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# Characterisation of carbapenem-resistant Gram-negative organisms from clinical specimens in Yola, Nigeria

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ABSTRACT

*Objectives:* This study aimed to identify carbapenem-resistant Gram-negative bacteria from clinical specimens of patients in Yola, Nigeria.
*Methods:* Routine clinical specimens were screened for the presence of carbapenem-resistant Gram-negative bacteria using chromogenic agar plates. Susceptibility of all presumptive isolates to carbapenems was tested by MIC and disk diffusion methods. Real-time PCR was used to test for the presence of carbapenemase genes.
*Results:* Screening of 1741 clinical specimens yielded 119 (6.8%) presumptive carbapenem-resistant Gram-negative bacteria. Antimicrobial susceptibility testing confirmed carbapenem resistance in 105 of these isolates. New Delhi metallo-β-lactamase ($bla_{NDM}$) gene was detected in 26 isolates and Verona integron-encoded metallo-β-lactamase ($bla_{VIM}$) gene was detected in four. The mechanism of resistance could not be identified in approximately two thirds of the carbapenem-resistant isolates.
*Conclusion:* While $bla_{NDM}$ and $bla_{VIM}$ accounted for 28.6% of the resistance seen, further molecular-based studies are needed to characterise the other mechanisms of carbapenem resistance in these isolates.


## 1. Introduction

Carbapenems are β-lactam antibiotics that are considered to be one of the last lines of treatment for serious infections caused by multidrug-resistant organisms, especially Enterobacteriaceae [1]. This class of drugs is currently threatened by the emergence of resistant strains of bacteria that have been reported globally [2]. Resistance is primarily mediated by carbapenemases (i.e. enzymes that hydrolyse this class of β-lactams) and by non-enzymatic mechanisms such as efflux or porin changes in the presence of AmpC or extended-spectrum β-lactamases (ESBL) [3]. Carbapenemase-mediated resistance is particularly problematic because it is often encoded on transmissible plasmids and mobile genetic elements that are easily transferred between species of Enterobacteriaceae and even other Gram-negative bacilli, including *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) [4]. Carbapenemases also confer resistance to penicillins and cephalosporins [5]. Presently, $bla_{KPC}$, $bla_{OXA-48}$, $bla_{NDM}$, $bla_{VIM}$, and $bla_{IMP}$ are among the most commonly reported carbapenemase encoding genes in Enterobacteriaceae and *P. aeruginosa* around the world [4,5]. However, the most commonly reported carbapenemase genes in *A. baumannii* are $bla_{OXA-23}$, $bla_{OXA-24}$ and $bla_{OXA-58}$. In Nigeria, there have been few reports on carbapenemase-producing organisms [6,7]. However, identification of such organisms and knowledge about their epidemiology is essential for controlling the spread of resistance, thereby conserving the effectiveness of carbapenems. This study focused on the carbapenemase genes present in clinical specimens from a hospital in Yola, Nigeria.

## 2. Materials and methods

### 2.1. Screening for carbapenem resistance

All routine clinical specimens submitted to the Microbiology Laboratory of the Federal Medical Center in Yola from November 2017 to February 2018 were screened for the presence of carbapenem-resistant bacteria using a chromogenic screening agar, Chromatic™ CRE (Liofilchem, Roseto degli Abruzzi, Italy). Specimens were inoculated directly onto the chromogenic agar plates and incubated at 37 °C for 24–48 h in ambient air. A heavy inoculum of all presumptive carbapenem-resistant Gram-negative bacterial isolates from the agar plates was suspended in brain heart infusion broth (Oxoid, Basingstoke, United Kingdom), frozen at –20 °C and shipped to a central laboratory for further testing. A second

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round of screening for resistant isolates was performed using a chromogenic screening agar by a different manufacturer, Hardy-CHROM CRE plates (Hardy Diagnostics, Santa Maria, CA, USA) and also by a method involving the use of blood agar plates (Hardy Diagnostics) with the addition of a 10 μg meropenem disk (BD BBL™, Franklin Lakes, New Jersey, USA), as described by Tenover et al. [8,9].

### 2.2. Identification and antimicrobial susceptibility testing using the minimal inhibitory concentration method

Identification and antimicrobial susceptibility testing were conducted on all 119 presumptive carbapenem-resistant Gram-negative bacteria using the MicroScan WalkAway 40 SI system (Beckman Coulter, Inc., West Sacramento, CA, USA). Neg ID Type 2 panel was used for identification, while Neg MIC 43 panel was used for susceptibility testing (Beckman Coulter, Inc.). MIC results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendation [10]. Quality control organisms included *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218, and *Klebsiella pneumoniae* ATCC 700603.

### 2.3. Antimicrobial susceptibility testing and presumptive identification of ESBL and AmpC β-lactamases using the disk diffusion method

Beta-lactamase identification was performed using the 12-disk method described by Schreckenberger and Rekasius [11]. ESBL testing was performed on 105 confirmed carbapenem-resistant Gram-negative bacteria, according to CLSI guidelines, using ceftazidime and ceftriaxone disks with and without clavulanic acid [12]. Presumptive identification of AmpC β-lactamases was also performed on 105 confirmed carbapenem-resistant isolates using cefoxitin and cefepime disks, as described by Schreckenberger and Rekasius [11].

### 2.4. Polymerase chain reaction for detecting carbapenemase genes

All 119 presumptive carbapenem-resistant bacteria were tested for the presence of carbapenemase genes, using Xpert® Carba-R (Cepheid, Sunnyvale, CA, USA), which is a real-time polymerase chain reaction (PCR)-based qualitative diagnostic assay [5,13,14] for detecting five common carbapenemase gene families ($bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP}$, and $bla_{OXA-48}$).

## 3. Results

A total of 1741 clinical specimens were submitted to the microbiology laboratory for culture during the 4-month study period. Based on growth on chromogenic agar, 119 presumptive carbapenem-resistant Gram-negative bacteria were recovered from 119 specimens. The isolates were obtained from 55 urine specimens (46.2%), 26 wound specimens (21.8%), 15 stool specimens (12.6%), 13 sputum specimens (10.9%), seven female genital tract specimens (6.0%), two ear swabs (1.7%), and one pleural aspirate (0.8%). The bacterial identifications of the isolates recovered from the specimens are presented in Table 1. Disk diffusion testing was used to predict the presence of ESBL and AmpC enzymes, and PCR was used to test for the presence of five classes of carbapenem resistance genes (i.e. $bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{IMP}$, and $bla_{OXA-48}$, respectively). The data are shown by specimen type in Table 2 and by bacterial species in Table 3. Of the 119 presumptive carbapenem-resistant isolates, disk diffusion and MIC results showed that 14 were fully susceptible to both meropenem and imipenem and all were negative for carbapenem resistance genes by PCR. Nineteen isolates that demonstrated phenotypic carbapenem resistance were PCR negative but had disk diffusion profiles consistent with either ESBL (11 isolates) or AmpC (eight isolates) carriage. Thirty carbapenem-resistant isolates were positive for carbapenemase genes by PCR. This included 26 isolates that carried $bla_{NDM}$ and four isolates that carried $bla_{VIM}$ (Table 3). Finally, there were 56 carbapenem-resistant isolates that were negative by PCR for carbapenemase genes and did not have disk diffusion patterns consistent with either ESBLs or AmpC enzymes. This included 23 *P. aeruginosa* isolates, 15 *Stenotrophomonas maltophilia* isolates and 10 *A. baumannii* isolates for which other alternative mechanisms of resistance (e.g. chromosomal metallo-β-lactamases, efflux, or porin changes) are likely.

## 4. Discussion

Carbapenemase-producing organisms (CPO) have been reported from all continents; however, the molecular epidemiology of the resistance mechanisms varies by geographic region [15]. Of the 119 organisms tested in this study, 30 (25.2%) were confirmed to carry either $bla_{NDM}$ or $bla_{VIM}$. The predominance of $bla_{NDM}$ is unsurprising, as a previous multinational survey reported Nigeria to be among the countries with the highest number of $bla_{NDM}$-containing isolates [6]. It is likely that there are other carbapenem resistance genes that were not detected by the PCR assay used in this study, such as the chromosomal metallo-β-lactamases present in *S. maltophilia* as well as $bla_{SIM}$ and $bla_{GIM}$ in *A. baumannii* [16,17]. There were also a number of *P. aeruginosa* and *A. baumannii* isolates that were negative by PCR but likely contained either porin changes or efflux pumps that mediate carbapenem resistance, as these are mechanisms that have been reported in literature [18]. A study conducted in China revealed that the efflux pump-positive strains of *P. aeruginosa* were all negative by phenotypic carbapenem resistance testing [19].

In addition to the potential for both community and nosocomial dissemination of these organisms, medical tourism may also be a contributing factor to the spread of carbapenem-resistant strains

**Table 1**
Carbapenem-resistant bacteria recovered from each specimen type.

| Species | Ear swab | Endo-cervical swab | Vaginal swab | Pleural aspirate | Sputum | Stool | Urine | Wound | Total |
|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | | 2 | 1 | | 3 | 1 | 12 | 9 | 28 |
| *Providencia rettgeri* | | | | | 3 | 3 | 8 | 3 | 17 |
| *Acinetobacter baumannii* | | | | 1 | 2 | 1 | 6 | 6 | 16 |
| *Stenotrophomonas maltophilia* | 1 | | 1 | | | 2 | 9 | 2 | 15 |
| *Escherichia coli* | 1 | 2 | | | 1 | 1 | 8 | 2 | 15 |
| *Klebsiella pneumoniae* | | | | | 4 | 3 | 5 | 1 | 13 |
| *Enterobacter* spp. | | 1 | | | | 2 | 2 | 3 | 8 |
| *Pseudomonas* spp. | | | | | | 1 | 2 | | 3 |
| *Acinetobacter* spp. | | | | | | 1 | 1 | | 2 |
| *Aeromonas* spp. | | | | | | | 2 | | 2 |
| Total | 2 | 5 | 2 | 1 | 13 | 15 | 55 | 26 | 119 |

**Table 2**
Distribution of β-lactamase phenotypes and genotypes among isolates recovered by specimen type.

| Clinical specimen type | Carbapenem-resistant organisms: PCR negative | | | Carbapenem-resistant organisms: PCR positive | | Carbapenem-susceptible[b] | Total isolates recovered |
|---|---|---|---|---|---|---|---|
| | Mechanism unknown | Presumptive ESBL by disk diffusion[a] | Presumptive AmpC by disk diffusion[a] | PCR positive ($bla_{NDM}$) | PCR positive ($bla_{VIM}$) | | |
| Urine | 25 | 6 | 4 | 14 | 2 | 4 | 55 |
| Wound | 15 | 1 | 1 | 4 | 2 | 3 | 26 |
| Stool | 6 | 1 | 2 | 4 | | 2 | 15 |
| Sputum | 4 | 2 | | 3 | | 4 | 13 |
| Endo-cervical swab | 2 | 1 | 1 | | | 1 | 5 |
| Ear swab | 1 | | | 1 | | | 2 |
| Vaginal swab | 2 | | | | | | 2 |
| Pleural aspirate | 1 | | | | | | 1 |
| Total | 56 | 11 | 8 | 26 | 4 | 14 | 119 |

*Abbreviations*: PCR, polymerase chain reaction; ESBL, extended-spectrum β-lactamase.
[a] Excluding *Stenotrophomonas maltophilia*.
[b] Most organisms were ertapenem-resistant.

**Table 3**
Presumptive mechanisms of β-lactam resistance by bacterial species.

| Species | PCR negative for carbapenemase genes | | | PCR positive for $bla_{NDM}$ | PCR positive for $bla_{VIM}$ | Mechanism unknown | Total |
|---|---|---|---|---|---|---|---|
| | Carbapenem-susceptible | Presumptive AmpC by disk diffusion* | Presumptive ESBL by disk diffusion* | | | | |
| *Pseudomonas aeruginosa* | | | | 1 | 4 | 23 | 28 |
| *Acinetobacter baumannii* | 6 | | | 2 | | 10 | 18 |
| *Providencia rettgeri* | | | | 17 | | | 17 |
| *Escherichia coli* | 3 | | 7 | 4 | | 1 | 15 |
| *Stenotrophomonas maltophilia* | | | | | | 15 | 15 |
| *Klebsiella pneumoniae* | 5 | | 4 | 1 | | 3 | 13 |
| *Enterobacter* spp. | | 6 | | 1 | | 1 | 8 |
| *Pseudomonas* spp. | | | | | | 3 | 3 |
| *Aeromonas* spp. | | 2 | | | | | 2 |
| Total | 14 | 8 | 11 | 26 | 4 | 56 | 119 |

*Abbreviations*: PCR, polymerase chain reaction; ESBL, extended-spectrum β-lactamase.
* excluding Stenotrophomonas maltophilia.

in this region. There is a high reported rate of travel to India for medical treatment for Nigerian individuals, where $bla_{NDM}$ is endemic [2,5]. A previous study conducted in Nigeria also reported $bla_{NDM}$- and $bla_{VIM}$-containing organisms [7]. Both genes have been reported from multiple regions globally, although $bla_{NDM}$ predominates in India and $bla_{VIM}$ is mostly found in Europe and Latin America [20,21].

As previously noted, reduced susceptibility to carbapenems via mechanisms other than carbapenemase production has been reported. Other resistance mechanisms include the overexpression of AmpC enzymes or ESBLs in conjunction with porin changes, and possibly efflux pumps [22,23]. Disk diffusion results suggest that both ESBLs and AmpCs may have contributed to the carbapenem resistance of some of the current isolates. However, there was no capacity to test strains for porin changes, so this mechanism remains a hypothesis but it may explain some of the phenotypic carbapenem resistance that was observed.

In summary, $bla_{NDM}$ and $bla_{VIM}$ were the resistance genes most commonly detected among carbapenem-resistant isolates from patients in the current hospital, which is consistent with the previous reports of carbapenemases in Nigeria. Among the carbapenem-resistant PCR-negative isolates in this study, it is likely that resistance may be due to other carbapenemase genes such as $bla_{OXA-23}$, $bla_{OXA-24}$ and $bla_{OXA-58}$ in *A. baumannii* and $bla_{GES}$ lineages in *P. aeruginosa*. In addition, other mechanisms that may be responsible for resistance in these isolates include porin changes and efflux pumps. These microorganisms will be the focus of future studies.

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## Competing interests

Isabella A. Tickler, Caitlin M. dela Cruz and Fred C. Tenover are employed by Cepheid.

## Ethical approval

Obtained from the Health Research Ethical Committee of Federal Medical Centre, Yola, Nigeria. Approval number: FMCY/SUB/96N/T/44.

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