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PREVALENCE AND ANTIBIOGRAM OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *Klebsiella pneumoniae* FROM URINARY TRACT INFECTION IN GENERAL HOSPITAL, CALABAR

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ABSTRACT

The rise of multidrug-resistant *Klebsiella pneumoniae* poses an enormous challenge to public health sector, as this leads to treatment failure for nosocomial infections. This study therefore aimed to phenotypically characterize extended spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* from urinary tract infection in General Hospital, Calabar. A total number of 17 non repetitive clinical bacterial strains were isolated, out of which (35.29 %;6/17) isolation rate falls in the age range greater than 60 years and seconded to age range 51-60 years with isolation rate of (29.41 %;5/17). Extended spectrum beta lactamase production screening revealed 23.52 %;4/17 of isolates to be ESBL producer and (76.47 %;13/17) were non-ESBL producer. The ESBL producing isolates were characterized and identified as strains of *Klebsiella pneumoniae*. Antibiogram of EBSL producing strains revealed, they were resistant to cefoxitin, cefotaxime, tarivid and sensitive to pefloxacin and septrin. Nevertheless, this situation has become a heavy burden in developing countries, including Nigeria, and is associated with substantial morbidity, mortality, and increased healthcare expenses

INTRODUCTION

Antimicrobial resistance (AMR) has reached the agenda of global policy makers. Its importance was recently emphasized by the World Health Organization in a comprehensive report, including data from 114 countries. It is clear that AMR is no longer a potential, but a current major threat to global public health and it is time to take action (Bennett *et al.,* 2020). In particular, the increasing prevalence of multidrug resistant (MDR) Gram-negative bacteria, expressing extended-spectrum ù-lactamases (ESBLs) and associated resistance mechanisms, causing hospital outbreaks and difficult-to-treat human infections has been of concern since the turn of the millennium. The diverse use and misuse of antibiotics across sectors (in humans, animals and agriculture), is considered the primary driver of AMR. The global spread of MDR human pathogens is a multifaceted challenge, and also hugely influenced by migration and tourism, the lack of access to clean water, open rather than closed sewage systems, high population densities and inadequate healthcare in many parts of the world.

The pathogenicity of Klebsiella spp. may be associated with virulence factors, such as capsular antigens (O- and K-antigens), adhesins, siderophores and lipopolysaccharides (endotoxins). The capsule is considered essential to the virulence of Klebsiella, as it protects the bacterium from phagocytosis and prevents killing of the bacteria by bactericidal serum factors (Ferrari *et al*., 2019). Some serotypes or capsular types (K- types) of *K. pneumoniae*, e.g. K1, K2, K5, K54 and K57, have been associated with invasive human infectious diseases. K1 was observed among isolates causing Friedländer's pneumonia, and has more recently been associated with pyogenic liver abscesses (Gonzalez -Ferrer *et al*., 2021). Brisse et al. studied the association between K-type, sequence type (ST) and virulence gene content. The authors concluded that K-types are not associated with specific *K. pneumoniae* clones, and that K-types are distributed among unrelated clones by horizontal transfer of the cps operon, which encodes the synthesis of capsular polysaccharides. Furthermore, the virulence gene content was found to be associated with specific clones, rather than with K-types (Gu *et al*., 2018).

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During recent years, several genes encoding virulence factors in *K. pneumoniae* have been described: the plasmid-borne rmpA regulates the mucoid phenotype (Humphries & Hemarajata, 2017), wcaG is associated with enhanced bacterial escape from phagocytosis (Ko *et al*., 2002), kfu is involved in iron acquisition, fimH encodes type 1 fimbriae, mrkD encodes type 3 fimbriae and cf29A encodes the non-fimbrial adhesion factor CF29K (Lan *et al*., 2012). *K. penumoniae* ST23, which is frequently of serotype K1, is considered to be a particular virulent clone. Presence of allS is a marker for ST23 (Pendleton *et al*., 2013). Calhau *et al*. recently detected several virulence genes and pathogenicity islands (PAIs) in a collection of clinical ESBL producing *K. pneumoniae* isolates from renal transplant patients (Landman *et al*., 2008). In a recent Danish study, the virulence factors aerobactin, kfu and rmpA were detected in a hypermucoviscous *K. pneumoniae* ST23 blood isolate from a patient with a liver abscess (Lee *et al*., 2017). *Klebsiella* spp. are known to be inherent or intrinsically resistant to ampicillin, ticarcillin and piperacillin due to chromosomal SHV-1- production. Furthermore, all Enterobacteriaceae are intrinsically resistant to penicillin G, glycopeptides, fusidic acid, macrolides (with some exceptions), lincosamides, streptogramins, daptomycin and linezolid (Lin *et al*., 2012). Acquired resistance to other relevant antibiotic groups is increasingly reported in clinical *K. pneumoniae* isolates. Bacterial tolerance to biocides has been observed, and concern has been raised on their impact on the selection of antimicrobial resistance (AMR) in human pathogens (Mathers *et al*., 2020). This study was aimed at the phenotypic characterization of extended spectrum beta lactam.

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 (5µg), 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.

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 collection

Sampling was conducted in April-May, 2024. In all, 80 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.

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 hours. 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 24hours and were stored in the refrigerator for future use.

Screening of bacterial isolates for ESBL-production

Preparation of 0.5 McFarland's standard: 1. 1 % w/v sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. The solution was mixed well. 2. 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 x 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.

Double Disc Synergy Test (DDST)

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 370C 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

Isolation of bacterial strains

The distribution of *Klebsiella pneumoniae* isolates according to age range is shown in table 1. Results indicates the rate of isolation to be high in patient's urine in the age range of over 60 years (35.29 %), seconded to age range 51-60 years (29.41 %).

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	3	0	0
21-30	3	1	5.88
31-40	3	2	11.76
41-50	5	3	17.64
51-60	8	5	29.41
>60	8	6	35.29

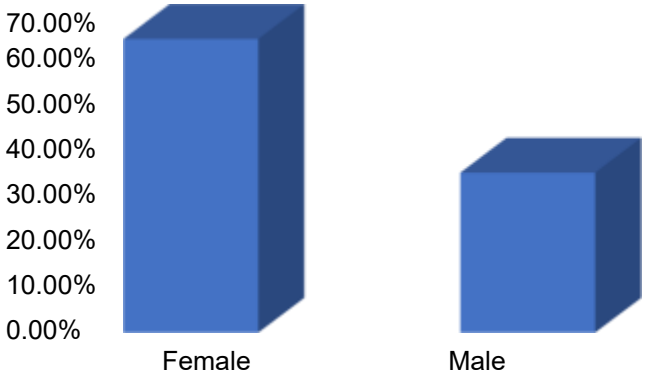

Figure 1: the distribution of *Klebsiella pneumoniae* isolates according to gender

Figure 1 shows the distribution of *Klebsiella pneumoniae* isolates according to gender. Results indicates that the female had 68 % rate of isolation while sample from the male were recorded at 42%.

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

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

CLSI Interpretation	No of Isolates	Percentage
Susceptible (≥21mm)	13	76.47%
Intermediate (18-20mm)	0	0%
Resistant (≤17mm)	4	23.52%
Total	**17**	**100**

Antibiotic susceptibility profile of ESBL-producing *Klebsiella pneumoniae* strains

Table 4 shows the antibiotics resistance profile of strains of ESBL-producing *Klebsiella pneumoniae*. It was observed that the isolates were resistant to cefoxitin(30µg), cefotaxime(30µg) and tarivid(10µg). Sensitivity was recorded for Pefloxacin(10µg) and septrin(30µg)

Table 4: Antibiotic resistance profile of ESBL-producing *Klebsiella pneumoniae* isolates

Antibiotic used	*Klebsiella pneumoniae* **UC**1	*Klebsiella pneumoniae* **UC**2	*Klebsiella pneumoniae* **UC**3	*Klebsiella pneumoniae* **UC**4
Streptomycin(30µg)	R	S	R	S
Ampicillin(30µg)	R	S	R	S
Ceporex(10µg)	R	S	S	S
Tarivid(10µg)	R	R	R	R
Pefloxacin(10µg)	S	S	S	S
Gentamycin(10µg)	R	R	S	S
Augmentin(30µg)	S	S	R	S
Ciprofloxacin(10µg)	S	S	S	I
Septrin(30µg)	S	S	S	S
Imipenem(10µg)	R	R	R	R
Cefoxitin(30µg)	R	R	R	R
Cefotaxime(30µg)	R	R	R	R
Ceftazidime(30µg)	R	R	R	R

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

DISCUSSION

Antibiotics are now widely utilized, and new antibiotics are constantly being developed for the treatment of illnesses. The widespread use of β-lactam antibiotics in hospitals and the community "has resulted in increased morbidity, mortality, and health-care costs (Mathers *et al*., 2020) Antibiotics must be used properly. Klebsiella infections are a major opportunistic infection in health-care settings, posing a global health-care concern. Its importance has grown as a result of its ability to survive in a variety of environments the development of drug resistance mechanisms, and the rise of multidrug resistant." The isolation, identification and performing antimicrobial susceptibility testing of Klebsiella infection to find the resistance patterns aids in the selection of appropriate antibiotic and it plays the important role in lowering patient mortality and morbidity and reducing the spread of resistant strains in the community. A total of 17 clinically consecutive, nonduplicate, significant isolates of *Klebsiella* species isolated from urine samples were included in the study. In the present study among 17 isolates, 11 isolates were from female patients and remaining 6 isolates were from male patients. Six (6) maximum number of *Klebsiella* isolates were obtained from age group greater than 60 years of age, similar finding was made by Henshaw *et al*., (2026), followed by 51-60 years of age, in contrast to the study done by Rice, (2008) showed a greater number of isolates were obtained from the age group 41-50 years of age, this may be due to a different in geographical location. The findings in this study are similar to a previous study that revealed most *Klebsiella* species were isolated from patients over 70 years old (Landman *et al*., 2008). Meanwhile, another study suggested that a greater number of *Klebsiella* species isolates were obtained from patients aged between 40 to 65 years old (Pendleton *et al*., 2013; Henshaw *et al*., 2026). The differences in terms of patient's age distribution could be related to the strength of the immune system response, which is expected to decline in senescence. Patients aged under 40 years tend to have stronger immune systems, thus giving more pressure to *Klebsiella* species to fight the immunity of the host (Lin *et al.,* 2012). On the contrary an increased age leads to a higher risk of *Klebsiella* spp. infection because of increased incident of comorbid illness increased age leads to a higher risk of *K. pneumoniae* infection because of increased incident of comorbid illness (Safder and Maki, 2002). In the present study, from the 17 isolates obtained only 4(23.52%) showed ESBL production, this is lower than the observation made in the study done by Mehrgan *et al*., (2009) 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.

CONCLUSION

This present study revealed, 23.52 %; 4/17 of isolates to be ESBL producer and (76.47 %; 13/17) to be non-ESBL producer. The ESBL producing isolates were resistant to cefoxitin, cefotaxime, tarivid and sensitive to pefloxacin and septrin.

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