Antibiotic Resistance Pattern of *Klebsiella pneumoniae* in Clinical Samples of Patients Attending Aisha Muhammadu Buhari General Hospital Jega, Kebbi State, Nigeria

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Abstract

Klebsiella pneumoniae is known as agent of nosocomial infection and its broad spectrum antibiotic resistance is of great concern to patient treatment outcome. The pathogen has showcase a public health significant as its incidence is rapidly increasing and consequently turn out to be among the major public health priority in the global perspectives. The present study was aimed to determine the antibiotic resistant pattern of *Klebsiella pneumoniae* in Clinical Sample of Patients Attending General Hospital Jega, Kebbi State, Nigeria. A clinical samples of sputum, blood and urine was aseptically collected and analyzed using standard microbiological techniques and molecular methods, phenotypic methods were also used for antibiotic sensitivity testing (AST) and Extended Spectrum Beta-Lactamase production (ESBL). Out of the total 138 clinical samples that were analyzed during the course of present study, only 13/138 (9.42%) yielded positive for *Klebsiella pneumoniae*. The AST study shows that most of the *Klebsiella pneumoniae* isolates were resistance to the tested drugs, the highest resistance was observed in Cefepime 12/13 (92.30%), and Cefoxitin 12/13 (92.30%), followed by Ceftazidine 11/13 (84.61%), and Cefpodoxime 11/13 (84.61%), then Tetracycline 10/13 (76.92%), Cefotaxime 8/13 (61.53%). While Imipenem 9/13 (69.23%), been the most sensitive drug then followed by Meropenem 8/13 (61.53%), Augumentin 7/13 (53.84%), and Ciprofloxacin 6/13 (46.15%) respectively. Our present study, reveal that ESBL phenotypes was only observed in 6/8 (75%), isolates, out of 8 (100%) suspected ESBL producers screened isolates. During the molecular analysis, among the total isolates analyzed using Polymerase Chain Reaction, only 7/8 (87.5%) isolates amplified the $\boldsymbol{Bla_{CTX\text{-}M}}$ gene, 6/8 (75%) $\boldsymbol{Bla_{SHV}}$ gene, and 4/8 (50%) $\boldsymbol{Bla_{TEM}}$ gene. The study concluded that *Klebsiella pneumoniae* harbors genes which confer antibiotic resistance on the isolates. The study exposes further the challenge of antibiotic resistance and need for concerted effort at stopping the challenge of antibiotics resistance.

Keywords: *Klebsiella pneumoniae*, Antimicrobial resistance, Blood, Urine and Sputum samples

1. Introduction

Klebsiella pneumoniae, was initially discovered in 1875 by Theodor Albrech Edwin Klebs, a German physician and bacteriologist, from the respiratory tract of a patient diagnosed with pneumonia. Later in 1882, Carl Friedländer provided a comprehensive description of the microorganism, resulting in its temporary designation as Friedlander's bacillus (Chang *et al*., 2021). Furthermore, in 1885, Trevisan V. paid tribute to Theodor Albrech Edwin Klebs by naming the genus as *Klebsiella* (Ning *et al*., 2022). The genus *Klebsiella* encompasses a group of immotile members of the enterobacteriaceae family, traditionally classified into *Klebsiella pneumoniae, K. ozaenae,* and *K. rhinoscleromatis* (Saif *et al*., 2020).

The bacterial species known as *Klebsiella pneumoniae*, which is characterized by its encapsulated Gram-negative nature, has a tendency to colonize various regions of the human body such as the gastrointestinal tract, respiratory tract, oral cavities, and skin (Badger-Emeka *et al*., 2021). In terms of its dimensions, this species typically measures between 1-2 µm x 0.5-0.8 µm, and it thrives under normal conditions at a temperature of 37ºC for a duration of 18-24 hours. When cultivated on MacConkey Agar, the colonies of *Klebsiella pneumoniae* display a distinctive appearance, being both large and mucoid, with a color ranging from pink to red (Grover *et al*., 2022). Known for its opportunistic nature, *K. pneumoniae* is a significant causative agent of hospital-acquired infections, which encompass bloodstream infections, urinary tract infections, and pneumonia (Medrzycka-Dabrowska *et al*., 2021).

Klebsiella pneumoniae exerts a significant impact on the healthcare sector, because it is one of the species recognized as part of the ESKAPE group, associated by their characteristic potential to escape or evade the action of antimicrobial agents (WHO, 2017). Additionally, the World Health Organization lists *K. pneumoniae* as one of the species of high priority and promotes the research and development of new antibiotics due to the growing global problem of antimicrobial resistance (WHO, 2017), and also According to a global antimicrobial resistance surveillance report conducted by the World Health Organization, *K. pneumoniae* is one of the nine bacteria implicated in antibiotic resistance (WHO, 2014). *K. pneumoniae* has demonstrated resistance against several third-generation cephalosporin antibiotics, notably cefotaxime, ceftazidime, and ceftriaxone (Effendia *et al*., 2018).

To withstand the lethal effects of antibiotics, *K. pneumoniae* has developed various resistance mechanisms, such as target site modification, drug inactivation, reduced cell permeability, and activation of efflux pumps (Ferreira *et a*l., 2019). However, certain strains of *K. pneumoniae* possess the ability to survive and overcome the impact of β-lactam antibiotics by producing extended-spectrum beta-lactamase (ESBL) enzymes. These ESBLs are capable of hydrolyzing and deactivating β-lactam antibiotics, including cephamycins and carbapenems (Jalal *et al*., 2023). And thus, the impact of ESBL enzymes can be surmounted by β-lactam inhibitors, such as clavulanic acid (Ferreira *et al*., 2019). ESBLs are coded by transferable plasmid-mediated genes, including TEM, SHV, and CTX-M (Jalal *et al*., 2023). The burden of infection caused by *K. pneumoniae*, as a consequence of its ability to withstand the impact of antimicrobial medications, is highly correlated with elevated morbidity and mortality, a correlation that may be attributable to the large number of resistance genes harbored by the bacteria (Orole *et al*., 2020). The bacterium

adheres to host cells using fimbriae and adhesins, thereby facilitating tissue infection. Prolonged hospital stays, prior antibiotic usage, and the type of ventilation are risk factors associated with colonization and infection by K. pneumoniae (Orole *et al*., 2020). In Kebbi State, however, there is paucity of data on the prevalence and antibiotic resistant pattern of *K. pneumoniae* (Danlami *et al*., 2019), and most of the few data available are established base on phenotypic methods. In view of that, this study aimed to use molecular method to determine the prevalence, and antibiotic resistance pattern of *K. pneumoniae* as well as its genetic diversity and spread of extended-spectrum-β-lactamases from clinical source of patients attending Aisha Muhammadu Buhari general hospital Jega (AMBGHJ) of Kebbi State, Northwestern, Nigeria.

2. Materials and Methods

2.1 Study area

This study was carried out at Jega Local Government Area of Kebbi State, which was situated at latitude of 12.3667° N and a longitude of 4.6333° E, encompassing a land area of 891km2. The population of this area is roughly 200,000 (NPC, 2006).

Figure 1, Map of Kebbi State showing the research study area; Jega Local Government, Last date visited Friday, 15/December/2023 - 4:57:55 PM (https://www.kebbistate.gov.ng).

2.2 Study Design

This is a descriptive hospital-based study were clinical samples was gathered from Aisha Muhammadu Buhari general hospital Jega. The samples then were handled at Department Microbiology of the Postgraduate Laboratory at Kebbi State University of Science and Technology (KSUSTA), Aliero. And Subsequent molecular analysis were also carry at Molecular Biology Laboratory, Faculty of Agriculture, KSUSTA.

2.3 Sampling and Collection of Samples

A total of 138 clinical specimens comprising sputum, blood, and urine were procured from the Aisha Muhammadu Buhari general hospital Jega, located in Kebbi State. The prevalence rate was assessed to be 10% according to Danlami *et al*. (2019). Prior to data collection, ethical approval was sought from the Kebbi State Health Research Ethics committee (KSHREC). The corresponding reference number for ethical approval was MOH/KSREC/VOL I/56, and the KSHREC registration number was 107:017/2023.

2.4 Inclusion and Exclusion Criteria

Samples were obtained from consented inpatients and out patients of both sexes and those of age that ranges between ≥ 10 and ≤ 90 were include. Those who patients didn't give they consent and who took antibiotics about two weeks prior to sample collection, were excluded from the study participate.

2.5 Isolation and Identification

The clinical samples were all subjected to inoculation on MacConkey agar plates using the streaked plate method. Subsequently, they were incubated at 37°C under aerobic conditions for a duration of 24 hours, facilitating the growth of distinct colonies. Then the colonies were subjected to phenotypic identification, whereby their characteristics such as colony morphology, staining behavior, and biochemical properties (including Oxidase, Urease, MR-VP, Simon citrate, and Indole) were carefully observed and recorded (Cheesbrough, 2010).

2.6 Determination of Antibiotic Susceptibility Profile of Isolated Klebsiella pneumoniae

The antibiotic susceptibility pattern of *Klebsiella pneumonia* isolates was determined through the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar. This determination was carried out according (CLSI, 2020).The antibiotics employed in this study included Tetracycline (TTR 30 µg), Augmentin (AMC 30 µg), Ciprofloxacin (CIP 5 µg), Cefepime (FEP, 30 µg), Cefotaxime (CTX 30 µg), Ceftazidime (CAZ 10 µg), Cefpodoxime (CPD 10 µg), Cefoxitin (CFT 30 µg), Imipenem (IMP, 10 µg), and Meropenem (MEM 10 µg). The test isolates were prepared as a suspension and adjusted to 0.5 McFarland turbidity standards. These suspensions were then aseptically inoculated onto Muller-Hinton agar plates using sterile swab sticks, and the antibiotic discs were subsequently applied. Within 15 minutes of inoculation of plates, then the Plates was incubated at 37ºC for 18 to 24 hrs. After incubation the diameter of the clear zone around the disc were measured under transmitted light and the results was interpreted according to (CLSI, 2020).

2.7 Screening Tests for extended spectrum β-lactamases (ESBL) Production

An isolate that showed resistance to any two or more of third generation cephalosporins antibiotics with zone size Cefpodoxime <17mm, Ceftazidime <22mm and Cefotaxime <27mm were identified as potential ESBL producer and further confirmed by the confirmatory test procedure according to CLSI guidelines (CLSI, 2020).

2.7.1 Confirmatory Tests for (ESBL) Production

The isolates resistant to two or more beta lactams antibiotics were assumed to be potential ESBL producers, and were subjected to phenotypic confirmation by Double Disk Synergy Test (DDST), briefly; a suspension of the test isolates were adjusted to 0.5 McFarland turbidity standards, and were aseptically inoculated on Muller-Hinton agar plate using sterile swab sticks. Augumentin

(AMC 30 µg), disk was placed at the center of the plate and Cefpodoxime (10µg), Cefotaxime (30µg) and Ceftazidime (30µg), were placed each on either sides of the central disk Augumentin (AMC 30 µg), at a distance of 15 mm apart and the plates were incubated for 18 to 24 h at 37°C. After 18 to 24 hours of incubation, an isolates that produce zone of inhibition ≥ 5 mm of any of the cephalosporins tested toward the central disk Augumentin (AMC 30 µg), was considered ESBL producer and positive for the test (Ahmed, *et al*., 2016)

2.8 Molecular Identification of *K. pneumoniae* Isolates by PCR

2.8.1 DNA Extraction

DNA was extracted by boiling method, briefly; three to five (3-5) pure and fresh colonies was introduce into a sterile micro centrifuge tube containing 1ml of distilled water, then the cells were lysed by heating in water bath at 100°C for 20 minute, immediately the cells was placed into ice for 30 min and the other cellular components were removed by centrifugation at 8500 rpm for 10 min. Finally the supernatant was used as the DNA template for PCR or stored at −20°C for further analysis (Ahmad *et al*., 2016).

2.8.2 Determination of Extracted DNA Concentration

The DNA concentration was determined by measuring the amount of light absorbed by the DNA at ~260 nm (A260) with a spectrophotometer, DNA was considered pure at the ratio of (A260/A280) which lies between ~1.8 - 2.0 for dsDNA while a ration of less than 1.7 will indicates protein or RNA contamination (Gupta, 2019).

2.8.3 Amplification of *bla*$_{TEM}$ Gene: Polymerase Chain Reaction

The amplification was performed from protocol adapted by Effendia *et al*. with little modifications, using Master Mix ready to load (Solis Biodyne, Estonia) with pure genomic DNA of *K. pneumoniae* as template, and primers, **Table 1**. PCR reactions were performed in a total volume of 20µl, containing 4µl of Solis Biodyne Master Mix ready to load (Solis Biodyne, Estonia), 0.6µl of forward and reverse primer, 1µl of DNA template, and 13.8µl of molecular grade water making 20µl of PCR solution. The temperature and time conditions of the amplification steps involved initial denaturation process at 95°C for 15 min, 30 cycles denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec, extension at 72°C for 4 min followed by the final extension on temperature of 72°C for 10 min. (Moenstein *et al*., 2007; Effendia *et al*., 2018). After the last cycle, the PCR products were stored at -20°C for further analysis (Dalia, *et al*., 2020).

Table 1: Primers used for amplification of extended spectrum beta-lactamase genes (ESBL)

Gene	sequence	Amplicons bp	References
BlaTEM	Forward; (5'-TCGCCGCATACACTATTCTCAGAATGA-3) Reverse; (5'-ACGCTCACCGGCTCCAGATTTAT-3')	445	Effendia *et al*., 2018
BlaSHV	Forward; (5'-TTAGCGTTGCCAGTGCTC-3') Reverse; (5'-GGTTATGCGTTATATTCGCC-3')	842	Mahrouki *et al*., 2014
BlaCTX-M	Forward; (5'-CGCTTTGCGATGTGCAG-3') Reverse; (5'-ACCGCGATATCGTTGGT-3')	550	Feizabadi *et al*., 2010

2.8.4 Electrophoresis of PCR Products

The amplication product was separated base on the protocol adapted by Effendia *et al*. (2018) with little modifications briefly as follows; ten microliter 10µl of the PCR products and 10µl DNA ladder ready to load (Solis Biodyne, Estonia) was used, then were analyzed by electrophoresis on 1.5% agarose gel containing 10µl of SYBR dye, pipetted into well created with comb. Electrophoresis was run at 90 volts for 30 minutes, after which DNA amplicon were then viewed on a UV trans-illuminator (Effendia *et al*., 2018).

2.9 Statistical Analysis

The data obtained during the course of the study were analyzed using Microsoft Office Excel (2013), then the data was presented by frequency tables, and charts

3. Results and Discussion

3.1 Results

3.1.1 Patient and Samples Demographics

Out of the total 138 clinical samples collected from patients attending (AMBGHJ), upon obtaining informed consent and have met the selection criteria. The age of the patients was from 10 to 90 years, and the study participants were majorly Males 78/138 (56.52%). The highest samples is urine with 48 (34.78%), then followed by sputum 46 (33.33%), and blood samples 44 (31.88%) respectively.

Table 2. Prevalence of *Klebsiella pneumoniae* infection in relation to sex and age of patients attending General Hospital Aliero and Jega

Variables	Negative samples (%)	Positive Samples (%)	Total samples (%)
Sex			
Male	70 (56)	8 (61.54)	78 (56.52)
Female	55 (44)	5 (38.46)	60 (43.47)
Age			
10-25	38 (30.4)	2 (15.38)	40 (33.58)
26-41	32 (25.6)	7 (53.85)	39 (29.43)
42-57	31 (24.8)	3 (23.08)	34 (22.64)
58-73	20 (24.8)	1 (7.69)	21 (11.32)
74-90	4 (3.2)	0 (0)	4 (3.01)
Total	**125(90.58)**	**13 (9.42)**	**138 (100)**

3.1.2 Prevalence of Klebsiella pneumoniae infection among patients attending General Hospital Jega

Out of one hundred and thirty eight (138) clinical samples that were collected from patients attending (AMBGHJ). Significant number of *Klebsiella pneumoniae* was observed in 13/138 (9.42%). However prevalence of *Klebsiella pneumoniae* was highest in the adolescent age group ranges from 26–41 with 7/13 (53.85%) as compared to the lowest value of 1/13 (7.69%) in the age group of 58-73 **Table 2**. *Klebsiella pneumoniae* infections was highest in males with 8/13 (61.54%) as compared to females with 5/13 (38.46%) **Table 3**. Sputum sample had the highest

number of yield with 8/13 (61.54%), then followed by Urine samples with 3/13 (23.08%) while the lowest value was observed in Blood with 2/13 (15.38%).

Table 3. Prevalence of *Klebsiella pneumoniae* infection in relation to Sample and gender of patients attending General Hospital Jega

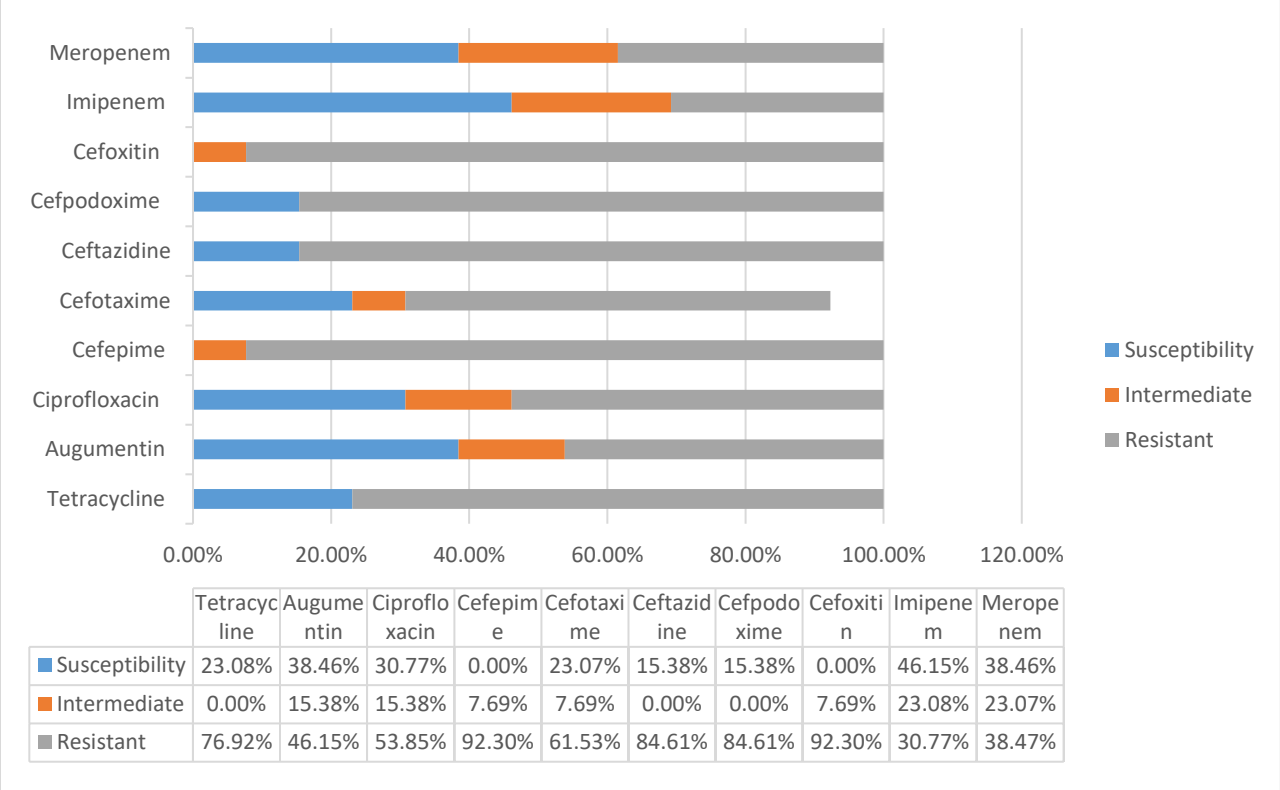
Sample	Male, n (%)	Female, n (%)	Total, n (%)
Sputum	5 (38.46)	3 (23.08)	8 (61.54%)
Blood	2 (15.38)	0 (0)	2 (15.38)
Urine	1 (7.70)	2 (15.38)	3 (23.08%)
Total	**8 (61.54%)**	**5 (38.46%)**	**13 (100%)**

3.2 Antibiotic Resistance Profile of Klebsiella pneumoniae Isolates

The antibiotic resistance profile of *Klebsiella pneumoniae* isolates was determined using nine (10) different antibiotics as depicted in **Table 4.** Cefepime 12/13 (92.30%), and Cefoxitin 12/13 (92.30%), followed by Ceftazidine 11 (84.61%), and Cefpodoxime 11/13 (84.61%), then Tetracycline 10/13 (76.92%), Cefotaxime 8/13 (61.53%). While Imipenem 9/13 (69.23%), been the most sensitive drug then followed by Meropenem 8/13 (61.53%), Augumentin 7/13 (53.84%), and Ciprofloxacin 6/13 (46.15%)

Table 4. Antibiotic Resistance pattern of *K. pneumoniae* isolated three clinical samples of patient in Aisha Muhammadu Buhari General Hospital and Jega.

Antibiotics	Disc potency (µg)	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
	Number of isolates (n=13)			
Tetracycline	30	2 (15.38)	1 (7.69)	10 (76.92)
Augumentin	30	4 (30.77)	2 (15.38)	7 (53.85)
Ciprofloxacin	5	3 (23.08)	4 (30.77)	6 (46.15)
Cefepime	30	1 (7.69)	0 (0)	12 (92.30)
Cefotaxime	30	3 (23.08)	2 (15.38)	8 (61.53)
Ceftazidine	10	1 (7.69)	1 (7.69)	11 (84.61)
Cefpodoxime	10	2 (15.38)	0 (0)	11 (84.61)
Cefoxitin	30	1 (7.69)	0 (0)	12 (92.30)
Imipenem	10	6 (46.15)	3 (23.08)	4 (69.23)
Meropenem	10	7 (53.85)	1 (7.69)	5 (38.46)



	Tetracycline	Augumentin	Ciprofloxacin	Cefepime	Cefotaxime	Ceftazidine	Cefpodoxime	Cefoxitin	Imipenem	Meropenem
Susceptibility	23.08%	38.46%	30.77%	0.00%	23.07%	15.38%	15.38%	0.00%	46.15%	38.46%
Intermediate	0.00%	15.38%	15.38%	7.69%	7.69%	0.00%	0.00%	7.69%	23.08%	23.07%
Resistant	76.92%	46.15%	53.85%	92.30%	61.53%	84.61%	84.61%	92.30%	30.77%	38.47%

Figure 2. Resistant pattern of *K. pneumoniae* isolated (n=13)

3.3 Phenotypic Screening and Confirmation of Extended Spectrum Beta-Lactamase production (ESBLs) among K. pneumoniae isolates n=13, based on Double Disk Synergy Test (DST)

Out of eight (8) *Klebsiella pneumoniae* isolates that are resistant to more than two drugs in third-generation cephalosporin antibiotics upon routing antibiotic susceptibility testing out of the total 13 *Klebsiella pneumoniae* isolates, that were isolated during the present study in (AMBGJ), and then 8 suspected isolated were further subjected to phenotypic screening and confirmation test, were reveal that only 6 (75%) out of the total 8 isolates was phenotypically confirm to be ESBLs enzymes producing *Klebsiella pneumoniae* isolates, while 2 (20%), were ESBLs enzymes producing negative as shows in **Table 5**.

Table 5. Screening and Confirmation of ESBLs production among *K. pneumonia*e isolates n=13, based on Double Disk Synergy Test (DDST)

Number of suspected ESBLsp (%)	Number of confirmed ESBLsp negative (%)	Number of confirmed ESBLsp positive (%)
8	2 (20)	6 (75%)

3.4 Detection of Extended Spectrum Beta-lactamases ESBL Resistance Genes

Out of 8 phenotypically confirm positive ESBL producing isolates tested strains, 7/8 (87.5%) isolates amplified the $\boldsymbol{Bla_{CTX\text{-}M}}$ gene, $\boldsymbol{Bla_{SHV}}$ gene 6/8 (75%) and 4/8 (50%) amplified $\boldsymbol{Bla_{TEM}}$ gene among the 8 *K. pneumoniae* isolates respectively.

3.5 Discussion

A total of 13 *Klebsiella pneumoniae* was isolate during the course of the study, out of total one hundred and eighty and eight (138), clinical samples that were culture, which comprises of Sputum, Urine, and Blood samples that were aseptically collected from Aisha Muhammadu Buhari General Hospital Jega (AMBGHJ) respectively. The prevalence of *Klebsiella* in this study was **13** 9.42% **Table 2**. A comparatively similar report was previously conducted at Enugu State, Nigeria on the prevalence *Klebsiella pneumoniae* isolated from Urine samples which is in consistence with our present study were report 77/735 (10.47%) (Aneke *et al*., 2022), and another report conducted at multicenter from Kebbi State were reported prevalence of 24/350 (6.85%) which isolated from Sputum samples (Zaharaddin *et al*., 2023) and also with a previous study reported from Kaduna with frequency of occurrence of 16/380 (4.21%) which was isolated from clinical samples (Iliya *et al*., 2021), from Niger State isolated from various clinical samples with prevalence 15 /390 (13.07%) (Oyedum *et al*., 2022), and similar to a report conducted among Students Residence in Nicon Hostel, Federal Polytechnic Bida in Niger State with 11/190 (5.7%) prevalence which were isolated from Urine source (Alfa *et al*., 2022), and also in consistent to a study reported from Osun State Southwestern Nigeria, isolated from clinical samples with the prevalence of 62/1056 (5.87%) (Akingbade *et al*., 2019). And consequently this finding is also in line with study done at Tertiary Care Hospital in Bangladesh were the pathogenic bacteria are yielded from various clinical specimens of Urine, Wound swab, Sputum, Endotracheal aspirates and Blood, 75/500 (15%) (Sonia *et al*., 2020). And another report which is in contrary to our study, conducted in Dalhatu Araf Specialist Hospital (DASH), Lafia, Nasarawa State, *Klebsiella pneumoniae* with 66/194 (34%) (Orole *et al*., 2020), and also study conducted at Tertiary Care Hospital, Jaipur, Rajasthan, India recorded a prevalence of *Klebsiella pneumoniae* (30.15%) (Ashina *et al*., 2021), and another report were *K. pneumoniae* accounted for 65 (14.5%) (Asati *et al*., 2013). The isolates of *Klebsiella pneumoniae* identified in clinical cultures of patient attending General Hospital Aliero and in (AMBGHJ) also exhibited varying degrees of resistance to the antibiotics tested as in **Table 4** and **Figure 2.** The highest resistance of isolates of *Klebsiella pneumoniae* was observed in Cefepime 12/13 (92.30%), and Cefoxitin 12/13 (92.30%), followed by Ceftazidine 11/13 (84.61%), and Cefpodoxime 11/13 (84.61%), then Tetracycline 10/13 (76.92%), Cefotaxime 8/13 (61.53%). On the other hand, some isolates exhibited susceptibility pattern to few drugs, Imipenem been the most sensitive with Imipenem 9/13 (69.23%), then followed by Meropenem 8/13 (61.53%), Augumentin 7/13 (53.84%), and Ciprofloxacin 6/13 (46.15%) as indicated in **Table 4** respectively, this study is comparatively similar with a findings in Kebbi State, with regard to Cefotaxime 18 (74%) and Ceftazidine 16 (66%) (Kalgo *et al*., 2022), and consequently its differs from a report conducted at kano Metropolis, Nigeria were they recorded (100%) resistant isolates in Cefotaxime, Ceftazidine and Augumentin toward 10 clinical isolated of *K. pneumoniae* from patient suspected of urinary tract infections by (Muhammad *et al*., 2019). A comparatively different results was

reported by a percentage of susceptibility of Meropenem (25%) and Imipenem with (18.75%) by (Aljanaby & Alhasani, 2016).

Prevalence of phenotypic ESBLs-Producing *K. pneumoniae* was asses, however the occurrence of ESBLs among clinical isolates greatly varies worldwide and geographically, and is rapidly changing over time (Turugurwa *et al*., 2019). The percentage of ESBL production in different *K. pneumoniae* isolate was cleared in **Table 5,** in our present study, ESBL phenotypes were found to be positive in 6/8 (75%), *K. pneumoniae* isolates demonstrating a high prevalence of ESBL production, our study is partially similar with a study reported by Zaghloul *et al.* (2021) with (56.3%) and another reported from Latin America were ESBL producing *K. pneumoniae* was recorded as (54.4%) (Aminazadeh *et al*., 2008), and with study conducted in Zaria which reported (40%) of ESBL producing *K. pneumoniae* (Giwa *et al*., 2018) and (40.7%) similarly also with a reported conducted at Port Harcourt Southern, Nigeria (Onanuga *et al*., 2019) respectively. However also about (26.5%) ESBLs producing *K. pneumoniae* has been reported in Ilorin, which quite differ with our report (Fadeyi *et al*., 2016). These observed differences could be due to regional and attitudinal behavior towards prescription and consumption of antibiotics especially the cephalosporins in both hospital and community settings.

Molecular analysis using polymerase chain reaction showed that most of the isolates contained ESBL genes, $\boldsymbol{Bla_{CTX\text{-}M}}$ 7/8 (87.5%) $\boldsymbol{Bla_{SHV}}$ 6/8 (75%) and $\boldsymbol{Bla_{TEM}}$ 4/8 (50%), after subjecting all ESBL positive upon screening confirmatory test by DDST respectively. Our report is comparatively similar to a research conducted by (Aljanaby & Alhasani, 2016) and with report by (Orole *et al*., 2020).

3.5Conclution

The prevalence of *Klebsiella* spp in our institute was 9.42% and was found to be resistant to many antibiotics and also possessed multiple resistance genes. Hence formulation of a good antibiotic policy and detection of drug resistance mechanisms should be done by all laboratories. A proper antibiotic stewardship program should be incorporated after consulting medical and surgical departments. This helps us to identify and

Combat emerging multi drug resistance.

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