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Research Article

Characterization and Plasmid Profile of Resistant *Klebsiella pneumoniae* Isolates in Patients with Urinary Tract Infection in Nasarawa State, Nigeria

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Abstract

Klebsiella pneumoniae has been identified as an urgent threat to human health based on its increasing antimicrobial resistance to the beta-lactamases and Carbapenemases. The pathogen has become a threat to both patients and healthcare providers as its incidence is on the increase, becoming a major global healthcare issue. The study was aimed at characterizing and determining the plasmid profile of resistant *Klebsiella pneumoniae* isolates from urinary tract of patients at Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria. Early morning mid-stream urine samples were collected from patients with urinary tract infections between April and May, 2019 and *Klebsiella pneumoniae* characterized on the basis of its antibiotic resistance pattern, and the plasmid DNA profile determined. Thirty-eight strains of resistant *Klebsiella pneumoniae* were obtained of which 33 showed resistance to more than three antibiotics. About 51.1% of the isolates were resistant to Tetracycline, while the isolates were least resistant to Azithromycin and Cefotaxime (30.3%) respectively. *Klebsiella pneumoniae* isolates showed 32 different resistance patterns, 24 of the strains had the capacity to produce Extended Spectrum Beta-lactamases enzymes: CTX-M 24(72.7%), SHV 19(57.6%) and TEM 16(48.5%) respectively. All the resistant *Klebsiella pneumoniae* isolated had the same plasmid size of 48.5 kilobases and only 1 plasmid each though they all obtained a multiple antibiotic resistance (MAR) index > 0.2. The study concluded that *Klebsiella pneumoniae* harbours genes which confer antibiotic resistance on the isolates. The study exposes further the challenge of antibiotic resistance and need for concerted effort at stopping the challenge of drug resistance.

Keywords: Resistance; *Klebsiella*; Plasmid; Beta-lactamases; Antibiotics

Introduction

Klebsiella pneumoniae and other bacteria of health importance are pathogens causing concern globally due to resistance acquired to more than two lines of antibiotic classes (Nordmann *et al.,* 2009). *Klebsiella pneumoniae* strains have the capacity to take up traits (genetic) leading to more virulence and resulting in multidrug resistance. These resistant bacterial strains produce enzymes like cephalosporinase or Carbapenemases that can deactivate antibiotic drug administered on patients (Long *et al*., 2018), and so integrons, plasmid genes, and transposons harboured by the pathogen induces resistance to antibiotics drugs (Paterson and Bonomo, 2005).

Klebsiella pneumoniae causes nosocomial infection and it is a prevalent bacterium that abound in health facilities with special ability to take-up resistance genes from the environment. It is implicated in developing states of the world and poor communities as one of the major cause of diseases. About 78 serogroups have been identified each with a different capsular K antigen in the bacteria (Janda,

2015; Pan *et al*., 2019). Spagnolo *et al*. (2014) reported that *K. pneumoniae* is responsible for some healthcare-associated and community-associated infections. Colonization of the gastrointestinal (GI) tract is usually a pre-requisite for induction of infection, and medical personnel are culpable reservoir of infection contagion (Calbo *et al*., 2011). *Klebsiella pneumoniae* exerts resistance using a) the capability to produce resistance enzymes that inactivates cephalosporins and monobactams, b) ability to express production of carbapenemases which confers resistance to all β-lactams antibiotics, including the carbapenems (CDC, 2015).

The burden of infection caused by the pathogen is associated with high morbidity and mortality which might not be unconnected with the high number of resistance genes the bacteria habours (CDC, 2013; Hoban *et al*., 2014). The bacterium attaches to host cells employing fimbriae and adhesins which promote tissue infection (Ong *et al*., 2010). Prolong hospital stay, prior use of antibiotics and ventilation type are risk factors associated with colonization and infection by *Klebsiella pneumoniae*. The study was aimed at characterizing and determining the plasmid profile of resistant *Klebsiella pneumoniae* isolates from urinary tract of patients at Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria.

Materials and Methods

Sampling and Collection of Samples

A total of 194 urine samples were collected from Dalhatu Araf Specialist Hospital (DASH), Lafia, Nasarawa State determined by a prevalence rate of 14.8% (Yusuf et al., 2014), after obtaining ethical approval from DASH, Ethical committee. Early morning Mid-stream urine samples were collected from patients with urinary tract infections with symptoms such as vomiting, fever lasting more than 7 days, and those who had lower abdominal pain.

Inclusion and Exclusion Criteria

Samples were obtained from patients of both sexes and all ages to include: inpatients and out patients. Patients already on antibiotics and intensive care unit were excluded from the sampled group.

Isolation and Identification

Urine samples was inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar plates and streaked to obtain discrete colonies which were then sub-cultured unto Mac-Conkey agar to obtain pure colonies and incubated overnight at 37°C. Identification was based on colonial appearance, Gram reactions and biochemical tests.

Determination of Antibiotic Susceptibility Profile of Isolated Klebsiella pneumoniae

Antibiotic susceptibility pattern of *Klebsiella pneumonia* isolate from urinary tract patients was determined using the methods of Clinical and Laboratory Standards Institute Guidelines CLSI (2012). Broth cultures containing the different isolates were separately prepared using sterile peptone water comparable to 0.5 McFarland's standard and inoculated using Kirby-Bauer disc diffusion method on Mueller Hinton agar, and tested against antibiotics. The antibiotics tested were Ceftriaxone/Cefotaxime (30/30 µg), Cefotaxime (30 µg), Ofloxacin (30 µg), tetracycline (30 µg), Gentamycin (10 µg), levofloxacin (5 µg), Imipenem (10 µg), Amoxicillin + Clavulanic acid (20/10 µg) Azithromycin (30 µg), and Ciprofloxacin (5 µg). Incubation was done at 37°C for between 18-24 h after which the inhibition zones around the discs were measured and interpreted according to Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI.

Determination of Plasmid Patterns in Resistant Klebsiella Pneumoniae Isolates

i) Extraction of DNA from resistant Klebsiella pneumoniae isolates

One thousand microliters of phosphate buffer saline (PBS) was added to each tube containing the isolates. It was then added into a tube and centrifuge at 2000 rpm for 5 minutes. Discard the supernant. Two- hundred and fifty µl of PBS was again added to the pelleted isolates and re-suspend 250 µl p2 buffer was then added and turn upside then down the tube for like four times (p2 is a lysis buffer). Three -hundred and fifty µl N3 buffer (N3 is a neutralization buffer). The tubes were turn upside then down for four times. It was then centrifuge at 1300 rpm for 10 minutes. Eight- hundred microliters of the supernant was now added into a column and was centrifuge at 13000 rpm for 1 minute. Five-hundred microliters of PB buffer was also added to the binding column and centrifuge at 13000 rpm for 1 min. Three- hundred microliters of wash buffer 1 was added and centrifuge for 1 min, 300 µL of wash buffer 2 was added and centrifuge for 1 min. The empty tube was centrifuge at 13000 rpm to remove residual. The tube was transferred to a new tube for elution in which 500 µL of elution was added. It was centrifuge at 8,000 rpm for 1 min to elute the DNA.

ii) Multiplex Polymerase Chain Reaction

A mixture of 20 mL in 0.5 mL tube containing 2 mL of Sulphydryl variable primer, 2 mL of Temoneria primer, 2 mL of Cefotaximase primer, 12 mL of H_2O and 2 mL of the extracted DNA was mix together. An addition of 50 mL of nuclease was added to avoid evaporation during the cycling period. The PCR begins with: Pre-denaturation at one cycle for 5 min at a temperature of 94°C then denaturation for 30 s at a temperature of 94°C, followed by annealing at a temperature of 52°C for 30 s and, extension at 72°C for 1 min at 35 cycles. Finally, extension for 5 min at a temperature of 72°C. At the end of cycling, the tubes were stored at – 20°C.

Primer sequences

Bla TEM- F: GTA TCC GCT CAT GAG ACA ATA ACC CTG

Bla TEM -R: CCA ATG CTT AAT CAG TGA GGC ACC

Bla SHV -F: CGC CTG TGT ATT ATC TCC CTG TTA GCC

Bla SHV- R: TTG CCA GTG CTC GAT CAG CG

Bla CTX-M -F: CGC TTT GCG ATG TGC AG

Bla CTX-M –R: ACC GCG ATA TCG TTG GT

iii) Electrophoresis

About 2 g agarose powder was dissolved in 1x TAE and boiled using a water bath until agarose was completely dissolved. It was allowed to cool in a water bath set at 50 – 55^{o}C. Gel casting tray was prepared by sealing ends of gel chamber with tape or appropriate casting system. Appropriate number of combs was then placed in gel tray. An addition of 5 uL of ethidium bromide was added to cooled gel and pour into gel tray. It was allowed to cool for 15-30 min at room temperature. Comb(s), was removed and placed in electrophoresis chamber and covered with buffer (TAE or TBE as used previously). DNA and standard (Ladder) was loaded onto gel. Plasmids were separated by agarose gel electrophoresis at a voltage 45 V for at least 1 h and then DNA bands were visualized using UV illumination.

Data Analysis

Data was processed using Statistical Package for Social Sciences (SPSS) program Version 20. Pearson Correlation coefficient test was used to check the relationship between two or more variables. All statistical testing were two tailed at 5% confidence interval and statistical significance was defined as $p<0.05$).

Results

Bacterial Population in Urinary Tract Infected Patients

Out of the total samples collected from patients, seven bacterial isolates were isolated from the urine samples. (Table 1). *Klebsiella pneumoniae*, was isolated the most with a percentage bacterial count of 33%, followed by *Staphylococcus sp* (9.0%), *Escherichia coli* (3.5%), *Proteus sp* (2.5%), *Streptococcus sp* (3.5%), *Pseudomonas aeruginosa* (1.5%) and *Chlamydia trachomatis* with a (1.5%) count.

Table 1: Bacterial count in urine samples from UTI patients

Isolates	Number of isolates	Percentage (%)
Klebsiella pneumoniae	66	33
Escherichia coli	6	3
Staphylococcus sp	9	5
Proteus sp	5	2.5
Pseudomonas sp	3	1.5
Streptococcus sp	**7**	3.5
Chlamydia trachomatis	3	1.5

Susceptibility Profile of Klebsiella pneumoniae Isolates

As shown in the Table 2 below, *Klebsiella pneumoniae* strains were most susceptible to Azithromycin and Cefotaxime (69.7%) respectively, followed by Gentamicin (68.2%), Imipenem, Ceftriaxone, Levofloxacin, and Ofloxacin (63.6%) each, while Augmentin and Ciprofloxacin (57.6%) respectively. The least sensitivity was observed in Tetracycline (34.8%). Hence, *Klebsiella pneumoniae* strains were most resistant to Tetracycline (65.2%) and least resistant to Azithromycin and Cefotaxime (30.3%).

Antibiotic Resistance Pattern of Klebsiella Pneumoniae Isolated from Urine

Klebsiella pneumoniae strains showed 32 different resistance patterns with Ceftriaxone (CRO), Ciprofloxacin (CIP), Imipenem (IMP) and Tetracycline (TET) been the most encountered (Table 3). Out of the 33 isolates, 2 isolates showed resistance to 9 antibiotics, 1 isolate showed resistance to 7 antibiotics, 3 isolates to 6 antibiotics while 14 isolates showed resistance to 5 antibiotics respectively. The multidrug resistant *Klebsiella pneumonia* strains identified in the present study all had one plasmid with 48.5 kilobases each, and a MAR index greater than 0.2 each as reported in Table 3. *Klebsiella pneumoniae* isolates with MAR index of 0.5 were 14 which was the highest recorded, while the least occurrence was a MAR index of 0.7.

Table 2: Antibiotic Susceptibility pattern of *Klebsiella pneumoniae* isolates from UTI patients

Antibiotics	Resistance (%)	Susceptibility (%)	Correlation coefficient
	Number of isolates = 66		**0.962**
Augmentin	28(42.4)	38(57.6)	
Ceftriaxone	24(36.4)	42(63.6)	
Cefotaxime	20(30.3)	46(46.9)	
Levofloxacin	24(36.4)	42(63.6)	
Ciprofloxacin	28(42.4)	38(57.6)	
Imipenem	24(36.4)	42(63.6)	
Ofloxacin	24(36.4)	42(63.6)	
Gentamicin	21(31.8)	45(68.2)	
Azithromicin	20(30.3)	46(69.7)	
Tetracycline	43(65.2)	23(34.8)	

Table 3: Antibiotic resistance pattern and plasmid profile of *Klebsiella pneumoniae* isolates in patients with urinary tract infection

Bacterial isolates	Number of plasmid	Plasmid size(kb)	Resistance pattern	Antibiotic resistance	MAR index
Kleb 1	-	-	Cro, Cip, Gn	3	0.3
Kleb 2	1	48.5	Lev, Cip, Ofx, Azn	4	0.4
Kleb 3	1	48.5	Aug, Lev, Cip, Imp	4	0.4
Kleb 4	1	48.5	Cro, Cip, Imp, Ofx, Gn	5	0.5
Kleb 5	1	48.5	Aug, Lev, Cip, Gn	4	0.4
Kleb 6	1	48.5	Aug, Cro, Zem, Lev, Cip, Imp, Ofx, Azn, Tet	9	0.9
Kleb 7	1	48.5	Aug, Cip, Ofx, Gn, Azn	5	0.5
Kleb 8	1	48.5	Cro, Lev, Cip, Imp, Tet	5	0.5
Kleb 9	1	48.5	Aug, Lev, Imp, Gn, Tet	5	0.5
Kleb10	1	48.5	Cro, Lev, Cip, Gn, Tet	5	0.5
Kleb11	1	48.5	Cro, Zem, Lev, Gn	4	0.4
Kleb12	1	48.5	Zem, Imp, Azn	3	0.3
Kleb13	1	48.5	Cip, Imp, Azn	3	0.3
Kleb14	1	48.5	Cro, Cip, Imp, Tet	4	0.4
Kleb15	1	48.5	Zem, Lev, Cip, Ofx, Gn, Tet	6	0.6
Kleb16	1	48.5	Aug, Zem, Lev, Tet	4	0.4
Kleb17	1	48.5	Aug, Zem, Lev, Ofx, Azn	5	0.5
Kleb18	1	48.5	Zem, Lev, Ofx, Azn, Tet	5	0.5
Kleb19	1	48.5	Cip, Imp, Gn, Azn, Tet	5	0.5
Kleb20	1	48.5	Aug, Lev, Cip, Gn, Azn	5	0.5
Kleb21	1	48.5	Aug, Zem, Tet	3	0.3
Kleb22	1	48.5	Aug, Zem, Ofx, Gn, Azn, Tet	6	0.6
Kleb23	1	48.5	Aug, Cro, Zem, Ofx, Gn, Azn, Tet	7	0.7
Kleb24	1	48.5	Zem, lev, Cip, Gn, Azn, Tet	6	0.6
Kleb25	1	48.5	Cro, Cip, Imp, Azn, Tet	5	0.5
Kleb26	1	48.5	Cro, Lev, Cip, Imp, Tet	5	0.5
Kleb27	1	48.5	Cro, Cip, Ofx, Gn, Tet	5	0.5
Kleb28	-	-	Aug, Cro, Lev, Ofx, Gn, Tet	6	0.6
Kleb29	1	48.5	Aug, Cro, Zem, Lev, Cip, Imp, Ofx, Gn, Tet	9	0.9
Kleb30	1	48.5	Zem, Ofx, Gn, Azn	4	0.4
Kleb31	1	48.5	Cip, Imp, Gn	3	0.3
Kleb32	1	48.5	Aug, Cro, Lev, Imp, Azn	5	0.5
Kleb33	-	-	Cro, Zem, Ofx, Gn, Tet	5	0.5

CRO: ceftriaxone, AUG: Augmentin, OFX: Ofloxacin, LEV: Levofloxacin, TET: Tetracycline, AZN: Azithromycin, CIP: Ciprofloxacin, GN: Gentamicin, IMP: Imipenem, ZEM: Cefotaxime

Table 4: Antimicrobial Resistance Gene Profiles of *Klebsiella pneumoniae* strains

ESBL-Enzyme	Strains	Number of isolates (%)
Temoneria	2,3,4,5,6,7,8,9,10,12,13,14, 15,17,20,29	16 (48.5)
Sulphydryl Variable	2,3,4,5,6,7,8,9,12,13,14,15, 17,19,22,24,26,29,30	19 (57.6)
Active on cefotaxime	2,3,4,5,6,7,8,9,10,11,12,13,14, 15,16,17,18,19,20,22,24,26, 29,30	24 (72.7)

Molecular Detection of Antimicrobial Resistance Genes

24 strains out of the 33 strains isolated contained extended spectrum beta-lactamase (ESβL) enzymes with CTX-M as the highest (72.7%), followed by SHV (57.6%) and TEM (48.5%) respectively. However, 9 strains such as strain 1, 21, 23, 25, 27, 28, 31, 32 and 33 did not produce such enzymes as shown in Fig. 1 and 2.

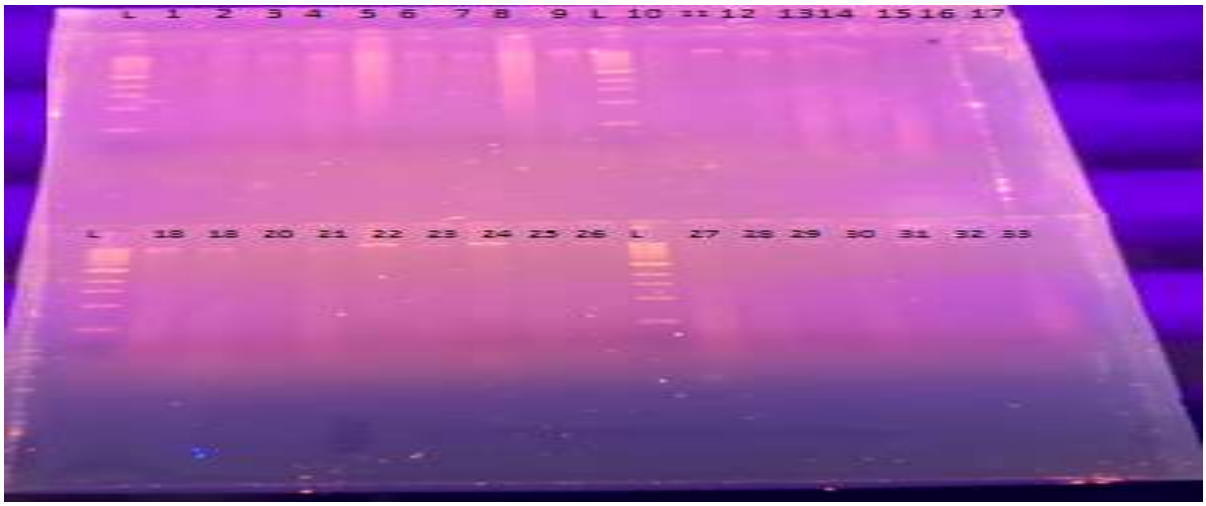

Fig. 1: Gel electrophoresis of plasmid profile of *Klebsiella pneumoniae* isolates

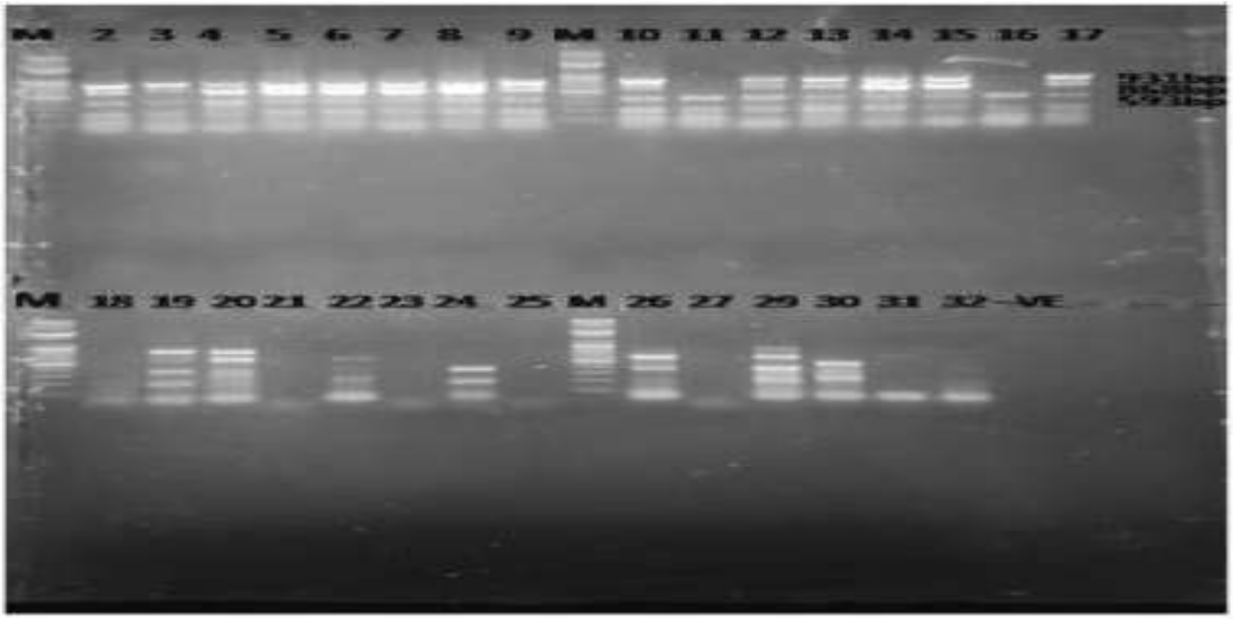

Fig. 2: Gel electrophoresis of antimicrobial resistance genes in *Klebsiella pneumoniae*

Discussion

Treatment of nosocomial infections is becoming a challenge as a result of resistance to antibiotics and the associated burden (Cao *et al*., 2014). *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus* spp, *Pseudomonas* spp., and other isolated bacteria have been implicated as contributing agents of nosocomial infections, and were also reported in the study by Kibret and Abera (2014), Derese *et al*. (2016), and Anejo-Okopi *et al*. (2015). The *Klebsiella pneumoniae* obtained in the study though had the highest incidence of occurrence when compared with other isolates at 33% was still low generally which aligns with other studies where the occurrence was reported to be low (Farajnia *et al*., 2009; Kamrul *et al*., 2012).

Isolates obtained in this study are known to be ubiquitous in nature, and are easily acquired and transmitted due to poor hygienic conditions in the hospital setting and community. High occurrence of *Klebsiella pneumoniae* in this study agrees with findings by Idomir *et al*. (2014) who reported that the pathogen can be readily isolated at high percentage in urinary diseased patients. High occurrence of *Klebsiella pneumoniae* in this study agrees with findings by Idomir *et al*. (2014) who reported that the pathogen can be readily isolated at high concentration in urinary diseased patients.

The prevalence obtained for *E. coli* in our study was 3.50% which was similar to the value recorded in the study by Derese *et al*. (2016) but, contrasted by the report of Kibret and Abera (2014) and Oladehinde *et al*. (2011). The presence of *Pseudomonas aeruginosa* agrees with the study of Oladehinde *et al*. (2011). The presence of these bacterial isolates could be result in multiple infections and were

likely introduced through sexual activities or poor hygienic conditions or disease conditions. The *Klebsiella pneumoniae* isolates showed varied susceptibility to the tested antibiotics and many of the isolates were susceptible to Azithromycin followed by Gentamicin. The result obtained in the study was similar to results recorded in the research carried out by Iyoha and Tula (2015).

Resistance recorded by the *K. pneumoniae* could be attributed to drug abuse through self-medication, over the counter access to drugs, and consumption of residual antibiotics present in food as meat (chicken, beef, pork, fish – growth promoters), and plants (roots, leaves, tubers, stems, grains). The resistance exhibited by the *Klebsiella pneumoniae* isolates against tetracycline corroborates findings by Olusola *et al*. (2013) who explained that the pathogens were ineffective against the antibiotic using mechanisms such as biofilm formation, evasion, and their capacity to acquire resistance gene and plasmid to overcome the stress factor.

Antibiotic resistance patterns exhibited by the *Klebsiella pneumoniae* isolates showed that 32 patterns were found with Ceftriaxone, Ciprofloxacin, Imipenem and Tetracycline being the most encountered. This finding agrees with the study of Arezoo *et al.* (2018) who reported several resistance patterns. The different antibiotic resistance patterns observed is an indication of a widespread unregulated use of these antimicrobials coupled with factors such as sexual activity, age, sex, poor immunity, personal hygiene, history of recurrent UTI's, and social behaviour.

Pathogenicity of MDR *Klebsiella pneumoniae* has been attributed to the possession and production of resistance genes, virulence genes, integrons, production of toxins (Li *et al*., 2013; Firoozeh *et al*., 2019). Extended Spectrum Beta-Lactamases genes from isolated resistant *Klebsiella pneumoniae* include TEM, SHV, and CTX-M. These genes promote virulence and resistance in the bacteria hence Akingbade *et al*. (2012) reiterated the importance of determining the plasmid profile of isolates along with the resistance pattern for effective and efficient diagnosis because plasmid harbouring these genes confers broad antibiotic resistance on the bacteria. Generally, it was observed in the study that all *Klebsiella pneumoniae* strains were multidrug resistant as they were resistant to three or more antibiotics. *Klebsiella pneumoniae* MDR isolates from this study produced extended β-lactamase. Further analysis using sequencing showed the isolates contained CTX-M, SHV and TEM Extended Spectrum Beta-Lactamases genes. CTX-M (Active on Cefotaxime) was the highest being harboured by 24 isolates. The three ESBL genes detected in the isolates have been reported to confer resistance on the bacterial isolates and peculiar to the Enterobacteriaceae family (Arcilla *et al*., 2017).

High prevalence of resistance genes obtained in the present study agrees with the report of Anes *et al*. (2017). The SHV and TEM both contributed to conferring resistance on the isolates which is transferable. The issues of *Klebsiella pneumoniae* resistance genes between human and animals have been raised by Wu *et al*. (2019). The authors investigated the mechanism of multiple resistance and dissemination of resistance genes by the pathogen, and recommended proactive surveillance of florfenicol-resistant strains in animals. The MAR index obtained in the study were many with only two isolates been ineffective against 9 antibiotics, 14 isolates scoring MAR index of 0.5, and one isolate scoring 0.7. The isolates were multidrug resistant. Our finding agrees with that of Christopher *et al.* (2013) who obtained a MAR index of 0.89 in their study. All MDR *K. pneumoniae* isolates analysed possess only one plasmid with the same size of 48.5 base pairs.

Conclusion

The study established the possession of Temoneria, Sulphydryl Variable, and Active on Cefotaxime genes in the isolated *Klebsiella pneumoniae* strains with only one plasmid (48.5bp). The finding affirms the fact that resistance is not only conferred by the presence of plasmid bearing genetic agents but that other factors are involved in resistance noted in pathogens. CTX-M genes was found more in the isolates and reports showed that the genes are also found in animals which calls for concern so as to avoid cross dissemination. The study also revealed that a host of antibiotics employed in the treatment of *Klebsiella pneumoniae* infections are becoming ineffective. Use of multiple antibiotics in treatment is not encouraged to reduce resistance but we are left with little option of surveillance to better manage the challenge of resistant pathogens. Based on the results obtained *Klebsiella pneumoniae* is the frequent pathogen associated with urinary tract infections (UTI) in DASH, Lafia.

Author's Contribution

O.O. Orole Author 1 conceptualize and designed the research, did critical revision of the manuscript, and participated in the interpretation of data. N.S. Hadi Author 2 designed the research, acquired data, drafted the manuscript, and carried out analysis of data. Final form of manuscript was approved by both authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

References

Akingbade OA, Ogiogwa IJ, Okonko IO, Okerentugba PO, Innocent-Adiele HC, Nwanze JC and Onoh CC (2012) Plasmid Profile of Isolated Klebsiella Species in a Tertiary Hospital in Abeokuta, Ogun State, Nigeria. *World Applied Science Journal* **3**: 371-378.

Anejo-Okopi JA, Okojokwu OJ, Ramyil SM, Bakwet PB, Okechalu J, Agada G, Bassi PA and Adeniyi SD (2015) Bacterial and antibiotic susceptibility pattern of urinary tract infection isolated from asymptomatic and symptomatic diabetic patients attending tertiary hospital in Jos, Nigeria. *Trends in Medicine* **17**(1): 1-5. DOI: 10.15761/TiM.1000108

Anes J, Hurley D, Martins M and Fanning S (2017. Exploring the Genome M and Phenotype of Multi-Drug Resistant *Klebsiella pneumoniae* of Clinical Origin. *Frontiers in Microbiology*, 8: 1913. 10.3389/fmicb.2017.01913

Arcilla MS, Van Hattem J.M, Haverkate MR, Bootsma MCJ, Van Genderen PJJ, Goorhuis A, Grobusch MP, Lashof AMO, Molhoek N, Schultsz C, Stobbering EE, Verbrugh HA, de Jong MD and Melles DC (2017. Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. *Lancet Infectious Diseases*, 17: 78-85. 10.1016/S1473-3099(16)30319-X

Arezoo SF, Jamileh N, Gita E and Azar S (2018) RAPD PCR Profile, Antibiobiotic resistance, Prevalence of armA Gene, and detection of KPC Enzyme in *Klebsiella pneumoniae* isolates. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 1: 1-7. 10.1155/2018/6183162

Calbo E, Freixas N, Xercavins M, Riera M, Nicolas C, Monistrol O, Sole MM, Sala MR, Vija AJ and Garau J (2011) Foodborne nosocomial outbreak of SHV1 and CTXM-15-producing *Klebsiella pneumoniae*: Epidemiology and control. *Clinical Infectious Diseases*, 52(6): 743-749. 10.1093/cid/ciq238

Cao X, Xu X, Zhang Z, Shen H, Chen J and Zhang K (2014) Molecular characterization of clinical multidrug-resistant *Klebsiella pneumoniae* isolates. *Annals of Clinical Microbiology and Antimicrobials*, 13(16): 1476-0711.10.1186/1476-0711-13-16

CDC (2013) Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR. *Morbidity and Mortality Weekly Report*, 62: 165-170.

CDC (2015) Notes from the field: Carbapenem- resistant Enterobacteriacea producing OXA-48-like carbapenemnases. MMWR. *Morbidity and Mortality Weekly Report*, 64(47):1315-1316. 10.15585/mmwr.mm6447a3

Christopher A, Broberg MP and Virginia LM (2013) Whole genome draft sequences of three multidrug- resistant *Klebsiella pneumoniae* strains available from the American type culture collection. *Genome Announcement*, 1(3): 312-313.10.1128/genomeA.00312-13

Clinical and Laboratory Standards Institute (CLSI) (2009) Performance standards for antimicrobial susceptibility: Nineteenth informational supplement. Wayne PA: Clinical and Laboratory Standards Institute (CLSI).M100, 19thed, January 2009. ISBN- 13:978-0012769010.

Derese B, Kedir H, Teklemariam Z, Weldegebreal F and Balakrishnan S (2016) Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending antenatal Clinic in DilChora Referral Hospital, Dire Dawa, Eastern Ethiopia. *Therapeutics and Clinical Risk Management*, 12: 251-260. doi: 10.2147/TCRM.S99831. 10.2147/TCRM.S99831

Farajnia S, Alikhani MY, Ghotaslou R, Naghili B and Nakhlband A (2009) Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. *International Journal of Infectious Diseases*, 13(2): 140-144. 10.1016/j.ijid.2008.04.014

Firoozeh F, Mahluji Z, Khorshidi A and Zibaei M (2019) Molecular characterization of class 1, 2 and 3 integrons in clinical multi-drug resistant *Klebsiella pneumoniae* isolates. *Antimicrobial Resistance and Infection Control*, 8(59), 10.1186/s13756-019-0509-3

Hoban DJ, Badal R, Bouchillon S, Hackel M, Kazmierczak K, Lascols C and Hawser S (2014) In vitro susceptibility and distribution of beta-lactamases in Enterobacteriaceae causing intra-abdominal infections in North America 2010-2011. *Diagnostic Microbiology and Infectious Disease*, 79: 367-72. 10.1016/j.diagmicrobio.2014.03.026

Idomir ME, Mateesu GG and Nemet C (2014) Etiological and therapeutically particularities of urinary tract infections in urological patients. Bulletin of the Transilvania University of Brasov, 7(56): 1.

Iyoha O and Tula MY (2015) Plasmid-mediated multiple antibiotic Resistance among *Klebsiella pneumoniae* and *Escherichia coli* in a tertiary hospital in Nigeria. *Journal of Annual Tropical Pathology*, 6(1): 23-32.

Janda MJ (2015) The Genus *Klebsiella*: An ever- expanding panorama of infections, Disease- associated syndromes, and problems for Clinical Microbiologists. *Clinical Microbiology and Case Reports*, 1: 4-22.

Kamrul L, Eliza R, Habibura R and Ranjith R (2012) Urinary Tract Infection in Children: An Update Bangladesh. *Journal of Child Healthcare*, 36(2): 90-97. 10.3329/bjch.v36i2.13085

Kibret M and Abera B (2014) Prevalence and antibiogram of bacterial isolates from urinary tract infections at Dessie Health Research Laboratory, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*, 4(2): 164-168. 10.1016/S2221-1691(14)60226-4

Li B, Hu Y, Wang Q, Yi Y, Woo PC, Jing H, Zhu B and Liu CH (2013) Structural diversity of class 1 integrons and their associated gene cassettes in *Klebsiella pneumoniae* isolates from a hospital in China. *PLoS One*, 8(9), e75805.dio:10.1371/journal.pone. 10.1371/journal.pone.0075805

Long SW, Linson SE, Ojeda Saavedra M, Cantu C, Davis JJ, Brettin T and Olsen RJ (2018) Discovery and whole genome sequencing of a human clinical isolate of the novel species *Klebsiella quasivariicola* sp. nov.bioRxiv. *Genome announcement*, 19(5): 42. 10.1128/genomeA.01057-17

Nordmann P, Cuzon G and Naas T (2009) The real threat of *Klebsiella pneumonia* carbapenemase producing bacteria. *Lancet Infectious Disease*, 9: 228-236. 10.1016/S1473-3099(09)70054-4

Oladeinde BH, Omoregie R, Olley M and Anunibe JA (2011) Urinary tract infection in a rural community of Nigeria. *North American of Journal Medical Sciences*, 3(2): 75-77. 10.4297/najms.2011.375

Olusola AA, Ogiogwa IJ, Iheanyi OO, Philip OO, Hope C, Innocent A, Jonathan CN and Charles CO (2013) Plasmid profile of Isolated *Klebsiella* species in a tertiary Hospital in Abeokuta, Ogun State, Nigeria. *World Applied Science Journal*, 21(3):371-378.

Ong CLY, Beatson SA, Totsika M, Forestier C, McEwan AG and Schembri MA (2010) Molecular analysis of type 3fimbrial genes from Escherichia coli, Klebsiella and Citrobacter species. *BMC Microbiology*, 10: 183. 10.1186/1471-2180-10-183

Pan JY, Tzu-lung L, Yi-yin C, Peng-Hsuan L, Yi-Tsung L and Jin-Town W (2019) Identification of three podoviruses infecting *Klebsiella* encoding capsule depolymerases that digest specific capsular types. *Microbial Biotechnology*, 12(3): 472-486. 10.1111/1751-7915.13370

Paterson DL and Bonomo RA (2005) Extended-spectrum beta-lactamases: A clinical update. *Clinical Microbiology Reviews*, 18(4): 657-686. 10.1128/CMR.18.4.657-686.2005

Spagnolo AM, Orlando P and Panatto D (2014) An overview of carbapenem-resistant *Klebsiella pneumoniae*: epidemiology and control measures. *Reviews in Medical Microbiology*, 25: 7-14. 10.1097/MRM.0b013e328365c51e

Wu F, Ying Y, Yin M, Jiang Y, Wu C, Qian C, Chen Q, Shen K, Cheng C, Zhu L, Li K, Xu T, Bao Q and Lu J (2019) Molecular Characterization of a Multidrug-Resistant *Klebsiella pneumoniae* Strain R46 Isolated from a Rabbit. *International Journal of Genomics*, 1-12. 10.1155/2019/5459190

Yusuf I, Arzai AH, Haruna M, Sharif AA and Getso MI (2014) Detection of multidrug resistant bacteria in major hospitals in Kano, North-west, Nigeria. *Brazilian Journal of Microbiology*, 45(3): 791-798. ISBN 1678-4405.