International Journal of Pathogen Research

Volume 13, Issue 6, Page 169-178, 2024; Article no.IJPR.126837
ISSN: 2582-3876

Prevalence and Antibiogram of *Klebsiella pneumoniae* in Urine and Sputum Specimen in Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpr/2024/v13i6331

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126837

Original Research Article

Received: 18/10/2024
Accepted: 21/12/2024
Published: 26/12/2024

ABSTRACT

Klebsiella pneumoniae is considered as the second leading cause of hospital-acquired and community-acquired infections globally and antibiotic resistance which has contributed to the high motality rate. This research focused on the isolation and antibiogram of *Klebsiella pneumoniae* isolates in urine and sputum specimen in Nasarawa State. From March to April 2024, a total of 210 urine and sputum samples were collected from one hospital in each of the three districts of Nasarawa State. Specimens were collected in sterile universal containers and cultured on MacConkey agar and cysteine lactose electrolyte deficient agar. The biochemical characterization

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Cite as: *I.G., Innocent, Sar, T.T., Aernan Tracy Paulyn., and Agada, E.O. 2024. "Prevalence and Antibiogram of Klebsiella Pneumoniae in Urine and Sputum Specimen in Nasarawa State, Nigeria". International Journal of Pathogen Research 13 (6):169-78. https://doi.org/10.9734/ijpr/2024/v13i6331.*

of the isolates were done. Antibiotic susceptibility testing was done by Kirby-Bauer disk agar diffusion method. The study identified *Klebsiella pneumoniae* in 28 out of 210 samples, resulting in a prevalence rate of 13.3%. Specifically, 18 (15.0%) of the urine samples tested positive for *Klebsiella pneumoniae*, while 10 (11.1%) of the sputum samples were also positive. Among the patients, 10 (10.2%) males were infected with *Klebsiella pneumoniae*, whereas 89 (89.8%) males were not infected. In contrast, 18 (16.1%) females had *Klebsiella pneumoniae* infections, while 94 (83.9%) females did not. Patients aged 30-39 had the highest 10(16.1%) prevalence of *Klebsiella pneumoniae*. Antibiotics susceptibility testing showed meropenem, amikacin and gentamicin exhibited the highest sensitivity outcomes as compared to other antibiotics while *Klebsiella pneumoniae* were highly resistant to ampicillin and amoxicillin. There were statistically significant difference in antibiotics resistance in *Klebsiella pneumoniae* isolates at 5% level of significance.

Keywords: Klebsiella pneumoniae; antibiotic; nosocomial; urine and sputum specimens.

1. INTRODUCTION

Klebsiella pneumoniae is a common Gram-negative bacterium found in the oral cavity, on the skin, in the intestines, and in natural environments such as soil and water (Siddhardha et al., 2020). Discovered in 1883 by German pathologist Carl Friedländer, it was later named after German bacteriologist Edwin Klebs (Sturchler, 2016). This bacterium is a facultative anaerobe, non-motile, encapsulated, lactose-fermenting, Gram-negative bacillus that belongs to the Enterobacteriaceae family. It has a rod shape, measuring approximately 2µm by 0.5µm, and forms mucoid colonies when grown on MacConkey agar (Calfee, 2017).

Klebsiella pneumoniae is a common opportunistic bacterium linked to both hospital-acquired and community-acquired infections (Frieri et al., 2017). It is capable of causing a variety of infections, including those affecting the respiratory system, urinary tract, lower biliary tract, soft tissues, bloodstream, surgical wounds, and liver, especially in patients with weakened immune systems (Gipson et al., 2020; Holt et al., 2015). The rise of multidrug-resistant strains of *K. pneumoniae* has become a significant clinical and public health concern, contributing to an increase in the prevalence of these infections (De Oliveira et al., 2020; Donelli & Vuotto, 2014; Gipson et al., 2020; Qiu et al., 2021).

This bacterium is commonly found across the globe and demonstrates atypical patterns of drug resistance. It is classified as part of the ESKAPE pathogen group, which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species* (De Oliveira et al., 2020; Riaz et al., 2012; Wyres & Holt, 2018). This research focused on isolation and antibiogram of *K. pneumoniae* isolates in urine and sputum specimen in Nasarawa State.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out in Nasarawa State at three key healthcare facilities for the period of Febuary, 2024 to May 2024: Dalhatu Araf Specialist Hospital Lafia (DASHL) located in the southern senatorial zone, General Hospital Akwanga in the northern senatorial zone, and Federal Medical Center Keffi (FMCK) situated in the western senatorial zone.

2.2 Study Population

Patient's at the study locations who exhibited symptoms of urinary tract infection and those exhibit respiratory tract infection, and who were on admittion at Dalhatu Araf Specialist Hospital Lafia (DASHL), General Hospital Akwanga, and Federal Medical Center Keffi (FMCK) were the eligible study participants.

2.3 Inclusion Criteria

Patients 15years of age and older and suspected of urinary tract infections and respiratory tract infections.

2.4 Exclusion Criteria

Repeated specimen from the same patient was not included in the study.

2.5 Sample Size Determination

The sample size was determined using the formula $n=Z^2p(1-p)/d^2$ (Naing et al., 2022).

Where:
n = sample size
z = statistic for level of confidence
p= prevalence rate of previous *Klebsiella pneumoniae* (12.78%) in Lafia, Nasarawa State (Ashefo and Habibu, 2024).
d = precision
$n = 1.96^2 x\ 0.1278 \times (1-0.1278)/ (0.05)^2$
= 171
≃ 210

2.6 Ethical Approval

Ethical approval was obtained from National Health Research Ethic Committee (NHREC) in the Nasarawa State Ministry of Health, Lafia prior to the commencement of the study**.** This is to ensure the rights, dignity, and autonomy of individuals are respected. This includes obtaining informed consent, maintaining confidentiality, and avoiding harm.

2.7 Collection of Samples

Sputum and urine specimen were collected from patients from the selected hospital each in three district of Nasarawa State. Patients who met inclusive criteria were given instructions on how to collect midstream urine samples and sputum specimens. Each patient was given a sterile container and instructed to fill up to 20ml of urine and small quantity of sputum specimens. Each container was labeled with the patient's identity codes, collection time, and date. The collected specimens were analyzed at the study locations.

2.8 Isolation and Identification of *Klebsiella pneumoniae* Isolates

Urine specimens were inoculated on CLED agar and sputum specimens on MacConkey agar plates. The inoculated plates were incubated for 24hours at 37°C, and bacterial growth on them were assessed. Presence of mucoid and lactose fermenting colonies are used preliminary identification of *Klebsiella pneumoniae.*

2.9 Biochemical Characterization

Isolates that displayed mucoid colonies were subjected to biochemical tests for the confirmation of *K. pneumoniae*, which included motility testing, citrate utilization as a carbon source, urease production, indole testing, and urease testing (Cheesbrough, 2016). The isolates confirmed as *K. pneumoniae* were preserved on nutrient agar slants and stored in a refrigerator at approximately 8°C.

2.10 Antibiotic Susceptibility Testing

The disc-diffusion method utilizing Mueller Hinton agar was employed to assess the antibiotic susceptibility of all *Klebsiella pneumoniae* isolates. Colonies of *Klebsiella pneumoniae* were sub-cultured onto Mueller Hinton agar using sterile swabs to create streaks on the agar surface. Up to ten commercially available antibiotic discs, including ampicillin (30 µg), amoxicillin (20 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), gentamicin (10 µg), and amikacin (30 µg), were placed on the Mueller Hinton media with sterile forceps. The plates were incubated for 24 hours at 37°C, and the zones of growth inhibition surrounding each antibiotic disc were measured to the nearest millimeter. The sensitivity and resistance to the antibiotic agents were determined based on the diameter of the zone of inhibition, following the criteria established by the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2022).

2.11 Statistical Analysis

Data were expressed in percentages, analyzed using statistical product and service solutions (SPSS) version 29 and Pearson's Chi Square analysis test for comparisons at $p>0.05$. Where p-values ≤ 0.05 were considered statistically significant while where p-values >0.05 were considered not statistically significant.

3. RESULTS

3.1 Occurrence of *K. pneumoniae* Isolates Based on Specimen

A total of 210 Clinical Specimen of Sputum (90) and urine (120) were collected from the health facilities and examined by standard procedures. Occurrence of *Klebsiella pneumoniae* isolates based on specimen showed that urine specimen had (15.0%) of *Klebsiella pneumoniae* while the sputum specimen had (11.1%) of *Klebsiella pneumoniae.* The overall incidence rate of *K. pneumoniae* isolates in the urine and sputum specimen was (13.3%) as shows in Table 1. There was no significant difference in the urine and sputum specimen at $p>0.05$.

3.2 Occurrence of *K. pneumoniae* Isolates Based on Gender

Occurrence of *Klebsiella pneumoniae* based on gender showed that females had the highest prevalence (16.1%), while males had (10.2%) as shows in Table 2. The occurrence of *Klebsiella pneumoniae* based on gender was not significant difference at p>0.05.

Table 1. Occurrence of *K. pneumoniae* isolates based on specimen

Specimen	No. examined	No. positive (%)	No. negative (%)
Urine	120	18(15.0)	102(85.0)
Sputum	90	10(11.1)	80(88.9)
TOTAL	210	28(13.3)	182(86.7)

Table 2. Occurrence of *K. pneumoniae* isolates based on gender

Gender	No. examined	No. positive (%)	No. negative (%)
Male	98	10(10.2)	89(89.8)
Female	112	18(16.1)	94(83.9)
Total	210	28(13.3)	182(86.7)

Table 3. Distribution of *K. pneumoniae* isolates based on age

Age (years)	No. examined	No. positive (%)	No. negative (%)
15-24	23	2(8.7)	21(91.3)
25-34	44	5(11.4)	39(88.6)
35-44	62	10(16.1)	52(83.8)
45 —54	53	8(15.1)	45(84.9)
56 above	28	3(10.7)	25(89.3)
TOTAL	210	28(13.3)	182(86.7)

3.3 Distribution of *Klebsiella pneumoniae* Based on Age

Distribution of *Klebsiella pneumoniae* based on age group that the highest number of isolates were examined from the patients in the age group of 35-44 yrs (16.1%), followed by 45-54 yrs (15.1%), 25-34 yrs (11.4), 56 yrs and older (10.7%), and 15-24 yrs had the least number of (8.6%). The distribution of the isolates based on age group were not significant difference at p>0.05.

3.4 Antibiotic Sensitivity Profile of *K. pneumoniae* at Dalhatu Araf Specialist Hospital, Lafia

On the basis of resistance to antibiotics, strains were categorized into two groups, i.e., susceptible (S) and resistant (R), according to CLSI guidelines. Among 11 *K. pneumoniae* isolates, meropenem showed the highest activity (90.9%) against the test bacteria, amikacin (81.8%), gentamicin and ciprofloxacin (72.7%), ceftriaxone and nitrofurantoin (63.6%), ceftazidime and cefotaxime (54.5%), amoxicillin (27.3%) while ampicillin had the least (18.2%). Conversely, ampicillin had the highest resistant of (81.8%), ceftazidime and cefotaxime (45.5%), ceftriaxone and nitrofurantoin (36.4%), amoxicillin (27.7) followed by gentamicin and ciprofloxacin (27.3%), amikacin (18.2%) and meropenem (9.1%) which was the least resistant. There was significant difference in antibiotic sensitivity profile of *K. pneumoniae* at Dalhatu Araf Specialist Hospital, Lafia at p>0.05.

3.5 Antibiotic Sensitivity Profile of *K. pneumoniae* Isolates at General Hospital Akwanga

The isolates had (100%) sensitivity to meropenem, amikacin (85.7%), gentamicin and ciprofloxacin (71.4%), ceftazidime, cefotaxime, and nitrofurantoin (57.1%), ceftriaxone and amoxicillin (42.9%), ampicillin (14.3) which was the least sensitivity. The isolates showed highest resistance was examined in ampicillin (85.7%), ceftriaxone and amoxicillin (57.1%), ceftazidime, cefotaxime, and nitrofurantoin (42.9%), ciprofloxacin and gentamicin (28.6%), amikacin (14.3%), and meropenem (0%), which had least resistant. There was significant difference in antibiotic sensitivity profile of *K. pneumoniae* at General Hospital, Akwanga at p>0.05.

3.6 Antibiotic Sensitivity Profile of *K. pneumoniae* Isolates at Federal Medical Center, Keffi

The isolates had (90.0%) sensitivity to amikacin and meropenem, followed by gentamycin (80.0%), followed by ciprofloxacin and ceftriaxone (70.0%), followed by ceftazidime and cefotaxime (60.0%), and nitrofurantoin and amoxicillin (50.0%), and followed by ampicillin (20.0%). On the basis of resistance, the isolates had (80.0%) resistant to ampicillin, followed by nitrofurantoin and amoxicillin (50.0%), followed by ceftazidime and cefotaxime (40.0%), followed by ciprofloxacin and ceftriaxone and ciprofloxacin (30.0%), followed by gentamicin (20.0%), amikacin and meropenem had the least resistant (10.0%). There was significant difference in antibiotic sensitivity profile of *Klebsiella pneumoniae* at Federal Medical Center, Keffi at p>0.05.

Table 4. Antibiotic sensitivity profile of *K. pneumoniae* isolates at Dalhatu Araf Specialist Hospital, Lafia

Antibiotics	Concentration (mcg/disc)	No. of *K. pneumonia* isolate n=11	
		Sensitive No. (%)	Resistance No. (%)
Ceftriaxone	30	7(63.6)	4(36.4)
Ampicillin	30	2(18.2)	9 (81.8)
Cefotaxime	30	6(54.5)	5(45.5)
Ceftazidime	30	6(54.5)	5(45.5)
Meropenem	30	10(90.9)	1(9.1)
Gentamicin	10	8(72.7)	3(27.3)
Ciprofloxacin	5	8(72.7)	3(27.3)
Amikacin	30	9(81.8)	2(18.2)
Nitrofurantoin	300	7(63.6)	4(36.4)
Amoxixillin	20	3(27.3)	8(72.7)

Table 5. Antibiotic sensitivity profile of *K. pneumoniae* isolates at General Hospital Akwanga

Antibiotics	Concentration (mcg/disc)	Number of *K. pneumonia* isolate n=7	
		Sensitive No. (%)	Resistant No. (%)
Ceftriaxone	30	3(42.9)	4(57.1)
Ampicillin	30	1 (14.3)	6(85.7)
Cefotaxime	30	4(57.1)	3(42.9)
Ceftazidime	30	4(57.1)	3(42.9)
Meropenem	30	7(100)	0(0)
Gentamycin	10	5(71.4)	2(28.6)
Ciprofloxacin	5	5(71.4)	2(28.6)
Amikacin	30	6(85.7)	1(14.3)
Nitrofurantoin	300	4(57.1)	3(42.9)
Amoxicillin	20	3(42.9)	4(57.1)

Table 6. Antibiotic sensitivity profile of *K. pneumoniae* isolates at Federal Medical Center Keffi

Antibiotics	Concentration (mcg/disc)	No. of *K. pneumonia* isolate n=10	
		Sensitive No. (%)	Resistant No. (%)
Ceftriaxone	30	7(70.0)	3(30.0)
Ampicillin	30	2(20.0)	8(80.0)
Cefotaxime	30	6(60.0)	4(40.0)
Ceftazidime	30	6(60.0)	4(40.0)
Meropenem	30	9(90.0)	1(10.0)
Gentamicin	10	8(80.0)	2(20.0)
Ciprofloxacin	5	7(70.0)	3(30.0)
Amikacin	30	9(90.0)	1(10.0)
Nitrofurantoin	300	5(50.0)	5(50.0)
Amoxicillin	20	5(50.0)	5(50.0)

4. DISCUSSION

Klebsiella pneumoniae is recognized as the predominant opportunistic pathogen in hospital settings, frequently encounters a wide array of antibiotics. This exposure leads to the evolution of resistance mechanisms and the rise of multidrug-resistant (MDR) strains. Nonetheless, the prevalence of antibiotic resistance in *Klebsiella pneumoniae* can differ significantly depending on geographic location, the specific population affected, and the strategies employed in antibiotic management (Asbell et al., 2018). This research focused on the isolation of *Klebsiella pneumoniae* isolates in urine and sputum specimen in Nasarawa State. The total of 28 out of the 210 specimen was positive for significant *Klebsiella pneumoniae* growth, which gave a prevalence of 13.3% lower compared to the prevalence of 18.2% found in Anambra by Ogbukagu et al. (2016), prevalence of 29.2% *K. pneumoniae* in Lafia, Nasarawa State by Innocent, et al. (2023), prevalence of 18.5% in Cameroon by Pieboji et al. (2004) and in Benin, Edo State, 33.3% by Osazuwa et al. (2010). In contrast, the findings of this study was higher compared to the 8.0% prevalence of *Klebsiella pneumoniae* in Kano state (Hamza et al., 2016), 8.33% and 10.0% in Anambra State by Vranic et al. (2017); Sokhn et al. (2020) and prevalence of 10.0% in Morocco (Lahlou Amine et al., 2009).The differences between this finding and others could be factors such as the source of isolation (hospital environment, community, soil, etc.), isolation techniques, growth conditions, or diagnostic methods and geographical location can all affect the phenotype and genotype of the bacterium.

The occurrence of *Klebsiella pneumoniae* isolates based on specimen showed high numbers of *K. pneumoniae* isolates (15.0%) were from urine samples while sputum had (11.1%). The findings of this study was coherent with studies by Riaz et al. (2015) and Akter et al. (2014) who reported urine samples as the most prevalent source of *Klebsiella pneumoniae* isolates, while Sah et al. (2015) and Varghese et al. (2016) identified *Klebsiella pneumoniae* as the second most commonly isolated organism from urine, following *Escherichia coli*. Urinary tract infections (UTIs), which arise from the colonization of urine and the invasion of various structures within the urinary tract by microorganisms such as bacteria, viruses, yeasts, and protozoa, are recognized as prevalent infections in humans (Almehdawi et al., 2017; Chijioke et al., 2016). *Klebsiella pneumoniae* is often associated with urinary tract infections (UTIs) due to its propensity to multiply in the urinary system, leading to increased bacterial concentrations in urine. On the other hand, while *Klebsiella pneumoniae* can cause respiratory infections, it may not always be present in substantial amounts in sputum, particularly in cases of milder infections.

This study showed that females had a greater incidence of *K. pneumoniae* than males. This is in agreement with the findings of Hamza et al. (2016), Ogbukagu et al. (2016) support this observation, Ostojic et al. (2021) observed that infection was more commonly confirmed in females than males and Shilpa et al. (2016) found that *Klebsiella pneumoniae* infection was the predominant organism isolated from females (45.45%) than males (31.57%). This might be because female urethras are significantly shorter and closer to the anus than male urethras, and they lack the bacteriostatic characteristics of prostatic secretions, which may account for the higher incidence of bacteria. According to Ogbukagu et al. (2016), unsanitary practices like cleaning the vagina with stored water that has been left out in the open for a while and cleaning the vagina incorrectly by going from back to front after urinating could also be the reason for the higher incidence. A woman's risk of getting a UTI may increase during pregnancy and during sexual activity, as bacteria can be massaged up the urethra into the bladder after delivery and germs can be push into the urethra during sexual contact (Varghese et al., 2016). In contrast, Otajevwo (2013) found a prevalence rate of *K. pneumoniae* 58.3% in males and 41.7% in females and Wanja et al. (2021) in Kenya showed that males 18(4.7%) were more affected than females 10(2.6%).

Klebsiella pneumoniae can impact individuals across all age demographics, suggesting that age does not significantly influence the transmission and infection rates of this disease; rather, it is primarily linked to exposure. A study indicated that individuals aged 35-44 exhibited the highest prevalence of *K. pneumoniae* at 16.1%. This finding aligns with the research conducted by Devrari & Pai (2018), which noted a greater prevalence of *Klebsiella spp*. among older individuals in the 41-50 age range. Similarly, Ndako et al. (2019) also reported a higher prevalence in older age groups. Conversely, these results contrast with those of Sule & Kumurya (2016), who found the highest prevalence of *Klebsiella species* in individuals aged 21-30. The data suggest that the most vulnerable populations include the elderly, young adults, and those with compromised immune systems, highlighting that infection with *Klebsiella pneumoniae* is linked to reduced immunocompetence (Devrari & Pai, 2018).

Antibiotic susceptibility testing of *K. pneumoniae* at Dalhatu Araf Specialist Hospital Lafia showed relatively high rate (90.9%) of susceptibility to meropenem followed by amikacin. The resistance effect of *Klebsiella pneumoniae* observed showed high resistant to ampicillin, followed by ceftazidime and cefotaxime, cefuroxime, followed by nitrofurantoin, followed by gentamicin and ciprofloxacin, followed by amoxicillin and meropenem. The findings of this study are in line with Giske et al. (2012) in Guinea- Bissau who showed that *Klebsiella pneumoniae* isolates exhibited good sensitivity to amikacin (100%) and meropenem (80%). Research in Kenya in 2010 on the prevalence *Klebsiella pneumoniae* reported a higher resistance of *Klebsiella pneumoniae* to third generation gentamycin and cephalosporin and a lower resistance to amikacin and meropenem (Ogalo et al., 2016). Arowosegbe et al. (2017) observed 83% resistance of *Klebsiella* sp to ampicillin and 67% resistance to ceftazidime in a study on the sensitivity pattern of *Klebsiella* sp isolated from neonatal septic patients in Abeokuta Nigeria. In Nigeria, as in many other countries, *Klebsiella pneumoniae* has been increasingly problematic due to its growing resistance to antibiotics.

The Antibiotic susceptibility patterns at General Hospital Akwanga is in agreement with those of Varghese et al. (2016) and Hamza et al. (2016) whose findings revealed that 100% of the *Klebsiella pneumoniae* isolated were resistant to Ampicillin and Amoxicillin. According to Varghese et al. (2016), *Klebsiella pneumoniae* isolates are naturally resistant to Ampicillin, due to a constitutively expressed chromosomal class β lactamase.

The findings of this study in relation to antibiotic sensitivity pattern at Federal Medical Center Keffi are in line with Giske et al. (2012) who showed that *Klebsiella pneumoniae* isolates exhibited 100% sensitivity to amikacin and 80% to meropenem.

In recent years, there has been a significant rise in *Klebsiella pneumoniae* strains exhibiting resistance to commonly prescribed antibiotics. This development poses an increasing challenge in managing infections caused by this bacterium (Cerceo et al., 2016). Antimicrobial resistance is a natural occurrence that is exacerbated by the inappropriate use of antibiotics (Nwafia et al., 2021; Ochei & Kolhatkar, 2000). The overuse and misuse of antibiotics, along with the proliferation of organisms that produce extended spectrum beta-lactamases (ESBLs), have led to a surge in multidrug resistance among *Klebsiella pneumoniae* strains in recent years (Cerceo et al., 2016). β-lactam antibiotics, which are typically employed to treat infections from the Enterobacteriaceae family, have become ineffective due to the emergence of ESBL (Eltai et al., 2018). The rise of antibiotic resistance in *Klebsiella pneumoniae* is increasingly recognized as a significant public health issue globally, resulting in adverse patient outcomes, extended hospital stays, and increased healthcare costs (Keke et al., 2005; Ohanu et al., 2018). This problem is particularly severe in developing countries like Nigeria, where counterfeit medications are prevalent, and there is widespread overuse and misuse of antibiotics (Keke et al., 2005; Nwafia et al., 2021). The use of broad-spectrum antibiotics in hospitalized patients has led to higher rates of *Klebsiella pneumoniae* colonization and multidrug resistance (Chakraborty et al., 2016). As noted by Naqid et al. (2020) *Klebsiella pneumoniae* has developed resistance not only to commonly used antibiotics but also to other classes, including third-generation cephalosporins, in various countries.

Due to the high prevalence of ESBLs, meropenem is considered the preferred antibiotic for the empirical management of severe, life-threatening infections. While amikacin may serve as a reasonable alternative for empirical therapy based on its cost and susceptibility profile, its use may be limited due to potential side effects, especially in neonates (Raji et al., 2015).

5. CONCLUSION

The prevalence of *Klebsiella pneumoniae* in Nasarawa State is 13.3%. *Klebsiella pneumonia* is a pathogen that colonize the urinary tract and respiratory tract in Nasarawa State with prevalence of 15.0% and 11.1% respectively. *Klebsiella pneumoniae* affects all age groups, affirming that age is not a contributing factor towards spread and infection of the disease, but infection rather depends on exposure. This study also concludes that females were more affected by *Klebsiella pneumoniae* than males. The higher resistance of *Klebsiella pneumonia* isolates were observed highest to Ampicillin and

Amoxicillin from the three facilities. Meropenem and Amikacin are the highly effective antibiotics in the study.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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