Antibiogram of *Klebsiella pneumoniae* isolates from Urine Samples of patients attending Hospital in Wukari, North-East Nigeria

[1,] Abhadionmhen O. A and [2.] Imarenezor E.P.K.

1. Department of Microbiology, Federal University, Wukari, Nigeria
2. Department of Microbiology, Federal University, Otuoke, Nigeria

Correspondence: abel2nice@gmail.com; +(234) 8062610523

Abstract:
This study investigated the presence of *Klebsiella pneumoniae* in urine samples obtained in Wukari metropolis. Sterile containers with boric acid were used to collect mid-stream urine samples from 20 hospitalised and 20 non-hospitalised patients aged 18-50. Additionally, sterile swabs were used to collect samples from doorknobs in patient wards. Using the streak-plate technique, samples were cultured on MacConkey agar at 37°C for 24 hours. Morphological and biochemical characterizations were used to make a presumptive identification of the bacterial isolates. The *khe* genes common to Klebsiella species were identified using Polymerase Chain Reaction (PCR) methods with fw (5'-TGATTGCATTCGCCACTGG-3') and rev (5'-GGTCAACCCAACGATCCTG-3') primers. The antimicrobial sensitivity assay was performed using the agar diffusion method. All isolates were sensitive to Chloramphenicol 30µg (≥21mm), Gentamycin 30µg (≥22mm), Streptomycin 30µg (≥22mm), and Ciprofloxacin 30µg (≥24mm). Additionally, the isolates showed multi-drug resistance to Augmentin 30µg (≤13mm) and Amoxicillin 30µg (≤11mm). *Klebsiella pneumoniae* was not isolated from any of the non-hospitalised patients' samples but was isolated from 25% of hospitalised patients' samples, indicating that *Klebsiella pneumoniae* is a hospital-acquired infection (HAI). Furthermore, *Klebsiella pneumoniae* was isolated from samples obtained from doorknobs in patient wards. *Klebsiella pneumoniae* has shown to be prevalent in the hospital environment, as evidenced by its isolation from samples obtained in patient wards and among hospitalised patients. Consequently, hospital equipment and environments should be kept clean at all times, and strict infection control protocols along with regular antimicrobial stewardship programs should be implemented in hospital settings to prevent the spread of multi-drug resistant *Klebsiella pneumoniae.*

Keywords: Antibiogram, Antimicrobial Resistance, Hospital Acquired Infection (HIA), *Klebsiella pneumoniae*, Urinary Tract Infection (UTI).

1. Introduction

Nosocomial" describes any disease acquired in hospital environment [1]. Recently, "healthcare associated infections" (HIA) is being alternatively used to describe whatever Nosocomial implies [2]. Nosocomial pathogens may be isolated from asymptomatic patients or even hospital staffs and visitors. [4], [5]. HAI accounts for increased stay in hospitals [6]. This shift in terminology from "nosocomial" to "healthcare-associated infections" signifies a broader understanding of how infections are acquired within healthcare settings. The high prevalence in low-income countries indicates a pressing need for comprehensive strategies to address and mitigate the transmission of these infections across various cohorts within healthcare environments.

Nosocomial infection is responsible for mortality and a number of socioeconomic challenges such as household poverty faced by millions of individuals globally [6]. According to a WHO report [7], about 15% of hospitalized individuals are estimated to be infected with nosocomial pathogens. There is variation in the rates of nosocomial infection across the globe, with Africa and Asia facing higher occurrences compared to western countries [8]. About 10% of all hospitalizations in the western world results in nosocomial infections, while in Africa and Asia about 40% hospitalizations presents with nosocomial infections [8]. More than half of all neonatal death are attributed to nosocomial infection with over about 80% of these deaths occurring in parts of Africa and Asia [8]. The negative impact of nosocomial infection among infants is particularly alarming as it impedes the human capital development of a nation and the world at large. The increase in the rates of infant mortality associated with nosocomial infection is not unconnected to their vulnerability due to their developing immune system [8]. The uneven distribution of the health outcome caused by nosocomial infection across nations of the globe is health inequality raising significant concern on the effectiveness of current management policies of nosocomial infection, highlighting the urgent need for an invested region-specific interventions strategy to safeguard the most vulnerable population of society.

Additionally, there are socioeconomic and cultural implications of nosocomial infections which cannot be ignored. Nosocomial infection is responsible for unnecessary stay in hospital incurring additional medical bill [9]. This aggravates the socioeconomic challenges of individuals whose household is already grappling with poverty [9]. In most part of underserved communities in rural parts of Africa,

there is a complete lack of healthcare infrastructure and resources. Even when there are, health care professionals lack basic trainings on infection control measures that can support effective prevention and treatment strategies [10]. Although, healthcare hesitancy based on cultural affiliations, beliefs, and practices has been identifies to contribute to the attitude of healthcare professionals and patients in adhering to specific infection prevention and management protocols [11]. The complete lack of universal health coverage and healthcare supports particularly in most part of Africa significantly worsens socioeconomic challenges in households who are frequently faced with prolonged stay in hospital due to nosocomial infection. The attitude of healthcare professionals towards hygienic and aseptic practices in hospital settings have been identified as a significant contribution to the prevailing rates of nosocomial infections [11].

Nosocomial pathogens are a panel of microorganisms having the capacity to either cause infection independently or collectively [4]. While fungi and viruses are less prominent in HAI, more than 90% of nosocomial infections are caused by bacteria [12]. Bacteria pathogens which are commonly isolated from cases of HIA includes but not limited to, *Klebsiella pneumonia, Acinetobacter, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirablis, Bacillus cereus, Escherichia coli and* Streptococcus *species* [4]. Unregulated and indiscriminate use of broad-spectrum antibiotics, in hospitals contributes significantly to the prevalence rates of nosocomial infections [6]. The dominance of bacteria in nosocomial infections is of significant concern due to their propensity to cause infections independently or collectively, compounding the challenges associated infection control particularly, antimicrobial resistance [4]. The varied spectrum of bacterial pathogens involved in nosocomial infection complicates chemotherapy. This is because, it is more difficult to treat infection caused by a single pathogen than multiple microorganisms based on the diverse sources and modes of transmission of the later within healthcare settings [11]. This occurrence demands rigorous infection control protocols and targeted antimicrobial stewardship initiatives [11].

Moreover, the overuse or unrecommended use of antibiotics particularly the broad-spectrum ones increase the potential of the emergence of antibiotic-resistant traits among microorganisms, creating a formidable challenge in managing nosocomial infections [13]. Discouraging overreliance on broad-spectrum antibiotics and adopting more judicious chemotherapy that is recommended is key in curbing the escalating rates of antibiotic resistance and, consequently, nosocomial infections [14][15]. Further, a collaborative effort which is aimed at refining antibiotic prescribing practices will mitigate the impact of nosocomial pathogens and safeguard patient well-being within healthcare environments [16][17].

Klebsiella is of the order *Klebsiellae,* a member of the family *Enterobacteriaceae* [18]. *Klebsiella pneumoniae* is a Gram-negative bacillus which normally exists as normal flora in the intestine but when they are introduced into the urinary tract and bile duct through the use of contaminated medical equipment during surgical operations or through the use of indwelling catheters, mechanical ventilators and complemented by sophisticated virulence factors [19]. Some of these factors include Pili, Capsular Polysaccharide, Serum Resistance, Siderophores, and Lipopolysaccharide. Nosocomial transmission of *Klebsiella pneumoniae*, particularly antibiotic-resistant strains, is a significant concern in healthcare settings. Hospitals provide an environment where pathogens like *Klebsiella pneumoniae* can survive and spread [20]. Fomites, which are inanimate objects like medical equipment, bed linens, door handles, and other surfaces, can become contaminated with *Klebsiella pneumoniae* [21]. Nosocomial pathogens including *Klebsiella pneumoniae* can persist on surfaces for extended periods, contributing to transmission. *Klebsiella pneumoniae* can colonize the gastrointestinal tract and respiratory tract of patients asymptomatically [22]. Colonized patients can serve as reservoirs for the bacteria, facilitating its spread within healthcare settings [23]. When patients become infected, *Klebsiella pneumoniae* can cause a range of healthcare-associated infections, including urinary tract infections, bloodstream infections, pneumonia, and surgical site infections [21]. When *Klebsiella pneumoniae* gains access to the body it adheres and colonise the cell with the help of its Pilli [24]. The proliferation of the pathogen leads to the overriding of body immune response which subsequently results to the proliferation of the organisms and translocate to other parts of the human system most notably, the urinary tract.

Klebsiella species are almost as ubiquitous as *E. coli* in nature and are readily isolated from sewage, soil and surface water [25]. *Klebsiella* can also be found in the mucosal surfaces of mammals such as humans. Globally, over 9% of all HAI are caused by *Klebsiella pneumoniae* [26]. This ranks *Klebsiella pneumoniae* as the 8th most significant pathogen in healthcare settings [27]. Transmission of *Klebsiella pneumoniae* within hospitals can occur through various routes. Direct contact with contaminated surfaces or healthcare workers' hands is a common mode of transmission [28]. Indirect transmission via

airborne particles or aerosols generated during medical procedures can also contribute to the spread of the bacteria [29]. Additionally, contaminated medical devices or equipment, such as urinary catheters, ventilators, or endoscopes, can serve as vehicles for transmission if not adequately cleaned and disinfected [30].

Hospitalized patients, especially those in intensive care units (ICUs) or with underlying health conditions, are particularly vulnerable to nosocomial infections [31]. Factors such as compromised immune systems, invasive medical procedures, prolonged hospital stays, and the use of medical devices like urinary catheters or ventilators increase the risk of acquiring *Klebsiella pneumoniae* [32]. Neonates are most at risk of *Klebsiella pneumoniae* infection owing to their immature body physiology [33]. More than 1.5 million neonatal deaths resulting from sepsis caused by *Klebsiella pneumoniae* are recorded annually in developing countries [34]. Also, immunocompromised individuals and the elderly are also at risk due to the increased hospital admission rates [35]. The global prevalence of *Klebsiella pneumoniae* demonstrates an alarming rise, with a retrospective study spanning 2008 to 2018 revealing an escalation from 2.5% to 15.8% in Carbapenem-Resistant *Klebsiella pneumoniae* cases, notably affecting hospitalized ICU patients and individuals aged 60 years or older [36]. The prevalence of *Klebsiella pneumoniae* (K. pneumoniae) in various regions of Nigeria has been extensively studied. In South West Nigeria, Lagos State showed the highest prevalence at 45.7% (32/70), followed by Oyo State at 35.7% (25/70), with Ekiti State exhibiting the lowest at 22.9% (16/70) [37]. In a study at Barau Dikko Teaching Hospital, Kaduna, focused on women with UTIs during antenatal care, *Klebsiella pneumoniae* accounted for 7.39% (17/230) of cases in Kaduna [38]. Another investigation in Lagos across four medical centers in 2014 identified *Klebsiella pneumoniae* in 43 patients, representing 34% of the cases [39]. These findings collectively indicate varied prevalence rates of *Klebsiella pneumoniae* across different regions and medical settings within Nigeria, emphasizing its significance as a causative agent in urinary tract infections and underscoring the importance of regional epidemiological studies for a comprehensive understanding of its prevalence and distribution patterns.

The *khe* gene, found in certain strains of *Klebsiella pneumoniae*, encodes a hypervirulence factor known as the *Khe* hemolysin which enhances pathogenesis [40]. Hemolysins are proteins produced by bacteria that have the capability to lyse (rupture) red blood cells, which can play a significant role in the pathogenesis of various infections [41]. The *khe* gene belongs to a family of pore-forming toxins that can cause damage to host tissues and contribute to the severity of infections [40]. The *khe* hemolysin aids the bacteria in evading the host immune response, facilitating tissue invasion and dissemination [41]. The *khe* hemolysin, specifically, has been implicated in the virulence of *Klebsiella pneumoniae* [42]. Studies have shown that strains of *Klebsiella pneumoniae* carrying the *khe* gene exhibit enhanced hemolytic activity, which correlates with increased virulence in various infection models [43]. Furthermore, the expression of the *khe* hemolysin has been associated with the ability of *Klebsiella pneumoniae* to cause severe clinical manifestations, including urinary tract infections (UTIs), pneumonia, and bloodstream infections [44]. The hemolytic activity of *khe gene* can induce tissue damage, facilitating bacterial invasion of the urinary tract [45]. Additionally, *khe* ability to trigger an inflammatory response through cell lysis and release of inflammatory mediators contributes to UTI symptoms such as pain, inflammation, and tissue damage [44]. Furthermore, *khe* may aid in immune evasion by disrupting immune cells and functions, facilitating bacterial persistence and recurrence of UTIs [45]. Moreover, there is emerging evidence suggesting a role for *khe* in biofilm formation, a critical virulence factor in UTIs, which can protect bacteria from host defenses and antimicrobial agents, leading to persistent infections [46].

The increased frequency of *Klebsiella pneumonia* isolates from reoccurring cases of urinary tract infection (UTI) and the emergence and spread of antibiotic-resistant strains of K. pneumoniae, including those producing extended-spectrum beta-lactamases (ESBLs) has instigated the need to study its trends particularly regarding its antimicrobial resistance pattern. Also, the socioeconomic burden of nosocomial infection to public health cannot be overemphasized in terms of its pressure on lean hospital resources necessitated by prolonged stay in hospital as well as money spent in managing unforeseen extended hospital attention [9]. Hence, this research set out to investigate the epidemiological isolation of *Klebsiella pneumoniae* among hospitalized and non-hospitalized patients and establish the antibiogram associated with the isolates. Also, this essay shall employ molecular procedure using Polymerase Chain Reaction (PCR) to characterize culturally and biochemically identified isolates according to virulence.

2. Materials and Methods

2.1 Study Area

The experimental research was conducted in Wukari, Taraba State, Nigeria. Wukari is the administrative hub of Wukari Local Government Area (LGA). The predominant languages spoken in Wukari are Jukun, Hausa, and English, while common occupations include farming, trading, artisanal work, civil service, business, and fishing. Wukari shares its borders with neighboring towns, including Takum to the northeast, Bali to the northwest, Donga to the southwest, and Ibi to the southeast. Key institutions within Wukari include the Federal University Wukari, pivotal in advancing tertiary education and fostering intellectual development, the Wukari General Hospital, a cornerstone of healthcare provision in the region, and the Wukari Local Government Secretariat, the administrative nucleus overseeing governance and service delivery. Additionally, the Wukari Main Market stands as a bustling center of commerce, facilitating trade among its predominantly trader and farmer populace.

2 Methods

2.2.1 Study design

The study, conducted in Wukari, Taraba State, Nigeria, involved 40 patients (20 hospitalized, 20 non-hospitalized) aged 10-42 receiving treatment in a hospital. Informed consent and ethical approval were obtained. Urine samples were collected in sterile containers, and swabs from doorknobs; negative controls were collected. Samples were transported with boric acid for preservation. Urine samples were streaked on MacConkey agar, swabs vortexed and plated; suspected Klebsiella pneumoniae colonies sub-cultured and analyzed. Antimicrobial sensitivity was tested using the agar-disc diffusion method. PCR was employed for molecular identification of the *khe* gene from isolates, confirming sizes via gel electrophoresis.

2.2.2 Study population

The study population consisted of patients in Wukari, Taraba State, Nigeria, who were receiving treatment in a hospital. Specifically, the study included both hospitalized and non-hospitalized patients within the ages of 10 to 42. The patients were actively receiving treatment, indicating that they were likely diagnosed with some form of illness or medical condition requiring medical attention. The study aimed to investigate potential nosocomial transmission routes of *Klebsiella pneumoniae* within healthcare settings, suggesting that the focus was on patients within hospital environments. Additionally, samples were collected from doorknobs in patient wards, further indicating the hospital setting of the study population

2.2.3 Sample size

The sample size for this study comprises 40 participants, including 20 hospitalized and 20 non-hospitalized patients.

2.2.4 Ethical clearance and considerations

Approval was sought from the Research Ethics Committee of the Department of Microbiology, Federal University Wukari. The research adhered strictly to ethical guidelines and regulations governing human subject research and sample acquisition. Patient and hospital data linked to the samples were anonymized to ensure confidentiality.

2.2.5 Inclusion criteria

The inclusion criteria for the study encompassed patients aged between 10 to 42 years old who were either hospitalized or receiving treatment outside hospital settings in Wukari. Patients who were not actively undergoing treatment at the time of the study and those who did not give consent to participate in the study were excluded.

2.2.6 Samples Collection and Transportation

Sterile universal containers were used to collect early morning mid-stream urine samples from study participants. Some of the urine samples were clear while some were cloudy (not clear). Samples were labelled appropriately with patient's data. One gram of boric acid powder was added to each urine sample container and were immediately transported to the laboratory. This was done in order to preserve the microbiological standard of the specimen and prevents overgrowth during transportation to the laboratory. Furthermore, sterile swabs moistened with Amies transport media were used to collect samples from doorknobs in patient wards. Negative control samples were collected from areas such as storage rooms with no patient contact or minimal human activity to assess background contamination. This is to further validate the investigation of potential nosocomial transmission routes of *Klebsiella pneumoniae* within healthcare settings.

2.2.7 Storage and Stability

Samples and reagents were stored and handled meticulously to maintain integrity and efficacy. Urine samples were preserved with boric, while swab samples were stored in buffer solutions. All specimens were promptly transported to the laboratory under suitable conditions to prevent degradation. Reagents were stored according to manufacturer guidelines to ensure stability. Quality control measures were implemented to monitor reagent effectiveness. Overall, stringent protocols were followed to ensure accurate results.

2.3 Laboratory Analysis

2.3.1. Isolation of *Klebsiella pneumoniae*

Using streak plate technique, a sterilized wire loop was used to collect a loop-full of urine sample and was applied to a small area of already prepared plates of MacConkey agar following the standard procedure of [47] and incubated at 37°C for 24 hours. Also, swabs were placed in tubes containing appropriate buffer solutions and vortexed to ensure homogenization of collected microbial contaminants. Serial dilutions of sample suspensions were prepared, and aliquots were plated in MacConkey agar. Inoculated plates were incubated aerobically at 37°C for 24-48 hours to allow for the growth of *Klebsiella pneumoniae* colonies. All suspected growth, which appeared pinkish in colour and mucoid on the line of streaking were picked and were sub-cultured into MacConkey agar plates and incubated for 24 hours at 37°C in order to get discrete colonies of isolates which were further subjected to Gram staining, biochemical analysis, gene amplification and antimicrobial susceptibility testing. Reference strain of *Klebsiella pneumoniae* ATCC 700603, was used as quality control and validate assay performance.

2.3.2. Antibiotic sensitivity test

Antibiotics sensitivity of *Klebsiella pneumoniae* to streptomycin 30µg, Gentamycin 30µg, Ciprofloxacin 30µg, Nalidixic acid 30µg, pefloxacin 30µg, ampicillin 30µg, Augmentin 30µg, Amoxicillin 30µg, Ofloxacin 30µg and Cephalexin 30µg was carried out using agar-disc diffusion method as described by Bauer and Kirby [48] A sterile cotton wool was used to collect pure isolates of *Klebsiella pneumoniae* mixed in peptone water and spread on the surface of the nutrient agar. Using a sterile forceps, the antibiotics sensitivity discs were placed on the surfaces of the nutrient agar in such a way that it did not touch the edges of the plate. This was done close to a bursen burner flame in order to avoid contamination. The forceps were sterilized by flaming and when cool, were used to press the disc tightly down so that they would be in contact with the nutrient agar plates. The lids were used to cover the plates and were labelled. The plates were inverted and incubated overnight at 37°C. *Klebsiella pneumoniae* strains that
were sensitive to the antibiotics were inhibited at a large distance from the discs, while those strains that were resistant to the antibiotics had no zone of inhibition, they grew to the edges of the discs or they had a very small zone of inhibition. This procedure was performed multiple times on each isolate to ensure the reliability of results, assess statistical significance, identify potential sources of variability, validate findings, and address outliers.

2.3.3. Molecular identification of the *khe* gene

Polymerase Chain Reaction was used to detect the *khe* hemolysin gene. The DNA of culturally and biochemical confirmed *Klebsiella pneumoniae* isolates was extracted by boiling method. The PCR procedure was carried out with mixture of reagents consisting of sterile distilled water, GoTaq PCR reaction buffer, GoTaq DNA polymerase, 1 µl DNA template, 0.2 mM PCR nucleotide mix, and 0.5 µl each of forward and reverse DNA primers shown in Table 1, giving a final volume of 50 µl. Specific primers selected to target 428 bp segment of the *khe* invasive gene of *Klebsiella pneumoniae* are shown in Table 1. Amplicons were subjected to electrophoresis to confirm amplicon sizes using 1% agarose gel prepared with tris-borate-ethylenediaminetetraaceticacid (TBE). Size of amplicons were determined with the aid of a 100 bp marker.

2.4 Statistical Analysis

Data was analyzed using Microsoft Excel.

Table 1: Primer set for *khe* gene

Target gene	Primer sequence	Amplicon size	Reference
Khe	fw (5'-TGATTGCATTCGCCACTGG-3') rev (5'-GGTCAACCCAACGATCCTG-3')	428bp	[49]

3.0 Results and Discussion

3.1 Results

3.1.1 Prevalence of *Klebsiella pneumonia*

No isolate of *Klebsiella pneumoniae* was identified in any of the 20 non-hospitalized patients' urine samples (Table 2). However, 5 of the 20 hospitalised patent's urine sample were positive for *Klebsiella pneumoniae.* A total of 2 of the male patients' urine samples had *Klebsiella pneumoniae* while 3 of the female patients' urine sample (Table 2). Out of the 10

hospitalized male patients' urine samples examined in this current study, *Klebsiella pneumoniae* contributed a percentage of 20% and out of the 10 hospitalized female patients' urine samples examined, *Klebsiella pneumoniae* contributed 30%, which means that *Klebsiella pneumoniae* occurred more in the female urine samples. Furthermore, analysis of the various groups in the table shows that between the ages of 10-20 in the hospitalized male and female patients' urine samples, *Klebsiella pneumoniae* contributed no percentage. While between the ages of 21-31 in the hospitalized male and female patients' urine samples examined, *Klebsiella pneumoniae* contributed 17% and 20% respectively. Also, between the ages of 32-42 in the hospitalized male and female patients' urine samples examined, *Klebsiella pneumoniae* contributed 33% and 50% respectively. This indicates that *Klebsiella pneumonia* is more prevalent in older patients than the younger ones, owing to more time spent in emergency and intensive care units. Also, *Klebsiella pneumoniae* was isolated from samples obtained from doorknobs in patient wards.

Table 2: Age Group and Sex of participants Examined for the Presence of *Klebsiella pneumoniae*

	Hospitalised patients						**Non-Hospitalised patients**						**Total**
AGE GROUPS	**10 -20**		**21-31**		**32-42**		**10 -20**		**21-31**		**32-42**		
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	
Samples Examined	1	1	6	5	3	4	3	2	3	5	2	5	**40**
Positive Sample	0	0	1	1	1	2	0	0	0	0	0	0	**5**
Positive %	0	0	17	20	33	50	0	0	0	0	0	0	**12.5**

Key: M: Male, F: Female

3.1.2. Microbial features of Isolate

Klebsiella pneumoniae isolate from this study exhibited distinct morphological characteristics, appearing pinkish and mucoid. Biochemical tests indicated positive results for urease, methyl red, lactose fermentation, sucrose fermentation, and glucose fermentation, while catalase activity was absent and does not produce indole. Additionally, motility and gas production were observed. Gram staining revealed the isolate to be a negative rod. These findings collectively confirm the identity of the isolate as *Klebsiella pneumoniae* based on its morphological, biochemical, and Gram stain characteristics.

Table 3: Features of isolates of *Klebsiella pneumoniae*

Isolate	**Morphological Characteristic**	**Biochemical test**								**Gram stain**
		CAT	**URE**	**MR**	**LAC**	**SUC**	**GLU**	**IND**	**GAS**	
Klebsiella pneumoniae	Pinkish and mucoid appearance	-	+	+	+	+	+	-	+	*Negative rod*

Key: +: Positive, -: Negative, CAT: Catalase, URE: Urease, MAL: Maltose, LAC: Lactose, SUC: Sucrose, GLU: Glucose, IND: Indole, GAS: Gas production.

3.1.3 Antibiogram of *Klebsiella pneumoniae* isolates

The antibiotic sensitivity test revealed that all the presumed 5 isolates of *Klebsiella pneumoniae* were sensitive to streptomycin, gentamycin, ciprofloxacin, nalidixic acid and pefloxacin, and resistant to ampicillin, Augmentin and amoxicillin (Table 4). Two (2) isolates were sensitive to ofloxacin and cephalexin.

Table 4: Antibiogram of *Klebsiella pneumoniae* isolates

	***Klebsiella pnuemoniae* isolates**				
Antibiotics 30µg	**A**	**B**	**C**	**D**	**E**
Streptomycin	+	+	+	+	+
Gentamycin	+	+	+	+	+
Ampicillin	–	–	–	–	–
Augmentin	–	–	–	–	–
Amoxicillin	–	–	–	–	–
Ciprofloxacin	+	+	+	+	+
Nalidixic Acid	+	+	+	+	+
Pefloxacin	+	+	+	+	+
Cephalexin	–	–	+	–	+
Ofloxacin	+	–	–	+	–

Key: +: Positive, -: Negative, A-E: Isolates of *Proteus mirabilis*

3.1.4 Molecular identification of *khe* gene

Molecular analysis as shown in fig 1 indicates that all 5 isolates of *Proteus mirabilis* possess the haemolytic *khe* gene (Fig 1). t indicates that the molecular analysis demonstrates the presence of the haemolytic *khe* gene in all five isolates. This finding suggests a shared genetic characteristic among the isolates, specifically regarding the presence of the khe gene associated with hemolysis.

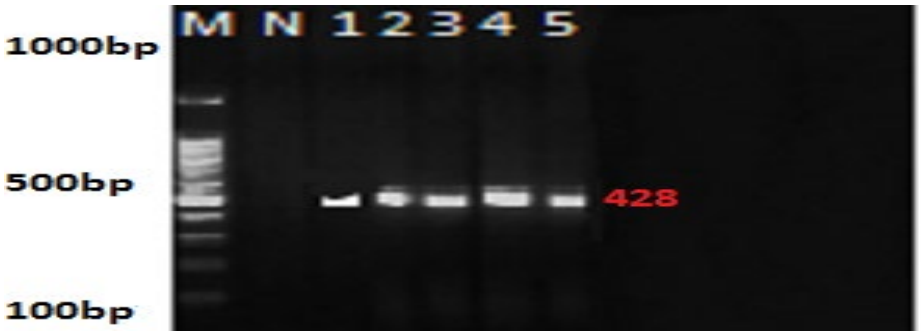

Figure 1: Agarose gel electrophoresis showing PCR amplification products of 428 bp *khe* gene in *Klebsiella pneumoniae* isolates.
Lane M: 100 bp marker, Lane N: Negative control, Lane 1-5: amplicons

3.2 Discussion of Results

The simultaneous presence of *Klebsiella pneumoniae* in hospitalised patient's samples and hospital environment samples suggests a potential link between patient colonization or infection and environmental contamination. Hospitalised patients may serve as reservoirs for the pathogen, shedding it into their surroundings and contributing to the persistence of the organism within the healthcare environment. This finding indicates that hospitalised patients stand more chance of contracting urinary tract infection caused by *Klebsiella pneumoniae* than outpatients. Hence, *Klebsiella pneumoniae* is a HAI. This agrees with [50] who discovered that following admission in the intensive care units (ICU) in hospital, there was an increased possibility of the colonization rates of resistant strains of *Klebsiella pneumoniae* among individuals especially those receiving broad-spectrum antibiotics. UTI may be persistent among out patients but *Klebsiella* is rarely isolated from UTI cases among healthy persons [51]. This explains the unique susceptibility of individuals within hospital settings to *Klebsiella pneumoniae* [52], a concerning trend that necessitates stringent infection control measures and judicious antibiotic

practices to mitigate the risk of hospital-acquired infections [53].

Findings from this study raises important implications regarding infection control and the spread of nosocomial infections within healthcare facilities. Firstly, the presence of *Klebsiella pneumoniae* in patient urine samples suggests a potential urinary tract infection (UTI). Secondly, the detection of *Klebsiella pneumoniae* on doorknobs in patient wards indicates environmental contamination within the hospital setting. Besides, Bedside furniture, bed railings, catheters, and other patient care equipment, can also harbour *Klebsiella pneumoniae* [21]. These fomites can also become contaminated with *Klebsiella pneumoniae* through direct contact with infected patients, and contaminated hands of healthcare workers [28], [29], [30]. Nonetheless, regular cleaning and disinfection of bedside furniture and bed railings are essential to minimize the risk of transmission to patients and healthcare workers [54]. Also, improper insertion, maintenance, or removal of catheters can introduce *Klebsiella pneumoniae* into the urinary tract, bloodstream, or other body sites, leading to infections [55]. If a patient is infected with *Klebsiella pneumoniae*, there is a risk of transmission to other patients or medical personnels [28]. This is possible when patients in healthcare facilities come in contact with one another through various interactions such as sharing rooms, communal areas, or during medical procedures [29]. Consequently, efforts to prevent and control nosocomial infections must capture both patient-centred interventions, such as hand hygiene, antimicrobial stewardship and infection prevention measures, as well as vigilant surveillance to prevent the escalation and spread of nosocomial pathogens [56].

The higher occurrence of *Klebsiella pneumoniae* in female urine samples compared to male urine samples in this study raises important considerations regarding gender-specific factors influencing susceptibility to urinary tract infections (UTIs) caused by this pathogen. While both male and female patients are susceptible to UTIs, there are several anatomical and physiological differences between the sexes which contributes to variations in infection rates. One key factor is the anatomical difference in the length of the urethra between males and females. Females typically have shorter urethras than males, which facilitates the ascent of bacteria into the bladder and increase the risk of urinary tract infections (UTIs) [57]. Additionally, the proximity of the female urethra to the anus increases the likelihood of fecal contamination, further predisposing women more to UTIs [57]. Furthermore, hormonal influences may also play a role in gender-based differences in infection rates. Estrogen, a hormone predominantly present in females, can influence the composition of the vaginal microbiota and the integrity of the urogenital epithelium [58]. Fluctuations in estrogen levels throughout the menstrual cycle affects the susceptibility of women to UTIs. Decreased estrogen levels during menopause can lead to changes in the vaginal environment, making women more prone to bacterial colonization and subsequent infection [59]. Furthermore, factors such as urinary catheterization can also predispose women to UTIs. About 40% of all infections that develop in hospitalized patients are in the urinary tract [60]. Hospitalized patients who are usually at higher risk of UTIs are those with indwelling urinary catheters and patients undergoing major surgical procedure [60]. The presence of urinary catheters creates a direct pathway for bacterial colonization, increasing the likelihood of infection [23]. Urinary catheterization, which is more commonly performed among female patients due to anatomical reasons, is a significant risk factor for healthcare-associated UTIs [61]. On the other hand, while male urethras are longer, they are not immune to UTIs [62]. Conditions such as benign prostatic hyperplasia (BPH) or urethral strictures can impede urinary flow and increase the risk of bacterial colonization and infection [63]. However, these conditions may not be as frequently encountered as the aforementioned risk factors in females. Importantly, an abrupt rise in the frequency of sexual activity among women increases the likelihood of UTIs, particularly in those employing a diaphragm as a contraceptive method [64]. When the diaphragm is incorrectly positioned, intensified sexual activity can cause physical irritation near the bladder, creating vulnerable sites for uropathogenic infiltration [65]. Moreover, this increased sexual activity can disrupt the natural microbial balance in the urogenital area [66]. Consistent use of spermicides contributes significantly to the prevalence of UTIs among women [67]. Spermicidal products, often contains chemicals like nonoxynol-9, which immobilize sperm, yet inadvertently disrupts the natural balance of bacteria in the vaginal and urinary tracts [68] [69]. This compromise of the body's immunological mechanisms, allowing for opportunistic infections caused by ubiquitous uropathogens such as *Klebsiella pneumoniae* and Escherichia coli [70]. Moreover, variations in healthcare-seeking behaviors between males and females significantly contributes to differences in infection rates. Women are observed to seek medical care more frequently than men, particularly for conditions such as UTIs [71]. This leads to an increased opportunities for urine sample collection, testing, and treatment. Social and cultural

factors have been argued to influence gender disparities in healthcare seeking behaviour [72].

The observed trend of higher prevalence of *Klebsiella pneumoniae* in older patients, as indicated by the increasing percentages of positive urine samples across age groups, suggests several potential explanations related to healthcare exposure and physiological factors associated with aging. Firstly, older patients have more frequent and prolonged interactions with healthcare settings, including emergency and intensive care units, due to age-related health conditions or comorbidities [73]. Prolonged hospital stays and exposure to invasive medical procedures, such as urinary catheterization or mechanical ventilation, increases the risk of acquiring nosocomial infections like UTIs [32]. Additionally, older adults may have compromised immune function or underlying medical conditions that predispose them to infections, making them more susceptible to colonization and infection by opportunistic pathogens [35]. Patients undergoing major surgeries often experience compromised immune systems and physiological stress, rendering them more vulnerable to bacterial infiltration [74]. Immunocompromised patients with other underlying conditions such as Tuberculosis, Diabetes and Acquired Immunodeficiency Syndrome (AIDS) who stay long in the hospital are usually predisposed to infections in their urinary tract [31]. Their weakened immune responses, diminishes their ability to combat bacterial infections effectively prompting longer hospital stay [75],[76]. Prolonged hospitalization exacerbates these risks, as it increases the exposure to nosocomial pathogens, necessitating tailored care and vigilant monitoring to prevent and manage infections including UTIs among these vulnerable populations [31]. The need for a policy review on hygiene and ascetic protocols in hospitals using evidenced based guidelines is crucial. Routine training for health workers is critical in the enforcement and adherence of hygienic and ascetic protocols [11]. Furthermore, physiological changes associated with aging, such as decreased bladder function, urinary retention, or alterations in the urinary microbiota, contributes to an increased susceptibility to UTIs in older patients [77]. These age-related changes disrupt the natural defence mechanisms of the urinary tract, allowing for the proliferation of uropathogenic bacteria [78]. The elderly have reduced immunological response capacity with even more challenging factor of antimicrobial resistance stemming from prolong use of antimicrobials over the years [79]. Hence, alternative treatment regimen should be prioritized for the elderly using antimicrobials from plant origin or even a combination of both antibiotics and antimicrobials of plant extracts [79].

The antimicrobial susceptibility in this study revealed important insights into the resistance profiles of the *Klebsiella pneumoniae* isolates and the effectiveness of various antibiotics tested against the pathogen. The resistance of all five isolates to ampicillin, amoxicillin, and augmentin emphasizes the prevalence of beta-lactamase-mediated resistance mechanisms among isolates, which render these antibiotics ineffective against the bacteria by hydrolysing the beta-lactam of these drugs [80]. This expresses specific challenge in treating infections caused by multidrug-resistant strains of *Klebsiella pneumoniae*, particularly in healthcare settings where antibiotic selection pressure is high. Extensive and unregulated use of antibiotics has repeatedly been blamed for this trend. Frequent antibiotic usage can disrupt the balance of bacteria in the body, eliminating both beneficial and harmful microbes [13]. This imbalance can lead to the overgrowth of pathogenic bacteria like Escherichia coli and Klebsiella pneumoniae, potentially causing infections and other health complications [80]. Consequently, the colonization of body sites, including the urinary tract, by uropathogens, poses a challenge for chemotherapy due to acquired antibiotic resistance. Therefore, judicious use of antibiotic is critical in preserving the host's defensive endogenic mechanisms preventing rapid microbial growth in the host. Conversely, the sensitivity of the isolates to streptomycin, gentamicin, ciprofloxacin, nalidixic acid, and pefloxacin indicates the potential efficacy of these antibiotics in treating *Klebsiella pneumoniae* infections. Streptomycin and gentamicin belong to the aminoglycoside class of antibiotics, which inhibit bacterial protein synthesis and are often used as second-line agents for treating Gram-negative infections. This corroborates the findings of [81], that the amino glycosides bind to the 30s ribosomal subunits of *Klebsiella pneumoniae* thereby inhibiting protein synthesis and the growth of the pathogen. The sensitivity of the isolates to ciprofloxacin, nalidixic acid, and pefloxacin, which are fluoroquinolone antibiotics, suggests the efficacy of this class of antibiotics in treating *Klebsiella pneumoniae* infections, particularly in cases of beta-lactam resistance. This finding is in accordance with earlier reports by [82, 83] that the effectiveness of fluoroquinolones against *Klebsiella pneumoniae* is due to their ability to inhibit the action of DNA gyrase enzyme, thereby inhibiting DNA replication and consequently the growth of the pathogen. The sensitivity of some isolates of *Klebsiella pneumoniae* to cephalexin and ofloxacin provides additional options for antibiotic therapy in cases where other commonly used antibiotics are ineffective due to resistance. Cephalexin is a first-generation

cephalosporin antibiotic that works by disrupting bacterial cell wall synthesis [82]. Ofloxacin, on the other hand, is a fluoroquinolone antibiotic similar to ciprofloxacin and pefloxacin, which inhibit bacterial DNA replication and transcription [82]. Some isolates' sensitivity to cephalexin suggests an absence of resistance mechanisms seen in beta-lactam antibiotics like ampicillin and amoxicillin. Thus, cephalexin could be considered for treating infections caused by *Klebsiella pneumoniae* isolates resistant to beta-lactam antibiotics. Similarly, the sensitivity of some isolates to ofloxacin provides an alternative fluoroquinolone option for treatment. Ofloxacin, like other fluoroquinolones, is effective against a broad spectrum of bacteria, including Gram-negative pathogens like *Klebsiella pneumoniae* [82]. However, as with all fluoroquinolones, the emergence of resistance is a concern, and appropriate use and monitoring are essential to prevent its development [82]. Hence, antibiotic selection should be guided by antimicrobial susceptibility testing results, patient factors, and local resistance patterns to ensure the most effective and appropriate therapy while minimizing the risk of resistance development.

As seen in this current study, the *khe* gene was isolated from HAI samples of patients who are already undergoing prolong antibiotic therapy. This finding is closely related with findings of [83] who discovered the *khe* invasive gene in more than 50% of samples analysed. The *khe* gene is considered a significant virulence component of Klebsiella pneumonia, aiding its manoeuvring of the host's immune structure [84]. It induces a major red blood cell breakdown, causing rapid infection in the human system [85]. This process defies the human immune response and may necessitate unnecessary chemotherapeutic regimens [86]. According to [84] *khe* gene is often associated with multidrug resistant *Klebsiella pneumoniae* isolates. This rapid invasion of the host immune system, by the pathogenic organisms harbouring the *khe* gene poses a serious challenge of antimicrobial resistance to conventional antibiotics, limiting the efficacy of standard treatments [85]. This outcome does not only intensify the sophistication of the pathogenetic mechanism of the pathogen and severity of clinical manifestations, but also complicates its management within healthcare environments where the pathogen dominates. This is because, rapid invasion of the immune system and subsequent reproduction of the pathogen requires prolonged antibiotic use leading to antimicrobial resistance. Another concern is the ability of *Klebsiella pneumoniae* harbouring the *khe* gene to transfer it to other strains via bacterial conjugation, a process enabling the exchange of genetic material between bacteria [86]. This facilitates the spread of virulence traits to other members of the human microbiome. This outcome necessitates the intensification of existing policies on infection control, surveillance, and the development of alternative treatment options to combat these virulent strains effectively [87].

Despite the valuable insights provided by this study, several limitations warrant consideration. These include the relatively small sample size, and the focus on a single geographic location. Future research endeavours should aim to address these limitations by conducting larger-scale epidemiological studies encompassing diverse patient populations and geographic regions. Expanding the sample size would allow for more robust statistical analysis and generalizability of findings. Moreover, broadening the geographic scope of studies can reveal regional variations in *Klebsiella pneumoniae* epidemiology and antimicrobial resistance patterns, informing tailored approaches to infection control and antimicrobial stewardship.

4. Conclusion

The study has shown that *Klebsiella pneumoniae* was isolated only from the urine samples of hospitalized patients, and on fomites in the hospital environment. This indicates that *Klebsiella pneumoniae* causes urinary tract infection in hospitalized patients. Hence, patients infected with *Klebsiella pneumoniae* should be quarantined, otherwise, they remain a transmitter of the pathogen to other patients who they share communal ward with. Apart from improved sanitary protocols, strict adherence to protocols for catheter insertion and care should be encouraged to reduce the risk of catheter-associated infections and subsequent transmission of pathogens. Also, advocating for a coordinated plans and actions aimed at judicious and ethical use of antibiotics should be explored to restrain the indiscriminate use of antibiotics. The use of plant extracts has been very effective in compacting infections caused by antimicrobial resistance stains of microorganisms. The effectiveness of research advancements in policy making decisions surrounding the management of nosocomial infections cannot be overemphasized. This is because, molecular evolution of microorganisms is rapid.

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