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Short Communication

Wide spread of carbapenemase-producing bacterial isolates in a Nigerian environment

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ABSTRACT

Objectives: The presence of carbapenemase-producing bacterial isolates is found not only in hospital and community settings but also in the environment. Carbapenemase production may be related to acquired, usually plasmid-borne, β-lactamase genes or to chromosomal genes intrinsic to various species. The aim of this study was to evaluate the occurrence of such carbapenemase-producing bacterial isolates among environmental samples from Nigeria.

Methods: A total of 122 environmental samples were plated on carbapenem-containing media. A total of 259 isolates were recovered, among which 124 were carbapenemase-producers according to the results of the Rapidec® Carba NP test.

Results: The majority of isolates ($n = 112$) recovered corresponded to natural producers of carbapenemases, i.e. *Stenotrophomonas maltophilia* ($n = 108$), *Burkholderia cepacia* ($n = 1$), *Shewanella* sp. ($n = 1$), *Sphingobacterium* sp. ($n = 1$) and *Chryseobacterium gleum* ($n = 1$). Ten isolates (mainly Enterobacteriaceae and *Acinetobacter baumannii*) produced an acquired carbapenemase, most commonly of the NDM type. In addition, two *Pseudomonas otitidis* isolates were identified as producing the Ambler class B carbapenemase POM-1, further confirming that this carbapenemase is naturally produced in this environmental species. Finally, several isolates co-producing 16S rRNA methylases (ArmA, RmtC) and/or extended-spectrum β-lactamases (CTX-M-9, CTX-M-15) were also identified.

Conclusion: This study revealed the presence and diversity of clinically-relevant antimicrobial-resistant bacteria in the environment in Nigeria.

1. Introduction

Carbapenems are last-resort antibiotics for managing multidrug-resistant bacterial infections but their effectiveness may be compromised by the production of carbapenemases [1]. Knowing the extent of the reservoir of carbapenemases is becoming important. The most clinically-relevant carbapenemases are of the KPC type (serine carbapenemase of Ambler class A), VIM-, IMP- and NDM-types (metallo-β-lactamases of Ambler class B) and OXA-48-type (oxacillinases of Ambler class D). Although extensive surveillance studies have been performed in Europe, America and Australia, data from Africa remain scarce. In Nigeria, the most populated country of Africa (ca. 200 million inhabitants), antibiotic use is widespread and largely unregulated [2]. Owing to a lack of sanitation and poor control of public health in many African countries, large amounts of antibiotics may end up in the

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environment and thus contribute to the selection and persistence of antimicrobial resistance. Therefore, the primary aim of this study was to evaluate the spread of multidrug-resistant bacterial strains among environmental samples collected in Nigeria, with a particular focus on carbapenemase-producers.

2. Materials and methods

2.1. Soil and water samples

A total of 122 environmental samples (water and soil) were collected during the same time period in 2017 from six different locations in Nigeria, namely Abuja (North Central), Kano (North West), Yola (North East), Nnewi (South East), Akwa Ibom (South) and Ibadan (South West). Water samples were recovered from different bodies of lakes.

2.2. Selective isolation of carbapenem-resistant Gram-negative bacteria

Selection of carbapenem-resistant isolates was performed following a selective enrichment step by culture in brain–heart infusion broth supplemented with ertapenem (0.25 μg/mL) overnight at 30 °C. Following enrichment, 100 μL of pure and diluted (1/100; 1:1000) cultures was plated onto selective media, including SuperCarba® (CHROMagar, Paris, France) [3] and Drigalski agar medium supplemented with imipenem (0.75 μg/mL), daptomycin (10 μg/mL) and amphotericin B (5 μg/mL). Plates were then incubated at 30 °C for 24 h.

2.3. Phenotypic and genotypic characterisation of carbapenem-resistant bacteria

Antimicrobial susceptibility testing was initially performed by the disk diffusion method and was interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (http://www.eucast.org), except for colistin for which susceptibility was evaluated by broth microdilution, as recommended.

Carbapenemase activity was assessed by the Rapidec® Carba NP test (bioMérieux) for each colony type growing on the selective media [4]. Identification of carbapenemase genes was performed by PCR using previously published primers [5]. The following carbapenemase genes were searched for: Ambler class A genes of bla_{KPC}, bla_{GES}, bla_{IMI} and bla_{FRI} types; Ambler class B genes of bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{GIM} and bla_{POM} types; and Ambler class D genes of bla_{OXA-48}, $_{-181}$, $_{-23}$, $_{-40\ and\ -58}$ types. In addition, the following genes were searched for: extended-spectrum β-lactamases (ESBLs), including $bla_{CTX-M-1,\ -2,\ -3,\ -8,\ -9\ and\ -15}$, bla_{PER}, bla_{TEM} and bla_{SHV} [6]; aminoglycoside resistance 16S rRNA methylase genes (*armA*, *rmtA–rmtH* and *npmA*) [7]; and plasmid-borne colistin resistance *mcr*-like genes (*mcr-1* to *mcr-9*) [8]. All positive PCR amplicons were sent for sequencing (Microsynth AG, Balgach, Switzerland). Bacterial identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (VITEK® MS; bioMérieux).

Whole-genome sequencing was performed for the two *Pseudomonas otitidis* strains for which identification was uncertain. DNA was extracted from isolates using a QIAamp® DNA Mini Kit and QIAcube Workstation (QIAGEN, Courtaboeuf, France), according to the manufacturer's instructions. Genomic libraries were generated using a Nextera XT DNA Sample Preparation Kit (Illumina Switzerland GmbH, Zurich, Switzerland) and sequencing was performed on an Illumina MiSeq benchtop sequencer. Reads from sequencing were assembled using CLC Genomics Workbench 7 (QIAGEN). Putative β-lactamase genes were then detected by ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/).

3. Results and discussion

From the 122 environmental samples, 259 distinct isolates were isolated on selective carbapenem-containing media. All carbapenem-resistant isolates were identified on both screening media. Of the 259 isolates, 124 were found to be carbapenemase-producers according to the results of the Rapidec® Carba NP test. The carbapenemase-negative isolates corresponded to 135 *Pseudomonas* spp. isolates (no acquired resistance genes) that were recovered only on the SuperCarba® medium owing to natural ertapenem resistance in this genus. The majority ($n = 112$) of the carbapenemase-producers identified corresponded to bacterial species that naturally produce carbapenemases such as *Stenotrophomonas maltophilia* (L1-like β-lactamase [9]) ($n = 108$), *Chryseobacterium gleum* (CGB-like carbapenemase [10]) ($n = 1$), *Burkholderia cepacia* (PenA chromosomal A penicillinase [11]) ($n = 1$), *Sphingobacterium* sp. ($n = 1$) and *Shewanella* sp. (OXA-181-like carbapenemase [12]) ($n = 1$).

Ten isolates harbouring an acquired carbapenemase gene were also identified (Table 1). A series of enterobacterial strains

Table 1
Information and genetic features of isolates with an acquired carbapenemase gene from environment samples from various geographical locations in Nigeria.

Strain no.	Type of sample[a]	Geographical location[b]	Species	Carbapenemase	ESBL	16S rRNA methyltransferase (s)	Non-susceptible phenotype[c]
1	Soil UO	Abuja (NC)	*Acinetobacter baumannii*	OXA-23	None	None	CIP, TET, SXT, CHL
2	Soil HE	Nnewi (SE)	*A. baumannii*	OXA-40	None	ArmA	GEN, AMK, CIP, SXT, CHL
3	Water	Nnewi (SE)	*A. baumannii*	OXA-40	None	ArmA	GEN, AMK, CIP, SXT, CHL
4	Soil HE	Abuja (NC)	*Aeromonas caviae*	NDM-1	CTX-M-9	ArmA, RmtC	GEN, AMK, SXT
5	Soil HE	Abuja (NC)	*Citrobacter freundii*	NDM-5	None	None	GEN, CIP, TET, SXT, CHL
6	Soil HE	Abuja (NC)	*Enterobacter cloacae*	NDM-5	CTX-M-15	None	GEN, CIP, TET, SXT
7	Soil HE	Akwa Ibom (S)	*E. cloacae*	NDM-7	None	None	SXT
8	Soil HE	Akwa Ibom (S)	*Klebsiella pneumoniae*	NDM-1	CTX-M-15	ArmA	GEN, AMK, CIP, TET, SXT
9	Soil HE	Akwa Ibom (S)	*K. pneumoniae*	NDM-1	CTX-M-15	ArmA	GEN, AMK, CIP, TET, SXT
10	Soil HE	Kano (NW)	*Pseudomonas putida*	VIM-5	None	None	CIP, TET, SXT, CHL

ESBL, extended-spectrum β-lactamase.
[a] UO, urban outskirt; HE, hospital environment.
[b] NC, North Central; SE, South East; S, South; SW, South Wes; NW, North West.
[c] CIP, ciprofloxacin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol; GEN, gentamicin; AMK, amikacin.

producing different NDM-type carbapenemases was found in different locations in Nigeria. This finding highlights the environmental spread of the NDM-type carbapenemase in Nigeria [13]. The large spread of NDM-producers has already been identified in many other African countries [14], including Angola [15] and Togo [16], indicating that this continent may act as a secondary reservoir after the Indian subcontinent for spreading NDM-producing isolates to Europe. The *Acinetobacter baumannii* isolates produced either OXA-23 or OXA-40, which are extensively reported in that species [17]. In addition, a bla_{VIM-5}-positive *Pseudomonas putida* isolate was identified, as previously identified from polluted wetlands in Nigeria [18]. Genome sequencing allowed the identification of a *Sphingobacterium* sp. isolate that produced a naturally occurring Ambler class B carbapenemase [19] and two *P. otitidis* isolates that harboured the bla_{POM-1} gene. This latter gene encodes a class B carbapenemase, with no associated mobile genetic element, which has been previously identified as intrinsic to that bacterial species [20].

Regarding the non-β-lactamase resistance determinants, it is worth mentioning that the 16S rRNA methylase determinants conferring pan-aminoglycoside resistance were frequently associated with NDM-producers, as previously shown [21]. Noteworthy, the spread of 16S rRNA methylases in Gram-negative bacteria in Africa appears to represent a major problem, with recent reports showing their occurrence in, e.g., Enterobacterales in Angola, Libya, Egypt and Algeria as well as *A. baumannii* in Algeria and Egypt. Finally, three colistin-resistant isolates were recovered among the acquired carbapenem-resistant isolates (*A. baumannii* and *Enterobacter cloacae*) and natural carbapenem-resistant isolates (*Sphingobacterium* spp.) but were not positive for MCR-encoding genes, suggesting that the resistance was mediated by other mechanisms of resistance such as chromosomal mutations. These results may indicate that acquired colistin resistance has not yet spread among environmental isolates in Nigeria.

Overall, this study revealed the presence and diversity of clinically-relevant antimicrobial-resistant bacteria in the environment and demonstrates the need for further investigation.

Competing interests

None declared.

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