



## Original article

# Phenotypic detection of carbapenem resistant Enterobacteriaceae and characterization of *Klebsiella pneumoniae* carbapenemase (KPC) producing *Klebsiella pneumoniae*

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## ABSTRACT

**Background:** Carbapenem-resistant Enterobacteriaceae (CRE) pose a great threat to the public health sector as a result of its high communicability, high morbidity and mortality rates. This study investigated the occurrence of carbapenem resistant Enterobacteriaceae and the prevalence of *Klebsiella pneumoniae* carbapenemase (KPC) producing *Klebsiella pneumoniae* as a mechanism of resistance. **Method:** One hundred and ten Enterobacteriaceae isolates were cultured from different samples of patients in Intensive Care Units of two hospitals. Using Clinical and Laboratory Standards Institute (CLSI) Guidelines of 2017. Carbapenemase production was determined phenotypically using Brilliance CRE agar, Carbapenem inactivation method and confirmed with Modified Hodge test (MHT). Modified Hodge test positive isolates were screened for KPC-producing *Klebsiella pneumoniae* using boronic acid-based inhibition test. **Result:** Phenotypic results revealed an occurrence of 10.9% (12/110) isolates for carbapenem resistant Enterobacteriaceae while KPC-producing *Klebsiella pneumoniae* prevalence was 3.6% contributing 33.3% to the burden of carbapenem production. The occurrence of carbapenem-resistant Enterobacteriaceae in this study was relatively high and KPC-*Klebsiella pneumoniae* had a significant contribution to the burden of CRE. **Conclusion:** Hence, a sinewy antibiotic stewardship is needed in this regard.

## Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) are highly pathogenic and antibiotic-resistant Gram negative organisms producing nosocomial infections because of its innate capacity for fast transmission within healthcare facilities and its environs [1]. Carbapenemases are beta-lactamase enzymes that inactivate carbapenems, and other beta-lactam antibiotics [2]. Carbapenem-resistant Enterobacteriaceae pose a great threat to the public health sector due to its high rate of transmission which causes high morbidity and mortality coupled with very minute chances of treatment [3,4].

Currently, hospitals remain the major sites of transmission for CRE infections. Poor hygienic practices remains the largest cause of CRE transmission and includes insufficient sterilization of drug shelves, contact points in wards, and movable health care instruments, such as X-ray and ultrasound machines useful for both CRE and non-CRE patients [5]. Carbapenemase-producing Enterobacteriaceae (CPE) are predominant in the clinical isolates of CRE where there is high burden of CRE [6]. The difference between CRE and CPE is very simple and clear. Majority (with the exception of very few) of

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Enterobacteriaceae that are resistant to carbapenems produce carbapenemase (that is, there are some CRE with a carbapenem resistance mechanism apart from carbapenemase production). Likewise, most but not all CPE, exhibit resistance to carbapenems (i.e. some display minimal carbapenem MICs and remain phenotypically irresistible to carbapenems) [1].

In line with Ambler classification system, classes which require serine at their active site, are classified as A and D. Class A serine  $\beta$ -lactamases family (e.g. KPC-type enzymes) consist of the *Klebsiella pneumoniae* carbapenemase (KPC), IMI, SME and GES enzymes. The group which requires zinc at their active site is classified as B, and this latter class is also referred to as Metallo- $\beta$ -lactamases (MBLs). This includes the IMP, VIM, NDM, and SPM enzymes. The class D serine  $\beta$ -lactamases (OXA), most notable of which is the OXA-48 enzyme, which hydrolyzes oxacillin, provide a good example of the variety of mechanisms that can be used to transfer resistance [7,8]. Each of these classes have their peculiar functional characteristics in relation to their phenotypic observation and hospital care. Boronic acid compounds are known as the best inhibitors of class A carbapenemases and this includes the KPC. Hence, these inhibitors can be employed in their detection [9]. The *bla*OXA genes which encode OXA  $\beta$ -lactamases are found on both chromosomes and plasmids and they have their natural reservoir in environmental bacteria and deep-sea microflora.

Based on the foregoing, this study aimed at determining the occurrence of CRE and clinical significance of KPC producing *Klebsiella pneumoniae* in Ogun State, Nigeria.

## Materials and Methods

### Study area

The study was conducted at Sacred Heart Hospital and Federal Medical Centre both in Abeokuta, Ogun State, Nigeria.

### Ethical considerations

This study was approved by the Federal University of Agriculture, Abeokuta, Nigeria through the Department of Microbiology and presented to the two concerned health review boards for ethical approval. Ethical clearance was obtained from the institutional Review Boards of Federal Medical Centre, Idi Aba, Abeokuta and Sacred Heart Hospital, Lantoro, Abeokuta, the two hospitals involved in this study who ensured that the study was performed in

compliance with the ‘The Code of Ethics of the World Medical Association’.

### Selection and description of participants

The sampled populations were patients on acute care having spent minimum of 3 days on admission in different wards, with most of them having recently undergone surgical operations. The patient must have spent minimum of 3 days on admission in the same hospital as an inclusion criterion in this study. The patients had various disease conditions including urinary tract infections (UTIs), gastrointestinal disorders, diabetes, acute nephritis and jaundice. A total of 170 samples (including cerebrospinal fluid, stool and urine obtained through catheter) were obtained between July 2019 and February 2020, and transported to the laboratory according to standard methods [10].

### Bacterial identification and characterization

Samples were inoculated on Brilliance CRE agar (Oxoid, UK), Blood agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) and incubated at 37°C for 24 h aerobically. Typical color appearance on Brilliance CRE agar, colonial morphology and attributes on other agars were observed and distinct colonies were Gram stained in line with standard methods [10]. Gram negative rods were subjected to further biochemical tests.

All Gram negative rods were further confirmed using the Microbact Gram-negative identification system™ (Oxoid, UK) 24E according to the manufacturer’s instructions.

### Carbapenem inactivation method (CIM)

Carbapenem-resistant Enterobacteriaceae isolates from Brilliance CRE agar were subjected to CIM test. With the CIM, a 10- $\mu$ g meropenem disk was first incubated in a suspension of the test strain at 37°C for 2 hours. After incubation, the meropenem disk was removed from the solution and placed on a Mueller Hinton agar plate inoculated with susceptible *E. coli* ATCC 25922 as with the Modified Hodge test (MHT). Carbapenemase production was indicated by the inactivation of meropenem, thus allowing for uninhibited growth of *E. coli*. If no carbapenemase is produced, a clear inhibition zone was observed.

### Phenotypic carbapenem detection

Carbapenem-resistant Enterobacteriaceae isolates from Brilliance CRE agar were further subjected to MHT test for confirmation of carbapenem production. Modified Hodge test (considered the gold standard), carbapenem inactivation method and boronic acid inhibition test were employed in

carbapenem production detection. In the MHT, a tenth dilution of 0.5 McFarland suspension of *E. coli* ATCC 25922 was used to evenly inoculate a Mueller-Hinton agar plate, and a 10- $\mu$ g imipenem disk (Oxoid, UK) placed in the center of the plate. The suspected CPE strain was then streaked from the edge of the disk to the edge of the plate to form a straight line of thick inoculum and incubated for 24 hrs. The growth of susceptible *E. coli* in the background towards the disk, creating a cloverleaf-like appearance indicated carbapenemase production by the test isolate according to CLSI [11]. Quality control was performed with control strains using MHT positive *Klebsiella pneumoniae* ATCC BAA-1705 and for negative control *Klebsiella pneumoniae* ATCC 700603.

#### Boronic acid inhibition test

A 350 $\mu$ g of 2-aminophenyl boronic acid was applied to an ertapenem or meropenem disk. An increase in the zone of inhibition of  $\geq 5$  mm compared to the ertapenem or meropenem disk alone (control) indicated KPC production after incubation for 24hrs at 37°C.

#### Data Analysis

Data analysis was carried out using Descriptive Statistics in Statistical Analysis software (SAS).

#### Results

From the 170 clinical isolates collected in this study, only 110 belonged to the family Enterobacteriaceae. These 110 isolates were tested for carbapenem production and only 12 (11.0%) exhibited carbapenem production using MHT as the gold standard. Brilliance CRE agar also revealed 12 isolates were positive for carbapenem production

while CIM had 11 positive isolates for carbapenem production (**Table 1**).

From the 110 Enterobacteriaceae, 52.7% (58) were *Klebsiella pneumoniae*, followed by *E. coli* 38 (35.5%) and *Proteus mirabilis* 2(1.8%). *Proteus vulgaris* and *Pseudomonas aeruginosa* were 1 (0.9%) and 10 (9.1%) respectively (**Table 1**). Out of the 110 screened for carbapenem production, 56 were from patients that had gastroenteritis from which five (4.6%) produced carbapenem (2 *Klebsiella pneumoniae*, 2 *E. coli* and 1 *Pseudomonas aeruginosa*). While none of the 2 isolates from acute nephritis and 1 from *diabetes mellitus* also known as sugar diabetes was negative for MHT. These isolates from acute nephritis and sugar diabetes didn't not show any resistance to carbapenem, hence were not amongst the isolates further studied in this research.

Only 7 (6.4%) out of the fifty-one UTIs isolates were MHT positive. Note that there was no growth from CSF samples cultured (**Table 2**).

More (4/5) of the KPC-Enterobacteriaceae from this present study were from *Klebsiella pneumoniae* followed by *E. coli* (1/5) shown in **table (3)** below.

The most isolated organism in this study was *Klebsiella pneumoniae* and it was mostly isolated in patients with UTIs (**Table 3**). From the total 110 Enterobacteriaceae, *Klebsiella pneumoniae* was 58 (52.7%) out of which seven (12.1%) were positive for carbapenemase production. Five (41.6%) isolates, consisting of 4 (33.3%) *Klebsiella pneumoniae* and 1 (8.3%) *E. coli* isolates, out of the seven were positive for KPC by boronic acid-based inhibition test.

**Table 1.** Percentage frequency of detection of CRE in three phenotypic methods.

Isolates	Total Screened (%)	MHT +ve (%)	Brilliance CRE agar +ve (%)	CIM +ve (%)
<i>Klebsiella pneumoniae</i>	58(52.7)	7(6.4)	7(6.4)	6(7.3)
<i>E. coli</i>	38(35.5)	4(3.6)	4(3.6)	4(3.6)
<i>Proteus mirabilis</i>	2(1.8)	0(0)	0(0)	0(0)
<i>Proteus vulgaris</i>	1(0.9)	0(0)	0(0)	0(0)
<i>Pseudomonas aeruginosa</i>	10(9.1)	1(0)	1(0)	1(0)
Total	110	12(10.9)	12(10.9)	11(11.8)

**Table 2.** Incidence of CRE in different isolates in relation to different disease condition.

Isolates	Gastroenteritis (CRE)	Acute nephritis (CRE)	UTI (CRE)	Diabetes (CRE)	CSF (CRE)	Total
<i>Klebsiella pneumoniae</i>	31 (2)	1(0)	26(5)	0(0)	0(0)	58(7)
<i>E. coli</i>	18(2)	0(0)	20(2)	0(0)	0(0)	38(4)
<i>Proteus mirabilis</i>	2 (0)	1(0)	0(0)	0(0)	0(0)	3(0)
<i>Proteus vulgaris</i>	1(0)	0(0)	0(0)	0(0)	0(0)	1(0)
<i>Pseudomonas aeruginosa</i>	4(1)	0(0)	5(0)	1(0)	0(0)	10(1)
	56(5)	2(0)	51(7)	1(0)	0(0)	110(12)

**Table 3.** Percentage frequency of KPC – Enterobacteriaceae.

Isolates	Frequency of MHT+VE	Frequency of BAI test +ve (%)
<i>Klebsiella pneumoniae</i>	7	4(33.3)
<i>E. coli</i>	4	1(8.3)
<i>Proteus mirabilis</i>	0	0(0)
<i>Proteus vulgaris</i>	0	0(0)
<i>Pseudomonas aeruginosa</i>	1	0(0)
Total	12	5(41.6)

### Discussion

Global CRE infection rates have been reported to range between zero and 89%, but mostly between 7.6–44.4%. This broad range might be attributed to varied populations studied [12]. This present study is not exceptional by revealing a prevalence of 10.9% carbapenem production in Ogun State. This is obviously high for a kind of drug that is considered the drug of last resort. However, when compared with other similar studies, it is relatively low. **Arena et al.** recently revealed high rates of CRE carriage (28.4%) among patients from a long-term acute-care rehabilitation facility (LTACRF) in central Italy [13].

**Beena et al.** reported 66% of isolates to be carbapenemase producers [12]. **Aseem et al.** developed the approach to carbapenemase detection in *Klebsiella pneumoniae* in routine diagnostic laboratories and reported 35.3% carbapenem resistance in the isolates studied [14]. The prevalence in the current study was also low in comparison with the phenotypic prevalence report of 22.4% Enterobacteriaceae resistance to third generation cephalosporins in Uganda [15]. In Nigeria, **Yusuf et al.** reported 33.5% carbapenemase production in Kano and Kaduna States [16]. However, in a neighboring State, Lagos, Nigeria, **Oduyebo et al.** determined the prevalence of carbapenem resistance

among clinical isolates of Enterobacteriaceae to be 15.2% [17], a range similar to the present study where our carbapenemase production prevalence is 10.9%. This might be attributed to the absence of a considerable boundary between Lagos and Ogun States, allowing for population interception. Studies carried out around the globe revealed that there was a gradual increase in carbapenem resistance all over the world between 2012 and 2013. For instance, in China, (1.2%) as reported by **Xia et al.** [18], Enugu-Nigeria (2.5%) revealed in the study of **Ejikeugwu et al.** [19], Morocco (2.8%) reported by **Wartiti et al.** [20], New Delhi-India (6.9%) as revealed by **Gupta et al.** [21] and Taiwan (8.6%) reported by **Lai et al.** [22].

Variation in the prevalence rates may be attributed to some factors such as, the varied sampled population, seasonal factors, iatrogenic outcome, carbapenem resistance mechanism sought for in different study and the use and misuse of drugs.

The rate of KPC-producing isolates in the current study was 41.6% on the average of the total CRE production recorded. This contradicts the studies of **Conte et al.** and **Nagaraj et al.** [23, 24]. **Zogorianou et al.** also reported a very high (97%, 75% and 66.4%) contribution of *bla*KPC, *K. pneumoniae* KPC producing *Klebsiella pneumoniae* isolates respectively to the total burden of CRE [25]. In contrary, **Netikul et al.** and **Ho et al.** reported a

very low incidence of KPC-producing isolates within their CRE isolates [26, 27]. The study of **Nayak et al.** also reported a slightly low (16.6%) prevalence of KPC producing *Klebsiella pneumoniae* as its quota to 54.8% of the entire CRE in their study [28].

The variation in MHT and CIM result was found among *Klebsiella pneumoniae* isolates, although, this was not statistically significant ( $p=0.005$ ).

This present study is in total conformity with the study of **Gupta et al.** who also reported the same prevalence of 33.3% as the total contribution of KPC-*Klebsiella pneumoniae* by modified Hodge's test [21]. This study also agrees with the review of **Ssekatawa et al.** who highlighted that CP bacteria have a predominant occurrence in the respiratory tract (23%), Blood (22%), urinary tract (19%) and wounds/pus (18%) in East Africa [29]. The prevalence of resistance to carbapenem in this study is relatively high, being the last drug of resort and KPC-Kp has a significant contribution to the burden of CRE.

### Conclusion

In conclusion, this study found 110 Enterobacteriaceae isolates from different samples of patients in the intensive care units of two public hospitals in Abeokuta, Ogun State, Nigeria out of which 10.9% exhibited carbapenem-resistance. *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* prevalence was 3.6%, contributing 33.3% to the burden of carbapenem production. The occurrence of carbapenem-resistant Enterobacteriaceae in this study was relatively high and KPC-*Klebsiella pneumoniae* had a significant contribution to the burden of CRE. Hence, a sinewy antibiotic stewardship is needed in this regard.

### Conflicts of interest:

The authors hereby declare that there are no conflicts of interest with respect to this article.

### Financial disclosure:

There is no specific financial interest, relationship and affiliations relevant to the subject of the manuscript.

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