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Carbapenemase-producing *Klebsiella pneumoniae* Isolated from Environmental Sources in a Tertiary Health Institution in Nigeria

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Abstract The acquisition of carbapenemase-producing organisms in healthcare settings is a significant threat and has dire implications for public health. Previous reports regarding carbapenemase-producing *Enterobacteriaceae* from fomites are limited. This study aimed to analyse the antimicrobial resistance patterns and prevalence of carbapenemase-producing *Klebsiella pneumoniae* in the ward environments of a tertiary health institution in Nigeria.One hundred and forty-two bacteria were isolated from 534 fomites in the hospital wards, and out of these, 15(10.6%) were *K. pneumoniae*. Therefore, the prevalence of *K. pneumoniae* in all the samples was 15/534(2.8%), while that of carbapenemase-producing *K. pneumoniae* was 8/534(1.5%). Multi-drug resistance was detected in 15/15(100%) of the *K. pneumoniae* isolated. All the *K. pneumoniae* isolates were resistant to ampicillin, trimethoprim-sulfamethoxazole, cefuroxime, and tetracycline. Although 8/15(53.3%) of the isolates were confirmed positive for carbapenemase production using the modified Hodge test, no *Klebsiella pneumoniae* carbapenemase gene (bla_{KPC}) was detected. The most frequent sites that harboured carbapenem-resistant *K. pneumoniae* were the beds 6/15(40%). Hence, the prevalence of carbapenemase-producing *K. pneumoniae* fomite colonisation in the NAUTH ward environment was low.

Keywords: *multidrug-resistant, Klebsiella pneumoniae, carbapenemase, oxacillinase, fomites*

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1. Introduction

Klebsiella pneumoniae are Gram-negative, non-motile, encapsulated bacilli belonging to the family of bacteria called the *Enterobacteriaceae* [1]. They are considered the second most common cause of healthcare-associated sepsis, remaining for long periods in hospital environments and equipment. They may be spread to patients by contact with these environmental surfaces [2,3]. They develop resistance by various mechanisms, but by far, the most troublesome of these are the carbapenemases which make the organisms resistant to all of the Beta lactam antibiotics such as penicillins and cephalosporins. They are often associated with other resistance mechanisms, giving resistance to other antibiotics such as the quinolones (e.g. Ciprofloxacin) and aminoglycosides (Gentamicin). They cause resistance to carbabenem antibiotics, (Ertapenem, Meropenem, Imipenem and Doripenem) which are often the last line in the fight against Gram-negative infections [4,5]. These enzymes are also resistant to the carbapenems which have been considered as agents of last resort in the treatment of infections caused by MDR Gram-negative bacilli [4,5].

It is important to note that resistance to carbapenems in some species is intrinsic. For example Stenotrophomonas maltophilia possesses the endogenous metallo-beta-lactamase (MBL) L1 [6], thus, the use of carbapenem antibiotics as a treatment for such infections should not be considered. Intrinsic resistance to carbapenems, however, is not a common finding among clinically important bacteria and for most of them carbapenem resistance is acquired from gene acquisition through horizontal gene transfer or some other mutational events.

Many Gram-positive bacteria acquire resistance to carbapenems and other beta-lactams through mutation-derived changes of their penicillin binding proteins (PBPs), while Gram-negative bacteria commonly recruit other mechanisms to overcome the effect of the carbapenems (e.g. efflux pumps, diminished expression or loss of outer membrane porins leading to a decrease in the permeability of outer membranes [6].

The most clinically important acquired mechanism of carbapenem resistance is the enzyme-mediated resistance through the production of carbapenemases. These are beta-lactamases that are able to inactivate carbapenems together with other beta-lactam antibiotics [7]. The impotance of these enzymes are due to the fact that they hydrolyze all or almost all beta-lactam antibiotics, confer high levels of carbapenem minimum inhibitory concentrations, are encoded by genes that are horizontally transferable by plasmids or transposons and are commonly associated with genes encoding for other resistance determinants.

The burden of antimicrobial resistance (AMR) in developing countries has increased remarkably in recent years [8,9]. In a 2017 review of AMR in Africa, only about 60% of the countries had available data on AMR. There was a strikingly high median resistance (MR) rate for the *Enterobacteriaceae* to ampicillin (MR= 88.1%) [8]. Resistance was however uncommon for the carbapenem group of antibiotics. In particular, 34.2% of the *Klebsiella spp.* were resistant to ceftriaxone, while 46.7% exhibited resistance to cefotaxime. This observation suggested a high-level extended-spectrum beta-lactamase (ESBL) production. However, the median resistance rate for *K. pneumoniae* against imipenem, a carbapenem was 3.0% [8]. In another survey involving Africa and Asia, high resistance rates were also observed for ampicillin (67.2%) and ceftriaxone (25.9%) [9].

The most frequently detected carbapenemases include class A- *Klebsiella pneumoniae* carbapenemase (KPC) types), class B-metallo-β-lactamases (MBLs) viz Verona integron-encoded metallo-β-lactamase (VIM) and NewDelhi metallo-β-lactamase (NDM) types, and class D-oxacillinases (OXA-48-like enzymes) [10]. Furthermore, KPCs are major causes of nosocomial outbreaks [11,12,13].

Several studies done previously on carbapenemase detection focused more on isolates from clinical specimens of patients. Still, limited information is available in the literature on the prevalence of carbapenemase-producing *K. pneumoniae* in the hospital environment. One environmental study worthy of note was that in which the presence of carbapenemase-producing *K. pneumoniae* was determined in environmental sites of Intensive Care Units (ICUs) in Cairo, Egypt [14]. This study, therefore, aimed at assessing the occurrence of carbapenemase-producing *K. pneumoniae* in the ward environments of a tertiary health institution in Nigeria.

2. Materials and Methods

2.1. Bacterial Isolation Sources

One hundred and forty-two bacterial isolates were isolated from 534 environmental specimens obtained in the wards of NAUTH, Nnewi, a major referral centre serving individuals from most parts of South-East, Nigeria. The bacteria were collected from January to June 2018. The specimens included swabs collected from; patients beds, bedside tables, bedside cupboards, trolleys, sphygmomanometers, water taps, antiseptics, disinfectants, hand wash solutions, hand sanitisers, forceps, wheelchairs, kidney dishes, door handles, drip stands, drug mortars, methylated spirits, suction tubes, nurses desks, doctors desks and pulse oximeters.

2.2. Bacterial Isolation

Duplicate swabs were collected by rolling moistened sterile swab sticks over the sites mentioned above for about 5 seconds. These swabs were sent to the laboratory immediately after collection and cultured on chocolate and Mac Conkey agar (Oxoid, UK) and incubated at 35-37°C for 24 hours [12,14]. The isolates were Gram-stained, and the Gram-negative rods were subjected to confirmatory identification of *K. pneumoniae* using the Microbact™ Gram-negative bacteria identification kit (Oxoid, UK) [12].

2.3. Antimicrobial Susceptibility Testing

The Modified Kirby-Bauer antimicrobial susceptibility testing technique was performed on all isolates confirmed as *K. pneumoniae* [15,16]. A lawn of each bacterial inoculum equivalent to 1.5 X 10^8 CFU/ml, was made on the surface of a Mueller-Hinton agar (Oxoid, UK) plate using a sterile swab stick and left to dry for 3-5 minutes. Antibiotics were then placed on the lawn, and the plates incubated aerobically at 35-37^{o}C for 16-18 hours. The zones of growth inhibition around each antibiotic disc were measured and reported based on the guidelines of the CLSI [16].

2.4. Screening for Suspected Carbapenemase Production

This involved placing 10μg carbapenem discs viz meropenem and ertapenem (Oxoid, UK) on the surface of Mueller Hinton agar (Oxoid, UK) plates inoculated with each isolate. Following incubation for 16-18 hours at 35-37°C, zones of growth inhibition around each antibiotic were read off.

K. pneumoniae isolates that showed a zone of inhibition ≤ 22mm in diameter for meropenem or ≤ 21mm for ertapenem were considered as suspected carbapenemase producers and were subjected to phenotypic confirmation by the modified Hodges test (MHT) [13,16].

2.5. Phenotypic Confirmation of Carbapenemase Production (MHT)

In this method, a suspension of *E. coli* ATCC 25922 equivalent to 0.5 McFarland turbidity standard was prepared. The *E. coli* suspension was then diluted 1:10 by adding 0.5 ml of the *E. coli* suspension to 4.5 ml of saline. A lawn of the 1:10 dilution of *E. coli* ATCC 25922 was evenly streaked onto Mueller Hinton agar plates using sterile cotton swabs and then allowed to dry for 3-5 minutes. One disc of meropenem (10µg), was placed on the centre surface of the MHA plate. In a straight line, using a sterilised wire loop, the test organisms were streaked from the edge of each Meropenem disc to the edge of the plate. The plates were incubated at 37°C for 24 hours. After incubation, they were examined for a cloverleaf type indentation at the intersection of the test organism and *E. coli ATCC 25922* within the zone of inhibition of the meropenem disc as described by the CLSI. [16] *K. pneumoniae* ATCC 1705 and *K. pneumoniae* ATCC 1706 were used as positive and negative controls [16].

2.6. Molecular Detection of bla$_{KPC}$

Bacteria DNA from the *K. pneumoniae* isolates was extracted using a previously described boiling method for DNA extraction with slight modifications [17]. The extracted DNA was quantified and tested for purity using the NanoDrop® ND-1000 spectrophotometer. The bla$_{KPC}$ gene was detected using a conventional PCR reaction that was based on the protocols and primer sequences previously published by Shanmugam *et al.*, with slight modifications [18] (Table 1).

The PCR conditions for bla$_{KPC}$ detection were as follows: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, then final extension at 72°C for 5 minutes. The products were then resolved at 130V for 25 minutes on 1.5% agarose gel stained with 0.5µg/ml ethidium bromide solution (Nippon Genetics, Europe GmbH) in an electrophoresis tank containing one mMol Tris-Borate EDTA (TBE) buffer. The gels were observed under UV gel Transilluminator (UV DOC, England) at 280nm, and the band pattern observed.

2.7. Data Analysis

Statistical analysis was done using STATA version 13 (Stata Corp LP, Texas, USA). Prevalence was determined using frequency distribution tables.

3. Results

One hundred and forty-two bacteria were isolated from 534 fomites in the hospital wards, and out of these, 15(10.6%) were *K. pneumoniae*. Thus, the prevalence of *K. pneumoniae* in the entire sample population was 15/534(2.8%).

The male surgical ward had the highest proportion of *K. pneumoniae* isolates 5/15(33.3%), followed by the male and female medical wards which had 3/15(20%) each.

The highest resistance pattern (100% resistant) was observed against ampicillin, trimethoprim-sulphamethoxazole, cefuroxime and tetracycline. In comparison, the least amount of resistance was seen in the carbapenem class of antibiotics, including imipenem (26.7%), meropenem (40.0%) and ertapenem (46.7%) (Table 2).

All the *K. pneumoniae* isolates were at least multi-drug resistant, and out of the 15 isolates, 8 (53.3%) were confirmed phenotypically as carbapenemase producers. The carbapenemase producers were those *Klebsiella pneumoniae* isolates that showed a clover leaf appearance on modified Hodges test.

Table 1. Primer Sequences used in the Study

Name	Primer	Gene	Product size	Reference
KPC (F)	5'-GCT CAG GCG CAA CTG TAA G-3'	bla$_{KPC}$	100bp	[18]
KPC (R)	5'-AGC ACA GCG GCA GCA AGA AAG-3'			

G= guanosine, C= cytocine, A= adenosine, T= thymidine, bla$_{KPC}$= *Klebsiella pneumoniae* carbapenemase beta-lactamase gene.

Table 2. Antibiogram of the *Klebsiella pneumoniae* Isolates

Antibiotic Class	Antibiotic	Disk content	Susceptible n(%)	Resistant n(%)
Penicillins	Ampicillin	10µg	0(0.0)	15(100.0)
β-lactam/β-lactamase Inhibitor	Amoxicillin-clavulanate	20/10µg	1(6.7)	14(93.3)
Folate Inhibitor	Trimethoprim-sulfamethoxazole	1.25/23.75µg	0(0.0)	15(100.0)
Cephalosporins	2nd gen: Cefuroxime	30µg	0(0.0)	15(100.0)
	3rd gen: Cefotaxime	30µg	4(26.7)	11(73.3)
	3rd gen: Ceftazidime	30µg	6(40.0)	9(60.0)
	4th gen: Cefepime	30µg	7(46.7)	8(53.3)
Aminoglycosides	Gentamicin	30µg	5(33.3)	10(66.7)
Carbapenems	Ertapenem	10µg	8(53.3)	7(46.7)
	Meropenem	10µg	9(60.0)	6(40.0)
	Imipenem	10µg	11(73.3)	4(26.7)
Quinolones	Ciprofloxacin	5µg	6(40.0)	9(60.0)
Tetracycline	Tetracycline	30µg	0(0.0)	15(100.0)

Key: µg= microgram, n= number, %= percentage, gen= generation.

Table 3. Distribution of carbapenemase production in the *Klebsiella pneumoniae* isolated from the sample sources

Sample Source (n)	MDR Isolates, n(%)	Carbapenemase Production	
		Yes, n(%)	No, n(%)
Beds (6)	6(40.0)	4(26.7)	2(13.3)
Bed Tables (2)	2(13.3)	1(6.7)	1(6.7)
Chlorhexidine (1)	1(6.7)	1(6.7)	0(0.0)
Cupboards (4)	4(26.7)	2(13.3)	2(13.3)
Hand Wash (1)	1(6.7)	0(0.0)	1(6.7)
Forceps (1)	1(6.7)	0(0.0)	1(6.7)
Total (15)	**15(100.0)**	**8(53.3)**	**7(46.7)**

Key: n= number, %= percentage, MDR= multi-drug resistant.

The largest proportion of these phenotypic carbapenemase producers were seen in *K. pneumoniae* isolated from bed surfaces 4 (26.7%). (Table 3). The bla_{KPC} gene was undetected in the *K. pneumoniae* isolates.

4. Discussion

Klebsiella pneumoniae is a frequent cause of infections, accounting for up to 10% of all nosocomial infections [19]. Carbapenems are the drugs of choice for the treatment of infections caused by drug-resistant *Enterobacteriaceae* [20]. Unfortunately, rising bacterial resistance to carbapenems has been well documented [21]. Previous studies have shown that *K. pneumoniae* strains of environmental origin are similar to those of clinical origin in terms of biochemical patterns, virulence, and pathogenicity. However, clinical *K. pneumoniae* have been observed to be significantly more resistant to antibiotics when compared with environmental *K. pneumoniae* [22].

K. pneumoniae was isolated from 15/534 (2.8%) of the study population. A slightly lower rate was obtained in environmental isolates of *K. pneumoniae* in an Egyptian hospital, where 4/100 (0.04%) of the study population was found to harbour *K. pneumoniae* [23]. Out of 142 isolated organisms, 15 (10.6%) were confirmed to be *K. pneumoniae* with 8(53%) of these observed to be producing carbapenemases. A higher rate was observed in the northern region of Brazil, where 25/25 (100%) of the *K. pneumoniae* isolates were confirmed as carbapenemase producers [24], but much lower values were observed for clinical isolates of *K. pneumoniae* in a Chinese study 4/153 (2.6%) [25]. In Kano, Nigeria, a low prevalence of carbapenemase-producing *K. pneumoniae* was also observed 6/73 (8.2%) [13]. The varying prevalence of carbapenemase production could be a result of different selection pressures from different antibiotic prescribing preferences in other countries. These inconsistent observations were highlighted in a statement by Oduyebo *et al.*, that carbapenemase production among the *Enterobacteriaceae* has been widely reported with prevalence ranges between 2.8% and 53.6% [12].

The most frequent site of isolation was in beds 6/15 (40%), followed by bedside cupboards 4/15 (26.7%), and then bedside tables 2/15 (13.3%). This finding was similar to that observed in Egypt, where the *K. pneumoniae* isolated from several ICUs were found more in beds, bedside tables, suction tubes, and ventilator tubes [14]. However, no *K. pneumoniae* was isolated from the ICU in this study. This variation in the detection of the organisms from the ICUs of the different hospitals could be attributed to the maintenance of strict infection control measures in the ICU of NAUTH, Nnewi.

The antibiotic susceptibility patterns of the *K. pneumoniae* isolates revealed that the organisms had maximum resistance (100%) to Ampicillin, Sulfamethoxazole-Trimethoprim, Cefuroxime, and Tetracycline, but were most susceptible to the Carbapenem class of antibiotics, in which imipenem showed the most sensitivity (73.3%). Contrasting findings were observed in an Egyptian study which revealed 100% resistance to meropenem [14]. The reduced rates of resistance to the carbapenems in this study could be attributed to the limited use of carbapenems due to the high cost of purchase of these antibiotics in the country.

None of the 15 isolates of *K. pneumoniae* produced bla_{KPC}. Although this was similar to findings observed in previous Nigerian studies which dealt with clinical isolates of *K. pneumoniae* [12,26], contrasting observations were seen in Maiduguri, Nigeria (6.5%) [13]. A significantly different finding was also observed in a Brazilian study that revealed that 100% of the *K. pneumoniae* isolates carried the bla_{KPC} gene [24]. The contrasting rates may be due to long term high use of carbapenems in Brazil, which in Nigeria, have only recently been introduced.

The *K. pneumoniae* isolates were phenotypically positive for carbapenemase production on modified Hodge test but were negative for bla_{KPC} gene on PCR. This could be because these isolates harboured other carbapenemase-producing genes (including bla_{NDM}, bla_{VIM}, bla_{OXA-48} etc.), which were not searched for in this study.

5. Conclusion

Although the prevalence of carbapenemase production in the *K. pneumoniae* isolates was high, the rate of colonisation of fomites with these pathogens in the NAUTH ward environment was still relatively low. However, the existence of fomites colonization with carbapenemase producing *Klebsiella pneumoniae* in the hospital environment poses a major risk for the for acquisition of health care associated infections with these resistant pathogens.

6. Limitations

All the genes responsible for carbapenemase production were not searched for. Although this limitation did not adversely affect the aim of this study, which was to determine carbapenemase production in the organisms, it would have been more accurate to detect all the genes responsible for its production. The phenotypic detection method (MHT) used in this study helped to curb this limitation. Larger sample size may also have helped to improve the accuracy of the survey.

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Competing Interests

The authors declare that they have no competing interests.

Abbreviations

ESBL: Extended Spectrum Beta-lactamase
ICU: Intensive Care Unit
KPC: *Klebsiella pneumoniae* Carbapenemase
MBL: Metallo-β-lactamase
MDR: Multi-drug resistant
MHT: Modified Hodges Test
NAUTH: Nnamdi Azikiwe University Teaching Hospital
NDM: New Delhi metallo-β-lactamase
OXA-48: Oxacillinases-48
PCR: Polymerase Chain Reaction
VIM: Verona integron-encoded metallo-β-lactamase

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Research and Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, with reference number NAUTH/CS/66/VOL.9/143/2016/11. Also, all isolates used in this study were obtained from inanimate materials in the wards of NAUTH, Nnewi. Hence permission/consent to participate in the study was given by the Chairman Medical Advisory Committee on behalf of the NAUTH Board of Management, with reference number NAUTH/CS/152/VOL. 2/224.

Consent for Publication

Not applicable.

Availability of Data and Materials

The necessary data generated or analysed during this study are included in this article.

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