SHORT COMMUNICATION

High prevalence of NDM genes among Carbapenemase-producing clinical Gram-negative bacilli in Benin City, Nigeria: Pseudomonas aeruginosa - a leading culprit

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Carbapenemase producing organisms (CPOs) have become a global health concern because they are multidrug resistant thus limiting therapeutic options for clinical management of infections (1). Carbapenemases are capable of inactivating the carbapenems, cephalosporins, monobactams and penicillins and their genetic determinants are mostly spread by horizontal transmission on mobile genetic elements such as transposons and/or conjugative plasmids (1,2). This facilitates their spread between Enterobacteriales and non-fermenters, a worrying fact for regions with fragile healthcare systems characterised by poor infection prevention and control practices, absence of policies on antibiotic stewardship, and absence of surveillance on antimicrobial resistance (3). Such favorable conditions for the spread of CPOs are rife in most African countries.

In Nigeria literature is emerging on the role of carbapenemase genes such as NDM, $OXA_{48\text{-like}}$, $OXA\text{-}_{181}$, IMP, KPC, GES, VIM and CPOs across various regions with varying prevalence rates (2,4,5). However, owing to paucity of data in the South-South and our locality (Benin City), this study was carried out to determine the prevalence of carbapenemase genes among clinical Gram-negative bacterial isolates in Benin City, Edo state, Nigeria.

The study was cross-sectional and was conducted at the University of Benin Teaching Hospital. Gram-negative rods recovered from consecutive non-repetitive routine specimens between February 24th 2020 and July 21st 2020 were identified using the Microbact 24E identification system. Antimicrobial susceptibility tests were conducted according to CLSI (2018) (6), using the following antibiotics as appropriate: Meropenem (10µg), Imipenem (10µg), Ceftazidime (30µg), Cefuroxime (30µg), Cefoxitin (30µg) Levofloxacin (5µg), Tazobactam-piperacillin (110µg), Amoxicillin-clavulanate (30µg) and Amikacin (30µg). Bacterial strains that were multidrug resistant (resistant to ≥ 3 classes of antibacterial drugs and/or resistant to the carbapenems) were screened using the simplified carbapenemase inactivation method (sCIM) (7). Isolates that were carbapenemase producing were further screened for carbapenemase genes by PCR.

PCR was used to identify genes encoding various known carbapenemases. DNA extracts were obtained by boiling method and multiplex PCR amplification for the simultaneous detection of bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{VIM}, β-lactamase genes was carried out on a Veriti 96-well thermal cycler instrument (Applied Biosystems,Life Technologies, Foster City, CA) with the AmpliTaq Gold PCR master mix (Applied Biosystems, Life Technologies, Hammonton, NJ) as previously described (8). The PCR products were analysed by electrophoresis with 1.5% agarose gels in 0.5X Tris-Borate-EDTA (TBE) buffer. The data obtained was analysed with appropriate statistical tool (chi square) using the statistical software INSTAT®.

A total of 451 Gram-negative bacilli were recovered from clinical specimens comprising 329 (72.9%) of Enterobacteriales and 122 (27.1%) of non-fermenters (Table 1). A total of 189 (41.9%) of these isolates were MDR and 39 (20.6%) MDR strains were carbapenemase producing using phenotypic method. Of this number, 37 (92.5%) were confirmed by PCR (Figure 1), with two (7.5%) isolates being negative. *Pseudomonas aeruginosa* was more likely to be carbapenemase producing in comparison with any other isolate ($p<0.0001$). The most detected carbapenemase gene was NDM (67.5%), with *P. aeruginosa* showing the highest prevalence of the gene (71.9%). Verona integron metallo-β-lactamase (VIM) gene ranked next (17.5%). Three isolates harbored two carbapenemase genes with one of these combinations NDM + VIM and NDM + $OXA_{48\text{-like}}$). Wounds had the highest prevalence of CPOs (35.9%) while cerebrospinal fluid and stool samples had the least prevalence (2.6%).

In this study the most detected carbapenemase gene was NDM (67.5%), with *P. aeruginosa* showing the highest prevalence of the gene while KPC gene was strikingly absent among CPOs, including *Klebsiella* spp. This finding is in contrast to the prevailing narrative in Europe and North America where KPC and its variants are the dominant carbapenemase genes found mostly in Enterobacterales (4). It also differs from few studies in Nigeria where KPC and GES were the dominant genes (2,4). Our finding is, however, similar to findings on the Indian subcontinent where an increased prevalence of metallo-beta-lactamase MBL-producing CPOs has been observed, and a recent study in Northwestern Nigeria where NDM gene dominated among CPO (1,4,9). New Delhi Metallo-beta-lactamase-1 was first detected in a *K. pneumoniae* isolate from a Swedish patient of Indian origin in 2008. Since then, it has been detected in bacteria from patients in India, Pakistan, the United Kingdom, Canada, the United States, and in several Asian and African nations (1,10). Although previous studies have highlighted indiscriminate use of antibiotics (especially beta-lactamases) in Nigeria as contributing to selective pressure leading to the survival and proliferation of beta-lactamase producing organisms (2), the cost of the carbapenems is prohibitive and its abuse not as rampant. A significant number of Nigerian citizens of Edo extraction visit Europe and Asian countries, including the Indian subcontinent, for economic reasons and medical tourism. They therefore, inadvertently, may have played key roles in the spread and distribution of CPOs harboring MBL genes. Further research exploring this facet would give clarity.

Strikingly, VIM type MBL-producing *Pseudomonas* spp were incriminated in infections in this study, as 17.5% of bacterial strains harboured the gene. Although previous studies in Southwestern and Northern Nigeria had reported VIM gene from Enterobacterales and *P. aeruginosa* (3,4,9), we for the first time report VIM-producing isolates from Edo state, South-South Nigeria.

Summarily, the prevalence of CPO was 8.9% with 80% being *P. aeruginosa*. Majority of CPOs were MBL-producing, harboring NDM, VIM and NDM + VIM genes; all CPOs were multidrug resistant. There is an urgent need to deeply entrench infection prevention and control practices as well as enact institutional and national policies on antibiotic stewardship.

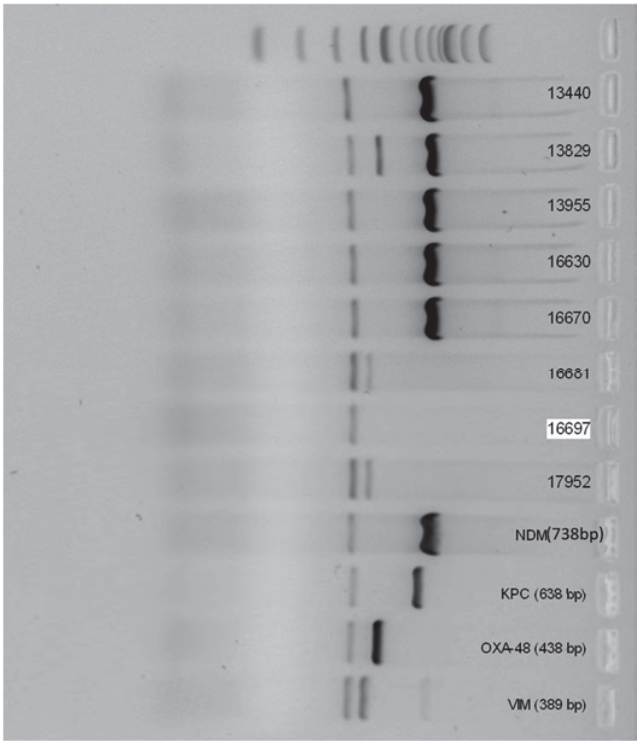

Figure 1. Agarose gel of PCR products (amplified carbapenemase genes): Isolates number 13440, 13955, 16630 and 16670 are NDM positive, while 13829 is NDM + $OXA_{-48\text{-like}}$ positive. Isolate 16681 and 17952 are VIM positive while 16697 is negative for all genes. Positive control genes are seen in the last four wells (NDM, KPC, $OXA_{-48\text{-like}}$ and VIM).

Table 1. Distribution of Gram-negative bacterial isolates in relation to carbapenemase genes and clinical specimens.

Organism	No. of isolates	Phenotypic detection (CPO)	Molecular detection				Clinical specimens					
			NDM	VIM	NDM ++VIM	NDM + OXA_{48}	Aspirate	Catheter tip	CSF	Stool	Wound	Urine
E. cloacae	27	1 (3.7)	0	1 (100)	0	0	0	0	0	0	1 (100)	0
K. oxytoca	26	1 (3.8)	0	0	0	1 (100)	0	0	0	0	1 (100)	0
K. pneumoniae	47	4 (8.5)	3 (75)	0	0	1 (25)	0	1 (25)	0	0	2 (50)	1 (25)
Providencia retgerri	4	1 (25)	1 (100)	0	0	0	0	0	0	1 (100)	0	0
P. aeruginosa	81	31 (38.3)	23 (71.9)	5 (15.6)	1 (3.1)	0	4 (12.9)	6 (19.4)	1 (3.1)	0	10 (32.3)	8 (25.8)
P. putida	4	1 (25)	0	1 (100)	0	0	0	0	0	0	0	1 (100)
Total	189	39 (20.6)	27 (67.5)	7 (17.5)	1 (2.5)	2 (5)	4 (10.3)	7 (17.9)	1 (2.6)	1 (2.6)	14 (35.9)	10 (25.6)

Number of isolates vs CPO: $p < 0.0001$, CPO- carbapenemase producing organism, NDM- New Dehli metallo-β-lactamase, VIM- Verona integron metallo-β-lactamase, OXA- Oxacillinase-like carbapenemase, CSF-cerebrospinal fluid.

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