

Molecular Detection of Extended Spectrum Beta-Lactamase Gene in *Klebsiella* Species from Clinical Isolates Obtained From Plateau State Specialist and Jos University Teaching Hospital

ABSTRACT

Aims: The aim of the study was to determine the antimicrobial susceptibility of *klebsiella species*, prevalence of Extended Spectrum Beta-Lactamases (ESBLs) and the gene coding ESBL production in *klebsiella* isolates obtained from clinical specimens of patients attending Plateau State Specialist and Jos University Teaching Hospitals both in Jos North Central of Nigeria.

Study Design

This was hospital based study conducted in the Department of Medical Microbiology unit of Plateau State Specialist and Jos University Teaching Hospital (JUTH) between the periods of January to April 2020.

Methodology: Out of 156 non-motile, oxidase negative gram negative organisms collected, 137 isolates were identified to be *Klebsiella species* using oxoid Microbact 24E kit for Enterobacteriaceae identification and Microsoft for bacteria identification. Antimicrobial Susceptibility Testing (AST) and ESBL screening was done by Kirby-Bauer disk diffusion method using oxoid Single disk antibiotics which include Ceftazidime (30µg), Cefotaxime (30µg), Imipenem (10µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ampicillin (10 µg), Aztreonam (30 µg), and Azithromycin (15 µg) on Mueller Hinton Agar (oxid). Break point was determined by CLSI AST guidelines. The isolates that showed resistance or reduced susceptibility to Ceftazidime and Cefotaxime are suspected to be ESBL positive *Klebsiella*. Phenotypic confirmation of ESBL positive isolates was carried out using Double Disk Synergy Test (DDST) with Ceftazidime 30µg (oxid), Cefotaxime 30µg (oxid) and Amoxicillin Clavulanic acid 20/10ug (Oxid).

Results: Antimicrobial Susceptibility Test showed Imipenem to be highly sensitive to ESBL positive *klebsiella pneumonia* with 100% sensitivity. Gentamicin, Ampicillin, and Azithromycin showed reduced susceptibility. Genotypic detection of ESBL genes was done by PCR: Genotypic results showed prevalence of bla TEM, bla SHV gene at 868bp and 972bp respectively with no occurrence of bla CTX-M gene. There was multiple occurrence of gene in some of the isolates tested while some recorded no ESBL gene. The ESBL prevalence of 15.3% was recorded in this study.

Conclusions The prevalence of 15.3% recorded in this study is low compared to other studies from some other states within the country. Efforts should be put in place to ensure non-proliferation of this ESBL *Klebsiella* strain as it creates not only a therapeutic challenge but health problem.

Keywords: *Klebsiella pneumoniae*, ESBL, Bla TEM gene, Bla CTX-M gene and Bla SHV gene.¹

INTRODUCTION

Antimicrobial resistance (AMR) is an urgent global public health threat, killing at least 1.27 million people worldwide [1]. Over the years, the misuse of antimicrobials in humans, animals and plants, poor prescribing practices, lack of patient adherence to prescriptions' and global travel have accelerated the threat of AMR worldwide [2]. According to World Health Organization the resistance is fueled by lack of access to clean water, poor hygiene, poor sanitation and inadequate infection prevention and control practices. These factors promote the spread of microbes some which harbour resistant gene. Extended Spectrum Beta- Lactamase (ESBLs) are enzymes produced primarily by the Enterobacteriaceae family of Gram-negative organisms with particular reference to *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus* and these enzymes are located on plasmids and can be transferred among strains and between bacteria [3]. ESBL is one of the major factors contributing to treatment Failures in the continents when new generation antibiotics are used. [4] ESBL hydrolyzes and cause resistance to beta- lactam antibiotics which include Cephalosporins, Monobactams and Aztreonam. They are inactive towards Cephamycins, Carbapenems and are inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [5,6,7]. The frequently encountered genes coding for ESBL production are Cefotaximases (CTX-M), Temonira (TEM), Sulfhyly (SHV), and oxacillinases (OXA) [8]. Risk factors associated with infection or colonization of ESBL producing microorganisms are prolonged and /or recent hospitalization, stay in nursing homes, catheterization, use of invasive medical device and inappropriate use of antibiotics especially those belonging to third-generation cephalosporins [9]. Other underlying conditions are persons with compromised immune system such as alcoholism, diabetes mellitus, cirrhosis, chronic renal failure, cancer, and transplants. ESBL producing *Klebsiella species* are distributed worldwide and are of increasing prevalence since its detection. A large number of outbreaks of infection caused by ESBL-producing organisms have been described on every continent of the globe except Antarctica [10]. There is a high rate of resistance among antibiotics commonly used to treat bacterial infections and it's been observed globally. The annual cost for Antimicrobial Resistance (AMR) in USA healthcare system has been estimated to be from \$21 to \$34 billion and estimated 23, 000 deaths recorded annually, with more than two million persons infected with resistant bacteria [11]. Deaths due to drug-resistant infections are projected to increase from 700, 000 to 10 million annually and the cost may reach as high as US\$100 trillion worldwide by 2050 [12]. In Africa, the dearth of information regarding Antimicrobial Resistance (AMR) is hampered by inadequate data. This is because surveillance of drug resistance is limited to a few countries, thereby resulting in incomplete data on the true nature of this problem [13]. A recent surveillance report in 22 countries published by the WHO has shown a high level of AMR, reporting *E. coli* and *K. pneumoniae* as the two most common resistant bacteria [14]. Unregulated use of antibiotics, coupled with over the counter sales of antibiotics without prescription in Nigeria has led to an increased emergence of ESBL producing gram negative organisms [15]. A systematic review reveals that lack of data on the occurrence of multi drug resistant Gram-negative bacteria especially *Klebsiella pneumoniae* is greatest in West Africa including Nigeria [16]. In an attempt to fill this epidemiological gap hence this study. The study was aimed at providing information on the prevalence of ESBL producing *klebsiella Species* and molecular detection of the gene responsible for the production of the ESBL in Jos plateau state. The molecular

detection of the gene would be a reliable approach in designing and implementing effective diagnostic procedures, infection control and treatment policy in Jos, Plateau State Nigeria.

2.0 METHODOLOGY

2.1 Study design

This was hospital based study conducted in the Department of Medical Microbiology of Plateau State Specialist and Jos University Teaching Hospital (JUTH). Both hospitals are located in Jos. Jos is a city in the middle belt of Nigeria with a population of about 900,000 residents based on the 2006 census. JUTH is a federal tertiary institution while PSSH is a state tertiary health institution. The two health institutions pursue excellence in the provision of service, training and research. Their services are not only to patients of the hospitals but to other public and private hospitals within Plateau state and its surrounding states. The study design was to investigate the antimicrobial susceptibility pattern of *Klebsiella* species, prevalence of ESBL producing *Klebsiella* species and to identify the gene coding the production of Extended Spectrum Beta Lactamases.

2.2 Determination of sample size

The sample size was determined by the formula defined by IFAD, 2013.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Where

n= required sample size

t= confidence level at 95% = 1.96 (standard value)

p= established local prevalence = 9.25% [10]

m=margin of error at 5%=0.05

$$n = \frac{1.96^2 \times 0.092(1-0.092)}{0.05^2} = 129$$

156 samples were collected for this study.

2.3 Isolate collection

156 non-motile, oxidase negative gram negative bacilli suspected to be *Klebsiella* species were obtained from Samples of inpatients and outpatients attending Plateau State Specialist and Jos University Teaching Hospitals for various infections during the period of study, January to April 2020. The isolates were subcultured on a fresh nutrient agar slant and incubated for 24hours at 37°C. The slant with growth were removed the next day and stored in the refrigerator. Sources of samples include urine, sputum, blood culture, wound swab. Ear swab throat swabs, Catheter and Hvs.

2.4 Laboratory analysis

The isolates were further identified using oxoid Microbact 24E kit for Enterobacteriaceae identification.

2.4.1 Preparation of inoculum

An isolated colony from an 18 – 24 hours culture was emulsified in 5ml of 0.85% sterile saline solution and mixed thoroughly to get homogenous suspension.

2.4.2 Standardization of inoculum

The homogenous suspension was later adjusted to 0.5 McFarland's standard which was prepared from 1.175% barium chloride and 1% sulphuric acid. McFarland's solution was used to match the turbidity (cloudiness) of the test organisms.

2.4.3 Inoculation

Automated micro pipette was used to aseptically transfer 100µl aliquot of the bacterial suspension into each well in the Oxoid Microbact 24E kit tray. Microbat mineral oil was used to overlay wells 20 and 24 according to manufactures instruction.

2.4.4 Incubation

Incubation was done at 24 hours. The purity of each inoculum was determined by inoculating the test suspension on freshly prepared MacConkey agar plate to act as a culture purity check.

2.5 Reading the test strip

The plates were read after 24 hours. The reactions were evaluated as positive or negative by comparing them with a color chart. Specie identification was done using software for bacterial identification.

2.6 Antimicrobial susceptibility testing/ Screening of the Isolates

Disc-diffusion antibiotic susceptibility test also known as Kirby Bauer test was used to carry out the sensitivity test. Muller Hinton Agar (Oxoid) was prepared according to the manufacturer's instructions and autoclaved at a temperature of 121°C at 15 psi of pressure for 15 minutes. The prepared Mueller Hinton was aseptically poured into a 100mm diameter petri dish, allowed to set. The plates were dried inside the incubator. Sterile cotton swab was dipped into inoculum and excess liquid removed by pressing against the tube. The swab stick was used to streak the plate lightly and uniformly in three directions rotating the plate at an angle of 90°C each time to ensure even distribution. A total of 6 Single Antibiotics disks (oxoid) were placed not less than 24mm apart on an agar plate of 100mm according to CLSI guide lines [17].The inoculated plates were incubated at 37°C for 24 hours. The plates were read by measuring in millimeter zones of inhibition using a meter rule. Antibiotics disks used in this study include Imipenem (10µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ampicillin (10 µg), Aztreonam (30 µg), Azithromycin (15 µg), Ceftazidime (30 µg) and cefotaxime (30 µg).

The following precautions were taken:

- Too much antibiotic disk on the plate was avoided to prevent overlapping which made reading the zone of inhibition difficult.
- To ensure reliability of test result, purity checks were done on the inoculum using a non-selective media.

The isolates that showed reduced susceptibility or resistance to Ceftazidime and Cefotaxime after antimicrobial sensitivity tests were suspected to be ESBL producers [1].

2.7 Confirmation of ESBL producers by double disk synergy (DDS) Test

Detection of Extended Spectrum Beta- lactamase (ESBL) producers was done by double- disk synergy. Amoxicillin-clavulanic acid disk (20/10 µg) was placed at the Centre of the freshly inoculated MHA plate. Third generation cephalosporin Ceftazidime (30 µg) and cefotaxime (30 µg) were placed 15 mm apart from the center of the amoxicillin-clavulanic acid disk. The plates were incubated at 37°C for 24 hours; a clear extension of the zone of inhibition towards amoxicillin-clavulanic acid disk was interpreted as ESBL production.

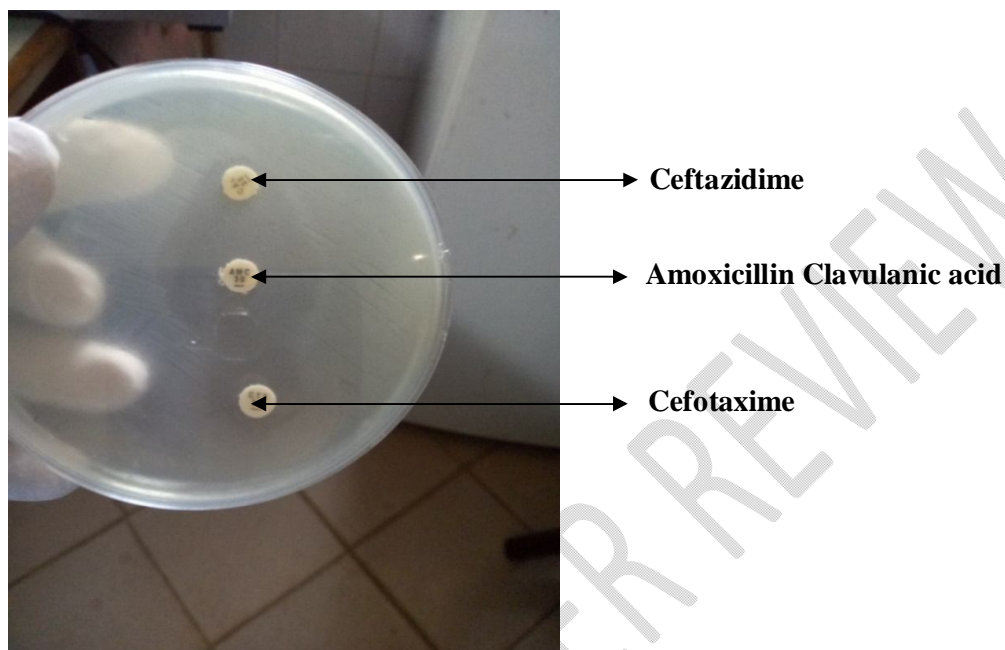


Fig 1: Plate showing extended clear zone of inhibition towards Amoxicillin-Clavulanic acid.

MOLECULAR ANALYSIS

Molecular analysis was done at National Veterinary Research institute (NVRI) Vom Plateau State Nigeria.

2.8 DNA extraction

The DNA extraction was done with QIA amp® DNA mini kit following the manufactures instructions.

2.9 Polymerase chain reaction amplification of bla TEM, CTX-M, SHV gene

The genes were amplified under the following temperature conditions. Initial denaturation 95°C for 5min. Denaturation 95°C for 30 seconds 30 cycle, primer annealing for SHV 58°C for 1 minute, TEM primer 56^o for 1min. Extension 72°C for 1 minute 30 seconds Final extensions 72°C for 10 minutes.

Chart 1. Primer Sequence

TARGET GENE	PRIMER NAME	PRIMER SEQUENCE (5'-3')	APPLICON SIZE
Bla _{TEM}	TEM-F	5'TCCGTCATGAGACAATAACC3' (Forward)	972bp
	TEM-R		

5TTGGTCTGACAGTTACCAATGC3'

(Reverse)

Bla _{SHV}	SHV-F	5'TGGTTATGCGTTATATTCGCC3'	868bp
	SHV-R	5'GGTTAGCGTTGCCAGTGCT3' (Reverse)	
Bla _{CTX-M}	CTX-F	5'ATGTGCACCAGTAARGT3' (Forward)	
	CTX-R	5'TGGGTRAARTARGTSACCAGA3' (Reverse)	

2.10 AGAROSE GEL ELECTROPHORESIS

Gel electrophoresis is a method for separation and analysis of bio macromolecules and their fragments, based on their size and charge by running them through a sieve like matrix using electricity. Agar Rose powder was mixed with an electrophoresis buffer to 1.5% concentration inside a conical flask and heated in the microwave to melt. The gel was allowed to cool to about 55°C. 5ul of ethidium bromide was carefully added to facilitate visualization. The gel was poured into the electrophoresis tray sample containing the comb. The gel was let left to solidify at room temperature for 1 hour. The comb was removed and solidified agar placed horizontally into the electrophoresis chamber and covered with a buffer. The extracted DNA was mixed with a loading buffer and pipetted into the sample wells. The DNA ladder was placed next to the samples to serve as reference. It was connected to electric current and was set to separate at 150volts for 60 minutes. The stained gel was observed at the expected band position and photographed using an ultraviolet light illuminator. The results showed bla-SHV gene present at band size of 868bp and bla-TEM gene at 972bp

2.11 STATISTICAL DATA ANALYSIS

Statistical data analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) Version 23A. (P value = 0.05) was considered as statistically significant. Results was presented in tables, charts and figures as appropriate.

3.0 RESULTS AND DISCUSSION

Table 1 shows the distribution of *klebsiella* isolates. According to the table *Klebsiella Pneumonia* had the highest isolation rate of 109 (79.6%), *Ozaenae* 17 (12.4%) and *Klebsiella Planticola* 11 (8.0%).

Table 1: Distribution of *klebsiella* Isolates according to *Species*

Organisms	Frequency	Percentage (%)
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<i>K pneumoniae</i>	109	79.6
<i>K. planticola</i>	11	8.0
<i>K. ozaenae</i>	17	12.4
Total (N)	137	100.0

Fig 2 shows the anti-biogram of *Klebsiella* species from clinical specimens obtained from JUTH and PSSH. The chart represents the overall profile of the antimicrobial Susceptibility testing result of *klebsiella* species to the selected antibiotics. According to the chart the highest sensitivity was recorded with Imipenem with 98.5% while Ampicillin had the lowest sensitivity of 0.7%.

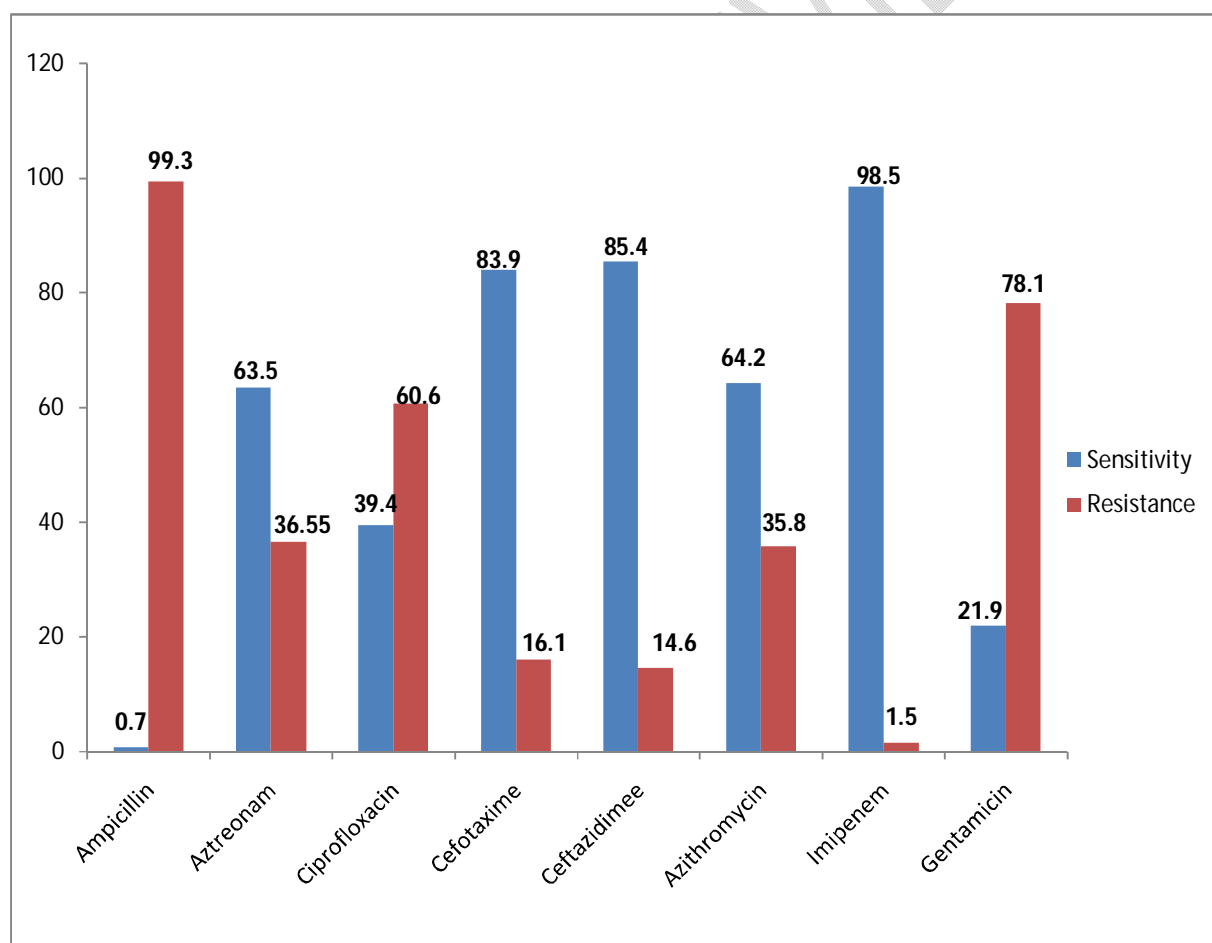


Figure 2: Anti-biogram of *Klebsiella* species clinical isolates obtained from JUTH and PSSH

Table 2 shows the antibiotics sensitivity and resistance pattern of ESBL producing isolates. Antibiotic sensitivity to the ESBL positive *Klebsiella* isolates revealed Imipenem to be 100%.

Table 2: Antibiotics sensitivity and resistance pattern of ESBL producing *klebsiella* isolates among the study population

Antibiotics	Sensitivity	Resistant
Ampicillin	0(0.0)	21(100.0)
Aztreonam	1(4.8)	20(95.2)
Ciprofloxacin	11(52.4)	10(47.6)
Cefotaxime	1(4.8)	20(95.2)
Ceftazidime	2(9.5)	19(90.5)
Azithromycin	0(0.0)	21(100.0)
Imipenem	21(100.0)	0(0.0)
Gentamicin	0(0.0)	21(100.0)

Table 3 shows The Distribution of ESBL and non-ESBL producing *klebsiella species*. The occurrence of ESBL according to this study was from *klebsiella pneumonia* with ESBL prevalence rate of 21(15.3 %) while *planticola* and *Klebsiella Ozaenae* had no incidence of ESBL.

Table 3: Distribution of ESBL and Non-ESBL *Klebsiella Species* from the Clinical Specimen

Organisms	Number of Isolates	ESBL Producers (%)	Non ESBL Producers (%)	P Value
K. Pneumonia	109	21(19.3)	88(80.7)	0.41
K. Planticola	11	0(0.0)	11(100.0)	
K. Ozaenae	17	0(0.0)	17(100.0)	
Total	137	21(15.3)	116(84.7)	

Table 4 shows the prevalence of ESBL and Non ESBL *Klebsiella* isolates in relation to hospitals. (JUTH) had prevalence of ESBL positive *Klebsiella* of 13 (14.1%) 92 while Plateau State Specialist Hospital (PSSH) had 8(17.5%) 45.

Table 4: Prevalence of ESBL producing and Non-ESBL producing *klebsiella* isolates in relation to Hospital.

Hospital	Number of Isolates	ESBL Producer (%)	Non ESBL Producer (%)	P Value
JUTH	92	13(14.1)	79(85.9)	0.58
PSSH	45	8(17.8)	37(82.2)	
Total	137	21(15.3)	116(84.7)	

Table 5 shows the distribution of ESBL positive and ESBL negative *klebsiella* according to gender. The results revealed that female gender had more ESBL positive *Klebsiella* than male gender.

TABLE 5: Distribution of ESBL-Positive and ESBL-Negative klebsiella isolates according to gender of patients.

GENDER	NUMBER OF ISOLATES	ESBL POSITIVE	ESBL NEGATIVE	P VALUE
MALE	75	14(18.7)	61(81.3)	0.23
FEMALE	62	17(27.4)	55(88.7)	
TOTAL	137	21(15.3)	166(84.7)	

P value 0.23= Not statistically significant.

Fig 3 shows the distribution of ESBL positive *Klebsiella* isolates according to units and wards. The Female Orthopedic Ward (FOW) and Male Medical Ward (MMW) recorded higher rates of 50% respectively while General out Patients Department (GOPD) recorded the least 7.2%.

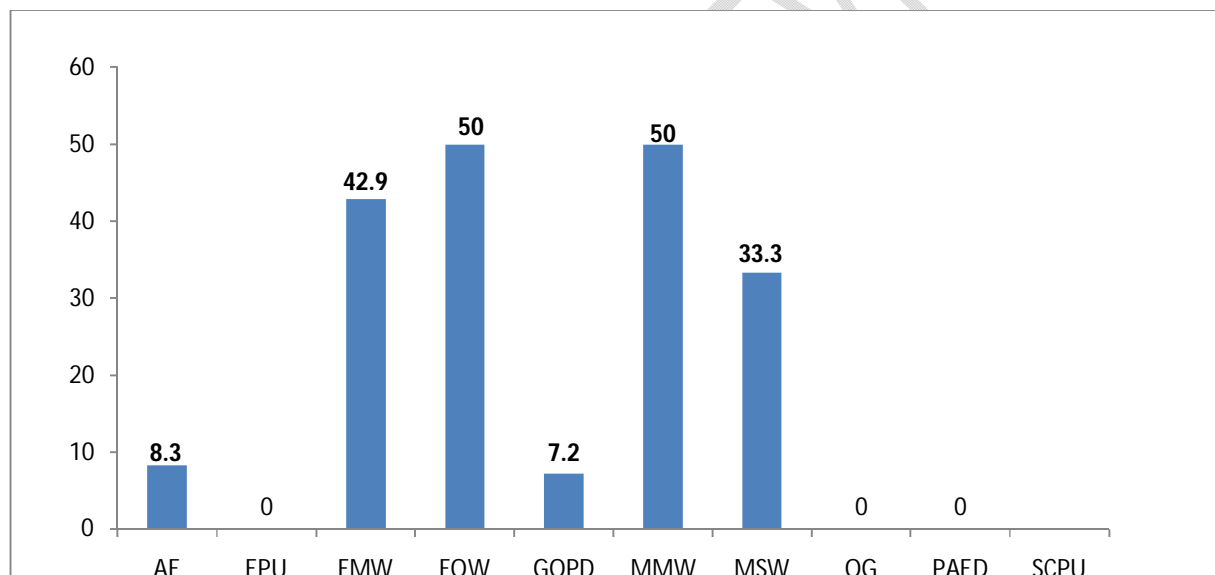


Figure 3: Distribution of ESBL positive *Klebsiella* isolates according to units and wards A&E (accident and emergency ward), Female Medical Ward (FMW), Female Orthopedic Ward (FOW), General out Patients Department (GOPD), Male Medical Ward (MMW), Male Surgical Ward (MSW), Obstetrician & Gynecology (O&G), Pediatrics (PAED), Special Care Baby Unit (SCBU), and Emergency Pediatrics Unit (EPU).

Fig 4 shows the Pictures of ESBL Genes Bla TEM (above) and Bla CTX-M (below) on 1.5% agarose gel electrophoresis. The TEM gene was present at band size of 868 base pair. There was no CTX-M gene in the isolates tested.

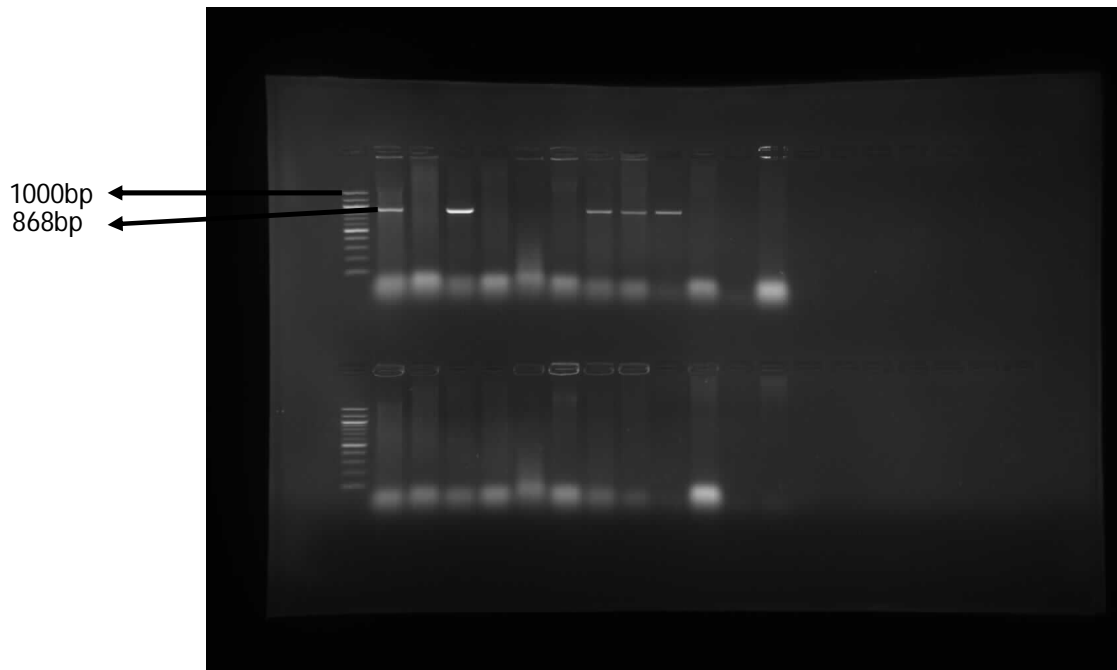


Figure 4: Pictures of ESBL Genes *Bla TEM* (above) and *Bla CTX-M* (below) on 1.5% agarose gel electrophoresis. Lane 1: 100-bp Maker, lane 1 to 10 *klebsiella Pneumoniae* isolates and lane 11.Nuclease free water

Figure 5 shows the Picture of ESBL gene *Bla SHV* on 1.5% agarose gel electrophoresis. The result revealed the presence of SHV gene at 972 base pair.

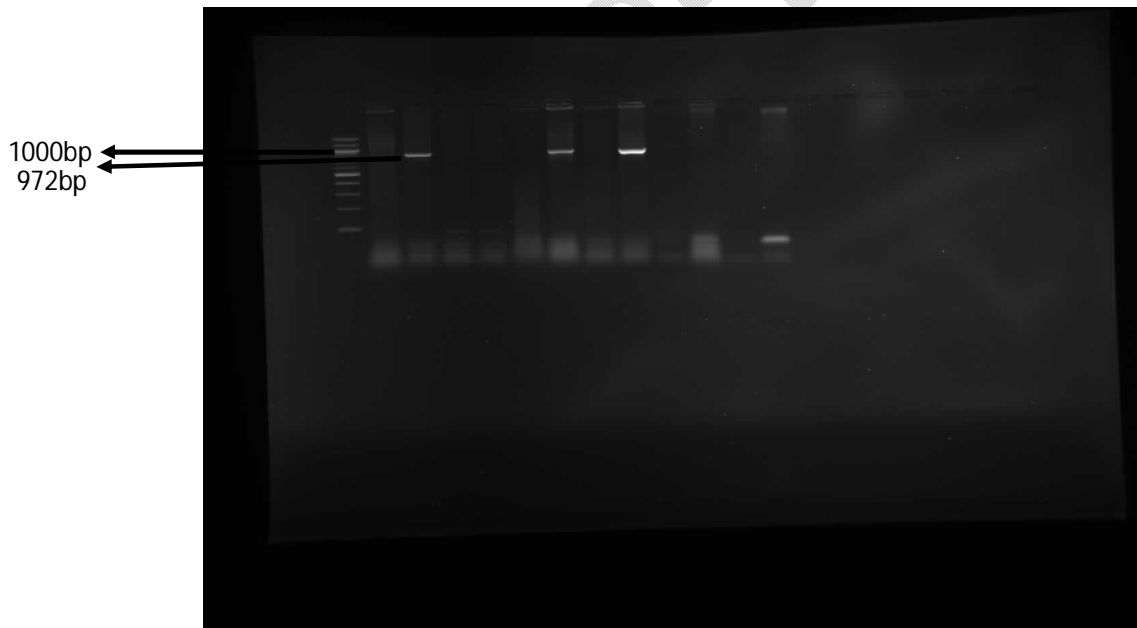


Figure 5: Picture of ESBL gene *Bla SHV* on 1.5% agarose gel electrophoresis. Lane 1:100-bp Maker, lane 1 to 10 *Klebsiella pneumoniae* isolates and lane 11. Nuclease free water

DISCUSSION

A total of 137 *Klebsiella* species consisting of *K.Pneumoniae* 109(79.6%) *K.Ozaenae* 17(12.4%) and *Planticola* 11(8%) were identified. This study recorded *Klebsiella pneumoniae* with highest isolation rate this could be attributed to the fact that among the three medical important species of the genus *Klebsiella* associated with illness in humans, *K.Pneumoniae*, *K.Oxytoca* and *Granulomatis*, *K.Pneumoniae* accounts for significant proportion of hospitals and community acquired infection.

Antimicrobial susceptibility of the *Klebsiella* species showed Imipenem antibiotics to be highly sensitive with 98.5% sensitivity. Ampicillin had the least (21.9 %) the reason could be that ampicillin was used in the past for treatment of most bacterial infections and this might have led to microbial resistance.

The Antibiotic sensitivity and resistance pattern of ESBL producing *Klebsiella Pneumoniae* showed Imipenem to be highly sensitive at the rate of 21(100%). Increased antibiotic resistance was recorded in the following antibiotics Ampicillin, Gentamicin, Azithromycin, Cefotaxime and Ceftazidime. The reason for this finding might be attributed that ESBL hydrolyses Beta-lactam antibiotics ring such as Penicillin, Monobactams, and Cephalosporin's and are inactive towards Carbapenems and Cephamycins. The ESBL genes which are plasmid located confers resistance to other classes of antibiotics which include Aminoglycosides, Macrolides and fluoroquinolones [5,6,7].

This research finding is in agreement with the work done at Ile-Ife and Kano where Imipenem showed 100% sensitivity to ESBL positive *K. pneumoniae* with more resistance to penicillin, Aminoglycosides and third generation Cephalosporins antibiotics [18] [19].The prevalence of 15.3% ESBL was recorded in this study and that was found only in *klebsiella pneumoniae* .Reason *K.Pneumoniae* and *E.coli* is the major producers of ESBL in the family Enterobacteriaceae [3].

Comparisons of the prevalence rate of ESBL *Klebsiella pneumoniae* found in this study with other studies using the same method of screening and confirmation of ESBL (Double Disk Synergy Test): A higher prevalence of 36.4% was recorded in Kano and 40% in Zaria North West region of the country [18][3] Other studies done outside the region which reported higher prevalence rate include 56.6% (South East) [19]33.9% (South West) [20] and 43.8% (South South) [15] at Enugu, Ile-Ife and Benin in 2008, 2010 and 2015 respectively. Some studies conducted outside Nigeria had also shown variations in ESBL prevalence's. Studies with higher prevalence include the study done at Ethiopia in 2018 where it recorded a prevalence rate of ESBL *klebsiella Pneumonia* of 23.5% [21], 53.8% in Saudi Arabia [22] and 39.5% in Iran [23].The present study is higher when compared to previous studies done in Kano in 2010 where ESBL was reported to be 9.25% [10] . Similarly a lower ESBL prevalence rate of 9.1% was recorded in Akwa [24] and 5% in Ogun State. It may be difficult to make a valid comparison of the prevalence of ESBLs because of the variation in study design.

The prevalence of ESBL producing *Klebsiella* species in relation to hospital showed that PSSH had a higher rate 17.8% than JUTH 14.1% though not statistically significant. Plateau State Specialist Hospital is located in the city center and because of its proximity receives many patients with severe health challenges from referrals and different private hospitals within the city. These might lead to

increased use of antibiotics by the clinicians without screening for ESBL thus contributing to proliferation of the ESBL *klebsiella* strain.

The distribution of ESBL positive *klebsiella* in hospital units showed that female orthopedic ward and male medical ward had 50% ESBL *K. pneumoniae* with G.O.P.D having the least 7.2 %. This could be as a result of long hospitalization. Risk factors associated with infection or colonization of ESBL is prolonged or recent hospitalization, stay in nursing homes, catheterization or use of other invasive medical device, and prolong use of antibiotics [9]. The ESBL strain may have been acquired from the contaminated hospital environment and also increased use of antibiotics by clinicians.

According to gender distribution, this report showed that females have a higher ESBL positive *Klebsiella Pneumonia* than male with a prevalence rate of 27.4% while male 18.7% the reason might be women take more antibiotics than men. A systematic review and data-analysis on Gender differences in antibiotic prescription reported that the amount of antibiotics particularly Cephalosporins and Macrolides prescribed to women in primary care is higher than that given to men [25].

This study recorded the presence of ESBL genes blaTEM at 972bp, blaSHV at 868bp and no incidence of CTX-M gene among the studied isolates. This finding is in agreement with study in Lagos, in 2003: only bla TEM and SHV ESBLs were detected [26]. Another study in Borno North East reported blaTEM as the commonest gene followed by SHV with low CTX-M [27]

The report was in contrast with findings in Benin where CTX-M gene was the most prevalent [15]. The use of antibiotics could be the reason for the variation in gene prevalence's. It was also observed that there were multiple occurrences of genes in some of the isolates similar to work done in Borno North East Nigeria, India and Turkey [27], [28] and [29] The clinical significance is that patients with multiple ESBL genes are more likely to have multi-drug resistance to their infections thus leading to therapeutic failure which might present a great challenge in severe illness. Some of the isolates tested had none of the ESBL genes. This is in agreement with a work done in Iran which recorded non-detection of genes in some isolates [31].

4.0 CONCLUSION

Antimicrobial susceptibility of ESBL positive *Klebsiella pneumoniae* according to this study shows imipenem to be highly sensitive and should be considered in treatment of ESBL positive strain.

A prevalence rate of 15.3% of ESBL producing *K. pneumoniae* was reported in this study and is lower when compared to research findings from some states within the country. There is presence of ESBL gene Bla TEM and Bla SHV with no incidence of CTX-M.

This Research finding recorded multiple genes among some isolates. The report also shows that some isolates confirmed phenotypically as an ESBL producers neither have any of the ESBL gene tested.

Contribution to Knowledge

- This research work has contributed to knowledge by filling the gap in providing data on the Occurrence of Extended Spectrum Beta- lactamase *Klebsiella pneumonia* and the gene coding for it in Plateau State Specialist and Jos University Teaching Hospitals
- The study has also established with empirical evidence the gene responsible for the production of ESBL.
- The study is significant as an improved understanding of the prevalence of *ESBL Klebsiella species* is important in designing and implementing effective diagnosis, Treatment, Infection prevention and control policy in Jos Plateau state.

Ethical Approvals

Approvals were obtained from Ethical Research Committee of Plateau State Specialist and Jos University Teaching Hospitals.

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