multidrug-resistant *K. pneumoniae* in Nigerian hospitals.

The MARI values obtained in the present study ranged from 0.1 to 1.0. Several isolates had MARI values of 1.0, indicating resistance to all ten antibiotics tested. Other isolates showed MARI values between 0.5 and 0.9, indicating resistance to a substantial proportion of the antibiotics included in the test panel. A MARI value greater than 0.2 is commonly interpreted as an indication of exposure to environments where multiple antibiotics are frequently used. The predominance of MARI values above 0.2 in this study therefore suggests considerable antibiotic selection pressure among the isolates.

The occurrence of isolates with MARI values approaching or reaching 1.0 is particularly important from an infection-control perspective. Such isolates have very limited susceptibility to the antibiotics tested and may create difficulties in selecting effective empirical treatment. The presence of highly resistant isolates in clinical settings also raises concern about transmission between patients and the potential dissemination of resistance determinants (Osundiya *et al*., 2013; Das *et al*., 2019).

The findings indicate a serious antibiotic resistance problem by *K. pneumoniae* studied. The combination of high cephalosporin resistance, substantial fluoroquinolone resistance, low susceptibility to Augmentin and high MARI values indicates that antibiotic resistance among *K. pneumonia*e in the selected hospitals in Akure warrants continued surveillance.

Overall, the findings indicate that *K. pneumoniae* is an important clinical isolate in the selected hospitals and that a substantial proportion of the isolates exhibit multi-drug resistance. Regular antibiotic susceptibility testing, rational antibiotic prescribing, effective infection-prevention practices and continuous surveillance are therefore necessary to limit the emergence and spread of resistant *K. pneumoniae* in healthcare facilities in Akure and the wider Southwestern Nigerian region.

Conclusion

This study demonstrated the occurrence o*f K. pneumoniae* among the clinical samples collected from selected hospitals in Akure, Ondo State, Nigeria. Out of 120 clinical samples examined, 105 isolates were identified as *K. pneumoniae*, with the highest occurrence recorded in sputum samples, followed by urine, stool and wound swabs. Phenotypic identification based on colony morphology and biochemical characteristics was supported by molecular confirmation of three representative isolates using 16S rRNA

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sequencing, with all three isolates showing 100% sequence identity with *K. pneumoniae* reference sequences.

The isolates demonstrated substantial antibiotic resistance, particularly against cefuroxime, ceftriaxone and ceftazidime, while ofloxacin and pefloxacin demonstrated the highest inhibitory activity among the antibiotics tested. A high proportion of the isolates were multidrug-resistant, and MARI values ranged from 0.1 to 1.0, with several isolates resistant to all ten antibiotics tested. These findings indicate considerable antibiotic selection pressure and highlight the clinical importance of multidrug-resistant *K. pneumoniae* in the study area.

The study emphasises the need for continuous antibiotic susceptibility surveillance, rational antibiotic use, antibiotic stewardship, and strengthened infection-prevention and control measures in healthcare facilities. Further studies should investigate carbapenem susceptibility and characterise ESBL and carbapenemase genes, including *bla*KPC, *bla*NDM, *bla*OXA-48 and related determinants in *K. pneumoniae* isolates from Akure. Such molecular surveillance would provide a clearer understanding of the mechanisms responsible for the observed resistance and help guide appropriate therapeutic and infection-control strategies.

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