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Isolation and Molecular Detection of Multidrug Resistant *Klebsiella Pneumoniae* Harboring BlaSHV Gene, from Suspected Respiratory Disease Patients Attending Some Hospital in Dutse Metropolis, Jigawa State.

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Abstract
Klebsiella pneumoniae is a Gram-negative opportunistic bacterium that causes infections in hospitalized and immune compromised individuals. Currently, K. pneumoniae is showing a high resistance to a broad spectrum of drugs including beta-lactam antibiotics, Penicilline, fluoroquinolones, and aminoglycosides. This resistance is resulting in a growing worldwide problem regarding the choice of effective antibiotic treatment for hospital-acquired infections. Meanwhile, the current study was carried out for the isolation and molecular detection of multidrug resistant Klebsiella pneumoniae harboring blaSHV gene, in Federal University Dutse Teaching Hospital and Dutse General Hospital respectively, in Jigawa State, Nigeria. In this research a total of 100 sputum samples were collected and analyzed using standard microbiological methods. Antibiotic susceptibility test was carried out using modified Kirby-Baur disk diffusion method, the blaSHV gene was detected using PCR. The results showed 16% confirmed K. pneumoniae. Dutse General Hospital were recorded with 9(56.25%) while Federal University Dutse Teaching Hospital 7(43.75) isolates. A 50-59 years and above 60 years age groups had the highest occurrence of 4(25%) . Males had higher occurrence of 11(68.75) compared to female gender (31.25). The K. pneumoniae were found to be more resistant to Ampiclox 62.5%, Cefuroxime and nalidaxic acid 56.26% each, it was highly susceptible to imipenem 100%, levofloxacin 100%, cefotaxime 93.75, nitrofurantoin 75% and gentamycin 75%.

Keywords: Klebsiella pneumoniae, fluoroquinolones, harboring, blaSHV, beta-lactamase

INTRODUCTION

A notable polysaccharide-based capsule is present in the gram-negative, oxidase-negative, rod-shaped bacteria belonging to the genus *Klebsiella*. There are *Klebsiella* species everywhere in the natural world. It is believed that this is because different sub lineages have evolved unique niche adaptations along with corresponding biochemical adjustments that improve their suitability for a given environment. They are present in soil, water, plants, and other animals, including humans. The typical flora found in the nose, mouth, and intestines of humans and animals include species of the genus *Klebsiella*. All *Klebsiella* species are gram

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negative and often nonmotile. Compared to other members of the Enterobacteriaceae family, they are typically thicker and shorter.

The facultative anaerobic bacteria *Klebsiella pneumoniae* are non-motile, lactors fermenting microorganisms. On MacConkey agar, they manifested as mucoid, lactors fermenters. Despite certain strains being capsulated, they are nonsporing. The three most significant species are *Klebsiella pneumoniae, K. rhinoscleromatis,* and *Klebsiella ozaenae*. They exist in soil, plants, and water and are typical intestinal and upper respiratory tract inhibitors (Ochei and Kolhatkar, 2007).

Penicillins, cephalosporins, monobactams, and carbapenems are among the β-lactam antibiotics that are often prescribed globally (Rahman *et al.,* 2018). The primary indicators of β-lactam antibiotic resistance are the active expulsion of β-lactam molecules from bacteria or the generation of β-lactamase enzymes due to the presence of β-lactam-insensitive cell wall transpeptidases (Wilke *et al*., 2005).

A diverse class of bacterial enzymes driven by plasmids, known as extended-spectrum beta-lactamases (ESBLs), significantly increases resistance to cephalosporin, monobactam, and other antimicrobials (Canton and Novais., 2008). The exact definition of ESBLs is up for debate. A class of enzymes known as ESBLs degrades and renders useless drugs from the cephalosporin and penicillin families (Bonomo and Patterson, 2005).

The Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification are the two general schemes that are typically used to classify beta-lactamases (Ambler, 1980; Bush *et al*., 1995). Based on the protein homology of the enzymes, the Ambler scheme divides β-lactamases into four classes. Class B enzymes are metallo-β-lactamases, while class A, C, and D beta-lactamases are serine β-lactamases. The substrate and inhibitor profiles of enzymes serve as the foundation for the Bush–Jacoby–Medeiros functional scheme. TEM, NDM, GER, PER, CTX, OXA, and SHV are a few examples.

The functional group known as Sulfhydryl Variant (SHV) is made up of sulfur bound to an atom of hydrogen (thiol). Vitamin B1 and cysteine functioned as a marker for protein in the example of membrane function. Worldwide cephalosporin resistance has expanded, and different bacterial species have been found to harbor many ESBL types (Shah *et al*., 2004). The treatment of infectious diseases is severely impacted by the global issue of harmful organisms becoming resistant to antibiotics. The main cause of the issue is the increased use and abuse of antibiotics in veterinary, agricultural, and human medicine. According to earlier research conducted in Nigeria's tertiary health facilities, the condition is common there and accounts for 2.5% to 5.7% of medical admissions as well as between 15.3% and 24.9% of respiratory admissions. Additionally, 7.4% to 26% of patients in the nation died from pneumonia (Onyedum and Chukwuka, 2011). According to reports, pneumonia mortality is particularly high in northern Nigeria. With an estimated 35 deaths per 1000 live births, the disease's present status in Jigawa is higher than the national average (19 deaths per 1000 live births of children under five years old) (Carina *et al.,* 2022). According to a study done in 2020 in Jigawa by Eze *et al*., (2020) the most common isolated uropathogens were *Alcaligenes* sp. (58.1%) and *Klebsiella pneumoniae* (28.3%). In the meantime, Muhammed *et al*. (2017) Jigawa study on bacteriuria in expectant mothers revealed that among other bacteria, *K. pneumonia*e (31.0%) and E. coli (41.4%) were the most common.

Pneumonia is fourth in terms of deaths among all age groups, accounting for 1.4 million deaths in 2010 and 3.0 million in 2016. The world's most prevalent regions are Sub-Saharan

Africa and South Asia (WHO, 2018). The three most burdened countries are China (21 million), Pakistan (10 million), and Asia (43 million). Sub-Saharan African epidemiological statistics also indicated significant rates of morbidity and mortality from the illness, with an estimated 4 million cases and 200,000 deaths from pneumonia every year. An estimated 450 million individuals worldwide are thought to contract pneumonia each year, which accounts for 7% of all fatalities worldwide (WHO, 2019).

The aim of the research is to isolate, determine antibiotic profile and molecularly detect Multidrug-Resistant *Klebsiella pneumoniae* harboring bla*SHV* gene from suspected respiratory disease patients attending Federal University Dutse Teaching Hospital and Dutse General Hospital, Jigawa State.

MATERIAL AND METHODS

Study Disign

The study is experimental design, the data were collected from different individuals at a single point in time, the subjects are the patients reporting to the hospital. They were directed to the hospital laboratory for sputum and other screening of bacterial pathogens.

STUDY AREA

The research was carried out at Federal University Dutse Teaching Hospital and General Hospital Dutse, Jigawa State. Dutse is a city located in northwest geopolitical zone of Nigeria. It is the capital city of Jigawa State with an estimated population of 153,000 people in 2009. It is currently the largest city in the State. (43,000). (National Bureau of Statistics., 2006). The city has one General hospital, one Teaching Hospital a number of primary health centers and a few private hospitals. high temperatures are normally recorded between the months of April and September. The daily minimum and maximum temperatures are 15^{o}C and 35^{o}C. The rainy season lasts from May to September with average rainfall of between 600 millimeters to 1000 millimeters (Ocheje and Dogara, 2016).

Ethical Issues: The study was approved by ethical review committee of the Federal University Dutse Teaching Hospital Jigawa State.

Collection of Samples

The Sputum samples of 50 patients were collected from each hospital between September to November for the analysis and determination of bacterial profile. December for the molecular detection of blaSHV gene. Sputum samples was collected in sterile containers within the first hour of hospital admission in all the individuals who had productive cough and isolated appropriately. This was done in all recruited patients prior to commencement of the first dose of antibiotics. The subjects was instructed to take deep breaths and cough forcefully from the lungs with expectoration of at least two milliliters of sputum. (SMIs. 2010).

Sample size determination

The required sample size was obtained using the Fisher's statistical formula for estimating minimum sample size in descriptive studies in populations >10, 000. (Salisu *et al.*, 2021) The formula;

$$n = \frac{Z^2p}{d^2}$$

Where n = sample size,
Z = standard normal deviate which was set at 1.96 (corresponding to 95% confidence level.
P = previous prevalence figure of 5.7 % (Fiberesima and Onwuchekwa. 2008).

Q=1-p, d= observe precision (0.05) constant.
Therefore, Z^2=3.84, P= 0.57, Q=0.94, d^2=0.025).
Substitution of values in to the formula

$$n = \frac{3.84 \times 0.57 \times 0.94}{0.025}$$

n= 82.28
But 100 samples was used for proper distribution and result.

Examination of sputum samples
Collected sputum were first inspected macroscopically at the microbiology unit of federal university Dutse teaching hospital. It was observed as Salivary, mucosalivary, mucoid, mucopurulent, purulent and bloodstained.

Isolation of *K. peumoniea*
The sputum were inoculated onto MacConkey agar and incubate at 37^0C for 24hrs a large, mucoid and red colony were presumably taken for microscopic examination.

Microscopy
This was achieved using gram staining procedure and appeared pinkish Cheesbrough., (2012).

Biochemical tests
Biochemical tests such as indole test, citrate utilization test, urease test, vogous-proskaeur and motility test were carried out to confirme *K. pneumonia* positive cases.

Antibiotic susceptibility tests
Preparation of inoculum
A sterile ware loop were used to collect a pinch of colony wich was suspended in 2 ml of sterile saline. The saline tube were Vortex to create a smooth suspension. The turbidity of the suspension was adjusted to 0.5 McFarland standard by adding more colony of *K. peumoniae* when the suspension was too light and diluting with sterile saline when the suspension was too heavy (Jan., 2016).

Inoculation of the Mueller Hinton (MHA)
A sterile ware loop were dip into the inoculum tube. The dried surface of a MH agar were inoculated by streaking the wire loop three times over the entire agar surface; the plate were rotated approximately 60 degres each time to ensure an even distribution of the inoculum. Leaving the lid slightly ajar, this allow the plate to sit at room temperature at least for 3 minutes, for the surface of the agar plate to dry. (Jan., 2016).

The lid of the petri dish was partially removed and Placed on the dry Muller Hinton ager, at the center. It was gently pressed with the forceps to ensure complete contact with the agar surface. The lid was replaced to minimize exposure of the agar surface to contamination. The lid were replaced and inverted the plates in an incubator at 35°C for 24 hours (Jan., 2016).

DNA Extraction
SaMag bacterial DNA extraction kit is used with SaMag-12 automatic nucleic acid extraction system for the extraction of genomic DNA from study isolates (Samaga, Cepheid, Italy). The extraction process consisted of steps of lysis, binding, washing, and elution. First, a bacterial suspension was done by taking five colonies from bacteria growth on the MacConkey agar and added to 2 ml brain heart infusion broth and incubated for 24 h at 37°C, 1 ml from bacterial suspension was transferred to 1.5 ml micro centrifuge tube at ×5000 g for 5 min, then discard

supernatant and added 220 µl buffer BL2 to pellet and mix by vortexing for 5–10 s. After that, 200 µl suspensions had been taken to sample tube (supplied in the kit) and added 10 µl from control positive to all tubes of the device, finally insert these samples tube to automated extraction DNA device (Samag-12 .(Roumayne *et al* 2020)

Molecular method for the detection of *blaSHV* gene

The genomic DNA was used as templates in the specific PCR amplification for the detection of the *blaSHV*, blaSHV-F (5'- CGC CTG TGT ATT ATC TCC CTG TTA GCC -3') (GC 54.55%), (TM 68°C), and blaSHV-R (3'- TTG CCA GTG CTC GAT CAG CG -5') (GC 52.38 %), (TM 64°C) having product size 842 bp. PCR was performed according to the Maxime PCR Pre-Mix kit, i-Taq DNA Polymerase 5U/µl, dNTPs 2.5 mM, reaction buffer (×10), and gel loading buffer ×10. About 5 µl from Taq PCR Premix, 10 picomols/µl (1 µl) from forwarding primer, 10 picomols/µl (1 µl) from the reverse primer, 1.5 µl DNA, 16.5 µl distill water to reach final volume 25 µl then mixed well by vortex. All tubes transferred into the thermal cycler. The optimum condition for the detection SHV gene as follows: Initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, amplification at 72°C for 30 s, and the final extension at 72°C for 5 min. The amplified products were separated in 1.5% agarose gel and the electrophoresis was performed according to standard procedure. (Roumayne *et al* 2020)

Ethanol precipitation

A labeled sterile 0.5ml tube was prepared for each sample. Prepare fresh stop solution/glycogen mixture as follows per sequencing reaction: 2ul of 3M Sodium acetate, 2ul of 100mM Na2-EDTA and 1ul of 20mg/ml of glycogen (provided in the kit).To each of the labeled tubes add 5ul of the stop solution/glycogen mixture. The sequencing reaction was transferred to each of the appropriately labeled tube and were mix thoroughly. 60ul cold 95 % (v/v) ethanol from -20^{0}C freezer was added and mix thoroughly. It was immediately centrifuge at 14,000rpm at 4^{o}C for 15min. the supernatant was carefully removed with a micropipette. (The pellet should be visible). Rinse the pellet with200ul 70 % (v/v) ethanol from -20^{0}C freezer, centrifuge at 14,000rpm at 4C for a minimum of 2minutes. The supernatant were aseptically removed using a micropipette. Vacuum dry for 10min or until dry. Suspend the sample in 40ul of the sample loading solution (provided in the kit). The resuspended samples were transferred to the appropriate wells of the sample plate**.** Overlay each of the resuspended sample with one drop of mineral oil from the kit, the prepared samples were loaded in to instrument and ran to obtained results.

RESULTS

This research shows result on isolation and nolecular detection of gene in *K. pneumonia* from the two hospitals. Table 1 shows details of the number of sample collected, processed and isolates obtained per week in each of two hospitals where Dutse general hospital obtained the highest number of *K. pneumoniae* with 9 isolates in 50 samples compared to Federal University Dutse Teaching Hospital with 7 isolates in 50 samples. The differences may be due to the hygienic level of staff, patients, equipment and the hospital environment. Poverty and educational level of the patients may be incorporated.

Table 1: showing the number of visits per week and isolates obtained from the hospitals

Week in GHD	Samples(%)	K. *pneumoniae* %	Week in RSTH	Samples%	*K. pneumoniae* %
1	7(14.0)	0(00.0)	1	7(14.0)	1(14.3)
2	10(20.0)	3(33.3)	2	6(12.0)	2(28.6)
3	8(16)	1(11.1)	3	7(14.0)	1(14.3)
4	6(12)	0(00.0)	4	8(16.0)	0(00.0)
5	9(18)	3(33.3)	5	9(18.0)	1(14.3)
6	10(20.0)	2(22.2)	6	8(16.0)	2(28.6)
7	0(00)	0(00)	7	2(28.3)	0(00.0)
Total	**50(100)**	**09(100)**	**07**	**50(100)**	**07(100)**

Key: GHD= General Hospital Dutse, RSTH= Federal University Dutse Teaching Hospital, %= percentage

Table 2 showed the Distribution of *K. pneumoniae* based on age variations. The sixty and above years and 50-59 years showed the highest occurrence of the organism in 4(25%) each. 30-39 years group had the second occurrence with 3(18.79%), which immediately followed by age group 40-49 and 20-29 which has 2(12.5%) each. The age group 10-19 has the least occurrence with 1(6.25%), all in to 15, 24, 21, 20,17and 3 samples respectively. This variations of K. pneumoniae occurrences these may probably be due to the number of samples collected and immune competent level

Table 2: The Distribution of *K. pneumoniae* in Relation to Age of patients

Age group (Years)	Positive samples (%)	Negative samples (%)	Total (%)
10-19	1 (6.25)	2 (2.4)	3 (3)
20-29	2 (12.5)	15 (17.9)	17 (17)
30-39	3 (18.79)	18 (21.4)	21 (21)
40-49	2 (12.5)	18 (21.4)	20 (20)
50-59	4 (25)	20 (23.8)	24 (24)
>60	4 (25)	11 (13.0)	15 (15)
Total	**16 (100)**	**84 (100)**	**100 (100)**

Chi-Square (X^2) =18.02; p-value=11.071 (P<0.05). Key: % = percent, > = Greater than.

Table 3 Showed the distribution of *K. pneumoniae* in relation to gender group i.e. male and female. There were 11 occurrences in male with 68.75% which showed the highest compared to female which has 7 occurrence with 31.25%. This could be due to constant exposure to the outdoor activities for men.

Table 3: The Effect of gender on the occurrence of *K. pneumoniae* in Relation to patients attending the two hospitals.

	Sex		
Age group	**Male (%)**	**Female (%)**	**Total**
10-19	1 (6.25)	0 (0.0)	01 (6.25)
20-29	1 (6.25)	1 (6.25)	02 (12.5)
30-39	2 (12.5)	1 (6.25)	03 (18.75)μ
40-49	1 (6.25)	1 (6.25)	02 (12.5)
50-59	2 (12.5)	2 (12.5)	04 (25.0)
>60	4 (25.0)	0 (0.0)	04 (25.0)
Total	**11 (68.75)**	**05 (31.25)**	**16 (100)**

Chi-Square (X^2) =9.14; p-value=11.071 (p>0.05). : % = percent, > = Greater than.

The antibiotic susceptibility pattern of sixteen isolates were determined following the measured zone inhibition from each antibiotic disc. Ampiclox showed the highest resistance with 62.5% immediately followed by cefuroxime and nalidixic acid with 56.25% each. cefexime indicate intermediary relationship with 50% susceptibility. Levofloxacin and Imipenem was recorded with highest Sensitivity antibiotics with 100% as indicated in Figure

1. Table 4.4 shows the possible drugs of choice for curing pneumonia infections course by *Klebsiella pneumoniae.*

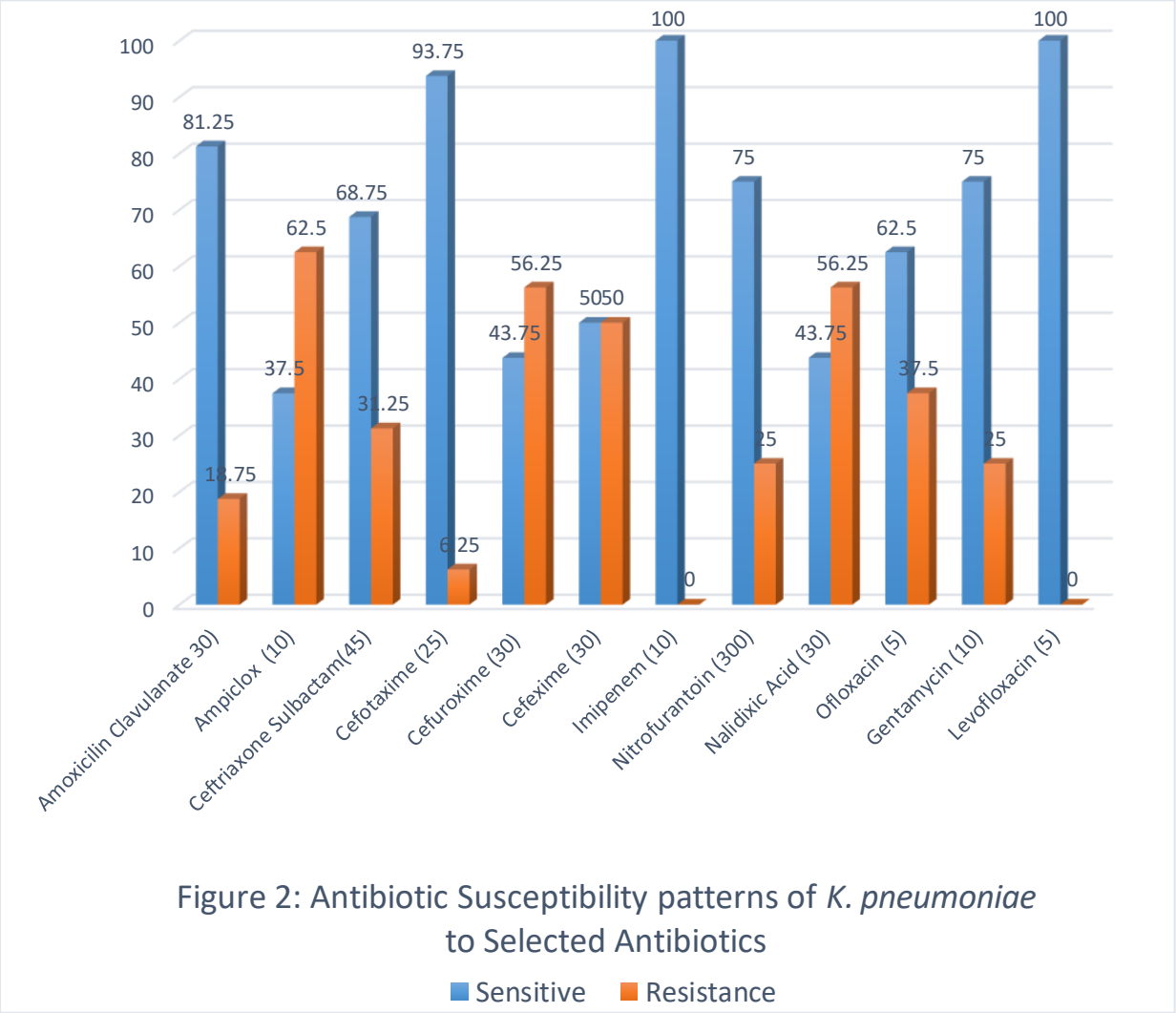

Figure 2: Antibiotic Susceptibility patterns of *K. pneumoniae* to Selected Antibiotics

Table 6: The Distribution of Isolates Showing Antibiotics Susceptibility and Resistant Pattern

Antibiotics (µg)	Sensitive (%)	Resistance (%)
Amoxicilin Clavulanate 30)	13 (81.25)	3 (18.75)
Ampiclox (10)	6 (37.5)	10 (62.5)
Ceftriaxone Sulbactam (45)	11 (68.75)	5 (31.25)
Cefotaxime (25)	15 (93.75)	1 (6.25)
Cefuroxime (30)	7 (43.75)	9 (56.25)
Cefexime (30)	8(50.0)	8(50.0)
Imipenem (10)	16 (100)	0 (00.0)
Nitrofurantoin (300)	12 (75)	4 (25)
Nalidixic Acid (30	7 (43.75)	9 (56.25)
Ofloxacin (5)	10 (62.5)	6 (37.5)
Gentamycin (10)	12 (75)	4 (25)
Levofloxacin (5)	16 (100)	0 (00.0)

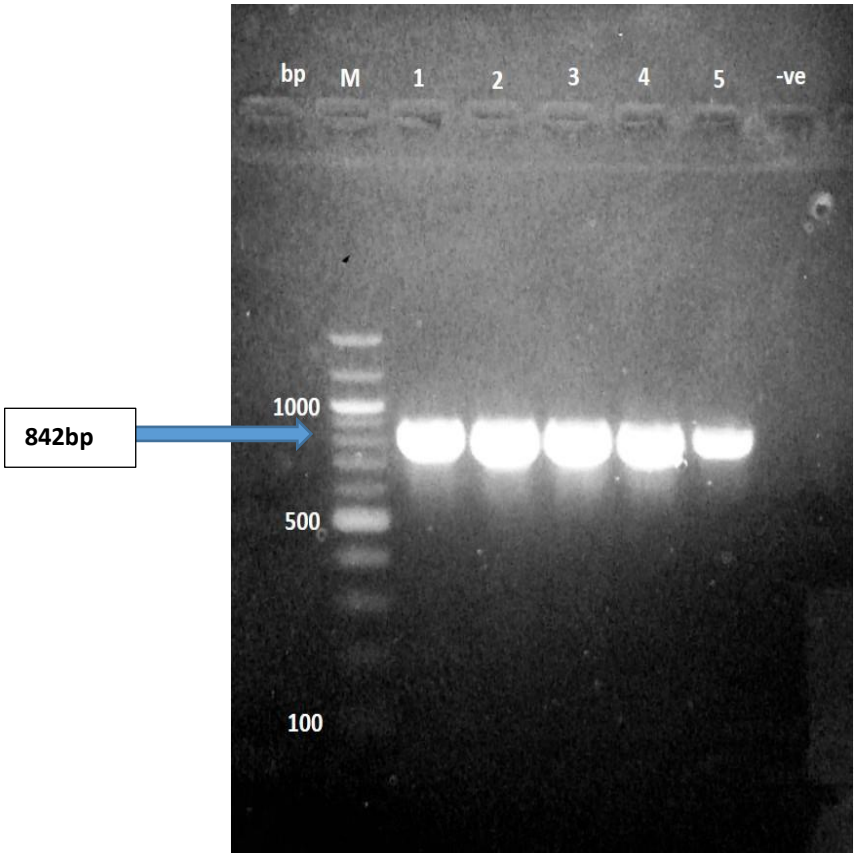

Figure 3: SHV gene amplified from *K. pneumoniae* on 1.5% agrose gel electrophoresis
Key: M=Lane, Bp =Base Pairs, +V =Positive Control,, -V =Negative C, Sample 1-5 are positive *Klebsiella pneumoniae*.

Agarose gel Electrophoresis showing 5 plasmid band located at 842bp obtained from *K. Pneumoniae* isolated in the sputum samples of patients suspected with respiratory diseases at Federal University Teaching Hospital and Dutse General Hospital Jigawa State.

DISCUSSION

The study was designed for the isolation of multidrug resistance *K. pneumoniae,* antimicrobial susceptibility profile and detection of the *blaSHV* gene, from the isolates found in the sputum of patients suspected with respiratory diseases attending Federal university Dutse Teaching Hospital and Dutse General Hospital Jigawa State. A total of 100 sputum samples were collected and analyzed. Out of these 16% ware confirmed positive. Thus, the research shows the lower rate of *Klebsiella pneumonia,* with 16 strains in 100 samples, compared to that conducted from AL-Ramadi Teaching Hospital and AL-Karkh Hospital in Baghdad shows 38.4% of *Klebsiella pneumoniae* in 100 samples (Khalaf and Al-Ouqaili .,2018). However similar research was reported in India by shilpa *et al*., (2016) with 23% in 200 samples, and Rakshya *et a.l,* (2018), with 40% in 264 samples The differences could probably be due to the sample variations, number of targeted organisms during the study period as well as geographical location Osagie *et al.,* (2017). It also showed the highest involvement of the bacteria in the nosocomial infections as well as underlying respiratory tract infections. (Abdullahi *et al*., 2018).

Meanwhile, this research showed higher result compared to that found in Kaduna State by Umar *et al*., (2018) North central of Nigeria 5 isolates in 50 samples. In Jigawa Muhammad *et al*., (2017) reported 31.0% in 116 samples. Edo State by Osage *et al*., (2017) South-South of Nigeria 31% in 500 samples, also in north west of Nigeria Kano state by Oyinloye *et al*., (2020) with 30% in 150 samples, A report from Jigawa showed prevalence of *Klebsiella pneumoniae*

from uropathogen as 28.3 % (Eze *et al.*, 2022). Moreover, Abdullahi *et al.*, (2018) report lower occurrence of the bacteria from School of Health technology Kano had 12% in 300 samples. This variation may be due to the high level of sanitation in the hospital. It should be noted that, the antibiotic susceptibility of *K. pneumoniae* is not constant but dynamic thus, varies with time and environment. This demand the need for periodic screening of common bacterial pathogens for their antibacterial drug susceptibility profile in different communities. It is a notorious fact that the use of antibiotics can lead to the emergence and dissemination of resistance *K. pneumoniae,* which can then be passed on to people via food, through direct contact or other means. In recent years the wide spread use of antibiotics in in the field of veterinary medicine has result in the development of increase number of bacterial strains possessing resistance to many antibiotics. The property of multiple antibiotics resistance could be shared via conjugation from resistance strains to other bacteria by means of plasmid. This gave chance to the emergence of newly bacterium with resistance to one or more antibiotics. Moreover the highly resistance of *K. pneumoniae* isolates to commonly used antibiotics could be probably due to some factors ranging from the use of fake antibiotics, abuse and misused of those antibiotics found commonly in circulation among general populace and health resources centers Abdullahi *et al.*, (2018) .

The highest occurrence of *K. pneumoniae* was obtained from 60 years and above with 36%, likewise 50-59 years also reveals 36%, though the correlations may be due to their difference in the number of samples collected 6 and 10 respectively. As you can see highest occurrence of *K. pneumoniae* in 60 and above years was reported severally for instant in Nigeria by Oyinloye *et al.*, (2020), Shresh *et al.*, (2013) and in India by Serchan *et al.*, (2007) a report for the patients attending Tribhuvan University Teaching Hospital reported the something. This could probably be due to their engagements in tedious works and stressed in the past and are currently immune depressed. Looking in to their food, some age people dietary balance is not the issue while others are struggle for three square meal due responsibility and health issues. Irrespective of living standard of individual at this age is occupied by a lot of responsibilities as an elder, they consider the problems of their sons and daughters including grandsons and granddaughters as their own and sisters concern are their concern. Also they could be community elder, always looking forward to see the members of the society are united and progressive. Therefore, this responsibilities made them loosed the energy gain from the meals rapidly and provide some with hypertension. Some finding reveals their highest occurrence in lower age like that of Abdullahi *et al.*, (2018) and Osagie *et al.*, (2017) were they reported as 21-30 and 20-29 years group respectively. This could probably due to special disease, as the people of this age group are expected to be immune competent.

Male gender was found with the highest occurrence of *K. pneumoniae* than female gender with 68.75% - 31.25%, similar results can be seen from Abdullahi *et al.*, (2018) 52.58% - 47.14% and Osagie *et al.*, (2017) 54.8% - 45.2% respectively. Others include Akingbade *et al.*, (2012), Gauchan *et al.*, (2006) and Oyinloye *et al.*, (2020) reported the highest occurrence in male than female gender which could be due to the high risk of smoking, use of tobacco, alcohol consumption that causes decrease local immunity in the respiratory tract due to the defective mucociliary clearance, mucus plugging, airways collapse, respiratory muscle fatigue and the effect of medications Abdullahi *et al.*, (2018). Present studies shows that there is divers' resistance in the class of antibiotics. Among the Penicillin, Ampiclox were 62.5 resistance this agreed with the studies of Umar *et al.*, (2018) which shows 100% resistance, this may be due to the overuse of the drug in the treatments of infectious diseases. Fluoroquinolones tested levofloxacin is the most effective against the tested organisms with 100%, which is followed by ofloxacin 62.5%.This finding is consistent with a report from similar studies of shilpa *et al.*, (2006) and Oyinloye *et al.*, (2020) which shows these antibiotics are highly effective against the

tested bacteria. Gentamycin in the aminoglycosides class is 75% effective, also a report from Umar *et al*., (2018) shows 100% effective against *K. pneumoniae* this may be due to the proper use of antibiotics in the area thus, can be used as alternative medication of the disease. Oyinloye *et al*., (2020). Oyintola in his research reported it as 71.1%. Three out of five tested Cephalosporin are also highly effective on *klebsiella pneumonia,* as such, can be used as the drugs of choice, these are Imipinem, cefotaxime and ceftriaxone sulbactam as 100%, 93.75% and 68.75% respectively. In contras according to Khalaf and Al-Ouqaili (2018) in their study conducted in Bagdad *K. pneumoniae* were resistance to ceftriaxone 88%, ceftazidime 84% this could probably be due to the difference in geographical area and rampant treatment of the drug.

This current study showed 31.25% of *blaSHV* gene from *Klebsiella pneumoniae* and can be compared well with the 30% Mohammed *et al.*, (2016) and 31.6% reported by Raji *et al.*, (2015) in Lagos. The study from Enyinnaya *et al*., (2021) shows a slightly lower *blaSHV* occurrence of 28.5%. In the research conducted at Ebonyi State. 7.0 *blaSHV* genes was reported by Ugbo *et al*, (2020) on urine samples in 84 isolates. This could be attributed to the variation in antibiotic usage, sensitivity and specificity of test methods compared to the other studies. The research differs widely from 60.8% reported by Aibinu *et al.,* (2003) in Lagos. Many resistance genes among ESBL producing *K. pneumoniae* were found from studies done in Kano Emmanuel *et al*., (2015) and Ibadan Makanjuola *et al*., (2018). This could be due to previous exposure to antibiotics resulting from self-medication that is widely prevalent in this part of the world due to easy access and purchase of antibiotics without prescription.

CONCLUSION

In this research the data generated the positive isolates found among male gender was significantly higher than that of their female counterpart. Currently the high antibiotics resistance is among the major contribution which result in long hospitalization of individuals, increase infections, increase cost of therapy, morbidity, mortality as well. Thus, the most probable drugs of choice shows in this study were Levofloxacin and Imipenem, Cefotaxime, Amoxicilin Clavulanate, Nitrofurantoin and Gentamycin, Ofloxacin and Ceftriaxone Sulbactam as arrange in decrease effective order.

Recommendations

Considering the fact above we therefore, recommended the following

1. There should be an active infection control committee to support and create awareness to the general public on drug abuse. This may reduce rampant used of some drugs.
2. There should be a strong body to checkmates thoroughly the hygiene level of the patients and hospital environment, as it might probably lead to the reduction of *K. pneumonia* transmission.
3. Regular surveillance of antibiotics susceptibility disc, laboratory equipment, media and reagent to ensure proper isolation of multi drug resistance *K.pneumonia*.
4. Molecular detection of ESBL-producing organism and their genes is necessary to curtail more public health problem.
5. There is also need to formulate the local antibiotics polices that will assist clinicians in the choice of antibiotic therapy.

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