RESISTANCE PROFILE OF KLEBSIELLA PNEUMONIAE ISOLATES FROM A TERTIARY HOSPITAL IN SOUTH-SOUTH NIGERIA.

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ABSTRACT

Introduction:

Klebsiella pneumoniae is notoriously drug resistant, possessing hundreds of antimicrobial resistance genes. It is also capable of transferring these genes to other gram-negative bacteria, especially in settings with suboptimal infection prevention and control practice, thereby propagating antimicrobial resistance within the environment. This study aimed at detecting the presence and distribution of resistant Klebsiella pneumoniae isolates in our institution.

Methods:

Two hundred and twenty-five (225) Klebsiella pneumoniae isolates were recruited from samples received from patients admitted to various wards in the hospital. Antimicrobial susceptibility was done using the modified Kirby Bauer technique. Isolates non-susceptible to third generation cephalosporin and carbapenems were screened for extended-spectrum beta-

lactamase (ESBL) and carbapenemase production using the double-disk synergy method and modified carbapenem inactivation method respectively, multidrug-resistant (MDR) isolates were also identified. The distribution of resistant isolates across various wards were determined based on information in accompanying laboratory request forms.

Results:

Average resistance rates to cephalosporins and carbapenems were 32% and 7.8% respectively with cefuroxime showing highest resistance (39.1%) among cephalosporins and ertapenem (8.4%) among carbapenems. Resistance to ciprofloxacin was 34.7% while 24.0% and 14.7% of isolates showed resistance to gentamicin and amikacin, respectively. While 12 (5.3%) isolates were multidrug resistant, 65 (28%) and 14 (6.2%) isolates produced ESBL and carbapenemase enzymes, respectively. 41 (63.1%) of the ESBL producing and 7 (50%) carbapenemase producing isolates were recovered from urine specimens. The isolates possessing ESBL and carbapenemase genes were mostly recovered from specimens from surgical wards, while the MDR organisms were on the other hand seen the most in specimens recovered from medical wards.

Discussion:

Although the general resistance rate of Klebsiella pneumoniae to last-line antibiotics was low in the study compared to many reports in-country and across the world, the rate of ESBL expression is worrisome. This is a cause for concern as both resistance mechanisms have similar predisposing factors. As a result, widespread difficult-to-treat infections are imminent if adequate measures such as optimal infection prevention and control practices and rational antibiotics use are not put in place.

Key words*: Klebsiella Pneumoniae, ESBL, Resistance, MDR*

INTRODUCTION

Klebsiella pneumoniae is a key component of the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*) which are considered the greatest threat to patients' care, due to the emergence of strains from this group capable of expressing resistance to all or most available antibiotics.[1] Accumulation of antimicrobial resistance genes in these organisms is attributed primarily to horizontal gene transfer (HGT) aided by plasmids and other mobile genetic elements.[2] Hundreds of mobile Antimicrobial resistance (AMR) genes have been found in *Klebsiella pneumoniae* and most of the AMR genes found in other clinically relevant gram-negative organisms have their origin traceable to *Klebsiella pneumoniae.*[3] While *Klebsiella pneumoniae* is intrinsically resistant to some antibiotics such as ampicillin due to the penicillinase encoded in its chromosome, and to a few others through chromosomal mutation,[4] most antimicrobial resistance associated with *Klebsiella pneumoniae* are through the acquisition of resistance genes via plasmid-mediated horizontal gene transfer.[5,6] This organism, in a similar manner, disseminates these resistance genes to other members of the Enterobacteriaceae family and gram-negative organisms such *Pseudomonas aeruginosa* and *Acinetobacter baumannii* especially in settings with suboptimal infection prevention and control practices.[3] The most notorious resistance mechanisms such as production of extended spectrum β-lactamase and carbapenemases and mobile quinolone resistance genes qnrA and qnrB were first detected in *Klebsiella pneumoniae.*[3,7] The promiscuity attributes of *Klebsiella pneumoniae* are linked to plasmid permissive traits which enhance the ability of the organism to amplify and disseminate resistance genes across different ecological niches resulting in broad dissemination amongst hospital,

human, commensal, environment and animal associated microbial populations.[8,9] *Klebsiella pneumoniae*, is a member of the *Enterobacteriaceae* family, and a normal commensal of the gastrointestinal tract of healthy animals and humans. It is often described as a common opportunistic healthcare-associated pathogen, found to account for about one third of all gram-negative infections.[10] These common healthcare-associated infections include ventilator-associated pneumonia, urinary tract infections, cystitis, surgical wound infections, and other life-threatening infections, such as sepsis and endocarditis. It is also an important cause of serious community-acquired infections such as pyogenic liver abscesses, necrotizing pneumonia, and endogenous endophthalmitis.[11] This study became necessary as most determinants known to predispose to *Klebsiella pneumoniae* resistance and spread, such as antibiotic misuse and suboptimal infection control, are found in our setting.

METHODS

This laboratory-based cross-sectional study was conducted in the Department of Medical Microbiology and Parasitology, University of Port Harcourt Teaching Hospital, Rivers State, Nigeria between January and June 2018. From a total of six hundred and fifty (650) clinical specimens submitted to the laboratory for cultures, *Klebsiella pneumoniae* isolates were recruited consecutively till the minimum sample size of 225 was achieved. Source specimens included urine, wound swabs, body fluid aspirates, blood, and cerebrospinal fluid. Samples were inoculated unto MacConkey agar and incubated at $35\text{-}37^0$C for 16-18 hours in aerobic conditions. All cultures with discreet colonies were subjected to Gram staining, motility test and biochemical tests. Lactose-fermenting Gram-negative, non-motile, citrate positive and indole negative bacilli were presumptively identified as *Klebsiella pneumoniae*. These were confirmed using MICROBACT 12A

identification kits (Oxoid, UK) according to the manufacturer's instructions. Only one isolate per patient was recruited.

Antimicrobial susceptibility testing was performed on all the isolates of *Klebsiella pneumoniae* using the disk diffusion technique according to CLSI guidelines.[12] The following antibiotics were tested: ertapenem (10µg), imipenem (10µg), meropenem (10µg), amoxicillin/clavulanic acid (20/10µg), ceftazidime (30µg), cefuroxime (30µg), ceftriaxone (30µg), gentamycin (10µg), ciprofloxacin (5µg), amikacin (30 µg), piperacillin/tazobactam (100/10µg), tigecycline (15µg), colistin (10µg) polymyxin (300units) and fosfomycin (200µg). Incubation conditions included aerobic environment for 16-18hours at 35-37°C. Thereafter, the zones of inhibition were measured and interpreted as resistant, intermediate or susceptible using criteria recommended by CLSI.[12] *Klebsiella pneumoniae* ATCC BAA-1706 was used as negative control while *Klebsiella pneumoniae* ATCC BAA-1705

was used as a positive control. For the purpose of the study, all isolates with intermediate susceptibility were regarded as resistant.

All *Klebsiella pneumoniae* isolates which were found to be resistant to third generation cephalosporins, and carbapenems using Ertapenem as surrogate, were screened for ESBL and carbapenemase production using double disk synergy method and modified carbapenem inactivation method respectively according CLSI interpretative criteria.[12] Briefly, the double disk synergy method was performed on fresh test isolates (isolates intermediately susceptible or resistant to third generation cephalosporins) which were exposed to combination discs of Ceftazidime/Clavulanic acid and single disc of each Ceftazidime(30µg) on the same Mueller Hinton agar plates and incubated at 37°C for 24 hours for the detection of ESBL enzymes. The zone diameter around each disc was measured

and if the diameter around the Ceftazidime/Clavulanic acid was 5 mm or more greater than the zone diameter around the single disc of Ceftazidime respectively, the bacterial isolate was said to be an ESBL-producing organism. For the modified carbapenem inactivation method, a 10μL loopful of the test organism (carbapenem resistant isolates) was emulsified in 2mL of peptone water. A (10μg) meropenem disk was then immersed in the suspension and incubated for 4 hours at 35°C. Thereafter the disc was removed and applied to Muller Hinton plates inoculated with a 0.5 McFarland suspension of *E. coli* ATCC 25922, and the plates were incubated in ambient air at 35°C for 18−24 hours. An inhibition zone diameter of 6–15 mm or colonies within a 16–18 mm zone was considered to be a positive result, and a zone of inhibition ≥19 mm was considered to be a negative result.

Multidrug-resistant (MDR) *K. pneumoniae* isolates were defined by non-susceptibility to at least one agent in three or more antibiotic categories such as beta-lactam antibiotics, fluoroquinolones, aminoglycosides etc.[13]

Basic demographic information was obtained from the laboratory forms. These included age, sex, ward/clinic, and provisional diagnosis.

DATA ANALYSIS

Data obtained was analyzed using the statistical package for social sciences (SPSS) version 25. Data was presented using tables. All categorical data were expressed as percentages and chi square test was used as a test for association. All analyses were done at a 95% confidence interval and a p value < 0.05 was considered significant.

RESULTS

225 isolates were tested against various antibiotics. The isolates were mostly resistant to cefuroxime (n=88, 39.1%), Ciprofloxacin (n= 78, 34.7%) and ceftriaxone (n=72, 32%). Isolates showed the least resistance to colistin (6%), fosfomycin (4%) and polymyxin B (4%).

No isolates showed resistance to tigecycline (Table 1)

Table 1: Antimicrobial Susceptibility Profile of *Klebsiella pneumoniae* isolates

	Number tested	Number resistant	Percentage (%)
Amikacin	225	33	14.7
Amoxicillin/Clavulanate	225	67	29.7
Cefuroxime	225	88	39.1
Ceftriaxone	225	72	32
Ceftazidime	225	62	27.5
Ciprofloxacin	225	78	34.7
Colistin	50	3	6.0
Ertapenem	225	19	8.4
Fosfomycin	50	2	4.0
Gentamicin	225	54	24.0
Imipenem	225	17	7.5
Meropenem	225	17	7.5
Nitrofurantoin	115	34	29.6
Piperacillin/tazobactam	225	53	23.6
Polymyxin B	50	2	4.0
Tigecycline	50	0	0.0

The distribution of ESBL-producing, carbapenemase-producing and multidrug resistant isolates is shown below. 30.8% of ESBL and 42.9% of carbapenemase producers were found in specimen from surgery wards (Table 2).

Table 2: Distribution of *K. pneumoniae* isolates expressing ESBL, carbapenemase and multidrug resistance by Hospital wards.

Source	ESBL	Carbapenemase	MDR	Chi-square (p-value)
Surgery	20(30.8)	6 (42.9)	3 (25)	
Medicine	16 (24.6)	5 (35.7)	4(33.3)	
Paediatrics	8(12.3)	0 (0.0)	1(8.3)	6.11 (0.6346)
ICU	15 (23.1)	3 (21.4)	4 (33.3)	
SCBU	6(9.2)	0 (0.0)	0(0.0)	
Total	**65**	**14**	**12**	

ESBL: Extended-spectrum beta-lactamase, SCBU: Special care baby unit. ICU: Intensive care unit, MDR: Multidrug resistant

Urinary isolates contributed the most to the overall resistance isolates found in the study accounting for 63.1%, 50% and 66.7% of the total ESBL-producing, carbapenemase-producing, and multidrug resistant morphotypes respectively. None of the resistant morphotype were detected in CSF *Klebsiella pneumoniae* isolates (Table 3)

Table 3: **Distribution of *K.pneumoniae* isolates expressing ESBL, Carbapenemases and Multidrug resistance by specimen type**

Specimen type	ESBL Producers	Carbapenemase Producers	MDR	Chi-square (p-value)
Urine	41(63.1)	7 (50.0)	8(66.7)	
Wound swab	15(23.1)	5 (35.7)	3(25.0)	
Blood	5(7.7)	0 (0.0)	0(0.0)	
Sputum	3(4.6)	1 (7.1)	1(8.3)	5.39 (0.7147)
Body fluid aspirate	1(1.5)	1 (7.1)	0(0.0)	
CSF	(0.0)	0 (0.0)	(0.0)	
Total	**65**	**14**	**12**	

CSF: Cerebrospinal fluid

DISCUSSION

Klebsiella pneumoniae has over the years emerged as a significant cause of both community- and hospital-acquired infections. The pathogen is notoriously drug resistant and associated with increased morbidity and mortality.[11]

In our study, resistance of *Klebsiella pneumoniae* isolates to all tested antibiotics was below 50%. However, the highest rates of resistance were generally noticed among the commonly used group of antibiotics such as the beta lactams particularly the cephalosporins. Cefuroxime, a second-generation cephalosporin, was the most affected with 39.1% followed by 32% and 27.5% respectively for ceftriaxone and ceftazidime. The level of resistance observed to these antibiotics could be related to the rate of use of these drugs in our centre as first line empirical antibiotics in most units. Interestingly, there was a lower level of resistance to β-lactam/β-lactamase inhibitor combination drugs,

compared to the cephalosporins, with amoxicillin-clavulanate and piperacillin-sulbactam having rates of 29.7% and 23.9% respectively. This may be attributable to the high rate of ESBL production among the isolates and probably low rate of AmpCgenes.[14]

Another class of first line antibiotics that recorded a comparatively high level of resistance among isolates were the fluoroquinolones where resistance was 34.7%. From the data, the average rate of quinolone-resistance in this study is greater than 20%, therefore this class of antibiotics should be used with caution as empirical treatment for *K. pneumoniae* infection in our environment, pending laboratory results of culture and sensitivity, because the risk of treatment failure is higher at resistance rates in excess of 10% to 20%.[15]

Our results are comparable to the findings from a study done by Ayandele et al,[16] where *Klebsiella pneumoniae* isolates resistance rate to third generation

cephalosporins was on average, about 38% [16] but differed markedly from results of another Nigerian study which reported resistance rates of over 50% to fluoroquinolones and aminoglycosides, and 94% of the isolates resistant to ceftazidime and 100% resistant to ceftriaxone.[17] This marked difference in the above findings compared to this study is likely due to the fact that the all of the study isolates were later found to be ESBL producers.

Our finding of resistance rates of less than 50% suggests that in our environment these first line antibiotics can still be considered for empirical therapy for infections caused by *Klebsiella spp*, however susceptibility testing is still important.

Resistance of *Klebsiella pneumoniae* isolates to second line drugs such as ertapenem, imipenem, meropenem was, on the average, 8.4 %. While this figure is lower than 14.5% reported by Oduyebo et al[18] and 23.7% reported by Adesanya et al,[19] it is higher than that of an earlier study done in Nigeria which reported no resistance of *Klebsiella pneumoniae* to carbapenems.[16] Our findings therefore add to the evidence that carbapenem resistance among *Klebsiella pneumoniae* is on the increase in Nigeria therefore rational use of these antibiotics should be instituted. Resistance to last resort antibiotics colistin, tigecycline and polymyxin B were also recorded in this work but the rates were significantly low.

Production of ESBL and carbapenemases by isolates are findings that have implications for both antibiotic therapy and infection prevention and control. ESBL prevalence among the *Klebsiella pneumoniae* isolates was 28.8% and this is comparable to prevalence rates of 22%, 26%, 29%, and 30% recorded in South South, North Central, North West and North East Nigeria, respectively.[20-23] This similarity across different health institutions in different zones may reflect a similar pattern of use of cephalosporin across Nigeria. Carbapenemase production in this study was low among the

carbapenem-resistant organisms (6.2%). This finding however differs from the higher rates of 12.4%,[17] 23%,[24] and even 63.4%[25] reported in other Nigerian studies. Such similarities and differences in prevalence of ESBL and carbapenemase production by clinical isolates in various centers may reflect the rate of use of third generation cephalosporins and carbapenems as their use or misuse select for isolates producing these enzymes.

This study also showed that *Klebsiella pneumoniae* isolates from urine specimen and those from CSF had the highest rates and least rates of both ESBL and carbapenemase production, respectively. This apparent difference was however not statistically significant. Other Nigerian studies, however, also report that ESBL production was mainly seen in urinary *Klebsiella pneumoniae* isolates [25,26,27] while others report majority of resistant *Klebsiella pneumoniae* isolates from sputum [17,21] with the sputum isolates being the greatest ESBL producers.

Carbapenemase producers were also mainly isolated from urinary isolates in studies from northern Nigeria.[25]

The presence of these ESBL and carbapenemase producers, as well as MDR phenotypes on various wards, were also considered in this study. This presence of these phenotypes are very significant for the spread of antibiotic resistance, as *Klebsiella pneumoniae* is notorious for plasmid transfer of resistant genes across intra-species, inter-species and even genus lines.[28] In this study differences in the prevalence of resistant *K.pneumoniae* across various wards were noted. This difference in prevalence across wards was however found to be not statistically significant. The higher rates of ESBL and carbapenemase production seen in surgical ward, and the MDR rate in medical ward are however comparable to findings from other studies.[2,6] This could be due the long hospital stay associated with patients admitted to these units as well as the high

possibility of acquisition of healthcare associated infections.

CONCLUSION AND RECOMMENDATIONS.

The majority of *Klebsiella pneumoniae* isolates are susceptible to most available antibiotics, these can therefore be employed for empirical treatment of infections. However there exists a significant proportion of antibiotic resistant *Klebsiella pneumoniae* in our environment. These resistant isolates produce major antibiotic hydrolyzing enzymes such as ESBL and carbapenemase and a few of them were multidrug resistant. Considering this, we recommend susceptibility testing, and when necessary resistance testing on all isolates to guide antibiotic management. We also recommend the institution of rational antibiotic prescribing and infection prevention and control measures to reduce the rising prevalence and widespread distribution of these resistant isolates. Failure to do this could lead to outbreaks of multidrug resistant infections.

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