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ANTIBIOTICS RESISTANCE PROFILE OF CARBAPENEMASE PRODUCING *Klebsiella pneumoniae* FROM URINE SAMPLES IN GENERAL HOSPITAL, CALABAR

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ABSTRACT

Urinary tract infection is among the most common infectious disease ranking next to upper respiratory tract infection. Therefore, studying bacterial pathogens causing urinary tract infection and their antimicrobials susceptibility patterns are of the highest priority. Infections caused by *Klebsiella pneumoniae* have become a significant worldwide public health problem. This study was aimed at phenotypic characterization of extended spectrum beta-lactamase (ESBL)-and carbapenemase producing *Klebsiella pneumoniae* from urinary tract infection in a secondary health facility in Calabar, Southern Nigeria. One hundred and fifty (150) urine samples were collected, and analyze using standard microbiological techniques on chromogenic agar. A total number of 21 non repetitive clinical bacterial strains were isolated, out of which (9/12; 42.85%) isolation rate falls in the age range greater than 60 years significantly higher at p>0.05 than that recorded for age range 21-31 years (1/21; 5.56%). The female gender showed high 58% isolation rate. Results for the screening of *Klebsiella pneumoniae* isolates for ESBL- and carbapenemase production revealed (5/21; 23.80%) to be ESBL-producers and (1/5;20%) to be resistant to imipenem antibiotics and were characterized and identified as strains of *Klebsiella pneumoniae*. Antimicrobials susceptibility profile of ESBL-and carbapenemase producers showed them to be susceptible to streptomycin, imipenem and, sulfonamide, ofloxacin respectively. The findings in this study indicate that age group of over 60 years are prone to urinary tract infection cause by *Klebsiella pneumoniae* and that one has more of it in the female gender.

KEYWORDS: Carbapenemase, ESBL, urinary tract infection, *Klebsiella pneumoniae*,

INTRODUCTION

Klebsiella pneumoniae is a gram-negative, non-motile, opportunistic infection causing agent and one of the major causes of nosocomial infections such as urinary tract infections and pneumonia Majid *et al.,* (2016). Diseases cause by *K. pneumoniae* generally correlate with high rate of morbidity and mortality, particularly in the group of immunocompromised patients Muzahed *et al.,* (2008). At a global scale *K. pneumoniae* is among the most causative agents that frequently shows resistance to various classes of antibiotics Sehlain *et al*., (2013). This antibiotic resistance traits could be move from one bacterium to the other via vertical and horizontal gene transfer process using plasmid and transposon Sheemar *et al*., (2016).

Production of β-lactamase that can hydrolyze β-lactam ring containing antibiotics resulting in an inactive product that do not exhibit inhibitory effect on the bacterial transpeptidase synthesis Abdulaziz *et al*., (2015). The commonest form of β-lactamases are the extended spectrum β-lactamases (ESBL) Abdulaziz *et al*., (2015). This form of enzymes can hydrolyze extended-spectrum penicillins, cephalosporins, and monobactams, restricting their therapeutic alternative to carbapenems Ferreira *et al*., (2015). This has resulted in the use of carbapenem as an antibiotic of last resort in tackling infectious diseases arising from multidrug-resistant bacteria Mushi *et al*., (2014). Nonetheless, pressure arising due to the overuse and misuse of carbapenem has resulted in the formation of carbapenem-resistant Enterobacteriaceae (CRE).

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Carbapenem resistant *Klebsiella pneumoniae* (CRKP) strains are among the most widely distributed in the increasing numbers of reported cases of CRE globally CLSI, (2016). Resistance to carbapenem as shown in strains of CRKP do necessitate the biosynthesis of various classes of carbapenem degrading enzymes' along with hyperproduction of *AmpC* β-lactamases with mutation in the outer membrane porin and production of ESBL with either mutation in porin or drug efflux mechanism. The synthesis of carbapenem is the most studied mechanism of carbapenem resistance in CRKP Bradford *et al*., (2001). The β-lactam ring containing antibiotics, particularly carbapenems, monobactams, and extended- spectrum cephalosporins, can be degraded by carbapenemases Wilson *et al*., (2015). Over the past 20 years, *K. pneumoniae* has become known as a vital tool of antimicrobial resistance (AMR) through the production of ESBLs and carbapenemases, and has been acknowledged by WHO as one of the worlds' most important considered pathogens in critical need of future-generation antibiotics and novel approaches for controlling it Akindeti *et al*., (2012). This study aimed to characterize phenotypically and assess the antibiotic resistance profile of ESBL and carbapenem-resistant *Klebsiella pneumoniae* strains from urine samples.

MATERIALS AND METHODS

Materials

Instruments/equipment

Major Equipment that was used in the course of this research are; light microscope, autoclave, hot air oven, refrigerator, incubator, water bath, weighing balance, colony counter.

Chemicals/reagent and consumables

Major reagents that were used are; Crystal Violet, Safranine, Lugol's iodine, Oxidase reagents, Kovac's reagent, Hydrogen peroxide, Normal saline, Buffer solution, tetraoxosulphate (vi) acid, Barium chloride, Acetone reagent, Creatinine, Phenol peptone water, Sodium hydroxide, Immersion oil, Peptone pellets.

Media

The following media were used; Nutrient agar, MacConkey agar, Mueller-Hinton agar, Nutrient broth, (Oxoid, USA).

Antibiotics

The following antibiotic disc were used: aztreonam (50µg), amoxicillin/clavulanic acid (20/10µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30µg), imipenem (10 µg), , ertapenem (10µg), nalidixic acid (30 µg), ofloxacin (5 µg), piperacillin (64µg), streptomycin (25µg), sulfonamide (30µg) and tetracycline (30µg) (Oxoid, UK).

The following sugars were used: glucose, fructose, maltose, sucrose, lactose and sorbose (Oxoid, UK).

Inclusion criteria

1. All age groups were included
2. Both sexes were included.
3. Out patients with signs and symptoms suggestive of infections of the urinary tract.

Exclusion criteria

Patients already on antibiotics.

Sample collection and sampling sites

Sampling was conducted in January-March, 2024. In all, 150 urine samples were collected from patients attending General Hospital, Calabar. The samples were transported in an ice chest containing ice and analyzed immediately on arrival at the microbiology laboratory of the Department of Microbiology, University of Calabar.

Ethical Consideration

Approval from Cross River State Ministry of Health Ethic Committee was obtained prior to the conduct of the study (CRSMOH/HRP/REC/2024/537).

Sample processing and isolation of bacteria

Zero point one meals of urine sample were inoculated on MacConkey agar and incubated aerobically at 37^0C for 24 h. Presumptive

Klebsiella pneumoniae isolates were purified on Nutrient agar.

Pure culture maintenance

Pure bacterial cultures were obtained by streaking each colony that appeared on the MacConkey agar plates on Nutrient agar slants in MacCartney bottles. These were incubated at 37^0C for 24 hours and were stored in the refrigerator for future use.

Screening of bacterial isolates for ESBL-production

Preparation of 0.5 McFarland's standard: 1% w/v sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. The solution was mixed well. 1% w/v of barium chloride solution was prepared by adding 0.5 g of dehydrated barium chloride with 50 ml of distilled water. 0.5 Mc Farland's standard has an optical density equivalent to that of 1.5×10^8 colony forming units/ml.

Inoculum Preparation: morphologically similar 4-5 isolated colonies were taken from 24 hrs culture plate with the help of a sterile loop and transferred to a test tube that contains sterile peptone water. The peptone water was incubated at 37ºC for 4 hrs. The turbidity was adjusted to 0.5 McFarland turbidity standards.

All the isolates were subjected to ESBL screening test using cefotaxime (30µg, zone size≤ 22mm) and ceftazidime (30µg, zone size ≤ 17mm) discs, after preparing test isolates lawn culture on Mueller-Hinton agar, the ceftazidime and cefotaxime discs were placed on the MHA and incubated at 37^0C for 18hours after which the zone of clearance was measured and compared with CLSI interpretation to ascertain if it were susceptible, intermediate or resistant.

CLSI Phenotypic Confirmatory Combination Disc Test (CDT)

ESBL-production was confirmed with CLSI confirmatory test using both ceftazidime CAZ (30µg) (Liofilchem s.r.l, Roseto, Italy) and ceftazidime/clavulanic acid CAC (30µg/10µg) were aseptically placed 25mm apart on Mueller-Hinton agar plates which were inoculated with bacterial isolates lawn culture (turbidity adjusted to McFarland 0.5 standard). After incubation at 37^0C for 18hours, increase in zone of diameter of ≥ 5mm with CAZ/CAC disc compared with CAZ disc was considered a positive result for ESBL production (CLSI, 2014).

Characterization and Identification of ESBL-producing bacterial isolates

The characterization of carbapenem resistant isolates was performed by employing gram staining reaction, oxidase, catalase, citrate, methyl-red, Voges Proskauer test, urease, indole, H_2S test and oxidative fermentative test as described by Cheesborough, (2006).

RESULTS AND DISCUSSION

Klebsiella pneumoniae are the significant causes of nosocomial infections and carbapenems are considered as the drug of choice in such cases. Nowadays Carbapenem resistance in *Klebsiella pneumoniae* is an emerging problem and is a cause of concern because many of them are found to be resistant to most other antibiotics. Falagas *et al*., (2014) have reported that the number of deaths were significantly higher in patients with carbapenem resistant *Enterobacteriaceae* infections in comparison to those with carbapenem-susceptible *Enterobacteriaceae* infections. Carbapenem resistance is conferred by the production of enzyme carbapenemases. Carbapenemases are β –lactamases that can breakdown almost all β –lactam antibiotics and are being recognized as one of the major threats to effective management of patients in hospital, especially in developing countries Sathya *et al*., (2015). In this study a total of 21 non repetitive clinical isolates of *Klebsiella pneumoniae* from urine samples, collected from 150 out patients attending General Hospital, Calabar were included. Among the 21 isolates it was observed that maximum number (n=9; 42.85%) of *Klebsiella pneumoniae* were isolated from urine specimens of age range >60years. This was in accordance with the study conducted by Samar & Morsi Samar *et al*., (2016) in Egypt where 46% of *Klebsiella pneumoniae* were isolated from urine in a similar age range. Similar reports were made by Abdallah *et al*., (2015) in India and Nawshad *et al*., (2012) in Bangladesh. This is probably those within that age range have low immunity and are susceptible to infections Henshaw *et al.,* (2025). In this present study *Klebsiella pneumoniae* infection is higher in females (58%) when compared to males (42%) in harmony to the study conducted by Sathya *et al*., (2015) in India where the *Klebsiella pneumoniae* infection is higher (54.68%) in females than males (45.32%). In the present study, from the 21 *Klebsiella pneumoniae* isolates obtained only 5(23.80%) showed ESBL production, this is lower than the observation made in the study done by Wilson *et al*., (2015), which showed ESBL production among 70% of *Klebsiella pneumoniae* isolates. This increasing trend of ESBL producers were due to steadily increasing of ESBL producing strains among the clinical isolates and also it indicates the possibility of treatment failure caused by resistant organisms. The high occurrence of ESBLs in *Klebsiella pneumoniae* is of great concern since infections caused by this bacterium were very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easy spread of organism, efficient at acquiring and disseminating resistance plasmid. In the current study out of 21 *Klebsiella pneumoniae* isolates only one was found to be resistant to carbapenem (20%). This is consistent with the study of Sehlain *et al*., (2013) at Jaipur, India where the incidence of Carbapenem resistant *Klebsiella pneumoniae* is 17.64 %. But according to a study done in Puducherry, southern India by Sathya *et al.,* (2015) the carbapenem resistance in nosocomial isolates of *Klebsiella pneumoniae* is 43.6%. This is almost double the value we had in the current study. Our study revealed that CRKP strains showed high resistance to most antibiotics, including carbapenems, cefoxitin, cefotaxime, tarivid, ampicillin, streptomycin, gentamycin and ceftazidime. Previous reports revealed that two major types of resistance mechanisms related to CRKP drug resistance. One is the expression of *AmpC* enzymes or extended-spectrum β-lactamases (ESBLs) combined with upregulation of efflux pump system or mutation of outer membrane proteins or alteration of penicillin-binding proteins, which make CRKP resistant to cephalosporins and monobactams (Mushi *et al*., 2014). The other one is the expression of carbapenemases, which pose even more challenge, and cause CRKP resistant to almost all available β-lactams, including the carbapenems (Falagas *et al*., 2014).

Isolation of bacterial strains

The distribution of *Klebsiella pneumoniae* isolates according to age range is shown in table 1.

Table 1: Distribution of *Klebsiella pneumoniae* isolates from samples according to age range

Age Range(years)	No of samples	No of positive sample	Percentage (%)
10-20	25	0	0
21-30	25	1	5.26
31-40	25	2	10.52
41-50	25	3	15.78
51-60	25	6	31.57
>60	25	9	42.85

150 21

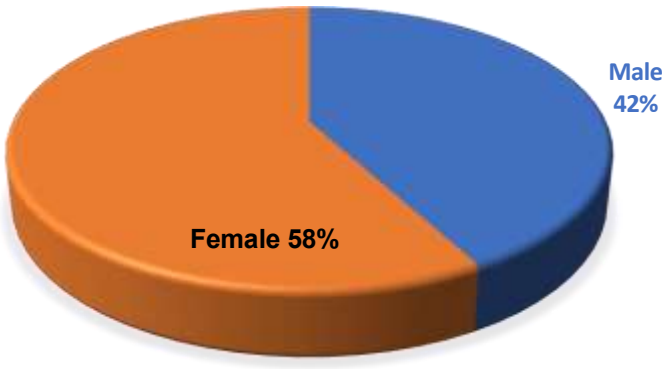

Figure 1: The distribution of *Klebsiella pneumoniae* isolates according to gender

Screening of bacterial isolates for extended spectrum β-lactam and carbapenem antibiotic resistant, characterization and identification

Table 2a: Extended spectrum β-lactam antibiotic (Ceftazidime) susceptibility among *Klebsiella pneumoniae* isolates by disc diffusion method

CLSI Interpretation	No of Isolates	Percentage
Susceptible (≥21mm)	16	76.19%
Intermediate (18-20mm)	0	0%
Resistant (≤17mm)	4	23.80%
Total	**22**	**100**

Table 2b: Imipenem(carbapenem) susceptibility among *Klebsiella pneumoniae* isolates by disc diffusion method

CLSI Interpretation	No of Isolates	Percentage
Susceptible(≥23mm)	4	80%
Intermediate(20-22mm)	0	0%
Resistant (≤19mm)	1	20%
Total	**5**	**100**

Table 3: Macroscopic, Microscopic, Biochemical and Sugar fermentation characteristics of ESBL- and carbapenemase producing *Klebsiella pneumoniae* isolates

Isolate Code	Macroscopic Morphology	Microscopic Morphology	Catalase	Oxidase	Indole	MR	VP	Nitrate Reduction	Citrate	Urease	H2S Production	Glucose	Lactose	Sucrose	Probable Organism
Ai1	Large, mucoid pink in colour	G-ve rod	+	-	-	-	+	+	+	+	-	AG	AG	AG	*Klebsiella pneumoniae*
Ai2	Large, mucoid pink in colour	G-ve rod	+	-	-	-	+	+	+	+	-	AG	AG	AG	*Klebsiella pneumoniae*
Ai3	Large, mucoid pink in colour	G-ve rod	+	-		-	+	+	+	+	-	AG	AG	AG	*Klebsiella pneumoniae*
Ai4	Large, mucoid pink	G-ve rod	+	-	-	-	+	+	+	+	-	AG	AG	AG	*Klebsiella pneumoniae*

Legend: +; positive, -; negative, MR; Methyl red, VP; Voges Proskauer,

Table 4: Antibiotic susceptibility profile of ESBL- and carbapenemase producing *Klebsiella pneumoniae* strains

Antibiotic used	*Klebsiella pneumoniae* **A**I1	*Klebsiella pneumoniae* **A**I2	*Klebsiella pneumoniae* **A**I3	*Klebsiella Pneumoniae* **A**I4
Piperacillin	R	S	R	S
Aztreonam	R	S	R	S
Amoxicillin-Clavulanic acid	R	S	S	S
Sulfonamide	R	R	R	R
Streptomycin	S	S	S	S
Ofloxacin	R	R	S	S
Chloramphenicol	S	S	R	S
Tetracycline	S	S	S	I
Nalidixic acid	R	R	R	R
Imipenem	S	S	S	S
Cefoxitin	R	R	R	R
Cefotaxime	R	R	R	R
Ceftazidime	R	R	R	R

Legend: Resistant: R, Sensitive; S, Intermediate; I

Table 4b: Antibiogram of carbapenem resistant *Klebsiella pneumoniae* isolate

Antibiotic used	*Klebsiella pneumoniae* **A**l5
Piperacillin	R
Aztreonam	R
Nalidixic acid	R
Amoxicillin-Clavulanic acid	R
Sulfonamide	S
Streptomycin	R
Ofloxacin	S
Chloramphenicol	R
Tetracycline	R
Imipenem	R
Cefoxitin	R
Cefotaxime	R
Ceftazidime	R

Legend: Resistant: R, Sensitive; S, Intermediate; I

CONCLUSION

In this study, four out of the 21 *Klebsiella pneumoniae* isolates obtained were resistance to beta lactam containing antibiotics and one strain was resistance to carbapenem(imipenem) and antibiotics susceptibility profile indicates ESBL- and carbapenem-resistant strains to be susceptible to streptomycin, imipenem and sulfonamide, ofloxacin respectively

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