JMSCR Vol||13||Issue||03||Page 01-12||March 2025

http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v13i03.01

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Antibacterial Susceptibility of Multidrug Resistant *Klebsiella Pneumoniae* from in-Patients in Jos University Teaching Hospital, Plateau State, Nigeria

Authors
Ishaya Jesse[1*], Lar, P.M[2], Alobu Walter Emeka[3], Aliyu Aishatu Mohammed[4], Lawrence Amuta[2], Dangana Martin[1] and Fwangmun Alhassan Damter[5]
[1]Department of Applied Biology, School of Applied Sciences, College of Science Technology, Kaduna Polytechnic, Tudun Wada, P.M.B. 2021, Kaduna, Nigeria
[2]Department of Microbiology, University of Jos, P.M.B. 2084, Jos, Nigeria
[3]Microbiology Department, Stepping Hill Hospital, Poplar Grove, Stockport, Cheshire, Manchester
[4]Department of Microbiology, Kaduna State University, P.M.B. 2339, Kaduna State, Nigeria
[5]Dermatophylosis Research Division, NVRI Vom, PMB 01, Vom.
*Corresponding Author
Ishaya Jesse

Abstract
Background: *Klebsiella pneumoniae is globally responsible for hospital- and community-acquired infections, but the problem of antibiotic resistance is increasing making treatment difficult. This study aimed to determine the prevalence of K. pneumoniae and to establish the antibiotic resistance profile among clinical specimens at Jos University Teaching Hospital (JUTH) in Plateau State, Nigeria.*
Methods: *A total of 344 clinical samples were collected aseptically from in-patients with bacteremia (122 blood samples), soft tissue abscess (50 superficial wound swab) pneumonia (50 sputum samples) and UTI's (122 urine samples). The samples were processed using standard microbiological methods for identification of K. pneumoniae. Samples were cultured on MacConkey agar and Cystine lactose electrolyte deficient agar. The resulting colonies of isolates were further sub cultured and Gram stained followed by biochemical test at Microbiology laboratory unit of Jos University Teaching Hospital. The antimicrobial susceptibility patterns were determined using Kirby-Bauer disc diffusion techniques.*
Results: *Following that, 39 (11.3%) K. pneumoniae isolates were obtained, where 23 (12.5%) and 16 (10.0%) were from males and females, respectively. The K. pneumoniae isolates were highly resistant to cefotaxime (94.9%), ceftazidime and cefepime (84.6%) but had low resistance for imipenem and meropenem (12.8% and 5.1%, respectively). 38 (97.4%) of the K. pneumoniae isolates were multidrug-resistant. PCR analysis confirmed 28 (93.3%) isolates to be Klebsiella using 16s rDNA having a product size of 1069bp.*
Conclusion: *The findings of this study show the prevalence of K. pneumoniae to be 11.3% and augmentin, imipenem, meropenem and ertapenem as the most effective antibiotics against the K. pneumoniae isolates. Antibiotic policies and regular surveillance of antibiotic susceptibility patterns may help to overcome the indiscriminate use of antibiotics which is a major cause of the emergence of drug resistance among pathogens.*
Keywords: *Klebsiella pneumoniae, PCR, Prevalence, Antibiotic-resistance, Kirby-Bauer.*

1.0 Introduction

Klebsiella pneumoniae is one of the most important members of the Enterobacteriaceae genera. It is a non-motile Gram-negative bacterium that ferments lactose and does not form spores. Under the microscope *K. pneumoniae* cells have edges, outward-curving edges with rounded ends, and a capsule that enhances pathogenicity[(1)].

Klebsiella pneumoniae is one such clinically significant organism that has caused public concern. It is a prominent opportunistic pathogen causing a broad spectrum of diseases including hospital-acquired (nosocomial infection) and community-acquired infections such as respiratory tract infection, bacteremia (blood stream infections), meningitis, rhinoscleroma and ozena, chronic genital ulcerative disease, colonization, pneumonia, urinary tract infections (UTIs), pyogenic liver abscess (PLA), burns inflammation, wounds inflammation, septicemia and diarrhea[(2)].

The symptoms of infections caused by *K. pneumoniae* are similar to those caused by other microbes[(3)]. For instance, meningitis caused by K. *pneumoniae is* characterized by all the classic signs and symptoms of bacterial meningitis. Due to the bacteria, tissues surrounding the brain will swell and prevent blood from reaching it. This can result in paralysis or stroke in some cases. Symptoms include high fever, headaches, a stiff neck, and sensitivity to bright light. A bloodstream infection (bacteremia or sepsis) triggered by *K. pneumoniae* can result in fever, chills, fatigue, light-headedness, and altered mental health. Infection with *K. pneumoniae* can cause fever and chills, flu-like symptoms, coughing with yellow, green, or bloody mucus, and breathing difficulties. In wounds and surgical sites, *K. pneumoniae* can cause skin or soft tissue infection. In most cases, this occurs after an injury or surgery. It can result in fever, blisters, fatigue, and pain at the surgical site or wound.

In Nigeria *K. pneumoniae* is among the most common causes of lower respiratory tract infections[(4)], neonatal septicemia, and bacteremia in children[(1)].

The usual antibiotic treatments for *K. pneumoniae* infections include beta-lactams such as penicillin (ampicillin, amoxicillin, carbenicillin, methicillin, etc.), cephalosporin (cefepime, cefixime, cefazolin, cefuroxime etc.), carbapenems (meropenem, imipenem, ertapenem, doripenem), aminoglycosides (gentamycin), and quinolones (ciprofloxacin, norfloxacin, levofloxacin etc.)[(5)], these treatments, however, are now ineffective against certain strains of *K. pneumoniae* because they have effective resistance mechanisms.

K. pneumoniae mechanisms of resistance, includes those shown by Gram-negative bacteria against various antibiotics, such as Aminoglycosides, Monobactams and Cephems. These are loss of porins, which reduce drug movement through the cell membrane; production of enzymes and the presence of beta-lactamases in the periplasmic space, which degrade beta-lactam[(6)]. They also involve increased expression of the trans-membrane efflux pump, which expels the drug from within the bacterium. They also involve target site mutations, which prevent the antibiotic from binding to its site of action; and ribosomal mutations or modifications, which prevent the antibiotic from binding and inhibiting protein synthesis. Other examples of such mechanisms include biofilm formation, which protects K. *pneumoniae* from antibiotic treatments [(7)]; metabolic bypass mechanisms, which use an alternative resistant enzyme to bypass the inhibitory effect of the antibiotic; and a mutation in the lipopolysaccharide, which renders the polymyxin class of antibiotics unable to adhere to the target site, *K. pneumoniae also* produces various enzymes that bind to and deactivate specific parts of drugs[(8)]. The enzymes produced usually target Beta-lactam type drugs, but some target other drug classes. These enzymes include extended spectrum beta-lactamases (ESBL),

metallo-beta-lactamases, oxacillinases, *K. pneumoniae* carbapenemases (KPC), and various others. *Klebsiella pneumoniae's* resistance to carbapenems (such as ertapenem, imipenem, meropenem and doripenem) is increasingly detected worldwide including Nigeria. This is worrisome because this drug class is usually the last resort for the treatment of serious infections caused by multidrug-resistant isolates[9].

Investigation of the prevalence of *K. pneumoniae* isolates among clinical samples of patients in four medical centers in Lagos, Nigeria, and the burden of ESBL and carbapenem-resistant *K. pneumoniae* (CRKP) strains in 2015. Different samples (stool, blood, urine, wound swabs, and nasal swabs) from 127 patients with suspected Gram-negative infections were analyzed and K. *pneumoniae* was identified in 43(34%) patients. The resistance rate of these 43 strains according to the CLSI breakpoints revealed cotrimoxazole (90.7%), cefuroxime (74.4%), ofloxacin (55.8%), ceftazidime (46.5%) and cefixime (35%). Three isolates (7%) were resistant to imipenem. All isolates were susceptible to amoxicillin/clavulanic acid and nitrofurantoin. The prevalence of ESBL-producing, MDR, and CRKP strains was found to be 69.8%, 62.8%, and 7.0% respectively[10].

The aim of this study was to assess the antibacterial susceptibility patterns and risk factors associated with multidrug-resistant *K. pneumoniae* from in-patients diagnosed with Pneumonia, Bacteremia, UTIs and soft tissue abscesses at Jos University Teaching Hospital (JUTH).

2.0 Material and Method

2.1 Sampling Site and Sample Collection

The research was designed as a hospital-based cross-sectional study was conducted at the Jos University Teaching Hospital (JUTH) Plateau State, Nigeria from January to June, 2023. The participants were recruited at the ward where clinical samples were collected. A total of 344 clinical samples were aseptically collected, including 50 sputum samples from patients diagnose with pneumonia, 122 blood samples from patients diagnoses with bacteremia, 122 urine samples from patients with urinary tract infection, and 50 superficial wound swabs from patients with wound sepsis. This study was approved by the JUTH Ethics Committee. A well-written informed consent form was used to obtain the patient's consent before the commencement of the study.

2.2 Culture/Microbiological Analysis

The clinical samples collected were streak separately onto cysteine lactose and electrolyte deficient (CLED) Agar for (urine) and MacConkey agar for (wound swab), Blood samples in inoculated vials were placed in the BD BACTEC fluorescent series instrument as soon as possible for incubation and monitoring. Vials were automatically tested for the duration of 7 days period. Positive vials were determined by the BD BACTEC fluorescent series instrument and identified as such “red” which means positive (there is growth) and “green” which means negative (no growth). The positive vials were removed and subculture in MacConkey agar plates. Purulent part of sputum samples was washed in 5 ml of sterile physiological saline and inoculated on MacConkey agar plates under aseptic techniques and incubated at 37°C for 24hours. The resulting colonies were further sub-culture for confirmatory onto Eosin Methylene Blue agar and incubated at 37°C for 24hours. The colonies were further identified using gram staining and biochemical techniques (Citrate utilization test, Oxidase, Triple Sugar Iron agar test, Indole production test, Urease test and Catalase test)[11].

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of confirmed *K. pneumoniae* isolates was performed according to Clinical Laboratory Standards Institute (CLSI) guidelines[12], using the Kirby-Bauer disc diffusion method to evaluate the

sensitivity/susceptibility of the test organisms. The turbidity of the test organisms was adjusted to 0.5 McFarland standard and the inoculum prepared by making a direct saline suspension of the isolated colonies from a 24hours culture and was inoculated on the surface of each Mueller Hinton agar plate using a sterile swab. Antimicrobial agents used includes Ciprofloxacin (10 µg), Augmentin (30 µg), Tazobactam-Piperacillin (10/100 µg), Cefotaxime (30 µg), cefepime (30ug), Gentamicin (10 µg), Aztreonam (30 µg), Cefpodoxime (10 µg), Ceftazidime (30 µg), Tetracycline (10 µg), Cefixime (OCF 5µg), Ertapenem (10 µg), meropenem (10ug), Imipenem (10 µg) and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg) (Oxoid, UK). The results were interpreted according to CLSI guidelines[12].

K. pneumoniae that were resistant to at least 3 different classes of antibiotics were considered multidrug-resistant strains[13].

2.4 Genotypic Confirmation of the Klebsiella

DNA Extraction for PCR Amplification

Isolates identified phenotypically to be *K. pneumoniae* and further screened as ESBL producers were used for DNA extraction using Qiagen QIAmp DNA Mini kit.

A sterile plastic loop was used to scoop the *K. pneumoniae* colonies into a sterile 1.5ml microtubes containing 200µl of phosphate buffer saline (PBS) and was briefly mixed with the help of a vortexer. After which, the tubes were centrifuged for 1 min at 8000 rpm. The supernatants were decanted and another 200µl of the PBS was added to the pellet and was dislodge by vortexing.

The mixture was transferred into a sterile 1.5ml microtubes containing 20µl of proteinase K solution and 200µl of buffer AL was added and was thoroughly mixed by vortexing for 15 sec. The tubes containing the mixture were incubated at 70^{o}C for 10 min and were briefly centrifuged to remove drops from the lid.

The mixture was transferred into the QIAam Mini spin column in a 2ml collection tube and was centrifuged at 8000 rpm for 1 min. The flow-through and the collection tube was discarded. The QIAam Mini spin column was placed in a new 2ml collection tube, a 500µl of buffer AW_1 was added and was centrifuge at 800 rpm for 1 min. The flow-through was discarded. A 500µl of buffer AW_2 was added and centrifuged at 14000 rpm for 3 min. The flow-through was discarded and the tubes were dry spin (tense) at 14000 rpm in order to eliminate the chance of possible buffer AW_2 carryover.

The QIAam Mini spin column was placed in a sterile 1.5ml microcentrifuge tube and a 150µl of AE (elution buffer) was added, incubated for 1 min at room temperature and was centrifuge at 8000 rpm for 1 min to elute the DNA. The eluted DNA extracts were immediately used for PCR analysis.

Amplification of bacterial DNA was done using 25µl total reaction volume containing 5µl of 5X master mix, 1µl of primer 16s rDNA, 13µl of nuclease-free water and 5µl of DNA. The thermocycling profile was as follows: Initial denaturation at 95^{o} C for 5 min, followed by 30 cycles of denaturation at 95^{o} C for 30 seconds, annealing at 56^{o} C for 1 min and extension at 72^{o}C for 90 seconds. hold for final extension at 72^{o}C for 10 min; hold for indefinite period at 4^{o}C. The amplified products were visualized on 1.5% agarose gel stained with 8ul ethidium bromide and was ran for 55 min at 90 volts.

Table 1: Primer Sequence Used in This Study

Primer	Sequence (5'-3')	Target Gene	Amplicon Size (bp)	Reference
Klebrib-1	5'-GTAATGTCTGGG AAACTGCC-3'	16S rDNA	1069	(14)
Klebrib-2A	3'- CCACC TTCCTCCAGTTTATC-5'	16S rDNA		(14)

2.5 Data Analysis

Analysis of data from this study was performed by OMEGA RESEARCH CONSULT using Statistical Package for Social Science (SPSS Version 20) software and Excel Office program. Descriptive analyses using percentages and frequencies were used for presence of antibiotic resistance pattern of multidrug resistant *K. pneumoniae*. Continuous variables were compared with Chi square and multivariate logistic regression analysis to identify associated risk factors to multidrug resistant *K. pneumoniae* infections.

3.0 Results

Table 2: Prevalence of *K. pneumoniae* Based on Gender and Sample Type

Variable	No. of Sample	No. of Positive	X^2	p-value
Gender				
Male	184	23 (12.3)	0.532	0.466
Female	160	16 (10.0)		
Total	3443	9 (11.3)		
Sample Type				
Blood	122	9 (7.4)	2.992	0.393
Urine	122	16 (13.1)		
Sputum	50	7 (14.0)		
Wound swab	50	7 (14.0)		
Total	344	39 (11.3)		

Median Age in years (IQR): 30 (31)
IQR: Interquartile range
*: statistically significant association exist at $p \leq 0.05$
**: statistically significant association exist at $p \leq 0.01$

Table 3: Antibiotic Susceptibility Profile of all *K. pneumoniae* Isolates from JUTH

	Number tested (n = 39)	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Cefotaxime (CTX 30)		37 (94.9)	2 (5.1)	0
Cefepime (FEP30)		33 (84.6)	3 (7.7)	3 (7.7)
Trimethoprin-Sulfamethoxazole (SXT 25)		28 (71.8)	2 (5.1)	9 (23.1)
Augmentin (AUG 30)		20 (51.3)	2 (5.1)	17 (43.6)
Cefpodoxime (CPD 30)		29 (74.4)	2 (5.1)	8 (20.5)
Ceftazidime (CAZ 30)		33 (84.6)	6 (15.4)	0
Meropenem (MEM 10)		2 (5.1)	9 (23.1)	28 (71.8)
Imipenem (IPM 10)		5 (12.8)	4 (10.3)	30 (76.9)
Ertapenem (ETP 10)		12 (30.8)	2 (5.3)	25 (64.1)
Cefixime (OCF 5)		32 (82.1)	4 (10.3)	3 (7.7)
Piperacilin -Tazobactam (TZP 100/10)		21 (53.9)	9 (23.1)	9 (23.1)
Aztreonam (ATM 30)		24 (61.5)	2 (5.1)	13 (33.3)
Gentamicin (CN 1 0)		16 (41.0)	1 (2.6)	22 (56.0)
Ciprofloxacin (CPT 10)		28 (71.8)	1 (2.6)	10 (25.6)
Tetracycline (TE 30)		32 (82.1)	4 (10.3)	3 (7.7)

Key:
% = Percentage
n = Number of isolates

Table 4: Distribution of Multi-Drug Resistance (MDR) Among *K. pneumoniae* Isolates in Relation to Clinical Samples (n=39)

Sample	No. of samples (%)	No. of isolates (%)	MDR (%)
Urine	122 (35.5)	16 (41.0)	15 (39.5)
Wound swab	50 (14.5)	7 (17.9)	7 (18.4)
Sputum	50 (14.5)	7 (17.9)	7 (18.4)
Blood	122 (35.5)	9 (23.1)	9 (23.7)
TOTAL	**344 (100)**	**39 (11.3)**	**38 (97.4)**

Key:
% = Percentage
n = Number of isolates

Table 5: Multi-Drug Resistant (MDR) Pattern of *K. pneumoniae* Isolates

Sample Types	No. of Isolates	Strains ID	Resistance Pattern
Urine, Blood and Wound swab	4	U2533, B250, B291, W2543	CTX-FEP-SXT-CPD-CAZ-OCF-TZP-ATM-CN-CPT-TE
Wound swab	1	W2621	ETP-TZP-CPT
Urine	1	U2531	CTX-FEP-SXT-CPD-CAZ-OCF-TZP-ATM-CN-TE
Urine	1	U2688	CTX-FEP-AUG-CPD-CAZ-ETP-OCF-ATM-CPT-TE
Wound swab	1	W275	CTX-FEP-AUG-OCF
Sputum	1	S1298	CTX-FEP-SXT-AUG-CAZ-TE
Sputum	1	S2645	CTX-FEP-AUG-CPD-CAZ-OCF-TE
Wound swab	1	W2963	CTX-FEP-SXT-AUG-CPD-CAZ-TZP-ATM-CN-CPT-TE
Sputum	1	S3358	CTX-FEP-SXT-AUG-CPD-ETP-OCF-TZP-ATM-CPT-TE
Urine, Sputum, Wound swab	3	U2965, S3350, W1808	CTX-FEP-SXT-CPD-CAZ-OCF-TZP-ATM-CPT-TE
Urine	1	U3941	CTX-AUG-CN
Urine	1	U2489	CTX-FEP-SXT-AUG-CPD-CAZ-IPM-OCF-CPT
Urine	1	U3012	CTX-FEP-AUG-CAZ-OCF-TZP-CN-CPT-TE
Urine	1	U1800	FEP-SXT-CPD-CAZ-ETP-OCF-TZP-ATM-CPT-TE
Blood	1	B453	CTX-FEP-SXT-CPD-CAZ-ETP-OCF-TZP-ATM-CPT-TE
Sputum	1	S3478	CTX-FEP-SXT-CPD-CAZ-OCF-ATM-CN-CPT-TE
Wound swab	1	W3584	CTX-AUG-CPD-CAZ-CN-TE
Blood	1	B459	CTX-FEP-SXT-CPD-CAZ-OCF-TZP-ATM-CN-CPT-TE
Blood	1	B365	CTX-FEP-SXT-AUG-CAZ-ETP-OCF-ATM-CN-CPT-TE
Urine	1	U3806	CTX-FEP-SXT-CPD-CAZ-MEM-ETP-OCF-ATM-TE
Blood	1	B396	CTX-FEP-SXT-AUG-CPD-CAZ-ETP-OCF-CPT-TE
Blood	1	B306	CTX-SXT-AUG-CAZ-OCF-ATM-CPT-TE
Urine	1	U1880	CTX-FEP-AUG-CPD-CAZ-ATM-CPT
Blood	1	B221	CTX-FEP-SXT-AUG-CPD-CAZ-OCF-ATM-CN-TE
Blood	1	B395	CTX-SXT-AUG-CPD-CAZ-IPM-OCF-CPT-TE
Urine	1	U3718	CT X-FEP-SXT-CPD-CAZ-ETP-OCF-TZP-CN-CPT-TE
Sputum	1	S3356	CTX-FEP-SXT-AUG-CPD-CAZ-OCF-CN-CPT
Urine	1	U3795	CTX-FEP-SXT-CPD-CAZ-MEM-IPM-ETP-OCF-TZP-TE
Urine	1	U2361	CTX-FEP-SXT-CAZ-OCF-TZP-ATM-CN-CPT-TE
Sputum	1	S3097	CTX-FEP-SXT-AUG-CAZ-OCF-TZP-ATM-TE
Wound swab	1	W2859	CTX-FEP-SXT-CPD-CAZ-IPM-ETP-OCF-TZP-CPT-TE
Urine	1	U2467	CTX-FEP-CPD-CAZ-IPM-ETP-OCF-ATM-CPT-TE
Urine	1	U3690	CTX-FEP-AUG-CPD-OCF-TZP-ATM-CPT-TE

Key:
CTX = Cefotaxime, **FEP** = Cefepime, **SXT** = Trimethoprin-Sulfamethoxazole, **AUG** = Augmentin, **CPD** = Cefpodoxime, **CAZ** = Ceftazidime, **MEM** = Meropenem, **IPM** = Imipenem, **ETP** = Ertapenem, **OCF** = Cefixime, **TZP** = Piperacilin-Tazobactam, **ATM** = Aztreonam, **CN** = Gentamicin, **CPT** = Ciprofloxacin, **TE** = Tetracycline

Table 6: Prevalence of *K. pneumoniae* Based on Associated Risk Factors

Variable	No. of sample	No. positive (%)	X^2	*p*-value
Previous hospital admission				
Yes	13	13 (100.0)	105.660	< 0.001**
No	331	26 (7.9)		
Total	**344**	**39 (11.3)**		
ICU admission				
Yes	7	7 (100.0)	55.881	< 0.001**
No	337	32 (9.5)		
Total	**344**	**39 (11.3)**		
Prolong antibiotic use				
Yes	3	3 (100.0)	23.668	< 0.001**
No	341	36 (10.6)		
Total	**344**	**39 (11.3)**		
Surgery				
Yes	2	2 (100.0)	15.732	< 0.001**
No	342	37 (10.8)		
Total	**344**	**39 (11.3)**		
Urethral catheter				
Yes	4	4 (100.0)	31.650	< 0.001**
No	340	35 (10.3)		
Total	**344**	**39 (11.3)**		
Diabetes mellitus				
Yes	2	2 (100.0)	15.732	< 0.001**
No	342	37 (10.8)		
Total	**344**	**39 (11.3)**		
Immunosuppression (severe illness)				
Yes	2	2 (100.0)	15.732	< 0.001**
No	342	37 (10.8)		
Total	**344**	**39 (11.3)**		
Burns				
Yes	3	3 (100.0)	23.668	< 0.001**
No	341	36 (10.6)		
Total	**344**	**39 (11.3)**		
Chronic liver disease				
Yes	1	1 (100.0)	7.843	0.005**
No	343	38 (11.1)		
Total	**344**	**39 (11.3)**		
Chronic lungs disease				
Yes	2	2 (100.0)	15.732	< 0.001**
No	342	37 (10.8)		
Total	**344**	**39 (11.3)**		

***: statistically significant association exists at $p \leq 0.05$**
****: statistically significant association exists at $p \leq 0.01$**

Table 7: Genotypic Confirmation of Klebsiella Specie

Sample Source	MDR No. of Isolates	Confirmed Klebsiella spp (%)	16s rDNA +ve (%)
Urine	16	12	11 (11.7)
Wound swabs	7	6	6 (100.0)
Sputum	7	5	5 (100.0)
Blood	9	7	6 (85.7)
TOTAL	**39**	**30**	**28 (93.3)**

Key:
MDR = Multidrug resistant

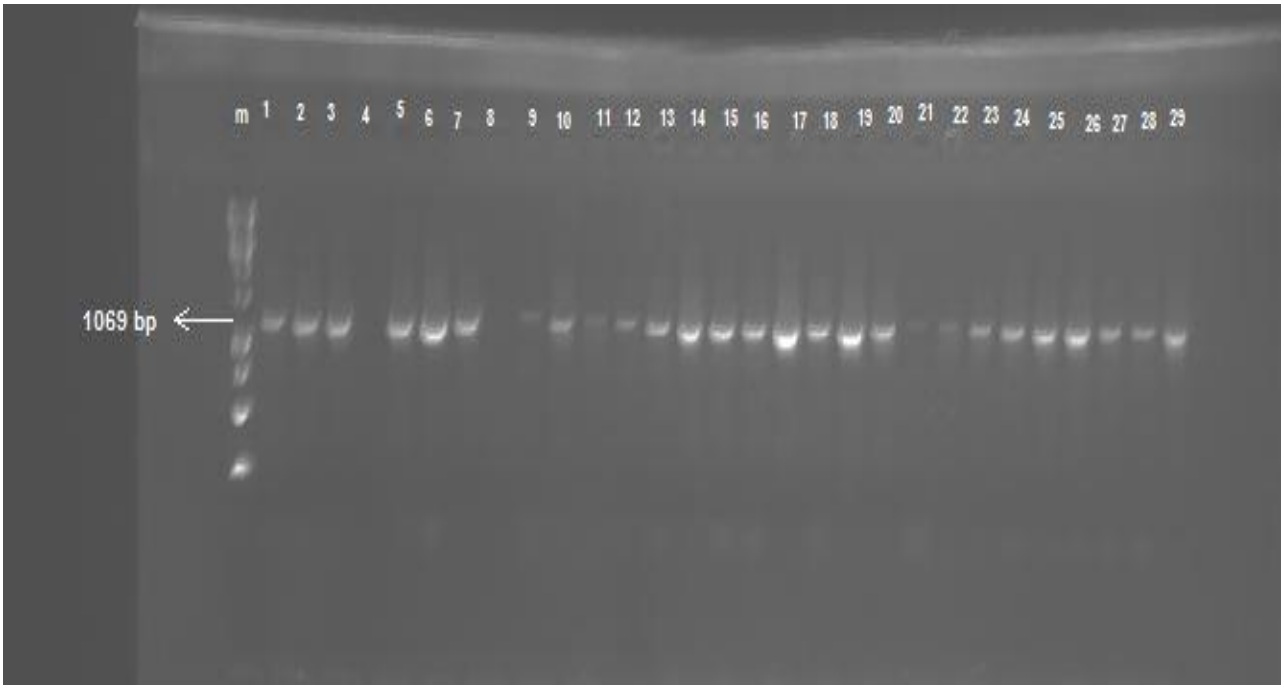

Plate-1: Agarose Gel Electrophoresis of PCR-amplified 16s rDNA Gene
Lane 1-30: Represents PCR product of *K.pneumoniae* 16s rDNA gene (1069 bp).
Lane M: Represents 1kb bp DNA ladder as a standard molecular size marker.

4.0 Discussion

In this study, among the 344 clinical samples collected 39 (11.3%) were positive as *K. pneumoniae* isolates, and among the 39 *K. pneumoniae* isolates, 23 (12.5%) isolates were from male patients and 16 (10.0%) isolates were from female patients. The male to female ratio of 1.44:1 is similar to the study done by Sunulkumar and Roopa[(15)] where they reported male to female ratio of 1.7:1.

The prevalence of *K. pneumoniae* (11.3%) in this study was similar to a prevalence of 11.4% in Nnewi in Anambra State,[(16)] and low compared to a prevalence of 35.3% in Zaria Kaduna State [(17)].

An isolate is said to be multidrug resistant if it is resistant to three or more antimicrobial classes.

Multidrug resistance in *Klebsiella* species varies in different parts of the world[[(18); (19); (20)]]. In this study, the prevalence of Multidrug resistance *K. pneumoniae* isolates was 38 (97.4), in comparison to India, 54% was reported[(21)] and in Nigeria 75.8%[(22)]. The high level of multidrug resistance in this study could be due to an interplay of several resistant mechanisms co-expressed by the isolates such as extended spectrum beta-lactamases, quinolone resistance genes etc., which hydrolyze quinolones and beta-lactams. Furthermore, prior antibiotic use in hospitals or through auto-medication, overuse of antibiotics in livestock and fish farming, poor infection control in health care facilitates and poor hygiene and sanitation exacerbates multidrug resistance.

Major risk factors for infection with Multidrug resistance organisms are long term antibiotic exposure, prolonged intensive care unit stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporins use and instrumentation or catheterization[(23)]. In this study, the high-risk factors for infection with Multidrug resistance *K. pneumoniae* isolates were predominantly repeated hospital admission-13, followed by other associated risk factors like ICU admission-7, urethral catheter-4, burns and prolong antibiotic use-3, surgery, diabetes mellitus, immunosuppression and chronic lung disease-2 and chronic liver disease-1. Several

studies done suggest these as the most common risk factors in patients[(24); (25)].

The Antimicrobial sensitivity of *K. pneumoniae* isolates was studied, in this study *K. pneumoniae* isolates were 37(94.9%) resistant to cefotaxime, 33 (84.6%) to cefepime and ceftazidime, 32 (82.1%) to cefixime and tetracycline, 29 (74.4%) to cefpodoxime, 28 (71.8%) to trimethoprim-sulfamethoxazole and ciprofloxacin, 24 (61.5%) to aztreonam, 21 (53.9%) to piperacillin-tazobactam, 20 (51.3%) to augmentin, 16 (41.0%) to gentamicin, 12 (30.8%) to ertapenem, 5 (12.8%) to imipenem and 2 (5.1%) to meropenem. The results were interpreted as per CLSI guidelines[(12)]. This correlates with the study done by Sasirekha *et al.,*[(26)] in India where they found 84% resistance to cefotaxime and 85% resistant to ceftazidime, as hospital acquired isolates were more resistant and it may be due to lack of antibiotic policy, irrational use of 3rd generation cephalosporins mainly ceftriaxone in the hospital.

In this study, resistance to ciprofloxacin was 71.8%. This finding is low compared to the study by Haque and Salam[(27)] from Bangladesh (90.9%). In another study by Sasirekha *et al.,*[(23)] from India, 68% strains were resistant to ciprofloxacin. In the present study, 56.0% isolates were susceptible Gentamicin, it was high 48% compared to Sasirekha *et al.,*[(26)]. This variation may be due to low use of gentamicin among patients in JUTH.

Carbapenems are the drugs of choice for many infections caused by MDR Gram positive and Gram-negative bacteria[(28)]. In this study *K. pneumoniae* isolates were sensitive to imipenem (76.9%), meropenem (71.8) and ertapenem (64.1%) respectively. This finding is similar to the findings done by Akinyemi *et al.,*[(10)] in Lagos with imipenem (93%). The low resistance of the isolates to these antibiotics could be explained by the fact that these drugs are reserved as last line treatment, they are expensive and they can be administered only via the intravenous route[(28)]. In this study most of the *K. pneumoniae* recovered were sensitive to Carbapenems.

Conclusion

In this study 39 (11.3%) *K. pneumoniae* isolates were obtained from the 344 clinical samples (23 [12.5%] and 16 [10.0%] from males and females, respectively).

The *K. pneumoniae* isolates were seen to have high resistance towards various types of antibiotics, especially cephalosporins such as cefotaxime (94.9%), ceftazidime and cefepime (84.6%), and showed low resistance to carbapenems such as imipenem and meropenem (12.8% and 5.1%, respectively).

The prevalence of multidrug-resistant *K. pneumoniae* isolates in this study was 97.4%.

The risk factors for *K. pneumoniae* in this study included Urethral catheterization, diabetes mellitus, immunosuppression, previous hospitalization, ICU admission, Burns, prolong antibiotic use, chronic lung disease, alcoholism/chronic liver disease and surgery.

Out of the 30 Multidrug resistant *K. pneumoniae* isolates, 28 were confirmed using 16s rDNA gene.

Acknowledgement: We acknowledge Mrs. Helen Luka of Biotechnology Laboratory Vom, Plateau State, Nigeria, for helping in conducting PCR analysis.

Author's contributions: Jesse Ishaya, P.M Lar and Emeka Walter conducted the research and all authors read and approved the final manuscript.

Funding: This study was sponsored by Tertiary Education Trust Fund (TET fund) through the Institutional Based Study Grant of Kaduna Polytechnic, Kaduna State, Nigeria.

Competing interest: The authors declare that they have no competing interest.

Ethical Approval: Approved

References

1. Brown, B., Dada-Adegbola, H., Trippe, C., and Olopade, O. Prevalence and Etiology of Bacteremia in Febrile Children with Sickle Cell Disease at a Nigeria Tertiary Hospital. Mediterr J Hematol Infect Dis.

2017; 9(1):e2017039. doi:10.4084/MJHID.2017.039

2. Zhang, S., Yang, G., Ye, Q., Wu, Q., Zhang, J., and Huang, Y. Phenotypic and Genotypic Characterization of *Klebsiella pneumoniae* Isolated from Retail Foods in China. *Front Microbiol*. 2018; 9:289. doi:10.3389/fmicb.2018.00289
3. Murray, P. R., Rosenthal, K. S., and Pfaller, M. A. Medical microbiology. Philadelphia: Elsevier/Saunders, 2013
4. Uzoamaka, M., Ngozi, O., Johnbull, O.S., and Martin, O. Bacterial Etiology of lower respiratory tract infections and their antimicrobial susceptibility. *Am J Med Sci*. 2017; 354(5):471-475. doi:10.1016/j.amjms.2017.06.025
5. Qureshi, S. *Klebsiella* Infections Treatment and Management (M. Bronze, Ed.). Retrieved November 29, 2015, from http://emedicine.medscape.com/article/219907-treatment.
6. Kumar, V., Sun, P., Vamathevan, J., Li, Y., Ingraham, K., Palmer, L., and Brown, J.R. Comparative Genomics of *Klebsiella pneumoniae* Strains with Different Antibiotic Resistance Profiles. Antimicrob Agents Chemother. 2011; *55*(9), 4267-4276. doi:10.1128/AAC.00052-11
7. Vuotto, C., Longo, F., Balice, M. P., Donelli, G., and Varaldo, P.E. Antibiotic Resistance Related to Biofilm Formation in *Klebsiella pneumoniae*. *Pathogens.* 2014; 3(3):743-758. doi:10.3390/pathogens3030743
8. Papp-Wallace, K.M., Endimiani, A., Taracila, M.A., and Bonomo, R.A. Carbapenems: Past, Present, and Future. Antimicrob Agents Chemother. 2011; 55(11):4943-4960. doi:10.1128/AAC.00296-11
9. Peirano, G., Seki, L.M., Val-Passos, V.L., Pinto, M.C., Guerra, L.R., and Asensi, M.D. Carbapenem-hydrolysing β-lactamase KPC-2 in *Klebsiella pneumoniae* isolated in Rio de Janeiro, Brazil. J Antimicrob Chemother. 2009; 63(2):265-268. doi:10.1093/jac/dkn484
10. Akinyemi, K.O., Abegunrin, R.O., Iwalokun, B.A., Fakorede, C.O., Makarewicz, O., Neubauer, H., Pletz, M.W., & Wareth, G. The emergence of *Klebsiella pneumoniae* with reduced susceptibility against third generation cephalosporins and carbapenems in Lagos hospitals, Nigeria. Antibiotics (Basel, Switzerland). 2021; *10*(2), 142. https://doi.org/10.3390/antibiotics10020142.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed, London: Cambridge University Press, 2006
12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Wayne PA: CLSI Document M100, Pennsylvania: Clinical and Laboratory Standards Institute, 2020
13. Magiorakos, A., Srinivasan, A., Carey, R., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D., Rice, L., Stelling, J., Struelens, M., Vatopoulos, A., Weber, J., and Monnet, D. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x
14. Arenas, N. E., P., Juan C., C., Sandra-Milena, D., Clara J. and Gómez, A. Design of a molecular method for subspecies specific identification of Klebsiella pneumoniae by using the 16S ribosomal

subunit gene. Colomb Méd. 2009; *40*(2), 194-201.

15. Sunilkumar, B., and Roopa, C. Isolation and Antibiogram of *Klebsiella Species* from Various Clinical Specimens. Int.J.Curr.Microbiol.App Sci. 2015; 4(9): 991-995
16. Akujobi, C.N., and Enwuru, C. Detection of extended spectrum β-lactamases in Gram negative bacilli from clinical specimens in a teaching hospital in South eastern Nigeria. Niger Med J. 2010; 51. 141-6.
17. Olonitola, O. S., Olayinka, A. T., and Inabo, H.I. Production of extended spectrum beta-lactamases of urinary isolates of Escherichia coli and *Klebsiella pneumoniae* in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Int. J. Biol. Chem. Sci. 2007; 1(2): 181-185
18. Tang, M., Kong, X., Hao, J., and Liu, J. Epidemiological Characteristics and Formation Mechanisms of Multidrug-Resistant Hypervirulent *Klebsiella pneumoniae*. *Front Microbiol*. 2020; 11:581543. Published 2020 Nov 20. doi:10.3389/fmicb.2020.581543
19. Kijineh, B., Alemeyhu, T., Mengistu, M., and Ali, MM. Prevalence of phenotypic multi-drug resistant Klebsiella species recovered from different human specimens in Ethiopia: A systematic review and meta-analysis. *PLoS One*. 2024;19(2):e0297407. Published 2024 Feb 9. doi:10.1371/journal.pone.0297407
20. Salawudeen, A., Raji, Y.E., Jibo, G.G. *et al*. Epidemiology of multidrug-resistant *Klebsiella pneumoniae* infection in clinical setting in South-Eastern Asia: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 12, 142 (2023). https://doi.org/10.1186/s13756-023-01346-5
21. Sikarwar, A.S., and Batra, H.V. Prevalence of Antimicrobial Drug Resistance of *Klebsiella pneumoniae* in India. International Journal of Bioscience, Biochemistry and Bioinformatics. 2011; 211-215. https://doi.org/10.7763/IJBBB.2011.V1.38
22. Chikwendu, C.I., Amadi, E.S., and Obi, R.K. Prevalence and Antimicrobial Resistance in *Pseudomona saeruginosa* and *Klebsiella pneumoniae* Isolates from Non-Clinical Urine Samples. New York Science Journal. 2010; 3, 194-200.
23. Sarma, J. B., Bhattacharya, P. K., Kalita, D., and Rajbangshi, M. Multidrug-resistant Enterobacteriaceae including metallo-β-lactamase producers are predominant pathogens of healthcare-associated infections in an Indian teaching hospital. Indian J Med Microbiol. 2011; 29(1):22-27. doi:10.4103/0255-0857.76519
24. Abhilash, K. P., Veeraraghavan, B., and Abraham, O. C. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing Escherichia coli and *Klebsiella spp*. in a tertiary care teaching hospital in south India. J Assoc Physicians India. 2010; 58 Suppl:13-17.
25. Namratha, K., Sreeshma, P., Subbannayya, K., Dinesh, P., and Champa, H. Characterization and Antibiogram of *Klebsiella spp*. Isolated from Clinical Specimen in a Rural Teaching Hospital. Sch. J. App. Med. Sci. 2015; 3(2E):878-883
26. Sasirekha, B. R., Manasa, P., Ramya, P., and Sneha, R. (2010). Frequency and antimicrobial sensitivity pattern of extended spectrum ß-Lactamases producing *E. coli* and *Klebsiella pneumoniae* isolated in a tertiary care

hospital. *Journal of the Medical Sciences*3. 2010; 265-271.

27. Haque, R., and Salam, M.A. Detection of ESBL Producing Nosocomial Gram-negative Bacteria from a Tertiary Care Hospital in Bangladesh. Pak J Med Sci. 2010; 26, 887-891.
28. Meletis, G. (2016). Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infectious Dis*. 2016; *3*(1), 15–21. https://doi.org/10.1177/2049936115621709.