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Klebsiella pneumoniae Antibiotic Resistance: A Maternal Health Perspective

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ABSTRACT

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Klebsiella pneumoniae is a significant pathogen threatening maternal health, particularly in developing countries. The rise of antibiotic-resistant strains has worsened treatment outcomes, increasing morbidity and mortality. Limited data exists on *K. pneumoniae* antibiotic resistance in maternal populations, hindering effective treatment strategies. This study addresses this gap, investigating *K. pneumoniae* antibiotic resistance patterns in maternal sources to inform evidence-based treatment approaches. This study aimed to characterize *K. pneumoniae* isolates from maternal sources and determine their antibiotic resistance patterns. Bacterial isolates were obtained from maternal sources and characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. Antibiotic susceptibility testing was conducted using the disc diffusion method. Three *K. pneumoniae* strains KP03, 2014C06-125 and KP2092 (KP03, KP2, and KPK2) were identified, exhibiting characteristic cultural and morphological features. The isolates showed varying levels of resistance to antibiotics, including sulphonamide, amoxicillin, and tetracycline. The overall prevalence of antibiotic resistance was 69.05%, with isolate Y (KP2) showing the highest resistance rate (81.08%). Statistical analysis showed significant differences in resistance patterns among the isolates ($p < 0.05$). The study highlights the importance of monitoring antibiotic resistance in *K. pneumoniae* isolates from maternal sources to inform effective treatment strategies. This study provides valuable data on the prevalence of antibiotic-resistant *K. pneumoniae* in maternal sources, emphasizing the need for judicious use of antibiotics and proper infection control practices.

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Antibiotic resistance, *Klebsiella pneumoniae*, maternal sources, multidrug resistance, opportunistic pathogen

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INTRODUCTION

Klebsiella pneumoniae is a formidable Gram-negative pathogen of significant medical importance, particularly within healthcare settings. As a member of the Enterobacteriaceae family, it is a common commensal of the human gastrointestinal tract but can transform into an opportunistic pathogen capable of causing severe infections, including pneumonia, bacteremia, and urinary tract infections (UTIs) (Patolla et al., 2019; Okeke *et al.*, 2017; Dim *et al.*, 2025a). The clinical management of these infections is increasingly complicated by the global surge in antimicrobial resistance (AMR). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *K. pneumoniae* have emerged as major public health threats, driven by the widespread dissemination of resistance genes encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases (Navon-Venezia et al., 2017; Amadi *et al.*, 2017; Dim *et al.*, 2025b).

This threat assumes critical dimensions in maternal healthcare. Pregnancy and the postpartum period induce physiological immunosuppression and involve frequent healthcare contact, elevating the risk of colonization and infection with nosocomial pathogens (Sharma et al., 2021). Maternity patients are particularly susceptible to UTIs, which can ascend to cause pyelonephritis, preterm labor, and neonatal sepsis if caused by a resistant organism. Infections with MDR *K. pneumoniae* in this population can lead to delayed effective therapy, prolonged hospitalization, increased healthcare costs, and adverse maternal and neonatal outcomes (Ahanja et al., 2017; Dim *et al.*, 2025c; Chude *et al.*, 2020).

The pathogenicity of *K. pneumoniae* is facilitated by an arsenal of virulence factors, including a thick polysaccharide capsule, siderophores for iron acquisition, and adhesins, which aid in immune evasion and tissue colonization (Paczosa & Mecsas, 2016). When coupled with AMR, these traits make infections exceptionally difficult to treat. Despite growing recognition of AMR as a global crisis, region-specific data on the resistance profiles of *K. pneumoniae* in maternity care units remains sparse, limiting the development of context-appropriate interventions.

While studies have characterized *K. pneumoniae* resistance in general hospital populations (Kim et al., 2016; El-Mahdy et al., 2018), there is a paucity of focused research on isolates from maternity patients in specific localities like Nnewi. Therefore, this study aims to evaluate the antibiotic resistance profile of *Klebsiella pneumoniae* isolated from maternity women in Nnewi.

MATERIALS AND METHODS

Sample collection, handling and transportation

A total of 150 urine samples were collected from different maternity women at University Teaching Hospital, Amaku, Awka, Anambra State using sterile capped rubber container. The inclusion and exclusion subjects were considered. The ethical clearance was sought. The samples were transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Culture and Isolation of Enteric Bacteria

This was carried out using the modified method of Cheesbrough. The swab sticks were stricked on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated in inverted at 37±2˚C for 24-48 h. (Egbe *et al*., 2025b; Egbe *et al*., 2025c; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al.* (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al.* (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al.*, 2017a; Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2023a; Iheukwumere *et al*., 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2^{0}C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al.* (2017d), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al.* (2017), Obianom *et al.* (2024), Ekechukwu *et al.* (2025c), Obiefuna *et al.* (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al.* (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025h), Ike *et al.* (2025d), Idigo *et al.* (2025e), Ezedianafo *et al.* (2025d), Ezedianafo *et al.* (2025e) and Iheukwumere *et al.* (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Prevalence and Distribution of the Isolates in the Frozen Meat Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated as a percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al* (2025u),

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010), Iheukwumere *et al* (2025u). The isolates were aseptically subcultured into a broth culture and incubated at 35 + 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$

and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* antibacterial susceptibility test:** This was carried out using the method described in the study published by Iheukwumere *et al*. (2025v). Each labeled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labeled and then incubated at 37±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al.* (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

RESULTS

The *Klebsiella* species isolates (X, Y, Z) exhibited characteristic cultural and morphological features, including red/mucoid appearance on MacConkey agar, smooth surface edges, and rod-shaped cells (Table 1). Molecular analysis confirmed the isolates as *Klebsiella pneumoniae* strains KP03, KP2, and KPK2, with 100% identity to reference strains (Table 2).

The occurrences of the isolates were analyzed, with KP2 being the most prevalent (44.05%), followed by KPK2 (33.33%) and KP03 (22.62%) (Table 3). The isolates' antibiotic susceptibility patterns were assessed, revealing that 69.05% of the isolates were resistant to conventional antibiotics (Table 4). The implicated antibiotics included sulphonamide, amoxicillin, and tetracycline, among others. Statistical analysis showed that the differences in resistance patterns were significant ($p < 0.05$).

The results indicate that the *K. pneumoniae* isolates exhibited high levels of antibiotic resistance, with isolate Y showing the highest resistance rate (81.08%). The p-values for the antibiotic resistance patterns were < 0.05, indicating statistical significance. Overall, the study highlights the importance of monitoring antibiotic resistance in K. pneumoniae isolates from maternal sources to inform effective treatment strategies.

Table 1: Cultural and morphological characteristics of *Klebsiella* species

Characteristics	X	Y	Z
Appearance on MacConkey	Red/mucoid	Red/mucoid	Pink/mucoid
Elevation	Slightly raised	Slightly raised	Revised
Surface edge	Smooth	Smooth	Smooth
Molility	-	-	-
Gram reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Oxidase	-	-	-
Indole	-	-	-
MR	-	-	-
VP	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Xylose	+	+	+
Galactose	+/-	+/-	+/-
Inositol	+	+/-	+/-
Sorbitol	-	-	+/-
Citrate	+/-	+/-	+/-
Dulcitol	+	+/-	-

Table 2: Molecular characteristics of the enteric bacterial isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
X	1681	1681	100	0.0	100	CP144373.1	*Klebsiella pneumoniae* strain KP03 chromosome complete genome (KP03)
Y	1552	1552	100	0.0	100	CP170972.1	*Klebsiella pneumoniae* strain 2014C06-125 (KP2)
Z	1552	1552	100	0.0	100	CP141801.1	*Klebsiella pneumoniae* strain Kp2092 (KPK2)

Table 3: Occurrences of the isolates

Isolate	Occurrences	Percentage
KP03	19	22.62
KP2	37	44.05
KPK2	28	33.33
Total	84	100.00

Table 4: Susceptibility of the bacterial isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
X	19	8 (42.11)	11 (57.89)	S, PER, SP, SXT, AU, CH
Y	37	7 (18.92)	30 (81.08)	AMX, AU, CH, S, PER, SXT, CN
Z	28	11(39.29)	17(60.71)	PER, S, CH, SXT, AU
Total	84	26 (30.95)	58 (69.05)	

DISCUSSION

The findings of this study underscore a significant public health threat within maternal healthcare settings: the circulation of multiple antibiotic-resistant (MAR) *Klebsiella pneumoniae* among maternity patients. Pathogenic bacteria, shaped by environmental factors and selective pressures from antimicrobial misuse, are particularly abundant and concerning in resource-limited regions where infection control can be challenging (Gebremedhin *et al.*, 2022). *K. pneumoniae* exemplifies this threat, causing debilitating infections that are especially severe for immunocompromised or vulnerable individuals, including pregnant and postpartum women (Sharma *et al.*, 2021). The MAR phenotype exhibited by the isolates in this study aligns with the global epidemiology of *K. pneumoniae*, which is increasingly defined by resistance to first-line antibiotics (Manjula *et al.*, 2014; Lorenzoni *et al.*, 2018). This resistance drastically complicates treatment, directly contributing to the high rates of maternal and neonatal morbidity and mortality from hospital-acquired infections.

Variation in the prevalence of *K. pneumoniae* colonization among the patient cohort suggests a complex interplay of risk factors. Studies have linked healthcare-associated infections to demographic and behavioral determinants, such as parity, history of previous hospitalization, and level of education affecting hygiene practices (Aneke *et al.*, 2022; Aljanaby and Alhasani, 2016). However, the relationship is not uniform, as other research indicates that younger, primigravid women may be at higher risk due to physiological and immunological factors (Chander and Shrestha, 2013). These conflicting observations highlight the need for local epidemiological studies to identify context-specific risk profiles and tailor prevention strategies accordingly.

Molecular characterization confirmed the presence of *K. pneumoniae* strains KP03, 2014C06-125, and Kp2092. Discrepancies in strain distribution compared to other studies (e.g., Aljanaby and Alhasani, 2016; Aneke *et al.*, 2022) are to be expected and likely reflect differences in regional antibiotic prescribing practices, infection control efficacy, and the specific clonal lineages circulating in different healthcare ecosystems (Nordmann *et al.*, 2017). The persistence of these resistant strains within the maternity clinic environment points to potential contamination reservoirs on surfaces, equipment, or among healthcare workers, facilitating ongoing transmission (Iregbu *et al.*, 2021). The corroborated resistance patterns, particularly to broad-spectrum agents, underscore the clinical reality that empirical treatment for suspected Gram-negative infections in this setting is increasingly prone to failure.

CONCLUSION

This study reveals a troubling prevalence of antibiotic-resistant *Klebsiella pneumoniae* among maternity patients, with over half of isolates demonstrating resistance and a substantial majority of those exhibiting a multiple antibiotic resistance (MAR) profile. These findings signify a direct and escalating risk for postpartum infections, neonatal sepsis, and treatment failures in a highly vulnerable population. To safeguard maternal and neonatal health, it is imperative to implement a multi-pronged strategy. This must include stringent antibiotic stewardship programs to curb inappropriate antimicrobial use, reinforced infection prevention and control (IPC) protocols within maternity wards, and routine surveillance of AMR patterns to guide clinical decision-making. Protecting mothers and newborns requires urgent action to break the chain of transmission and preserve the efficacy of existing antibiotics.

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Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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