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

Iloduba Nnaemeka Aghanya (aghanyailoduba@gmail.com)
Nnamdi Azikiwe University https://orcid.org/0000-0003-4481-4834

Comfort Nne Akujobi
Nnamdi Azikiwe University, Faculty of Medicine

Simon Nkpeh Ushie
Nnamdi Azikiwe University

Chika Florence Ubajaka
Nnamdi Azikiwe University

Ijeoma Maryrose Ajuba
Nnamdi Azikiwe University Teaching Hospital

Chibuike Jesse Ezeama
Nnamdi Azikiwe University

Nkechi Perpetua Maduekwe
Nnamdi Azikiwe University Teaching Hospital

Ngozichukwu Gertrude Uzoewulu
Nnamdi Azikiwe University

Chisom Godswill Chigbo
Nnamdi Azikiwe University

Research note

Keywords: Multidrug-resistant, Klebsiella pneumoniae, Carbapenemase, Oxacillinase, Fomites

Posted Date: September 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-42624/v2

Abstract

Objective The acquisition of carbapenemase-producing organisms in healthcare settings is a major threat and has serious implications for public health. Previous reports regarding carbapenemase-producing Enterobacteriaceae from fomites are limited. This study aimed at analysing the antimicrobial resistance patterns, and prevalence 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 fomites 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 carbapenemase production using the Modified Hodge Test. The commonest sites that harboured carbapenem-resistant Klebsiella pneumoniae were the beds 6/15(40%). Maximum resistance (100%) was observed against ampicillin, trimethoprim-sulfamethoxazole, cefuroxime, and tetracycline. In conclusion, the prevalence of carbapenemase-producing Klebsiella pneumoniae fomite colonization in the NAUTH ward environment is low, thus buttressing the need to reinforce strict infection control policies the hospital.

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, 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 carbapenems which have been considered as agents of last resort in the treatment of infections caused by MDR Gram-negative bacilli.[4] [5]

The burden of anti-microbial resistance (AMR) in developing countries has increased remarkably in recent years.[6][7] 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%).[6] Resistance was however uncommon for the carbapenem group of antibiotics. In particular, *Klebsiella spp.* which has intrinsic resistance to ampicillin, was resistant to 34.2% and 46.7% of ceftriaxone or cefotaxime respectively, suggesting high-level extended-spectrum beta-lactamase (ESBL) production. However, the median resistance rate for *K. pneumoniae* against Imipenem, a carbapenem was 3.0%.[6] In another survey involving Africa and Asia, resistance to ampicillin and ceftriaxone were reported as 67.2% and 25.9% respectively.[7]

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).[8] Furthermore, the KPCs have been documented as major causes of nosocomial outbreaks.[9][10][11]

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 (ICUs) in Cairo, Egypt.[12] 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 forty-two human bacterial pathogens 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 between January 2018 and June 2018, and the specimens included swabs collected from; patients beds, bedside 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.

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.[10][12] The isolates were Gram-stained, and the Gram-negative rods were subjected to confirmatory identification of *Klebsiella pneumoniae* using the Microbact^{TM} Gram-negative bacteria identification kit (Oxoid, UK).[10]

Antimicrobial Susceptibility testing

The Modified Kirby-Bauer antimicrobial susceptibility testing technique was performed on all isolates confirmed as *Klebsiella pneumoniae*.[13][14] 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 was measured and reported based on the guidelines of the CLSI.[14]

Screening for suspected carbapenemase production

Screening involved placing 10µg carbapenem (ertapenem and meropenem) discs (Oxoid, UK) on the surface of Mueller Hinton agar (Oxoid, UK) plates inoculated with each isolate. Following incubation of the plates for 16-18 hours at 35-37^{0}C, zones of growth inhibition around each antibiotic were read off. *Klebsiella 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)[11][14].

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 (10µg), was placed on the centre surface of the MHA plate. In a straight line, using 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^{o}C 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.[14] *K. pneumoniae* ATCC 1705 and *K. pneumoniae* ATCC 1706 were used as positive and negative controls.[14]

Molecular Detection of bla_{KPC}

Bacteria DNA from the *Klebsiella pneumoniae* isolates was extracted using a previously described boiling method for DNA extraction with slight modifications.[15][16] 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 a conventional PCR reaction that was based on the protocols and primer sequences previously published by Shanmugam *et al.*, [17] with slight modifications. (Additional file 2: Table S2).

The PCR conditions for bla_{KPC} detection were as follows: initial denaturation at 94ºC for 3 minutes, followed by 30 cycles of denaturation at 94ºC for 1 minute, annealing at 60ºC for 1 minute, extension at 72ºC for 1 minute, 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 0.5µg/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 280nm and the band pattern observed.

Data Analysis

Statistical analysis was done using STATA version 13 (Stata Corp LP, Texas, USA). Frequency distribution tables were used to determine rates.

Results

The prevalence of bacterial contamination in the total sample population was 142/534(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%). (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 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	2^{nd} gen: Cefuroxime	30µg	0(0.0)	15(100.0)
	3^{rd} gen: Cefotaxime	30µg	4(26.7)	11(73.3)
	3^{rd} gen: Ceftazidime	30µg	6(40.0)	9(60.0)
	4^{th} 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

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

The bla_{KPC} gene was undetected in the *Klebsiella pneumoniae* isolates (Figure 1).

Discussion

Klebsiella pneumoniae is a frequent cause of nosocomial infections, accounting for up to 10% of all nosocomial infections.[18] Carbapenems are the drugs of choice for the treatment of infections caused by drug resistant *Enterobacteriaceae.* [19] Unfortunately, rising bacterial resistance to carbapenems has been well documented.[20] Previous studies have shown that *Klebsiella pneumoniae* strains of environmental origin are similar to those 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*.[21]

Klebsiella pneumoniae was isolated from 15/534 (2.8%) of the study population. A slightly lower rate was obtained in environmental isolates of *Klebsiella pneumoniae* in an Egyptian hospital, where 4/100 (0.04%) of the study population was found to harbour *Klebsiella pneumoniae*.[22]

Out of 142 isolated organisms, 15 (10.6%) were confirmed to be *Klebsiella 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 *Klebsiella pneumoniae* isolates were confirmed as carbapenemase producers,[23] but much lower values were observed for clinical isolates of *Klebsiella pneumoniae* in a Chinese study 4/153 (2.6%).[24] In Kano, Nigeria, a low prevalence of carbapenemase-producing *Klebsiella pneumoniae* was also observed 6/73 (8.2%).[11] The varying prevalence of carbapenemase production could be a result of varying selection pressures from different antibiotic prescribing preferences in different countries. These varying 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%.[10]

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 *Klebsiella pneumoniae* isolated from several ICUs were found more in beds, bedside tables, suction tubes, and ventilator tubes.[12] However 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 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.[12] 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 *Klebsiella pneumoniae* produced bla_{KPC}. Although this was similar to findings observed in previous Nigerian studies which dealt with clinical isolates of *Klebsiella pneumoniae*,[10][25] contrasting observations were seen in Maiduguri, Nigeria (6.5%).[11] A significantly different finding was also observed in a Brazilian study that revealed that 100% of the *Klebsiella pneumoniae* isolates carried the bla_{KPC} gene.[23] The contrasting rates may be due to long term high use of carbapenems in Brazil, but the still recent introduction of these drugs in Nigeria.

The *Klebsiella 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.

Conclusion

Although the prevalence of carbapenemase production in the *Klebsiella pneumoniae* isolates was high, the rate of colonization of fomites with these pathogens in the NAUTH ward environment was still quite low, thus buttressing the need to reinforce strict infection control policies the hospital.

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 study.

Abbreviations

MHT: Modified Hodges Test

NAUTH: Nnamdi Azikiwe University Teaching Hospital

KPC: *Klebsiella pneumoniae* Carbapenemase

MBL: Metallo-β-lactamase

VIM: Verona integron-encoded metallo-β-lactamase

NDM: New Delhi 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.

Funding

This work and the subsequent article did not receive any form of financial support in the form of funding, grants or supplies.

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

Special appreciations go to Prof. Tatfeng Mirabeu (Coordinator, Molecular Biology Laboratory, Niger Delta University, Bayelsa, for his tutelage and assistance in the molecular analysis aspects of the work. We also acknowledge the technical assistance of Dr. Ikemefuna Onyeyili, as well as our research assistants; Cynthia, Favour, Mmesoma, and Matron Ezeji.

Author information

Affiliations

1. Department of Medical Microbiology/Parasitology, Faculty of Medicine, Nnamdi Azikiwe University, Awka.
2. Department of Community Medicine, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus.
3. Department of Nursing Sciences, School of Nursing, Nnamdi Azikiwe University Teaching Hospital, Nnewi.
4. Department of Medical Microbiology/Parasitology, Nnamdi Azikiwe University Teaching Hospital, Nnewi.
5. Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

Aghanya IN[1], Akujobi CN[1], Ushie SN[1], Ubajaka CF[2], Ajuba IM[1,3], Ezeama CJ[1], Maduekwe NP[4], Uzoewulu NG[1], Chigbo CG[5].

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Figures

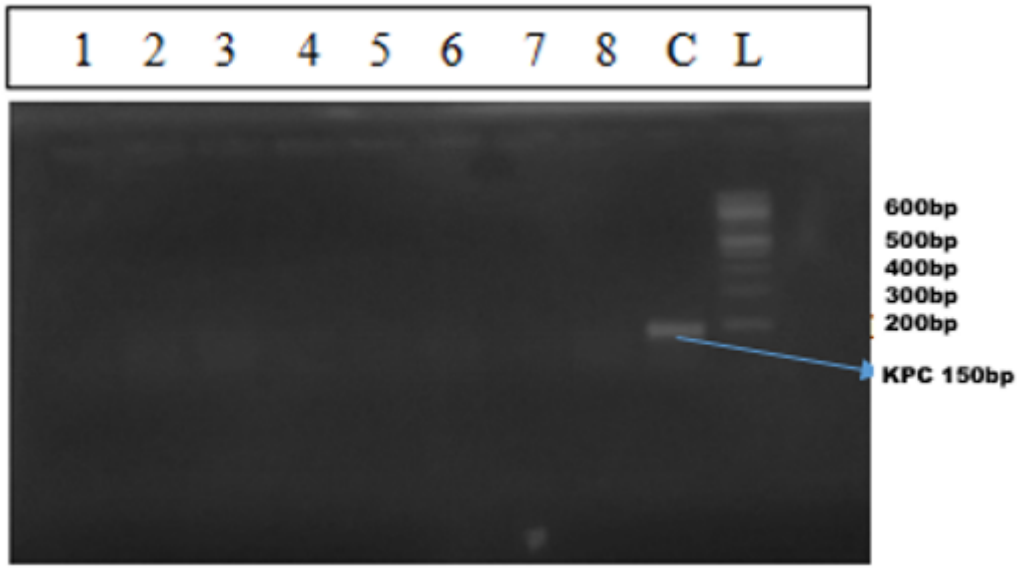

Figure 1

Agarose gel electrophoresis showing the amplified blaKPC gene from the bacterial isolates. Lanes 1-8 showed no amplification. Lane C represents the blaKPC positive control. Lane L represents the 100bp molecular ladder.

Supplementary Files

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