Detection of Carbapenemase producing Klebsiella pneumoniae isolated from Environmental Sources in a Tertiary Health Institution in Nigeria.

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Research note

Abstract

Objective: The acquisition of carbapenemase-producing organisms in healthcare settings is a major threat and has serious implications for public health. This study aimed at determining the occurrence of carbapenemase-producing *Klebsiella pneumoniae* in the ward environments of a tertiary health institution in Nigeria.

Results: A total of 142 bacteria were isolated from 534 formites in the hospital wards, and of the 142 isolates, 15(10.6%) were confirmed to be *Klebsiella pneumoniae*. The prevalence of *Klebsiella pneumoniae* in all the 534 samples was 15/534(2.8%), while the prevalence of carbapenemase-producing *Klebsiella pneumoniae* was 8/534(1.5%). Multi-drug Resistance was detected in 15/15(100%) of the *Klebsiella pneumoniae* isolated. Although no *Klebsiella pneumoniae* Carbapenemase (bla_{KPC}) gene was expressed in any of these isolates, 8/15(53.3%) of these isolates were confirmed positive for cabapenemase production using a phenotypic detection method- Modified Hodge Test. The commonest sites that harboured carbapenem-resistant *Klebsiella pneumoniae* were the beds 6/15(40%). The organisms showed maximum resistance (100%) to ampicillin, trimethoprim-sulfamethoxazole, cefuroxime and tetracycline, while the highest sensitivities were seen in the carbapenems especially imipenem (73.3%). It was concluded that carbapenemase-producing *Klebsiella pneumoniae* were present in the study site, thus, buttressing the need for reinforcements of infection control policies in hospital settings.

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 causes of health care associated sepsis, remaining for long periods in hospital environments and equipment, and 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 almost all forms of antibiotics, especially the carbapenems which have been considered as agents of last resort in the treatment of infections caused by MDR Gram-negative bacilli.[4][5]

The most frequently detected carbapenemases include class A carbapenemases (*Klebsiella pneumoniae* Carbapenemase [KPC] types), class B or 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).[6] Furthermore, the KPC's have been documented as major causes of nosocomial outbreaks.[7][8][9]

Several studies done previously on carbapenemase detection focused more on isolates from clinical specimens of patients, but limited information is found in the literature on the prevalence of

carbapenemase producing *Klebsiella pneumoniae* in the hospital environment. One environmental study worthy of note was that in which the prevalence of carbapenemase producing *Klebsiella pneumoniae* was determined in environmental sites of Intensive Care Units (ICU's) in Cairo, Egypt.[11] This study therefore, aimed at determining the occurrence of carbapenemase producing *Klebsiella pneumoniae* in the ward environments of a tertiary health institution in Nigeria.

Methods

Study Population

One hundred and fourty-two human bacterial pathogens were isolated from 534 environmental specimens obtained in the wards of Nnamdi Azikiwe University Teaching Hospital, Nnewi, a major referral centre serving individuals from most parts of South-East, Nigeria. The bacteria were collected between January 2018 and June 2018, and the specimens include swabs collected from; patients beds, bed side tables, bedside cupboards, trolleys, sphygmomanometers, water taps, antiseptics, disinfectants, hand wash solutions, hand sanitizers, forceps, wheel chairs, kidney dishes, door handles, drip stands, drug mortars, methylated spirits, suction tubes, nurses desks, doctors desks and pulse oximeters. The bacterial isolates were Gram-stained and the Gram-negative bacilli were subjected to confirmatory identification using the Microbact™ Gram-negative bacteria identification kit (Oxoid, UK).

Antimicrobial Susceptibility testing

The Modified Kirby-Bauer Susceptibility testing technique as described by the Clinical and Laboratory Standard Institute (CLSI)[12] was performed on all isolates confirmed as *Klebsiella pneumoniae*, and were tested against the following antibiotics: Amipicillin (AMP:10 µg); Cefuroxime (CXM:30 µg), Ceftazidime (CAZ:30 µg), Cefotaxime (CTX:30 µg), Cefepime (FEP:30 µg), Ertapenem (ETP:30 µg), Meropenem (MEM:10 µg), Imipenem (IPM:10 µg), Ciprofloxacin (CIP:30 µg), Gentamicin (CN:30 µg); Tetracycline (TE:30 µg), and Amoxicillin-clavulanate (AMC:20/10 µg), Trimethoprim-sulfamethoxazole (SXT:1.25/23.75 µg) (Oxoid-UK).

Screening for suspected carbapenemase production

The *Klebsiella pneumoniae* isolates were screened for carbapenem resistance, in view of detecting possible carbapenemase producers according to the 2017 CLSI guidelines.[9][12] In this method, 10 µg carbapenem antibiotics (ertapenem and meropenem) discs (Oxoid, UK) were placed on the surface of Mueller Hinton Agar (Oxoid, UK) plates inoculated with each isolate and then incubated for 24 hours at 35-37^{0}C after which zones of inhibition were read off. The *Klebsiella pneumoniae* isolates that showed a zone of inhibition ≤ 22 mm in diameter for meropenem or ≤ 21 mm for ertapenem were considered as suspected carbapenemase producers and were subjected to phenotypic confirmation by the Modified Hodges Test (MHT)[9][12]. For quality, *Escherichia coli* ATCC 25922 was tested using the same standards applied to the test isolates to control the screening test.

Phenotypic confirmation of carbapenemase production (Modified Hodges Test)

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 (10 μg), was placed on the center surface of the MHA plate. In a straight line, by means of a sterilized 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 37^0C for 24 hours. After incubation, they were examined for a clover leaf 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.[12] *K. pneumoniae* ATCC1705 and *K. pneumoniae* ATCC1706 were used as positive and negative controls.[12]

Molecular Detection of bla$_{KPC}$

Bacteria DNA from the *Klebsiella pneumoniae* isolates were extracted using previously described boiling method for DNA extraction with slight modifications.[13][14] The extracted DNA was quantified and tested for purity using the NanoDrop® ND-1000 spectrophotometer (Additional file 1: Table S1). The bla$_{KPC}$ gene was detected using conventional PCR reaction that was based on the protocols and primer sequences previously published by Shanmugam *et al*.[15] with slight modifications (Additional file 2: Table S2).

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

Results

A total of 534 samples were processed out of which, 142(26.6%) bacterial isolates were obtained, thus resulting to a sample site contamination rate of 26.6%. Out of these, 15(10.6%) were identified as *Klebsiella pneumoniae*, thus, the prevalence of the *Klebsiella pneumoniae* in the entire sample population was 15/534(2.8%). The distribution of organisms and their respective proportions were as follows: *Staphylococcus aureus* 74(52.1%), *Escherichia coli* 38(26.8%), *Klebsiella pneumoniae 15(10.6%)*, *Pseudomonas aeruginosa* 11(7.7%), *Acinetobacter baumanii* 2(1.4%), *Providencia spp.* 1(0.7%), *Salmonella typhimurium* 1(0.7%) (Additional file 3: Table S3).

The Male Surgical Ward had the highest proportion of *Klebsiella pneumoniae* isolates 5(33.3%), followed by the Male and Female Medical wards which had 3(20%) each. (Additional file 4: Table S4).

The highest resistance pattern (100% resistant) was seen against; Ampicillin, Trimethoprim-sulphamethoxazole, Cefuroxime and Tetracycline, while 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 1) (Additional file 5: Table S5).

Table 1
Antibiogram of the *Klebsiella pneumoniae Isolates*

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

All 15 (100%) of the *Klebsiella pneumoniae* isolates were at least multi-drug resistant, and out of the 15 isolates 8 (53.3%) were confirmed phenotypically as carbapenemase producers. The largest proportion of these phenotypic carbapenemase producers were seen in *Klebsiella pneumoniae* isolated from bed surfaces 4 (26.7%) (Table 2) (Additional file 6: Figure S1).

Table 2
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			

Polymerase chain reaction analysis revealed that none of the isolates possessed the bla_{KPC} gene (Fig. 1). Although the *Klebsiella pneumoniae* isolates were negative for bla_{KPC} gene, phenotypically they were positive for carbapenemase production using the Modified Hodge Test.

Discussion

Klebsiella pneumoniae is an important Gram-negative opportunistic bacterium and a frequent cause of nosocomial infections, accounting for up to 10% of all nosocomial infections.[16] Carbapenems are the β-lactam antibiotics of choice for the treatment of infections caused by MDR organisms especially the extended-spectrum beta-lactamase (ESBL)-producing bacteria such as *Klebsiella pneumoniae*, and these antibiotics are also considered the last resort for the management of life-threatening health-care-associated infections.[17] Unfortunately, rising bacterial resistance to carbapenems has been, and is well documented.[18] Previous studies have shown that *Klebsiella pneumoniae* strains of environmental origin are similar to those strains of clinical origin in terms of biochemical patterns, virulence, and pathogenicity; however, clinical *Klebsiella pneumoniae* have been observed to be significantly more resistant to antibiotics when compared with environmental *Klebsiella pneumoniae*.[19]

Out of the 534 sources sampled in this study, *Klebsiella pneumoniae* was isolated from 15 (2.8%) of the population. Similar but slightly lower values were obtained in a study conducted on environmental isolates of *Klebsiella pneumoniae* in an Egyptian hospital, where 4/100 (0.04%) of the study population was found to harbor *Klebsiella pneumoniae*.[20] In this study 142 organisms were isolated from the 534 samples, and out of these 15/142 (10.6%) were confirmed to be *Klebsiella pneumoniae* with 8/15 (53%) of these organisms observed to be producing carbapenemases. This finding corresponds with previously

documented studies that found the prevalence of *Klebsiella pneumoniae* in nosocomial infections to be about 10%.[17] Mohammed *et al.*, observed higher rates in Kano, Nigeria where 73/225 (32.4%) of the isolates were *Klebsiella pneumoniae*, with 6/73 (8.2%) of these *Klebsiella pneumoniae* producing carbapenemases.[9] High rates have also been recorded in the northern region of Brazil, where 25/25 (100%) of the *Klebsiella pneumoniae* isolates were confirmed as carbapenemase producers,[21] but much lower values were observed for clinical isolates of *Klebsiella pneumoniae* in a Chinese study 4/153 (2.6%).[22] The varying prevalence of carbapenemase production observed in these studies shows that several countries have different preferences for antibiotic prescription, and hence, varying selection pressures affecting antimicrobial resistance patterns. These varying observations were highlighted in a statement by Oduyebo *et al.*, that carbapenemase production among the *Enterobacteriaceae* has been widely reported with prevalence rates ranging from between 2.8% and 53.6%.[8]

Of the 15 *Klebsiella pneumoniae* isolates, the most frequent site of isolation was in beds 6 (40%), followed by bedside cupboards 4 (26.7%), and then bedside Tables 2 (13.3%). This finding was similar to that observed in a study involving several ICU hospital environment sites in Cairo, Egypt, where the *Klebsiella pneumoniae* isolates were found mainly in beds, bedside tables, suction tubes and ventilator tubes.[11] Also worthy of note is that contrary to the Abdallah *et al.* study, no *Klebsiella 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 *Klebsiella pneumoniae* isolates revealed that the organisms had the highest resistance rates to Ampicillin (100%), Sulfamethoxazole-Trimethoprim (100%), Cefuroxime (100%), and Tetracycline (100%), but were most susceptible to the Carbapenem class of antibiotics (Imipenem, Meropenem and Ertapenem), in which Imipenem showed the most sensitivity (73.3%). Contrasting findings were observed in an Egyptian study which revealed 100% resistance to Meropenem.[11] The reduced rates of resistance to the carbapenems in this study could be attributed to the limited use of carbapenems due to its high cost of purchase in the country.

None of the 15 isolates of *Klebsiella pneumoniae* produced bla$_{KPC}$. Although this was similar to findings observed in previous Nigerian studies which dealt with clinical isolates of *Klebsiella pneumoniae*,[8][23] contrasting observations were seen in Maiduguri, Nigeria (6.5%).[9] A significantly different finding was also observed in a brazillian study that revealed that 100% of the *Klebsiella pneumoniae* isolates carried the bla$_{KPC}$ gene.[21] The contrasting rates may be because of the long term high use of carbapenem antibiotics in Brazil, but still quite recent introduction of these drugs in Nigeria.

The *Klebsiella pneumoniae* isolates were phenotypically positive for carbapenemase production on Modified Hodge Test, but was negative for bla$_{KPC}$ genes on PCR. This could be because these isolates were harbouring carbapenemase genes other than those tested for in this study.

Conclusion

Carbapenemase producing *Klebsiella pneumoniae* is prevalent in the NAUTH ward environment, thus, *Klebsiella pneumoniae* colonization of formites in the hospital environment is a possible risk factor for acquisition of health care associated infections, thus, buttressing the need for reinforcements of infection control policies in hospital settings.

Limitations

This work was limited by the fact that 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. A larger sample size may also have helped to improve the accuracy of the study.

Abbreviations

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

Declarations

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 (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

AIN and ACN designed the experiments and performed the literature search. All authors made conceptual contributions. AIN, AIM, ECJ, MNP, and UNG performed the laboratory experiments, as well as data acquisition. CCG and AIN analyzed the data. AIN wrote the manuscript. USN, UCF, and ACN edited and reviewed the manuscript. All authors read and approved the final version of the manuscript. AIN was the project leader, while ACN was the project supervisor.

Acknowledgements

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Figures
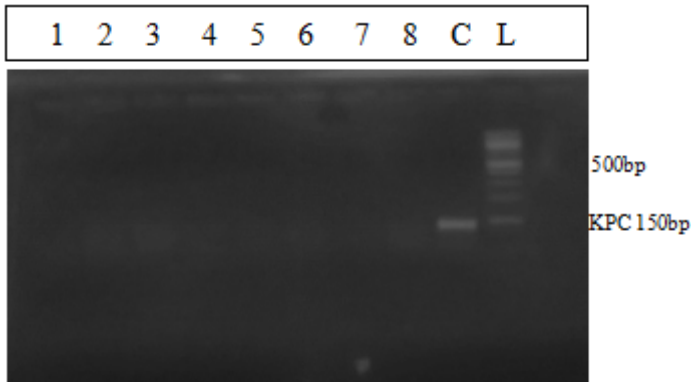
Figure 1

Agarose gel electrophoresis showing the amplified blaKPC gene from the bacterial isolates.

Supplementary Files

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