Original Research Article

Antimicrobial Resistance and Molecular Detection of ESBL *Klebsiella pneumoniae* in Urine of Suspected UTI Patients Attending Public Hospitals in Nasarawa South Senatorial District, Nasarawa State, Nigeria.

.

ABSTRACT

Aims: The aim of the current study was to determine antimicrobial susceptibility patterns and the incidence of resistance genes (β-lactamase-encoded genes) among clinical isolates of *K. pneumoniae*.

Study design: Investigative.

Place and Duration of Study: Department of Microbiology, Nasarawa State University, Keffi, between November 2022 and July 2023.

Methodology: A total of 375 clinical samples were randomly collected from patients and isolation and identification was done using standard microbiological methods. The antibiotic susceptibility testing for the isolates was carried out in accordance with the Clinical and Laboratory Standards Institute protocol. The detection of extended-spectrum beta-lactamase (ESBL) production in *K. pneumoniae* isolates was carried out using double disc synergy test. In addition, molecular detection of ESBL genes was carried out using Polymerase Chain Reaction (PCR) method.

Results: Resistance was high to ampicillin (94.74%) and amoxicillin-clavulanic acid (73.6%) but low to cefotaxime (13.16%), ceftriaxone (18.42%), ceftazidime and cefoxitin (23.60%) and gentamicin (28.94%). Most of the isolates were multi-drug resistant (MDR; 81.5%) and none was Pan Drug resistant. All 15 isolates jointly resistant to cefotaxime and/or ceftazidime and ciprofloxacin 100.0% were ESBL producers, distributed as follows: $bla_{CTX\text{-}M\text{-}1}$ 7(46.6%); $bla_{CTX\text{-}M\text{-}3}$4(26.6%); bla_{SHV}6(40.0%); bla_{TEM}6(40.0%); and bla_{OXA} 5(33.3%). **Conclusion:***K. pneumoniae* isolated from the urine of patients attending public hospitals in Nasarawa South were less resistant to cefotaxime, ceftazidime, gentamicin, ceftriaxone, and cefoxitin in the study location. This implies that the antibiotics are useful in the treatment of infection caused by *K. pneumoniae.* Also, ESBL-positive *K. pneumoniae* isolates harbored ESBL genes, with $bla_{CTX\text{-}M}$ as the most common.

Keywords: Klebsiella pneumoniae, Antimicrobial Resistance, β-lactamase, ESBL

1. INTRODUCTION

In recent decades, the global healthcare landscape has been significantly challenged by the escalating crisis of antimicrobial resistance (AMR), making it recognized as one of the major Global Health challenges of the 21st century [1]). This complex phenomenon has arisen due to the persistent overuse and misuse of antibiotics, and various cultural influence especially in the rural communities resulting in the diminished efficacy of these crucial therapeutics [2,3].

Among the myriad pathogens contributing to this crisis, *Klebsiella pneumoniae*, a Gram-negative bacterium known for its capacity to produce extended-spectrum beta-lactamases (ESBLs), stands out as a formidable adversary [4]. ESBL-producing *Klebsiella pneumoniae* strains have developed resistance to a wide array of antibiotics, rendering them increasingly difficult to treat [5]. The gravity of this threat is further compounded when considering their association with urinary tract infections (UTIs), one of the most common bacterial infections affecting individuals worldwide [6].

Nasarawa State, located in the north-central region of Nigeria, confronts a multitude of healthcare challenges, including a growing burden of infectious diseases such as UTIs [7]. Within this context, the emergence and spread of UTIs caused by ESBL-producing *Klebsiella pneumoniae* strains represent a particularly urgent concern [8]. Public hospitals, serving as the primary healthcare providers for a significant portion of the population, are at the forefront of diagnosing and treating these infections [9].

The surge in AMR, especially among pathogens causing UTIs like *Klebsiella pneumoniae*, has created a multifaceted and dire healthcare crisis, and the deficiency in comprehensive data regarding the prevalence, resistance patterns, and genetic mechanisms underpinning ESBL production in *Klebsiella pneumoniae* strains within Nasarawa South Senatorial District has made efforts to address the issue problematic.

Understanding the local epidemiology of ESBL-producing *Klebsiella pneumoniae* causing UTI is crucial for tailoring clinical interventions, optimizing resource allocation, and mitigating the risk of AMR propagation in Nasarawa South Senatorial District. This study investigates, the prevalence of *K. pneumoniae*, its antimicrobial susceptibility patterns and the incidence of resistance genes (β-lactamase-encoded genes) among clinical isolates of *K. pneumoniae*.

2. MATERIAL AND METHODS

2.1 Sample Collection

A total of 375 (175 from DASH and 50 from the other three hospitals) early morning mid-stream urine samples of patients with suspected cases of UTI were collected using appropriate sterile sample containers and transported to the Microbiology Laboratory at Dalhatu Araf Specialist Hospital (DASH), Lafia, for same-day analysis or stored in a refrigerator (Model PRN 1313 HCA, BEKO, Germany) at 5°C for later-day analysis. Three hospitals, designated GHA, GHD and GHK, are all major health facilities within the study area, Nasarawa South Senatorial District of Nasarawa State, in North Central Nigeria. GHA (General Hospital Awe) is a secondary health care hospital located in Awe, a town in Nasarawa State, 85km from the state capital, Lafia. The health facility is licensed by the

Nigerian Ministry of Health. GHD (General Hospital Doma), is a health facility located in Doma, a suburb of Nasarawa state and providing outpatient and inpatient services, amongst many others to its residents. GHK (General Hospital Keana) is a secondary health care facility and situated at Keana, a town and local government in Nasarawa State. Samples were collected between November 2022 and March 2023.

2.1.1 Isolation of *Klebsiella pneumoniae*

The samples collected were subjected to isolation procedure; presumptive *K. pneumoniae*was isolated from the urine as follows: a loopful of urine sample was streaked on MacConkey Agar (MCA: Oxoid Ltd., UK) and incubated in an incubator (Quincy Lab Inc. Model12-140E, USA) at 37ºC for 24hrs. Pinkish colonies from the 24-hrs MCA plates were selected as presumptive *K. pneumoniae*[10]

2.1.2Identification of *Klebsiella pneumoniae*

*Klebsiella pneumoniae*was identified done by morphological, cultural and biochemical characteristics using Gram staining, Motility Test and biochemical tests (Indole, Methyl Red-Voges-Proskauer, and Citrate.) as described in the Bacteriological Analytical Manual [11] and Cheesbrough [10]. The KB003 H125^{TM} Kit, a commercial kit designed for the identification of Enterobacteriaceae, was used to confirm the suspected isolates as described in the manufacturer's manual. The bacterium was stored in the refrigerator on nutrient agar (Oxoid Ltd, UK) slants and reactivated by sub-culturing on MCA for use in further research.

2.2 Antimicrobial Susceptibility Testing

The antibiotic susceptibility test for *K. pneumoniae*isolates from urine was carried outusing the Kirby-Bauer disc diffusion method as modified by the Clinical and Laboratory. Standards Institute (CLSI) [12]. Briefly, three (3) pure colonies of the isolates were inoculated in to 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension were adjusted to the turbidity equivalent to 0.5 McFarland's standard.. The 0.5 McFarland Standard was prepared as follows: 99.5 ml of 1% ($\frac{v}{v}$) H_2SO_4 + 0.5 ml of 1.172% ($\frac{w}{v}$) $BaCl_2.2H_2O$. A sterile cotton swab stick was dipped into the standardized *K. pneumoniae*suspension and streaked on Mueller-Hinton Agar (MHA: Oxoid Ltd, UK) plates. Antibiotics discs (Oxoid Ltd, UK) were gently placed 15mm apart on the MHA surface using a pair of sterile forceps and the plates were allowed to incubate at room temperature for 1 h before re-incubating at 37ºC for 17 hrs. The discs used include: Amoxicillin/Clavulanic acid (AMC: 10/20 µg), Sulphamethoxazole/ Trimethoprim (SXT: 25 µg), Ampicillin (AMP: 10 µg), Cefotaxime (CTX: 30 µg), Cefoxitin (FOX: 30 µg), Streptomycin (S: 10 µg), Gentamicin (CN: 10 µg), Ceftazidime (CAZ: 30 µg), Ciprofloxacin (CIP: 5 µg) and Ceftriaxone (CRO: 30 µg). After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler, and the result of the susceptibility test was interpreted using susceptibility breakpoint earlier described by CLSI [12].

2.3 Extended Spectrum β-Lactamase Production Test

The phenotypic confirmatory test for ESBL production by isolates resistant to cefotaxime and ceftazidime was carried out using Double- Disc Synergy Test (DDST) method earlier described by Giriyapur et al. [13]. Briefly, 10^5cfu/ml bacterial suspension was streaked on sterile Mueller-Hinton agar plates and amoxicillin/clavulanic acid (30 µg) disc was placed at the centre of the plate. Cefotaxime (30 µg) and ceftazidime (30 µg) discs were then placed 15 mm (edge-to-edge) from the disc at the centre. Enhancement of zone of inhibition in the

area between the amoxicillin-clavulanic acid disc and any one of the β-lactam discs compared with the zone of inhibition on the far side of the drug disc was interpreted as indicative of the presence of an ESBL in the tested strain.

2.3.1 Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance index (MARI) of the *K. pneumoniae*isolates were determined as described by [14]. MARI is defined as resistance to at least two (2) antibiotics, hence obtaining a MAR value higher than 0.2 indicated a significant and high risk source of acquiring the multidrug resistant *K. pneumoniae*from the tested samples

$$\textbf{MAR Index}=\frac{a\ (Number\ \ of\ \ antibiotics\ \ \ isolate\ \ is\ resistant\ \ \ to)}{b\ (Number\ \ of\ \ antibiotics\ \ \ tested\ )}$$

2.3.2 Molecular detection of ESBL resistance genes

2.3.2.1 DNA extraction

The DNA extraction of ESBL positive *K. pneumoniae*was performed by a method as described previously [15]. Following purification on MacConkey agar, bacterial DNA was isolated from a 24-hrs culture in Luria-Bertani broth (LB: Oxoid Ltd, UK) prepared according to the manufacturers' protocol. The bacterial cells were harvested by centrifugation at 1400 rpm in a microcentrifuge (Model 5417R, Lab-line Instrument Inc. USA) for 3 min at room temperature and the supernatant was discarded. The harvested cells were re-suspended in 1 ml of sterile normal saline transferred into 1.5 mL centrifuge tube and centrifuged at 14000rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 µL of sterile distilled water by vortexing. The tube was centrifuged again at 1400 rpm for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500µl of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice for 10mins and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^oC for other downstream reactions.

2.3.2.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. 2µl of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button [16].

2.3.2.3 Amplification of 16S rRNA Gene

The 16S rRNA genes isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers (Zymo research, USA) on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 µl for 35 cycles. The PCR mix included: X2 Dream Taq Master Mix (Taq polymerase, DNTPs, MgCl, Zymo research, USA), the primers at a concentration of 0.4 M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 min; denaturation, 95ºC for 30 sec; annealing, 52ºC for 30 sec; extension, 72ºC for 30 sec for 35 cycles and final extension, 72ºC for 5 min. The product was resolved on a 1% agarose gel and visualized on a UV transilluminator [17].

2.3.2.4 DNA Amplification by Polymerase Chain Reaction (PCR)

The single-plex polymerase chain reaction (PCR) was performed in 45 µl total volume using the ABI 9700 Applied Biosystems thermal cycler, containing 2.5 µl MgCl2 (50 mM), 2.5 µl 10 × buffer, 0.9 µl of each primer (2.7 µM), 8 mM of dNTPs, 0.5 µl Taq DNA polymerase (Zymo research, USA) and 5 µl of DNA template. Amplification was carried out in the following program: an initial denaturation step at 95°C for 5 minutes, followed by 40 denaturation cycles at 94°C for 1 min, annealing at 55°C for bla_{CTXM-1}, bla_{CTXM-3}, 56°C for bla_{SHV}, bla_{TEM} and bla_{OXA} for 30s and extension at 68°C for 30s, and a final extension step at 68 °C for 10 minutes.

2.3.2.5 DNA restriction analysis

The restriction enzymes analysis was carried out with 2–5 enzyme units (*EcoRI, smaI, xbaI, NdeI, XhoI and PstI*) and 1 µg DNA in the reaction mixture. The volume was made up to 20 µL with distilled water and appropriate enzyme buffer according to the recommendations of the manufacturer before incubation, with optimum temperature 30 ˚C for SmaI and 37 ˚C for the rest of the enzymes for 6 h. An aliquot of the digestion product was resolved by gel electrophoresis

2.3.2.6 Agarose Gel Electrophoresis

One percent (1%) agarose gel was used to resolve DNA fragment. This was prepared by combining 2g agarose in ten times concentration of tris-borate ethylene diamine tetraacetate (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved. Exactly 0.7µl of ethidium bromide was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs was inserted. The gel was allowed to set for 30 minutes. The casting combs were carefully removed after the agarose gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 8µl of gel tracking dye (bromophenol blue) was added to 10µl of each sample with gentle mixing. The sample was loaded onto the wells of the gel at a concentration of 10µl, the mini horizontal electrophoresis gel setup was covered and electrodes connected. Electrophoresis was carried out at 100-200mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and visualized under UV light and documented.

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Klebsiella pneumoniae*

The isolated bacterium had characteristics of *K. pneumoniae*earlier described in the “Materials and Methods” section in tandem with those of the genus *Klebsiella* [10]. Tested urine samples were positive phenotypically for *K. pneumoniae*in a percent of 10.13 (38/375).

3.2 Antibiotic Resistance Profile of the *Klebsiella pneumoniae*Isolates

The resistance profile of the isolates to common antimicrobial agents is as shown in Table 3. Resistance was high to ampicillin (94.74%) and amoxicillin-clavulanic acid (73.6%) but low (less than 30%) to cefotaxime (13.16%), ceftriazone (18.42%), ceftazidime and cefoxitin (23.60%) and gentamicin (28.94%).

3.3 Antibiotic Resistance Phenotypes of the *Klebsiella pneumoniae*Isolates

The antimicrobial resistance phenotypes of the isolates are shown in Table 4. Thirty-three (33) different phenotypes were observed, with the most (7.98%) frequent being AMP, AMT, CIP, SXT, S.

3.4 Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates is shown in Table 5. The highest (31.57%) MAR index is for resistance to three antibiotics; while the least (2.63%) is for resistance to one, seven or eight antibiotics.

3.5Classes of Antimicrobial Resistance

The confirmed *K. pneumoniae* isolates were classified into different categories of antibiotic resistance namely; Multi-drug resistance (MDR), Extensive-drug resistance (XDR) and Pan drug resistance (PDR) as shown in Table 6. Most of the isolates were multi-drug resistant (MDR; 81.5%).

3.6Molecular Detection of Extended Spectrum Beta-Lactamase

The molecular detection of ESBL production in *K. pneumoniae* isolates is as shown in Table 7. All 15 isolates jointly resistant to cefotaxime and/or ceftazidime and ciprofloxacin 100.0% were ESBL producers, distributed as follows: $bla_{CTX\text{-}M\text{-}1}$ 7(46.6%); $bla_{CTX\text{-}M\text{-}3}$ 4(26.6%); bla_{SHV} 6(40.0%); bla_{TEM} 6(40.0%); and bla_{OXA} 5(33.3%).

3.7 Restriction Fragment Length Polymorphism

Restriction Fragment Length Polymorphism (RFLP), a technique used to identify differences or variations among organisms in their DNA sequences at sites recognized by specific restriction enzymes. Such variations and similarity result in different sized, or length DNA fragments produced by digesting the DNA with a restriction enzyme(s) (Table 2). In this study, out of the overall bacteria species isolated, 15 DNAs of the *Klebsiella* species from the 16SrRNA gene identified by polymerase chain reaction (PCR) amplicons were subjected to RFLP.

Table 1. Primers and their sequences

Target Gene	Primer Name	Sequence (5' – 3')	Product Size (bp)
blaCTXM-1	CTXM1-F	GACGATGTCACTGGCTGAGC	500
	CTXM1-R	AGCCGCCGACGCTAATACA	
blaCTXM-3	CTXM825-F	CGCTTTGCCATGTGCAGCACC	300
	CTXM825-R	GCTCAGTACGATCGAGCC	
blaSHV	SHV-F	AGGATTGACTGCCTTTTTG	992
	SHV-R	ATTTGCTGATTTCGCTCG	

blaTEM	TEM-C	ATCAGCAATAAACCAGC	716
	TEM-H	CCCCGAAGAACGTTTTC	
blaOXA	OXA-F	ATATCTCTACTGTTGCATCTCC	619
	OXA-R	AAACCCTTCAAACCATCC	

F= Forward; R= Reverse; bp = Base pair

Table 2. Endonucleases used for the restriction analysis of *K. pneumoniae* DNA

Endonuclease	**Restriction Site (5' to 3')**
PstI	CTGCA/G
XhoI	C/TCGAG
XbaI	T/CTAGA
EcoRI	G/AATTC
SmaI	CCC/GGG
NedI	CA/TATG

Table 3. Antimicrobial Resistance Profile of the urinary *Klebsiella pneumoniae* isolated in Nasarawa South Senatorial District, Nasarawa State, Nigeria

Antimicrobial Agent	**Disc Content (µg)**	**Number (%) Resistance**
Ampicillin (AMP)	10	36 (94.74)
Amoxicillin-Clavulanic acid (AMT)	30	28 (73.60)
Cefotaxime (CTX)	30	4 (13.16)
Ceftazidime (CAZ)	30	9 (23.60)
Ceftriaxone (CRO)	30	7 (18.42)
Cefoxitin (FOX)	30	9 (23.60)

Ciprofloxacin (CIP)	5	15 (39.47)
Co-trimoxazole (SXT)	25	14 (36.84)
Gentamicin (CN)	10	11 (28.94)
Streptomycin (S)	10	18 (47.36)

Table 4. Antimicrobial Resistance Phenotypes of the urinary *Klebsiella pnuemoniae*isolated in Nasarawa South Senatorial District, Nasarawa State, Nigeria

Antibiotic resistance phenotypes	Number (%) *K .pnuemoniae*isolates (n=38)
SXT	1 (2.63)
AMP AMT	2 (5.26)
AMP, CAZ	1 (2.63)
AMP, CIP	1 (2.63)
AMP, FOX	1 (2.63)
AMP, FOX, S	1 (2.63)
AMP, FOX, CN	1 (2.63)
AMP, AMT, CIP	1 (2.63)
AMP, CAZ, S	1 (2.63)
AMP, AMT, S	1 (2.63)
AMP, CIP, CN	1 (2.63)
AMP, CIP, S	1 (2.63)
AMP, AMT, CAZ	1 (2.63)
AMP, AMT, CRO	1 (2.63)
AMP, AMT, CN	2 (5.26)
CXT, SXT, S	1 (2.62)
AMP, AMT, FOX, S	2 (5.26)

AMP, AMT, CRO, CN	1(2.63)
AMP, CTX, FOX, S	1 (5.26)
AMP, AMT, CIP, S	1 (2.63)
AMP, AMT, CIP, SXT, S	3(7.98)
AMP, AMT, CRO, SXT, CN	1(2.63)
AMP, AMT, CAZ, FOX, S	1 (2.63)
AMP, AMT, CAZ, CIP, SXT	1 (2.63)
AMP, AMT, CAZ, CIP,, S	1 (2.63)
AMP, AMT, CAZ, CRO, SXT	1 (2.63)
AMP, AMT, CAZ, CRO, FOX, S	1 (2.63)
AMP, AMT, CTX, CIP, SXT,S	1 (2.63)
AMP, AMT,CIP,SXT,CN, S	1 (2.63)
AMP, AMT, CTX,CRO,CN,S	1 (2.63)
AMP,AMT, FOX, CIP, CN, S	1 (2.63)
AMP,AMT, CAZ,FOX,CIP,SXT, S	1 (2.63)
AMP, AMT, CAZ, CRO, CIP, SXT, CN, S	1 (2.63)

AMP= Ampicillin; AMT= Amoxicillin-Clavulanic acid; CTX= Cefotaxime, CAZ: Ceftazidime, CRO= Ceftriaxone; FOX= Cefoxitin; CIP= Ciprofloxacin; SXT= Co-trimoxazole; CN= Gentamicin; S= Streptomycin

Table 5. Multiple Antibiotic Resistance (MAR) Indices of the urinary *Klebsiella pnuemoniae*isolated in Nasarawa South Senatorial District, Nasarawa State, Nigeria

Number of Antibiotics isolate is resistant to (a)	Number of Antibiotics tested(b)	MAR index (a/b)	Frequency (%)
8	10	0.8	1 (2.63)
7	10	0.7	1 (2.63)
6	10	0.6	5 (13.16)

5	10	0.5	6 (15.79)
4	10	0.4	4 (10.52)
3	10	0.3	12 (31.57)
2	10	0.2	6 (15.79)
1	10	0.1	1 (2.63)

Table 6. Classes of Antimicrobial Resistance in *Klebsiella pneumoniae* from the urine of the patients

Classes of Antimicrobial Resistance	**No. (%) Isolates (n=38)**
NMDR	1(2.6)
MDR	31(81.5)
XDR	17(44.7)
PDR	0(0)

NMDR= Non-multi-drug resistance; MDR= Multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) [18]. No.= Number, %= Percentage

ESBL Resistance Genes	**No. (%) Isolates (n=15)**
$bla_{CTX\text{-}M\text{-}1}$	7(46.6)
$bla_{CTX\text{-}M\text{-}3}$	4(26.6)

bla$_{SHV}$	6(40.0)
bla$_{TEM}$	6(40.0)
bla$_{OXA}$	5(33.3)

Table 7. Molecular detection of Extended Spectrum Beta-Lactamase Resistance Genes in phenotypically confirmed ESBL producing *Klebsiella pneumoniae* from the urine of the patients

No.= Number; %= Percentage

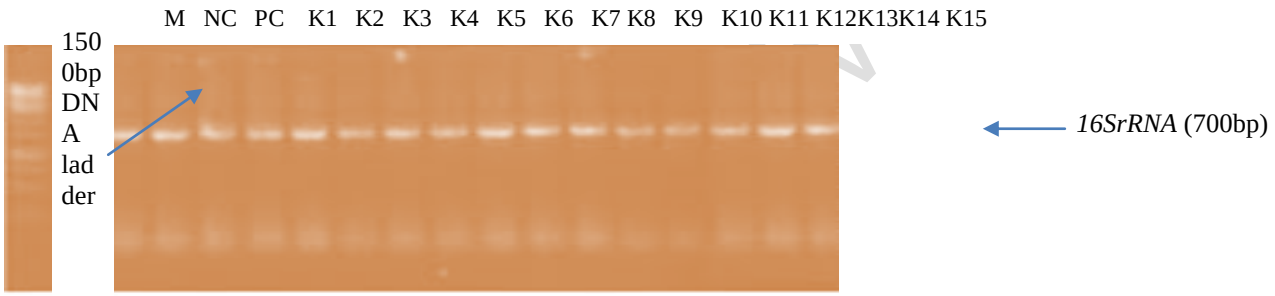

Plate 1: Agarose gel electrophoresis of the amplified *16SrRNA geneKlebsiella* species isolates. Lane NC represents Negative; Lane PC represents Positive control. Lane K1 K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14 and Lane K15 represent the expression of the *16SrRNA*(700bp) gene. Lane M represents 1500bp DNA molecular ladder.

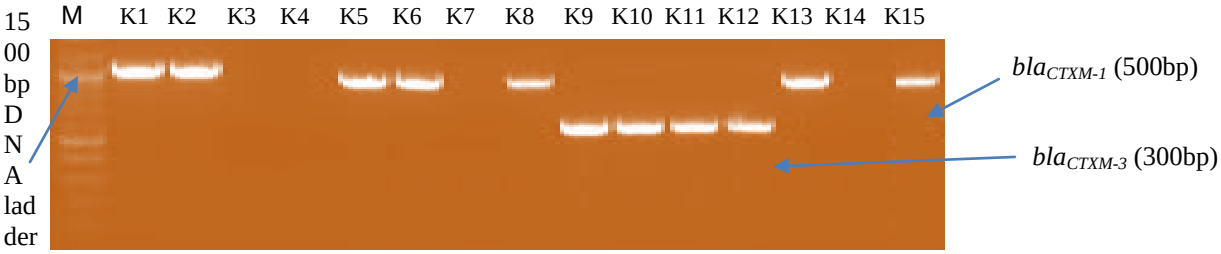

Plate 2: Agarose gel electrophoresis of the amplified ESBL genes in *Klebsiella* species. Lane K1 K2 K5 K6 K8 K13 and K14 represent the expression of the *bla*$_{CTXM-1}$ (500bp), Lane K9 K10 K11 and K12 represent the expression of the *bla*$_{CTXM-3}$ (300bp) gene, while Lane K3 K4 K7 and K14 did not show any band of any ESBL genes. Lane M represents 1500bp DNA molecular ladder.

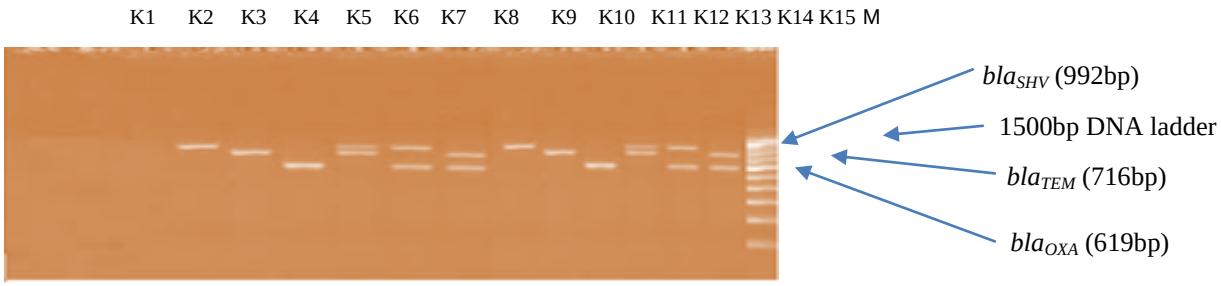

Plate 3: Agarose gel electrophoresis of the amplified ESBL genes in *Klebsiella*. Lane K4 K7 K8 K10K13 and K14 represent the expression of thebla_{SHV} (992bp), Lane K5 K7 K9 K11 K13 and K15 represent the expression of the bla_{TEM} (716bp) gene, Lane K6 K8 K9 K12 K14 and K15 represent the expression of the bla_{OXA} (619bp) while Lane K1 M K1 K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14 K15 represents 1500bp DNA molecular ladder.

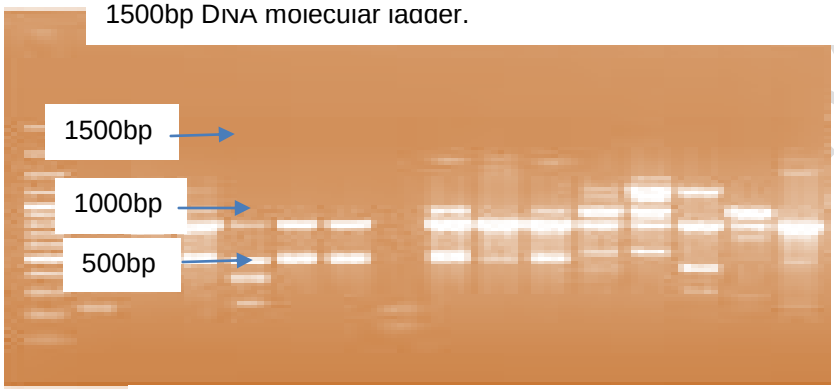

Plate 4: Restriction Fragment Length Polymorphism (RFLP) analysis of *Klebsiella* isolates cluster from different location. Each lane corresponds to an isolate' DNA. Identical and unidentical banding patterns were evident in all samples from Lane K1 to Lane K15.

The low prevalence rate for *K. pneumoniae* (10.13%) observed in this study is surprising, given that *K. pneumoniae* is commonly implicated in UTIs. However, the low prevalence rate is consistent with that of a study carried out in Kano State Nigeria, where 8.0% prevalence rate for *K. pneumoniae* was discovered [19]. Other studies reported similar results with prevalence rates of 8.33% and 10.0% [20, 21]. This result was somewhat lower than the percentage observed in different research performed in Anambra, which was 18.2% [22]. A Research in Benin, Edo State, however, found a much higher prevalent rate of 33.3% [23].

The antimicrobial resistivity testing reveal that Cephalosporins (Cefotaxime (86.84%), Ceftazidime (73.60%), Ceftriaxone (73.60%), and Cefoxitin (73.60%), together with gentamicin (71.05%), obtained acceptable sensitivity. This is in accordance with the findings of Ostojic *et al.* [24]. Co-trimoxazole, Ciprofloxacin [24] and Streptomycin [19] all had satisfactory therapeutic effects with sensitivity values of 63.16%, 57.89%, and 52.53%, respectively. Furthermore, *Klebsiella pneumoniae* isolated from the urine samples in the study areas were most resistant to Ampicillin (94.74%) and Amoxicillin-Clavulanic acid (73.60%) these findings are in agreement with those of Varghese *et al*., [25] and [19] whose findings reveals that 100% of the *K. pnuemoniae* isolated from urine are resistant to Ampicillin. As stated by [25], *K. pneumoniae* isolates are naturally resistant to Amoxicillin and Ampicillin, due to a constitutively expressed chromosomal class β lactamase.

The occurrence of MDR resistance isolates in this study (81.5%) was not different from the studies reported by [26] in India and [27] in Iraq where MDR *K. pneumoniae* was responsible for infection difficult to treat with antibiotics. The percentage occurrence of MDR isolates observed in this study was lower than 85.2% reported by [28]. The occurrence of XDR (44.7%) was not surprising as multiple reports have suggested XDR resistance is an emerging issue [29, 27] while absence of PDR resistance observed in this study was similar with the study described by [29]. This study addresses a lack of information and provides insight into the increasing incidence of MDR and XDR in hospitals, in Nasarawa State.

bla_{TEM}, $bla_{SHV,}$ $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}3}$ and bla_{OXA} ESBL genes were expressed in all the confirmed ESBL positive isolates. This finding is in agreement with the study described by [26], [27] and [30]. The occurrence of $bla_{CTX\text{-}M\text{-}1}$ gene (46.6%) was higher in this study location than other genes, and this finding is similar to a study by [28] where $bla_{CTX\text{-}M\text{-}1}$ was reported with a high occurrence of 64.8%. However, some studies seem to differ with this finding; [26] reported bla_{TEM} (49.4%) as the prevalent gene, [27] reported bla_{SHV} (65.8%) in his study as the prevalent gene, and [30] and [31] reported bla_{TEM}(38.0%, 63.1%) as the most prevalent gene.. The occurrence of bla_{OXA} ESBL gene observed in this study (33.3%) is higher than that reported by [26] and [30] (28.0%). An interesting observation from this study is the high occurrence of MDR and XDR *K. pneumoniae* isolates which points to a major antimicrobial resistance challenge. Also, the mechanism of resistance to *K. pneumoniae* isolates may not be due to the production of ESBL but may be due to other mechanisms of metabolic resistance.

4. CONCLUSION

The findings of this study underscore the alarming prevalence of multiple antibiotic and multidrug-resistant isolates within the study area, shedding light on a growing public health concern. It is evident that the emergence of resistance to commonly prescribed antibiotics has reached critical levels, necessitating urgent intervention. Intriguingly, despite the high resistance rates observed, there were pockets of hope in the form of low resistance to certain antibiotics, including cefotaxime, ceftriaxone, ceftazidime, cefoxitin, and gentamicin. Notably, these antibiotics remain valuable in the fight against ESBL-producing *Klebsiella pneumoniae* strains, representing essential options for clinical management. The identification of ESBL resistance, particularly in isolates concurrently resistant to cefotaxime and ceftazidime, further emphasizes the challenges faced in treating these infections. These ESBL genes, namely bla_{TEM}, bla_{SHV}, $blaCTX_{\text{-}M\text{-}3}$ and bla_{OXA} with $bla_{CTX\text{-}M\text{-}1}$ as the prevailing subtype, are fully expressed, contributing to the resistant phenotype observed.

ETHICAL APPROVAL

Authors obtained all necessary ethical approval from suitable Institutional or State or National or International Committees. The ethical clearance is as attached in the appendix 1.

REFERENCES

1. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N. & Cars O. Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases*, 2013:13(12), 1057-1098.

2. Borek AJ, Santillo M, Wanat M, Butler CC, & Tonkin-Crine S. How Can Behavioural Science Contribute to Qualitative Research on Antimicrobial Stewardship in Primary Care? *JAC Antimicrobial Resistance*. 2022: 4, dlac007.

3. Dionisio F, Baquero F, Fuertes M. Psychological and Cultural Factors Influencing Antibiotic Prescription. *Trends in Microbiology*. 2023: 29.

4. Bush K, & Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. *Cold Spring Harbor perspectives in medicine*. 2016: 6(8), a025247. DOI: 10.1101/cshperspect.a025247.

5. Li Y, Kumar S, Zhang L, & Wu H. Characteristics of antibiotic resistance mechanisms and genes of *Klebsiella pneumoniae*. *Open Medicine*. 2023: 18(1). DOI: 10.1515/med-2023-0707.

6. World Health Organization (WHO). (2021). Antimicrobial resistance. Website. Accessed on September 9th, 2023.

7. Ashefo DP, Ngwai YB, Ishaleku D. Isolation and antimicrobial resistance phenotype of Klebsiella pneumonia from the urine of suspected UTI patients attending public hospitals in Nasarawa south senatorial district, Nasarawa state, Nigeria. *FUDMA Journal of Sciences (FJS)*. 2023: 7(1), 119 – 125.

8. Onyedibe KI, Dibal NK, &Adesida SA. Extended-spectrum beta-lactamases production by *Klebsiella pneumoniae* and *Escherichia coli* isolates in a tertiary hospital in Nigeria. *The Pan African Medical Journal*. 2021: 35, 11.

9. Zilberberg MD, and Shorr AF. Secular trends in gram-negative resistance among urinary tract infection hospitalizations in the United States, 2000-2009. *Infection Control Hospital Epidemiology*. 2013:34, 940–946. PubMed

10. Cheesbrough M. District laboratory practice in tropical countries. 2006. Cambridge University Press

11. Bergey's Manual of Determinative Bacteriology. Identification Flow Charts. Available:http://mysite.science.uottawa.ca/jbasso/microlab/IDFlowcharts.pdf. Accessed on July 2023

12. Clsi C. Performance standards for antimicrobial susceptibility testing: 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute; 2015.

13. Giriyapur RS, Nandihal NW, Krishna BVS, Patil AB, Chandrasekhar MR. Comparison of disc diffusion methods for the detection of extended-spectrum Beta lactamase-producing enterobacteriaceae. Journal of Laboratory Physicians. 2011;3(1):33.

14. Krumperman PH. Multiple antibiotic indexing *Escherichia coli* to identifying risk sources of faecal contamination of foods. Applied Environmental Microbiology. 1983; 6(1):165-170.

15. Porteous LA, Armstrong JL, Seidler RJ, Watrud LS. An effective method to extract DNA from environmental samples for polymerase chain reaction amplification and DNA fingerprint analysis. Current microbiology. 1994;29(5):301-307

16. Abimiku RH, Ngwai YB, Nkene IH, and Tatfeng YM. Molecular detection of diarrheagenic pathotypes of *Escherichia coli* from diarrheic patients in Keffi, Nigeria. *Journal of Microbiology and Biomedical Researc*h. 2016: 2(3), 1-6.

17. Umeaku CN, Chukwudi CC, Njideka OC, Okwukweka NF, Chineze AC, &Ugonwa Chiagoziem, UU. Isolation of Multi-Resistant Bacteria of Patients Suffering from Urinary

Tract Infections in Owerri Metropolis, Imo State, Nigeria. *Acta Scientific Microbiology*. 2021: 4(9), 03-16.

18. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, & Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2012: 18(3), 268–281. DOI: 10.1111/j.1469-0691.2011.03570.x

19. Hamza S, Abdulhadi S, and Kumurya S. The Prevalence of Klebsiella Species Causing Urinary Tract Infections in Murtala Muhammad Specialist Hospital, Kano, Nigeria. *American Journal of Biomedical and Life Sciences*. 2016: 4(2), 11-15. DOI: 10.11648/j.ajbls.20160402.11

20. Vranic SM, Zatric N, Rebic V, Aljicevic M, and Abdulzaimovic A. The Most Frequent Isolates from Outpatients with Urinary Tract Infection. *Materia Socio Medica*. 2017: 29, 17–20.

21. Sokhn ES, Salami A, El Roz A, Salloum L, Bahmad HF, and Ghssein G. Antimicrobial Susceptibilities and Laboratory Profiles of *Escherichia coli*, *Klebsiella pneumoniae*, and Proteus mirabilis Isolates as Agents of Urinary Tract Infection in Lebanon: Paving the Way for Better Diagnostics. *Medical sciences (Basel, Switzerland)*. 2020: 8(3), 32. DOI: 10.3390/medsci8030032

22. Ogbukagu CM, Anakwenze VN, Ekwealor CC, Ezemba CC, and Ekwealor IA. Incidence of Urinary Tract Infections (UTI) amongst Patients Attending Primary Health Centres in Anambra State. *Advances in Microbiology*. 2016: 6, 537-547. DOI: 10.4236/aim.2016.67054

23. Osazuwa F, Mordi RM, Osazuwa E, Taiwo SS, Alli OAT, Ogbolu DO, Akanni EO, and Anukam KC. *Klebsiella* has taken lead among uropathogens in University of Benin Teaching Hospital, Benin City, Nigeria-An observation. *New York Science Journal*. 2010: 3(11), 61-64.

24. Ostojic M, Hubana M, Cvetnić M, Benić M, and Cvetnić Z. Antimicrobial resistance of *Klebsiella pneumoniae* strains isolated from urine in hospital patients and outpatients. *Archives of Biotechnology and Biomedicine*. 2021: 5, 001-007.

25. Varghese A, George S, Gopalakrishnan R. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from cases of urinary tract infection in a tertiary care setup. Journal of Evolutionary Medicine and Dental Sciences. 2016: 5(29): 1470-1474. DOI: 10.14260/jemds/2016/346.

26. Verma S, Kalyan RK, Gupta P, Khan MD, & Venkatesh V. Molecular Characterization of Extended Spectrum β-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae*Isolates and Their Antibiotic Resistance Profile in Health Care-Associated Urinary Tract Infections in North India*. Journal of Laboratory Physicians*. 2022: 15(02), 194-201.

27. Al-Hashimy AB, & Al-Musawy WK. Molecular Study and Antibiotic susceptibility patterns of some Extended Spectrum Beta-Lactamase Genes (ESBL) of Klebsiella pneumpniae in Urinary Tract Infections. *Journal of Physics: Conference Series.* 2021: 1660(1), 012017.

28. Bakr KI, Abdul-Rahman SM, &Hamasalih RM. Molecular detection of β-lactamase genes in *Klebsiella pneumoniae* and *Escherichia coli* isolated from different clinical sources. *Cellular and Molecular Biology*. 2021: 67(4), 170-180.

29. Farhadi M, Ahanjan M, Goli HR, Haghshenas MR, & Gholami M. High frequency of multidrug-resistant (MDR) *Klebsiella pneumoniae* harboring several β-lactamase and integron genes collected from several hospitals in the north of Iran*. Annals of clinical microbiology and antimicrobials*. 2021: 20(1), 1-9.

30. Mohammed AB, & Anwar KA. Phenotypic and genotypic detection of extended spectrum beta lactamase enzyme in *Klebsiella pneumoniae*. *PloS one*. 2022: 17(9), e0267221.

31. Haghighifar E, Norouzi F, &Dolatabadi RK. Molecular detection of Extended-Spectrum β-lactamases (ESBLs) and biofilm formation in uropathogen*Klebsiella pneumoniae* in Iran. *Medical Journal of the Islamic Republic of Iran*. 2021: 35, 72.