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Maternal Health and Antibiotic Resistance: *Klebsiella pneumoniae* Isolates Analysis

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Abstract

The increasing prevalence of antibiotic-resistant bacterial infections, particularly *Klebsiella pneumoniae*, poses a significant threat to maternal and neonatal health in maternity clinics. The high rate of antibiotic resistance complicates treatment options, leading to increased morbidity and mortality. This study aims to investigate the antibiotic resistance patterns of *Klebsiella pneumoniae* isolates from maternity patients, informing strategies to mitigate the spread of antibiotic-resistant bacteria and improve maternal healthcare outcomes. A total of 150 urine samples were collected and screened using standard microbiological techniques, revealing three strains of *Klebsiella pneumoniae*: KP03, KP2, and KPK2, with occurrences of 21.74%, 47.83%, and 30.43%, respectively. Antibiotic susceptibility testing showed that 54.72% of the isolates were resistant to conventional antibiotics, while 45.28% were susceptible. Notably, the resistant strains exhibited varying patterns of resistance, with 34.48% showing single antibiotic resistance and 65.52% displaying multiple antibiotic resistance (MAR). Statistical analysis using the student "t" test and one-way analysis of variance (ANOVA) confirmed the significance of these findings, with a p-value of ≤ 0.05 indicating a statistically significant difference. The high prevalence of antibiotic-resistant *Klebsiella pneumoniae* in maternity patients poses a significant risk to maternal and neonatal health, emphasizing the need for effective antibiotic stewardship and infection control measures in healthcare settings. The findings of this study highlight the importance of monitoring antibiotic resistance patterns and developing targeted interventions to mitigate the spread of antibiotic-resistant bacteria.

Keywords: Strains, Microbiological, Susceptibility, Occurrences, Bacteria

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Introduction

Klebsiella pneumoniae is one of the enteric bacteria that is of medical importance (Patolla *et al*., 2019). Enteric bacteria are aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria that are found in the gastrointestinal tract of both animals and humans (Dulm van *et al*., 2019). The gastrointestinal tract of human is gut natural habitat for various bacterial species, and most of them are involved in metabolic processes that restore energy and absorbable nutrients, thereby shielding the host against invasion by foreign microbes. The gastrointestinal tract harbours numerous numbers of aerobic and anaerobic bacteria, which may be in symbiotic relationship with the host but can confer negative impact via food borne gastroenteritis in humans, which poses threat to healthful living as shown in debilitating health condition of an infected

patient. These problems ensue due to emergence of antibiotics resistance to conventional antibiotics (El-Mahdy *et al*., 2018).

For several decades, antibiotics have been optimized in tackling disease-causing bacteria (Patolla *et al*., 2019). Antimicrobial resistance poses threat to public health globally (Patolla *et al*., 2019). In pathogenic bacteria, some of the mechanisms of antibiotic resistance are as follows; enzymatic modification (b-lactamases), reduced permeability, increased membrane transport via efflux pumps, altered binding site and metabolic bypass [Patolla *et al*., 2019]. The detection of multidrug resistance is, however, a principal step in effectively controlling antibiotic- resistant bacterial infections that can lead to clinical failure and additional antibiotic resistance (Patolla *et al*., 2019).

Research has shown that *Klebsiella pneumoniae* is one of the most incriminated bacterial pathogen in hospital-acquired infection, especially in developing countries [Kim *et al*., 2016]. Nosocomial infections that occur as a result of activity of multiple resistant *K. pneumoniae* have become an increasing public health concern. The pathogenic potential of the bacterium is facilitated by the presence of virulence factors such as capsular polysaccharide, lipopolysaccharide, fimbriae, siderophores and resistance gene

It is worthy to note that this bacterial pathogen is capable of infecting maternity women in hospital (Ahanja *et al*., 2017). This transmission is facilitated by hospitalization process, which predisposes the women to the infectious agents via contact with healthcare providers and equipment, which also harbor the infectious agent. Timely detection of the pathogen enhances prompt elimination of the pathogens using potent antibacterial agents (El-Mahdy *et al*., 2018)

Several researchers have worked on antibiotic resistance profile of *Klebsiella pneumoniae* isolated from patients in hospital such as Kim *et al*. (2016), Ahanja *et al*. (2017), El-Mahdy *et al*. (2018), and Lorenzon *et al*. (2018) but few studies are available on the antibiotic resistance profile of *Klebsiella pneumoniae* isolated from maternity women at Nnewi. Hence, the aim of this study is to evaluate antibiotic resistance profile of *Klebsiella pneumoniae* isolated from maternity women at 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 Ekesiobi *et al*. (2025a), and Ekesiobi *et al.* (2025b).

Isolation of organisms

The urine samples were plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar (MA/Biotech) using pour plate method. All the plates in triplicate were incubated inverted at 37±2˚C for 24-48 h.

Characterization and identification of the isolates

The isolates were sub-cultured 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*. (2018), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020), Iheukwumere *et al*. (2025c) and molecular characterization as described in the study published by Gabriela *et al.* (2014) and Ekesiobi (2015).

Prevalence and Distribution of the Isolates in the sampled women

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* (2021), Abiodum *et al*. (2024b), Ekesiobi *et al*. (2025c), and Ekesiobi *et al*. (2025d).

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), Ekesiobi *et al*. (2025e) and Ekesiobi *et al*. (2025f). 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*. (2018). 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 study at 95 % confidence level. Pairwise comparison of means was done by Student “t” test as described in the study published by Iheukwumere *et al* (2018), Ekesiobi *et al*. (2017), Abiodum *et al*. (2024a), Abiodum *et al*. (2024c), Iheukwumere *et al*. (2025d), Iheukwumere *et al*. (2025e), Iheukwumere *et al*. (2025f), Iheukwumere *et al*. (2025g), Iheukwumere *et al*. (2025h),

Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Egbe *et al*. (2025a), Egbe *et al*. (2025b) and Ekesiobi *et al*. (2025g).

Results

The characteristics of *Klebsiella* species are shown in Table 1. The isolates B1 and B3 had similar appearance on MacConkey agar except for B2, which appeared red and mucoid. Isolates B1 and B2 had similar elevations except for isolate B3, which was raised. All isolates had smooth surfaces, were non-motile and were Gram-negative rods. The isolates were all catalase and Voges-Proskauer positive, and were negative for oxidase, indole and methyl red. The test isolates were able to utilize glucose, maltose and xylose but showed varying utilization of galactose, inositol, sorbitol and dulcitol. The molecular characterization revealed the presence of *Klebsiella pneumoniae* strain KP03 (KP03), *Klebsiella pneumoniae stain K65921 (KPK65*), *Klebsiella pneumoniae* strain 2014C06-125, and *Klebsiella pneumoniae* strain Kp2092 Table 2.

The occurrences of the test isolates revealed that isolate B2 had the highest occurrence (47.83%), followed by isolate B3 (30.43%) and then isolate B1 (21.74%), which had the lowest occurrence, as shown in Table 3. The susceptibility of bacterial isolates to conventional antibiotics revealed 45.28 % of the bacterial isolates was susceptible to conventional antibiotics whereas 54.72 % was resistant (Table 4). The degree of resistance exhibited among the isolates revealed that 34.48% exhibited single antibiotic resistance to the conventional antibiotics whereas 65.52 exhibited multi-resistance as shown in Table 5.

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

Characteristics	B1	B2	B3
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
B1	1681	1681	100	0.0	100	CP144373.1	*Klebsiella pneumoniae* strain KP03 chromosome complete genome (KP03)
B2	1552	1552	100	0.0	100	CP170972.1	*Klebsiella pneumoniae* strain 2014C06-125 (KP2)
B3	1552	1552	100	0.0	100	CP141801.1	*Klebsiella pneumoniae* strain Kp2092 (KPK2)

Table 3: Occurrences of the isolates

Isolate	Occurrences	Percentage
Klebsiella pneumoniae B1	15	21.74
Klebsiella pneumoniae B2	33	47.83
Klebsiella pneumoniae B3	21	30.43
Total	69	100.00

Table 4: Susceptibility of the bacterial isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
C1	13	8 (61.54)	5 (38.46)	S, PN, CEP, SXT, AU, CN
C2	12	9 (40.91)	13 (59.09)	AMX, AU, CEP, S, PN, SXT, CN
D1	7	0 (0.00)	7 (100.00)	PN, S, CEP, SXT, AU
D2	11	7 (63.64)	4 (36.36)	AU, PN, S, CEP, SXT, CN
Total	53	24 (45.28)	29 (54.72)	

Table 5: Degree of resistance among the isolates

Isolates	NR	Single resistant strain (%)	Multiple resistant strain (%)
C1	5	1 (20.00)	4 (80.00)
C2	13	8 (61.54)	5 (38.46)
D1	7	0 (0.00)	7 (100.00)
D2	4	1 (25.00)	3 (75.00)
Total	29	10 (34.48)	19 (65.52)

Discussion

Research has revealed that pathogenic bacteria are abundant in the environment, due to certain environmental factors and human activities, especially in developing countries. These pathogenic bacteria cause highly debilitating diseases, especially among immunocompromised individuals. Understanding the pathogenic profile of bacterial pathogens is essential to reducing the alarming rate of morbidity and mortality.The characteristic features of multiple antibiotic resistant *Klebsiella pneumoniae* isolated in this study conform to the characteristic features of multiple antibiotic resistant *Klebsiella pneumonia*e isolated by several researchers (Manjula *et al*., 2014; Kim *et al.*, 2016; Jo et al., 2017; Ahanjan *et al.*, 2017; Lorenzoni *et al.*, 2018) in different hospital settings, especially among women attending antenatal care. The variation in the occurrences of the pathogens in the investigated women could be attributed to certain demographic factors such as age, occupation, and educational level. This observation corresponds to the findings of several researchers (Aljanaby and Alhasani, 2016; Frhadi *et al*., 2021; Aneke *et al.*, 2022). However, Chander and Shrestha (2013) reported that the occurrences of the multiple antibiotic resistant *Klebsiella pneumonia*e were not influenced by any of the demographic factors mentioned by other researchers, as lower aged women recorded the highest occurrence of the bacterial isolates.

The molecular characterization of the bacterial isolates revealed the presence of bacteria such as *Klebsiella pneumoniae* strain KP03 (KP03), *Klebsiella pneumoniae* strain 2014C06-125, *Klebsiella pneumoniae* strain Kp2092. However, there was variation in the bacterial strains reported by other researchers (Aljanaby and Alhasani, 2016; Frhadi *et al*., 2021; Aneke *et al.*, 2022), which could be attributed to the degree of contamination in the maternity clinics. The The resistance exhibited by the isolates corrobarated with the findings of other researchers (Aljanaby and Alhasani, 2016; Frhadi *et al*., 2021; Aneke *et al.*, 2022).

Conclusion

This study highlights a significant prevalence of antibiotic-resistant *Klebsiella pneumoniae* strains (54.72%) in maternity patients, with a high rate of multiple antibiotic resistance (65.52%). The findings emphasize the need for effective antibiotic stewardship, infection control measures, and targeted interventions to mitigate the spread of antibiotic-resistant bacteria, ensuring the health and well-being of mothers and newborns

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