Available: DOI: https://doi.org/10.54117/gjpas.v1i2.11 *Research article*

Gadau Journal of Pure and Allied Sciences
Gadau J Pure Alli Sci, 1(2): 121-132 (2022)
ISSN: 2955-1722
DOI: https://doi.org/10.54117/gjpas.v1i2.11

Phenotypic characterization, detection of virulence factors, and antibacterial susceptibility profile of clinical isolates of *Klebsiella pneumoniae*

Nazeef Idris Usman

Department of Microbiology, Bauchi State University Gadau, Bauchi State, Nigeria.

**Correspondence: iunazeef@gmail.com*

Abstract

Klebsiella pneumoniae has been one of the most occurring bacteria in clinical samples causing community-acquired and nosocomial infections. It has been developing resistance to most antimicrobial agents. These have increased the pathogenicity and the chance for the evolvement of more invasive *K. pneumoniae*. The study was aimed at the identification and detection of *Klebsiella pneumoniae* from clinical samples. Some selected virulence factors were detected and the antibacterial susceptibility profile of the isolates was conducted. The incubation period, infective dose, and mortality rate of *K.pneumoniae* were measured using Baggs albino rats (BALB/c) strain. The isolation and identification of 56 *Klebsiella pneumoniae* from 223 clinical samples were made. Out of 56 *K. pneumoniae* isolates, all manifested mucoid phenotype, 44(78.6%) capsule antigen and 13(23.2%) Siderophore. The antimicrobial test conducted identified Zinacef 51(91.1%), Rocephin 50 (89.3%), and Ampiclox 50 (91.1%) as the most resistant. Ciprofloxacin 49 (87.5%), chloramphenicol 35(62.5%), Gentamycin 32(57.1%), and Amoxicillin 28 (50%) were the most susceptible to *Klebsiella pneumoniae.* The mouse lethality test shows that *K. pneumoniae* hypermucoviscous strain can cause 41.7% lethality and 66.7% mortality in Baggs Albino rats. Meanwhile an infection dose 10^5cfu/ml, 10^7cfu/ml and 10^9cfu/ml produced an incubation period of 9 days, 7days and 5days. The chi-square test shows no significant association between the isolate and the gender of the patients, but there is a significant association between the samples and isolates identified $p < 0.05$. Likewise, the association between mucoid phenotype, capsule antigen, siderophore, and the type of infection is significant ($p<0.05$). It is finally concluded that *K. pneumoniae* is the second most prevailing bacterium causing community-acquired infection the resistant pattern recorded identified the test bacteria as a multidrug-resistant strain and manifestation of the virulent factor depends on the type and site of infection caused by the *K. pneumoniae.*

Article History

Received: 02/03/2022
Accepted: 19/09/2022
Published: 02/10/2022

Keywords
Klebsiella pneumonia;
Virulence factors;
Baggs Albino rat;
Ciprofloxacin;
Siderophore;
Hypermucoviscity

How to cite this paper: Usman, N.I. (2022). Phenotypic characterization, detection of virulence factors, and antibacterial susceptibility profile of clinical isolates of *Klebsiella pneumoniae*. *Gadau J Pure Alli Sci, 1(2): 121-132*. *https://doi.org/10.54117/gjpas.v1i2.11*.

1.0 Introduction

Klebsiella pneumoniae are gram-negative, rod-shaped non-motile bacteria belonging to the *Enterobacteriaceae* family. They are usually found in the microbiota of humans, the bacteria cause different types of healthcare-associated infections, including pneumonia, septicemia, surgical site infections, urinary tract infections, and meningitis (Yu *et al*., 2007). *K. pneumoniae* is a very common pathogen that is encountered by many healthcare providers. Other than being a hospital-acquired pathogen that causes several infections, the bacteria have been identified as community-acquired infections with fluctuating prevalence (Li *et al*., 2014). Community-Acquired *K.*

pneumoniae has been responsible for an increased number of bacteremia and liver abscess cases, especially in central and West Africa. Patients with Klebsiella liver abscess had higher rates of pulmonary emboli or abscess, brain abscess, pyogenic meningitis, endophthalmitis, prostatic abscess, osteomyelitis, septic arthritis, or psoas abscess (Yu *et al.*, 2007).

Multidrug-resistant bacteria are the serious causes of nosocomial and community-acquired infections that are hard to eradicate by using available antibiotics (Hussain *et al.*, 2015). Moreover, the extensive use of broad-spectrum antibiotics in hospitalized patients has led to the development of multidrug-resistant strains. Hospitalized patients who stay long in intensive care units receiving doses of broad-spectrum antibiotics or have additional health issues are at risk of contracting a nosocomial infection. Broad-spectrum antibiotics introduced into the microbiome, in turn, create resistance strains (Paterson, 2002). Antibiotic resistance is now a serious problem in hospitals, with increased mortality rates and fewer options for treating infection. However, choosing an antibiotic treatment for *K. pneumoniae* depends on the organ system that has been targeted. Cephalosporin, carbapenems, aminoglycosides, and quinolones contain high intrinsic activity against *K. pneumoniae* (Broberg and Palacios, 2014). These treatments are initially used as monotherapy or even as a combination. For patients who are severely ill, usually between 48-72 hours of combination aminoglycoside therapy is suggested. This should then be followed by an extended-spectrum cephalosporin (Chen *et al.*, 2012).

Recently, there has been an increase in the prevalence of *K. pneumoniae* in a clinical sample (Zhu et al.,, 2021) Hospital-acquired bacterial infections caused by *K. pneumoniae* can arise in different parts of the body and different forms of illness depending on transmission. *K. pneumoniae* is responsible for 6-17% of UTIs, 7-14% of pneumonia, 4-15% of septicemia, 2-4% of wound infections, 4-17% of nosocomial infections in intensive care units, and 3-20% of all neonatal septicemia cases (Pons *et al*., 2015). In Nigeria, people who suffer from alcoholism make up 66% of the people affected by community-acquired pneumonia. *K. pneumoniae* is now among the top eight pathogens in hospitals and is a rising issue among hospitals all around the world due to antibiotic resistance. Carriers rated in hospitalized patients were 19% in the pharynx, 77% in the stool, and 42% in the hands. These findings were linked to the overuse of broad-spectrum antibiotics rather than the delivery of care (Rice *et al.*, 2000).

This growing prevalence has been due to increasing resistance to drugs. The emergence of multi-drug resistant strains particularly those involved in nosocomial diseases and community-acquired infections is hard to eradicate by using available antibiotics (Prestinaci *et al.*, 2015). Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *K. pneumoniae* infection and the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (Dangre-mudey and Fule, 2012).

The regular use of antibiotics and lack of personal hygiene brings about infection with *K. pneumoniae* in hospitalized patients. The bacteria usually spread in different parts of the hospital. According to the Center for Disease Control (Li *et al*., 2014), this bacterium is the main reason for more than 8% of endemic and 3% of epidemic infections in hospitals. The virulence factors of *K. pneumoniae* are identified as factors contributing to the pathogenesis of the bacterium. Both *in vitro* and *in vivo* models have been established to investigate the interaction between bacterial cells and the host. The manifestation of some virulence factors of *K. pneumoniae* has contributed to its pathogenesis in several infections. The study focused on the detection of some major bacterial factors: Capsule type, Mucoid phenotype, and Siderophore as a measure of Pathogenicity and drug resistance (Zhu *et al.*, 2021).

The research was aimed at the identification and characterization of *K. pneumoniae* from clinical samples and the detection of selected virulence factors. Through isolation and characterization of *K. pneumoniae* from urine, sputum, and blood samples, detection of some phenotypic virulence factors of the isolates, determination of the antibacterial susceptibility pattern of the isolates, test the lethality profile of the identified isolates and estimate incubation period and infective dose during colonization.

2.0 Materials and methods

2.1 The study area

Three main hospitals (Abubakar Tafawa Balewa University Teaching, Hospital Bauchi, Bauchi Specialist Hospital, and Reemee Medicare) were selected within the study area for sample collection. Bauchi city is located 90 2′ 0″ north of the Equator and 120 19′ 0″ east of the Greenwich Meridian and has a population of 4, 676,465 people according to the 2006 census (NPC, 2006).

2.2 Sample collection

A total of 223 samples were used for this study; the samples were collected from Abubakar Tafawa Balewa Teaching Hospital Bauchi (ATBUTH), Bauchi State Specialist Hospital (BSSH), and Reemee Medicare (RM). 123(53.4%) urine samples were collected using a sterile screw-capped container, 36(16.1%) from RM, 62(27.8%) from BSSH, and 25(11.2%) from ATBUTH. 65(30.9%) sputum samples were collected using a wide-mouth screw-

capped container, 15(6.7%) from RM, 38(17.0%) from BSSH, and 12(5.4%) from ATBUTH. While 35 (16.1 %) blood samples using 5mls sterile plastic screw-capped container, 8(3.6%) were collected from RM, 21(9.4%) from BSSH and 6(2.7%) from ATBUTH. The samples were collected randomly from all ages and sexes in the pathology departments of the selected hospitals.

2.3 Sample processing

The clinical samples were inoculated on Cystine Lactose Electrolyte Deficiency (CLED) agar, Blood agar, and MacConkey agar (Oxoid) and incubated at 37^0C for 24 hours. After incubation, the resulting colonies were examined for typical *K. pneumoniae* cultural characteristics. Gram staining and biochemical confirmatory test were conducted.

2.4 Antibiotic susceptibility testing

The *K. pneumoniae* isolates identified were subjected to the antimicrobial susceptibility test. Antibiotic sensitivity of clinical *K.pneumoniae* isolates was done by Bauer's and Kirby's disc diffusion method (Chander and Shrestha, 2013). Organisms were grown in Brain Heart Infusion broth and inoculated on Mueller-Hinton agar plates with sterile swabs, and then antibiotic discs were placed on the media and pressed gently followed by overnight incubation. Ampiclox 10g, Augmentin 30g, Amoxicillin 20g, Ciprofloxacin 10g, Chloramphenicol 30g, Erythromycin 30g, Gentamycin 10g, Ofloxacin 10g, Pefloxacin 10g, Sparfloxacin 10g, Streptomycin 30g, Septrin 30g, Rocephin 20g, and Zinacef 10g were the antibiotic discs tested. Values obtained were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2014).

2.5 Mucoid phenotype test

The method of the International *Klebsiella pneumoniae* Study Group was adopted (Yu *et al*., 2007). Colonies grown on 5% sheep blood agar were touched with a sterile wire loop and lifted vertically upward from the surface of the agar plate. Mucoid isolates adhere to the loop as it is lifted from the plate. The length of the 5mm string of colonies was considered a positive result.

2.6 Capsular production

Capsular production was determined according to the method by Balows (2003); the bacterial cultures were mixed with a drop of Indian ink, and the smear was prepared in a circle of 2cm in diameter after drying. The stains were examined under a microscope for a capsulated shallow zone.

2.7 Siderophore production

The method of (Bhagat and Kumawat, 2014) was used to determine siderophore production, *K. pneumoniae* isolates were inoculated on prepared chrome azurol sulfonate (CAS) agar medium and incubated at 28^0C for 3 days. The development of yellow to an orange hollow zone around the spot due to iron removal from the dye is considered a positive siderophore production

2.8 Mouse lethality test

The modified method of Yu *et al.* (2007) was used where a standard inoculum of 10^7CFU/ml of *K. pneumoniae* isolates with hyper-mucoviscosity, capsule antigen, and siderophore were prepared separately. Each isolate with the distinct virulent marker was injected intravenously into the tail vein of 12 Baggs Albino Rats at 8 weeks with an average weight of 11.3g. The mortality rate of the mice was observed at 24 hours post-inoculation. The infection sign was recorded during the post-injection intervals.

2.9 Estimation of incubation period and infection dose

This was determined by using eight-week-old Baggs Albino Rat strain BALB/c, which were randomly assigned to three groups (4 animals per group) and a negative control group (4 animals) (Loynichan, 2005). *K. pneumoniae* colonies were inoculated intravenously at levels of approximately 10^5cfu/ml 10^7cfu/ml, and 10^9cfu/ml. The period between inoculation and manifestation of symptoms was recorded as an estimated incubation period; 24 hours after the manifestation of symptoms, a nasal swab was collected for the isolation of *K. pneumoniae*.

2.10 Statistical analysis

Using the SPSS 16.0 version, Pearson chi-square was used to test the association between the manifested virulence factors and the types of diseases and to correlate between the isolates identified in the three hospitals. The distribution of patient characteristics, the frequency of the isolates, and charts were designed using spss descriptive statistics.

3.0 Results

3.1 The frequency of *K. pneumoniae* isolates by hospitals

From Table 1, out of 223(100%) samples collected, 56(25.1%) of *K.pneumoniae* were isolated from the three selected hospitals. A High frequency of the bacterium was found in BASSH 27(48.2%) from 121 samples processed, followed by RM 16(28.6%), and then ATBUTH 13(23.2%).

3.2 The frequency of *K. pneumoniae* isolated from samples collected

Figure 1. shows the distribution of *K. pneumoniae* isolates among the types of samples collected; 25(53.5%) were isolated from urine, 22(30.9%) from sputum, and 9(16.1%) from blood samples. This makes a total of 56(100%) *K. pneumoniae*. However, the prevalence of the test bacteria is higher in urine samples, followed by sputum and blood.

3.3 The frequency of the occurrence of isolates by age group

Table 2 shows the association between the identified isolates and the age group of patients. A higher

frequency of the isolates was recorded within the age groups of 26-35 and 16-25. *E. coli* and *K. pneumoniae* were higher, with an occurrence rate of 63(28.1%) and 56(25.1%). The chi-square test shows a significant ($p<0.05$) association between the isolates and the age group of the patients.

3.4 Distribution of isolates with samples collected

As shown in Table 3, *E. coli* 63(28.2%), *K. pneumoniae* 56(25.1%), and *Staphylococcus aureus* 45(20.2%) were the most identified isolates among the samples collected. The rate of occurrence of *E. coli* was higher in urine and blood samples with a frequency of 31(13.9%) and 16(7.2%), while *K. pneumoniae* is higher in the sputum sample with a frequency of 22(9.9%). *Pseudomonas aeraginosa2 (0.9%) and Klebsiella oxytoca 6 (2.7%) were isolated exclusively from urine samples, while Salmonella species 3 (1.3%) was isolated exclusively from blood samples*. However, a total of 123(53.4%) isolates were identified from a urine sample, 65(30.9%) from sputum, and 35(16.1) from the blood sample. Thus the rate of the occurrence of the isolates is higher in urine samples**.** The chi-square test shows a significant association between the samples and isolates identified ($p < 0.05$).

3.5 Distribution of isolates by sex of the patients

The identified isolates were equally distributed between the sexes with a difference of 1(0.45%) (Table 4). *K. pneumoniae* was the most prevalent among the male patients with 29 (13.0%) while *E. coli* 35 (15.7%) is the highest among female patients. A total of 63(28.2%) *E. coli* were isolated from the tested population, making it the most prevalent in both sexes, followed by *K. pneumoniae* 56(25.1%). T*here were few occurrences of Neisseria species 8 (3.6%), Pseudomonas aeraginosa2 (0.9%), Salmonella typhi3 (1.3%), and Klebsiellaoxytoca6 (2.7%) in both sexes*. The chi-square test shows no significant association between the isolate and the gender of the patients ($p >0.05$).

3.6 Distribution of isolates by hospitals

Table 5 shows the distribution of identified isolates by hospitals where the samples were collected. The highest frequency of the bacteria was isolated from Bauchi State Specialist Hospital (BASSH) 121(54.3%), followed by Reemee Medicare (RM) 59(26.4%), and then Abubakar Tafawa Balewa Teaching Hospital Bauchi (ATBUTH) 43(19.3%). *E.coli* 63(28.2%), *K. pneumoniae* 56(25.1%) *and Staphylococcus aureus* 45(20.2%) were higher across the three hospitals. Thus, the correlation between isolates identified in the three hospitals is not significant ($p>0.05$).

3.7 Frequency and percentage of phenotypic virulence factors detected from *K. pneumoniae* isolates

Some selected phenotypic virulence factors were detected from the 56 *K. pneumoniae* isolates as shown in Figure 2. In all the isolates, 56(100%) manifested hypermucoviscous factor, 44(78.6%) capsular antigen, and 13(23.2%) Siderophore was detected. The frequency of hypermucoviscosity is higher, followed by capsule then siderophore.

3.8 The relationship between virulence factors and lethality in mice

The lethality of *K. pneumoniae* strains isolated from urine, sputum, and blood of the patients was tested on Baggs Albino rats BALB/c based on the source of the *K. pneumoniae* isolates and the virulence factor of the bacteria as shown in Table 6. The Highest lethality was recorded in strains with hypermucoviscosity isolated from blood samples 10(83.3%), followed by isolates from urine samples 8(66.7%), and then isolates from sputum samples 6(50%). However, the total lethality by isolates from all samples was thus higher in hypermucoviscous strains 24(66.7%) followed by capsule 11(30.5%) and then siderophore 1(2.8%). Overall, the mouse mortality rate was caused by strains isolated from the sputum of respiratory tract infection patients at 41.7%, followed by isolates from urine at 27.8%, and then isolates from blood at 5.5%. However, the association between the putative virulence factors and their lethality effect on mice was significant ($p< 0.05$).

3.9 The relationship between the infective dose and the incubation period of *K. pneumoniae* estimated on BALB/cRats

The volume of viable colonies forming a unit (CFU/mL) of *K. pneumoniae* used in these estimation tests shows that the higher the infective dose, the lower the incubation period and likewise the mortality (Fig. 4). Mice inoculated with 10^5 CFU/mL of *K. pneumoniae* strain manifested symptoms after 9 days and died after 12 days. A subsequent reduction in the incubation and mortality period was recorded when the mice were intravenously inoculated with 10^7 and 10^9cfu/mL as shown in Figure 3. At an inoculation volume of 10^7 CFU/mL, the incubation period of 7 days and the mortality period of 17 days was recorded, at an inoculation volume of 10^9 CFU/mL, the incubation period of 5 days and mortality period of 9 days were recorded.

3.10 Antimicrobial susceptibility profile of *K. pneumoniae*

All the *K. pneumoniae* isolates were subjected to an antibacterial susceptibility test; out of the 14 tested antibiotics, 35.7% of the *K. pneumoniae* strains were susceptible to the tested drugs while 64.3% were resistant. Ciprofloxacin 48 (85.7%), chloramphenicol 35 (62.5%), gentamycin 32 (57.1%), and amoxicillin

28 (50%) were the most susceptible, while Zinacef 51 (91.1%), Rocephin 50 (89.3%), and amoxicillin 50 (91.1%) were the most resistant, as shown in Table 7. Some strains were found to be resistant to more than two antimicrobial agents. The drug-resistant strains' pattern has revealed multi-drug strains.

Table 1: Frequency of *K. pneumoniae* isolates by hospitals

Hospitals	Number of samples collected(%)	Frequency (%)
ATBUTH	43(19.3)	13(23.2)
BASSH	121(54.3)	27(48.2)
RM	59(26.4)	16(28.6)
n = 3	**223(100)**	**56(100)**

Key
ATBUTH = Abubakar Tafawa Balewa University Teaching Hospital
BASSH = Bauchi State Specialist Hospital
RM= Reemee Medicare

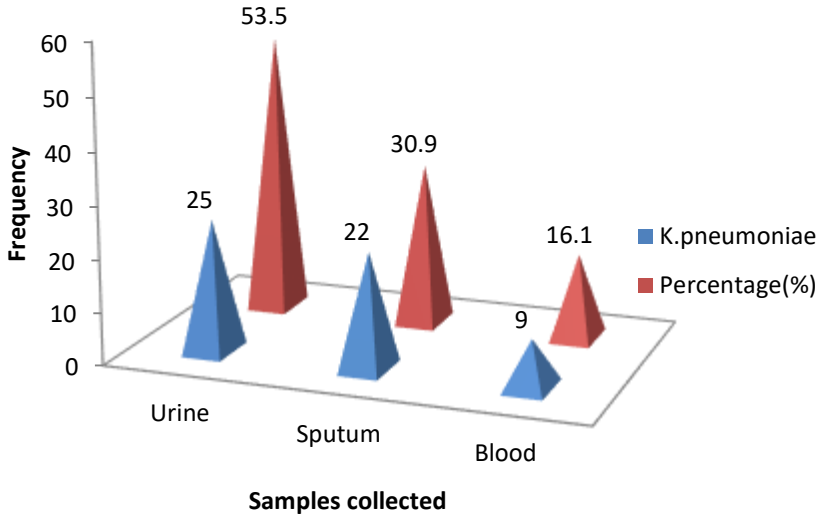

Figure 1: Frequency of *K. pneumoniae* isolated from samples collected

Table 2: Frequency of the occurrence of isolates by age group

Isolates	The age group of the patients								Total (%)
	6-15	16-25	26-35	36-45	46-55	56-65	66-75	76-85	
Klebsiella pneumoniae	8	10	13	10	10	1	1	3	56(25.1)
Escherichia coli	11	18	17	5	9	3	0	0	63(28.2)
Staphylococcus aureus	4	13	7	7	9	5	0	0	45(20.2)
Streptococcus species	3	1	6	2	1	4	0	0	17(7.6)
Enterococcus species	2	4	5	4	2	2	3	1	23(10.3)
Neisseria species	1	1	3	2	0	0	1	0	8(3.6)
P. aeraginosa	0	0	0	0	2	0	0	0	2(0.9)
Salmonella typhi	0	0	2	0	1	0	0	0	3(1.3)
Klebsiella oxytoca	1	1	1	0	0	3	0	0	6(2.7)
n = 9	30	48	54	30	34	18	5	4	223(100)

Table 3: Distribution of isolates with samples collected

Identified Isolate	Types of Samples collected			
	Urine	**Sputum**	**Blood**	Total (%)
Enterococcus species	16	6	1	23(10.3)
E. coli	31	16	16	63(28.2)
Neisseria species	7	0	1	8(3.6)
Salmonella species	0	0	3	3(1.3)
Staphylococcus aureus	28	13	4	45(20.2)
Streptococcus species	10	6	1	17(7.6)
Pseudomonas aeraginosa	2	0	0	2(0.9)
K. pneumoniae	25	22	9	56(25.1)
Klebsiella oxytoca	4	2	0	6(2.7)
n = 9	**123(53.4)**	**65(30.9)**	**35(16.1)**	**223(100)**

Table 4: Distribution of isolates by sex of the patients

Identified isolate	Male (%)	Female (%)	Total (%)
Enterococcus species	11	12	23(10.3)
E. coli	28	35	63(28.2)
Neisseria species	4	4	8(3.6)
Pseudomonas aeraginosa	2	0	2(0.9)
Salmonella typhi	0	3	3(1.3)
Staphylococcus aureus	25	20	45(20.2)
Streptococcus species	8	9	17(7.6)
K. pneumoniae	29	27	56(25.1)
Klebsiella oxytoca	4	2	6(2.7)
n = 9	**111(49.8)**	**112(50.2)**	**223(100)**

Note: Chi-square test is significant at 0.05

Table 5: Distribution of isolates by hospitals

	Hospitals			
Isolates	**ATBUTH(%)**	**BASSH(%)**	**RM(%)**	**Total(%)**
Enterococcus species	5(2.2)	12(5.4)	6(2.7)	23(10.3)
E. coli	10(4.5)	37(16.6)	16(7.2)	63(28.2)
Neisseria species	1(0.4)	5(2.2)	2(0.9)	8(3.6)
Salmonella species	0(0)	3(1.3)	0(0)	3(1.3)
Staph. Aureus	9(4.0)	26(11.6)	10(4.5)	45(20.2)
Streptococcus species	4(1.8)	7(3.1)	6(2.7)	17(7.6)
P. aeraginosa	0(0)	1(0.4)	1(0.4)	2(0.9)
K. pneumoniae	13(23.2)	27(48.2)	16(28.6)	56(25.1)
Klebsiella oxytoca	1(0.4)	3(1.3)	2(0.9)	6(2.7)
n = 9	**3(19.3)**	**121(54.3)**	**59(26.4)**	**223(100)**

Note: correlation not significant at 0.05

Key

ATBUTH = Abubakar Tafawa Balewa University Teaching Hospital

BASSH = Bauchi State Specialist Hospital

RM = Reemee Medicare

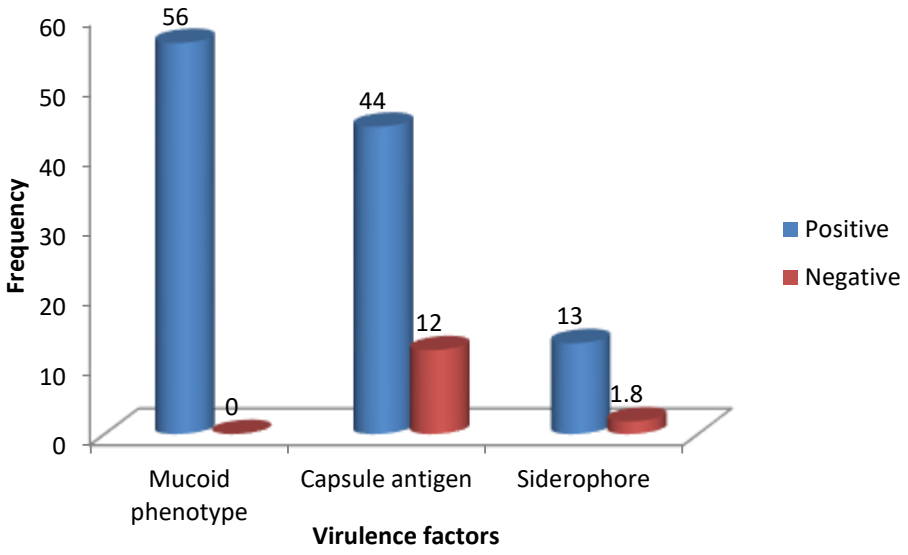

Figure 2: Frequency of virulence factors detected from *K. pneumoniae* isolates

Table 6: Relationship between virulence factors and lethality in mice

Sources of *K. pneumoniae* and Type of infection	Number of BALB/c Mice	Virulence factors			MLR %
		MP (%)	Capsule (%)	Siderophore (%)	
Urine					
UTI	36	8(66.7)	2(16.7)	0(0)	27.8
Sputum					
RTI	36	6(50)	9(75)	0(0)	41.7
Blood					
Bacteremia	36	10(83.3)	0(0)	1(8.3)	5.5
Total		**24(66.7%)**	**11(30.5%)**	**1(2.8%)**	

KEY:
MLR=Mouse Lethality Rate,
MP= Mucoid phenotype,
UTI=Urinary Tract Infection
RTI= Respiratory Tract Infection

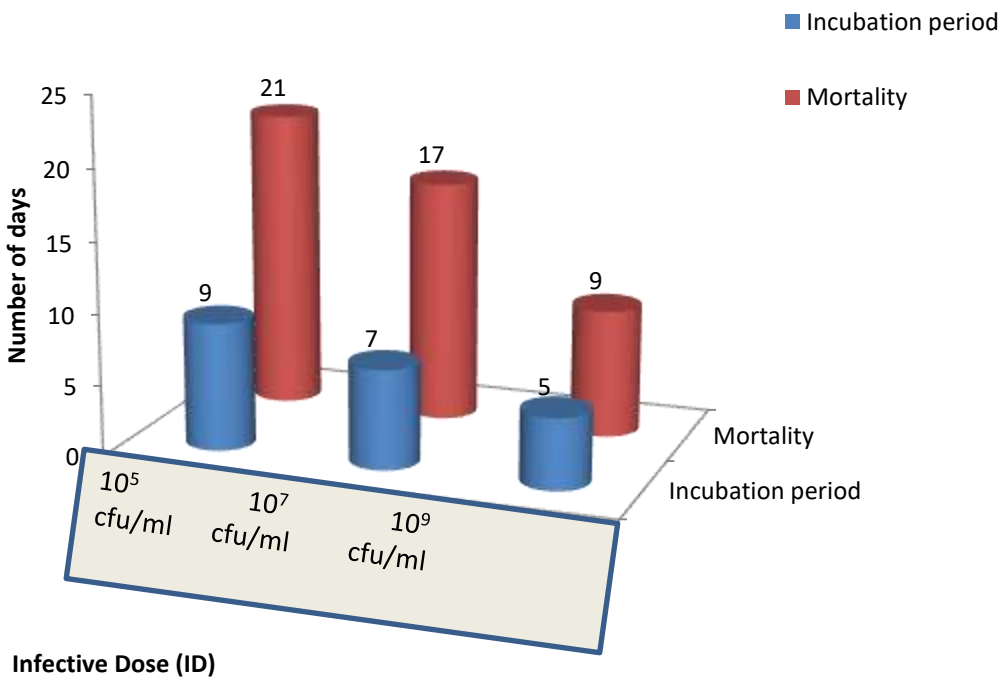

Figure 3: Relationship between the infective dose and incubation period of *K. pneumoniae* estimated on BALB/cRats

Table 7: Antibacterial susceptibility profile of *K. pneumoniae*

Antibacterial	Resistant (%)	Sensitive (%)
Gentamycin	24(42.8)	32(57.2)
Ciprofloxacin	8 (14.3)	48 (85.7)
Chloramphenicol	21(37.5)	35 (62.5)
Erythromycin	46 (82.1)	9 (16.1)
Streptomycin	35(62.5)	21(37.5)
Amoxicillin	28 (50)	28 (50)
Sparfloxacin	37 (66.1)	19 (33.9)
Pefloxacin	36 (64.3)	20 (35.7)
Septrin	35 (62.5)	21(37.5)
Rocephin	50 (89.3)	6 (10.7)
Ofloxacin	38 (67.8)	18(32.1)
Zinacef	51(91.1)	5(8.9)
Ampiclox	50(89.3)	6 (10.7)
Augmentin	33(58.9)	23 (41.1)

4.0 Discussion

4.1 Socio-demographic characteristics of the tested population

The rate of occurrence of the *Klebsiella pneumoniae* recorded in this study was close to the rate reported by Abubakar, (2009) in Yola, Nigeria. A similar result was also reported by Otajevewo, (2014), who explained that the presence of *K. pneumoniae* in those samples may be due to UTI, respiratory tract infection, and bacteremia. Considering the rate of the occurrence of bacteriuria in urine, it can be concluded that Urinary Tract Infection (UTI) is the most prevalent infection caused by *K. pneumoniae* followed by respiratory tract infection, then bacteremia (Jesmin, 2014). The prevalence of isolates recorded in this study is relatively higher than what was reported worldwide (Chander and Shrestha, 2013). These relatively high figures may be due to the reason that ATBUTH is a teaching hospital and most of the cases treated are usually referral cases from the hospitals in rural areas. From this study, it occurred that the highest frequencies of the isolates were recorded within the age groups 26-35year-48(21.5%) and 16-25 years-54(24.2%) which are mostly *E. coli and K. pneumoniae*. These age brackets consist of teenagers, adolescents, and young people. People within this age group are characteristically vulnerable to increased sexual activity, which predisposes them to UTIs, and this view is supported by reports of similar studies on *K. pneumoniae* diseases by earlier authors (Oluremi, 2011). The finding is not in agreement with reports of other workers (Shigemura, 2005).

4.2 The manifestation of virulence factors

The study has phenotypically detected the presence of some selected *K. pneumoniae* virulence factors. The bacteria possess many virulence factors of which one or more contribute to pathogenicity during infection processes. Alhasani (2016) also shows that the manifestation of these virulence factors depends on the type of infection, site of infection, and the presence of the specific genes that encode such virulence factors. This study has equally shown a variable manifestation of these virulence factors in *K. pneumoniae.* The mucoid phenotype, which is the most unique factor of *K. pneumoniae.* was detected in all the isolates across all sample types used in this study. The presence of capsule antigen was detected only on an isolate from urine and sputum while siderophore manifested in isolates identified from blood samples. Blum (2016) states that a siderophore is secreted by bacteria to acquire iron from the host, which helps the bacteria grow and spread. It is therefore apparent that the location of the bacteria is contributing to the manifestation of its virulence factors.

4.3 Antibacterial susceptibility profile of *K. pneumoniae*

The antimicrobial susceptibility test carried out in this study has identified the three most resistant and three most susceptible antimicrobial agents. Ampiclox 50 (89.2%), which is the most resistant, is a combination of cloxacillin and ampicillin, two antibiotics belonging to the beta-lactam group of antibiotics. Akova (2008) reports that K. pneumoniae producing beta-lactamase enzymes developed resistance to penicillin by hydrolyzing the beta-lactam ring. Zinacef (Cefuroxime) 51 (91.1%) and Rocephin (Ceftriaxone) 50 (89.2%), which are semi-synthetic second-generation cephalosporins, are ineffective against capsulated, mucoid, and *enterococcal* bacteria such as K. pneumoniae *and Streptococcus pneumoniae*. A similar result was explained by (NIPA, 2016). Aher *et al.*, (2012) listed erythromycin, which is resistant to 82.1% of the test organism, as one of the antibiotics that are resistant to many bacterial pathogens responsible for UTIs and respiratory tract infections. The rate at which these antimicrobial agents are resistant to bacteria has shown an increase in the emergence of multidrug-resistant strains.

Ciprofloxacin, which was found to be susceptible to 87.5% of the bacteria, has a wider spectrum of action on both gram-negative and gram-positive bacteria, This may be because of its ability to inhibit both DNA gyrase and type II, IV topoisomerase, which are necessary for cell division (Pommier, 2010). Hassan *et al*. (2014) have stated that Ciprofloxacin appears to be consistently more active than other quinolones against *K. pneumoniae.* Gentamycin was found to be efficient in the treatment of many infections (Bartlett, 2013), the composition of sisomicin, gallamine, gentamycin B1 and a 2-deoxystreptamine component of this new generation of gentamycin has made it susceptible to many bacteria (Karolina, 2010). Bacteria causing septicemia and ocular infections such as *K. pneumoniae, E. coli, Staphylococcus aureus, and Streptococcus pneumoniae* are susceptible to Chloramphenicol. The 62.5% susceptibility of *K. pneumoniae* to chloramphenicol was similarly reported (Carone *et al.,* 2014).

K. pneumoniae drug susceptibility profile has followed the same pattern as the result obtained by Hadis (2014). It is often resistant to multiple antibiotics which are usually encoded by plasmid transfer, as explained by Nathisuwan (2001). *K. pneumoniae*, with the ability to produce extended-spectrum beta-lactamase (ESBL), is resistant to many classes of antibiotics. The most frequent are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and Septrin.

4.4 *K. pneumoniae* virulence factors and their lethal effect in mice

In this study, isolates containing putative hypermucoviscous factor killed 24 albino rats (66.7%), followed by capsule 11 (30.5%) and siderophore 1 (2.8%). According to Blum (2016), mouse mortality rates revealed that mucoid was more closely associated with death than strains with capsule factor. A previous study by Osman *et al*., (2014) has shown that the mucoid phenotype may be due to a gene designated *rmpA* (regulator of mucoid phenotype). We found that the mucoid phenotype frequently coexists with siderophore production. The growth of bacteria in host tissues is limited not only by host defense mechanisms but also by the supply of available iron. The supply of free iron in the host may be extremely low; many bacteria attempt to secure their supply of iron in the host by secreting high-affinity iron chelators called siderophores.

4.5 The relationship between infection dose and the incubation period of *K. pneumoniae* estimated on BALB/cRats

In humans, the infectious dose is not known. As for the incubation period, it is also not fully understood but possibly arises from some days, as explained in the report by BMROHP (2012). Because clinically isolated K. pneumoniae are invasive and the rate at which they cause nosocomial infection is increasing, determining the incubation period, infection dose, and mortality of clinically isolated *K. pneumoniae* is critical. This research has found that a density of 10^7 colony-forming units per milliliter of *K. pneumoniae* is enough to colonize the body within 7 days and 10^9 CFU/ml within 5 days. Previous research by Janda (2006) confirmed that 10^8cfu/ml of *K. pneumoniae* can infect the host within 1–3 weeks; a similar result was reported by Medscape (2015b). Likewise, strains isolated from sputum manifest virulence markers that are lethal to 41.7% of the tested Baggs albino rats. It is also found that an infection with a strain-bearing hypermucoviscous factor causes 66.7% mortality.

From the characteristics of the patients tested, more female patients were recorded in this study with a frequency of 112 (50.2%). These may be due to UTIs, congenital abnormalities, and pregnancy, as explained in the studies of Sharma *et al.*, (2012). A similar report by Boyko *et al.*, (2005), explains that one in three women has a UTI by the age of 24, and around half of all women report at least one UTI sometime during their lifetime. The frequency of the tested patients was found to be highest in the 16-35 age bracket. As Kenneth and Ray, (2004) reported in India, this age group falls within the working population and normal distribution pattern of a patient found to be at risk of contracting *K. pneumoniae* infections.

5.0 Conclusion

The rate of occurrence of *Klebsiella pneumoniae* prevailed over most of the isolates except *E. coli*. This can be a result of the rate at which people are contracting *Klebsiella pneumoniae* and the asymptomatic nature of some of the infections, usually UTI and colitis. However, most of the isolates were isolated from female patients, thus the prevalence of the isolates is higher in female patients.

The most susceptible antibacterial agents that are effective in the treatment of *Klebsiella pneumoniae* infections were found to be semisynthetic antibacterial agents; the resistant drugs are usually synthetic first-generation antibacterial agents. It is concluded that first-generation antibacterial agents are already resistant to emerging and reemerging *Klebsiella pneumoniae* infections. Thus, there is a need to conduct relevant research that will correlate the susceptibility of enteric bacteria to synthetic and semi-synthetic antibacterial agents. Since enteric bacteria are the most isolated etiological agents, their susceptibility should be generally checked to identify the most resistant species; this will help in treating the phylogeny of their resistance to some classes of drugs. Among the virulence markers detected, the mucoid phenotype manifested in all isolates, regardless of the type of infection the isolates caused or the samples they were isolated from; this has thus qualified the

mucoid phenotype as the most virulent marker that is effective in the pathology of *Klebsiella pneumoniae*. Based on the rate at which mucoid factor manifests in all *K. pneumoniae* isolates, mostly from sputum and urine, it can be concluded that hypermucoviscosity is the most putative virulent factor of *K. pneumoniae.* Its manifestation depends on the type of infection and the site of the infection. Strains carrying iron chelating factors (Siderophore) were mostly isolated from blood samples, and their lethal effect on the mouse was limited compared to the strains bearing mucoid factor.

Declarations

Availability of data and material

Not Applicable.

Competing interests

Authors declare no competing interests.

Funding

There was no funding for the current report.

Acknowledgment

My appreciation goes to Prof. Fatima Tahir and Prof. Auwalu Uba for their guidance and advice despite their tied schedules. This work will not be successful without their effort.

References

Abubakar, E. M. (2009). Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa state, Nigeria. *Journal of Clinical Medicine and Research*, *1(1)*, 1–8.

Aher, T., Roy, A., *and* Kumar, P. (2012). Molecular detection of virulence genes associated with the pathogenicity of Klebsiella spp. isolated from the respiratory tract of apparently healthy as well as sick goats. *Israel Journal of Veterinary Medicine*, *67*(4), 249–252. https://doi.org/10.5455/vetworld.2012.676-681

Akova. (2008). Sulbactam-containing beta-lactamase inhibitor combinations. *Clinical Microbiology Infection*, *14 (1)*, 185–8.

Alhasani, A. A. (2016). Virulence factors and antibiotic susceptibility patterns of multidrug resistance *Klebsiella pneumoniae* isolated from different clinical infections. *African Journal of Microbiology Research*, *10(22)*, 829-843.

Balows A. (2003). Manual of clinical microbiology 8th edition: P. R. Murray, E. J. Baron, J. H. Jorgenson, M. A. Pfaller, and R. H. Yolken, eds., ASM Press, 2003, 2113 pages, 2 vol, 2003 + subject and author indices, ISBN: 1-555810255-4, US$ 189.95. *Diagnostic Microbiology and Infectious Disease*, *47*(4), 625–626. https://doi.org/10.1016/S0732-8893(03)00160-3.

Bartlett, J. (2013). Clinical Ocular Pharmacology (sed.). *Elsevier*, p.214. ISBN 9781483193915.

Bhagat, D., Sharma, P., Sirari, A., *and* Kumawat, K. C. (2014). Original Research Article Screening of Mesorhizobium spp . for control of Fusarium wilt in chickpea in vitro conditions Materials and Methods Procurement of cultures Isolation of Fusarium exosporium Biocontrol activities, *3*(4), 923–930.

Blum, K. (2016). *American Society of Microbiology*. Retrieved November 25, 2016, from ASM: https://www.asm.org/index.php/mbiosphere/item/308-a-role-for-siderophores-in-klebsiella-pneumoniae-pathogenesis

BMROHP. (2012). *Boston medical research occupational health program*. Retrieved November 25, 2016, from Rohm: Boston Medical Research Occupational Health Program. Available at http://www.bu.edu/rohp/files/2012/08/KPC-Klebsiella.pdf.

Boyko, E. J., Fihn, S. D., Scholes, D., Abraham, L., *and* Monsey, B. (2005). The risk of urinary tract infection and asymptomatic bacteriuria among diabetic and nondiabetic postmenopausal women. *American Journal of Epidemiology*, *161*(6), 557–564.

Broberg, C. A., Palacios, M., *and* Miller, V. L. (2014). Klebsiella: a long way to go towards understanding this enigmatic jet-setter. *F1000 Prime Reports*, *6*(August), 64.

Carone, B., Xu, T., Murphy, K., *and* Marinus, M. (2014). High incidence of multiple antibiotic resistant cells in cultures of in enterohemorrhagic Escherichia coli O157: H7. *Mutation research.* , 759: 1–8.

Chander, A., and Shrestha, C. D. (2013). Prevalence of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal. *BMC research notes*, *6*, 487. https://doi.org/10.1186/1756-0500-6-487.

Chen, L. F., Anderson, D. J., and Paterson, D. L. (2012). Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC) resistance. *Infection and drug resistance*, *5*, 133–141. https://doi.org/10.2147/IDR.S26613.

Clinical and Laboratory Standards Institute (CLSI) (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24, Wayne, 34(1).

Dangre-mudey, G., Tankhiwale, N. S., *and* Fule, R. P. (2012). Multidrug Resistance and Extended-Spectrum Beta-Lactamase Production in Klebsiella Species Isolated From Cases of Neonatal Septicaemia, Taylor and Francis *4*(1), 59–62.

Hadis Amraie, P. S. (2014). Prevalence assessment of the magA gene and antimicrobial susceptibility of Klebsiella pneumonia from clinical specimens from Shahrekord, Iran. *Iranian journal of microbiology IJM*, Volume **6** Number 5 (October 2014) 311-316.

Hassan, A. K., Shahata, M. A., Refaie, E. M., *and* Ibrahim, R. S. (2014). Pathogenicity testing and antimicrobial susceptibility of Helicobacter pullorum isolate from the chicken origin. *International Journal of Veterinary Science and Medicine*, *2(1)*, 72–77.

Hussain, M. B., Hannan, A., Akhtar, N., Fayyaz, G. Q., Imran, M., Saleem, S., and Qureshi, I. A. (2015). Evaluation of the antibacterial activity of selected Pakistani honeys against multi-drug resistant Salmonella typhi. *BMC complementary and alternative medicine*, *15*, 32. https://doi.org/10.1186/s12906-015-0549-z.

Janda, J.M. and Abbott, S.L. (2006). The Enterobacteria. ASM Press , Washington DC.

Jesmin, A. M. (2014). Study on Prevalence and Antibiotic Resistance Pattern of Klebsiella Isolated from Clinical Samples in the South East Region of Bangladesh. *American Journal of Drug Discovery and Development*, **4**:73-79.

Karolina, I. A. (2010). Determination of Gentamicin Sulphate Composition and Related Substances in Pharmaceutical Preparations by LC with Charged Aerosol Detection. *Chromatographia.*, **72**(11-12): 1225–1229.

Li, B., Zhao, Y., Liu, C., Chen, Z., *and* Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future Microbiology*, *9*(9), 1071–81.

Nathisuwan, S., Burgess, D.S., Lewis, J.S. (2001). "Extended-Spectrum β-Lactamases: Epidemiology, Detection, and Treatment". *Pharmacother.*, **21** (8): 920–928.

National Population Commission (NPC) (2006) Nigeria National Census: Population Distribution by Sex, State, LGAs and Senatorial District: 2006 Census Priority Tables (Vol. 3).

NIPA, (2016). *National information program on antibiotics*. Retrieved November 19, 2016, from http://www.antibiotics-info.org/cefuroxime.html: http://www.antibiotics-info.org/cefuroxime.html

Oluremi, I. A. (2011). Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a teaching hospital in Southwestern Nigeria. *Afr. Journal. Microbiol. Res. , 5(22)*: 3658-.

Otajevewo, F. A. (2014). asymptomatic urinary tract infection occurrence among students of western delta university. *World Journal of Medicine and Medical Science*, Vol. **2**, No, pp. 1 - 26, ISSN: 2330 - 1341.

Paterson, S. A. (2002). Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerg Infect Dis*, 8:160.

Pommier, L. E. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Chem. Biol., 17 (5)*: 421–433. doi:10.1016/.

Prestinaci, F, Pezzotti P, Pantosti A. (2015); Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109 (7):309-18. doi: 10.1179/2047773215Y.0000000030. Epub Sep 7. PMID: 26343252; PMCID: PMC4768623.

Rice, LB, C. L. (2000). High-level expressed chromosomes encoded SHV-1 beta-lactamases and an outer membrane protein change confers resistance to ceftazidime and piperacillin-tazobactam in a clinical isolate of Klebsiella pneumonia. *PUBMED*, **44**:362-367.

Sarathbabu, R. T. (2012). Antibiotic susceptibility pattern of Klebsiella pneumonia isolated from sputum, urine, and pus samples. *J. Pharm. Biol. Sci.*, **1**: 4-9.

Sharma, B. D., Bansal, R., *and* Gupta, B. (2012). Asymptomatic bacteriuria in diabetics. *Journal, Indian Academy of Clinical Medicine*, *13*(1), 55–59.

Shigemura, T. k.-F. (2005). Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during 20 years (1983-2002) at a single institution in Japan. *Japanese Journal. Infect. Dis , 58*,303-308.

Yu, V. L., Hansen, D. S., Ko, W. C., Sagnimeni, A., Klugman, K. P., von Gottberg, A., Goossens, H., Wagener, M. M., Benedi, V. J., and International Klebseilla Study Group (2007). Virulence characteristics of Klebsiella and clinical manifestations of K. pneumoniae bloodstream infections. *Emerging infectious diseases*, *13*(7), 986–993. https://doi.org/10.3201/eid1307.070187.

Zhu, J., Wang, T., Chen, L., Du, H. (2021) Virulence Factors in Hypervirulent *Klebsiella pneumoniae*. Front Microbiol. Apr 8; 12: 642484. doi: 10.3389/fmicb.2021.642484. PMID: 33897652; PMCID: PMC8060575.