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Short Communication

Clonal diversity of *Acinetobacter* clinical isolates producing NDM-type carbapenemase in Cuba, 2013–19

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ABSTRACT

Objectives: Our study aimed to elucidate the clonal diversity of carbapenem-resistant *Acinetobacter* clinical isolates producing NDM-type carbapenemase collected through national surveillance in Cuba during a 7-year period (2013–19).

Methods: A total of 199 isolates of *Acinetobacter* spp. from 37 hospitals in 12 provinces were genetically analyzed for their species, carbapenemase genes and genotypes. Sequence type (ST) and OXA-51-like gene type were determined for bla_{NDM}-positive isolates.

Results: Most isolates (95%) were identified as species of *Acinetobacter calcoaceticus-baumannii* complex, with *A. baumannii* being the majority. Acquired carbapenemase genes were assigned to bla_{OXA} or bla_{NDM} type; the most commonly detected gene was bla_{OXA-23}-like (49%), followed by bla_{OXA-24}-like (20%) and bla_{NDM} (15%). Twenty-nine bla_{NDM}-positive isolates (22 *A. baumannii*, 2 *A. pittii*, 2 *A. johnsonii*, 3 other species) were differentiated into 19 STs, including the most common, ST23. Though NDM genes were mostly typed as bla_{NDM-1}, a novel bla_{NDM-42} was identified in an ST79 *A. baumannii* isolate. $bla_{OXA-51-like}$ genes of NDM-positive *A. baumannii* were discriminated into 10 OXA types, including 2 novel ones.

Conclusions: Our study indicated the spread of bla_{NDM} to various clones of *A. baumannii* and other *Acinetobacter* spp. in Cuba.

Introduction

Acinetobacter species, primarily *A. calcoaceticus-baumannii* (Acb) complex, are one of the major nosocomial pathogens associated with drug resistance worldwide (Hamidian and Nigro, 2019). In particular, resistance to carbapenems, front-line antimicrobials for multidrug-resistant gram-negative bacteria, has been increasingly reported, posing a public health concern. Carbapenem resistance in *Acinetobacter* is principally mediated by the production of carbapenemases belonging to different types. In Latin America and the Caribbean, resistance rates to carbapenems in *A. baumannii* were more than 50% in many countries in 2014–16 with an increasing tendency in some countries (PAHO, Pan American Health Organization, 2020), with OXA type enzymes being the dominant carbapenemases (Yu et al., 2022a). In Cuba, 42% to 44% of *Acinetobacter* spp. in 2010–12 showed carbapenem resistance, primarily associated with OXA-23, despite the low incidence of NDM-1 (Quiñones et al., 2015). The present study was conducted to characterize recent carbapenem-resistant *Acinetobacter* (CRA), especially the prevalence and clonal diversity of NDM-producing strains, in Cuba.

Methods

We conducted a retrospective study for CRA clinical isolates collected from 37 hospitals in 12 provinces of Cuba (Fig. S1) as part of the national surveillance of carbapenemases in the National Reference Laboratory for Health Care-Associated Infections for a 7-year period starting January 2013. The clinical specimens were cultured on McConkey agar and then incubated at 37 °C for 18 to 24 h. For the presumptive pathogenic bacteria, species were identified by an automated analyzer (Vitek 2; bioMérieux, France). Partial 16S rRNA and *rpoB* gene sequences were determined for confirmation of *Acinetobacter* species. Susceptibility to 15 antimicrobials was determined using E-test or disc diffu-

Abbreviations: Acb complex, *Acinetobacter calcoaceticus-baumannii* complex; ST, sequence type; MICs, minimum inhibitory concentrations; CRA, carbapenem-resistant *Acinetobacter*; CRAb, carbapenem-resistant *Acinetobacter baumannii*.

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Table 1
Prevalence of OXA- and NDM-type carbapenemase genes among clinical isolates of *Acinetobacter* spp. in Cuba (2013-2019).

Acinetobacter species (Number of isolates)	Acquired carbapenemase genes			
	$bla_{\text{OXA-23-like}}$	$bla_{\text{OXA-24-like}}$	$bla_{\text{OXA-58-like}}$	bla_{NDM}
Acb complex (190)				
A. baumannii (186)	94	39	14	22
A. pittii (2)	0	0	2	2
A. calcoaceticus (2)	1	0	0	0
Non-Acb complex (9)				
A. junii (4)	3	0	1	1
A. johnsonii (2)	0	0	0	2
A. lwoffii (1)	0	1	0	0
A. haemolyticus (1)	0	0	1	1
A. bereziniae (1)	0	0	1	1
Total (199)	98	40	19	29

Acb complex: *Acinetobacter calcoaceticus-baumannii* complex.

Table 2
ST, OXA/NDM gene types of *Acinetobacter* isolates harboring bla_{NDM} in Cuba (2013-2019).

Acinetobacter species	ST (number of isolates)	Allelic profile (variant*[1])	OXA-like gene profile (51, 23, 24, or 58)	OXA-51-like gene type	NDM type	year	specimen / source
A. baumannii (n=22)	ST23 (6)	1-3-10-1-4-4-4 (SLV of ST10)	51, 58	OXA-68	NDM-1	2013, 2015, 2019	blood, respiratory tract, surgical site
	ST78 (2)	25-3-6-2-28-1-29	51	OXA-90	NDM-1	2015, 2018	catheter tip
	ST79 (2)	26-2-2-2-29-4-5	51, 24, 58	OXA-65	NDM-1	2013	surgical site
			51, 24	OXA-65	NDM-42*[2]	2019	respiratory tract
	ST85 (2)	5-2-4-1-3-3-4	51, 24, 58	OXA-94	NDM-1	2015	blood
			51, 24	OXA-94	NDM-1	2015	blood
	ST108 (2)	35-1-11-7-9-25-2	51	OXA-132	NDM-1	2015	respiratory tract, catheter tip
	ST2 (1)	2-2-2-2-2-2-2	51, 23	OXA-66	NDM-1	2014	respiratory tract
	ST10 (1)	1-3-2-1-4-4-4	51, 58	OXA-68	NDM-1	2019	surgical site
	ST32 (1)	1-1-2-2-3-4-4 (TLV of ST10)	51, 58	OXA-100	NDM-1	2016	catheter tip
	ST52 (1)	3-2-2-7-9-1-5	51, 58	OXA-1117*[2]	NDM-1	2018	skin
	ST368 (1)	1-62-3-2-40-1-4	51, 58	OXA-71	NDM-1	2018	blood
	ST905 (1)	1-1-2-2-30-4-4 (TLV of ST10)	51, 58	OXA-100	NDM-1	2019	cerebrospinal fluid
	ST921*[2] (1)	3-3-2-2-3-4-4 (TLV of ST10)	51, 58	OXA-424	NDM-1	2015	respiratory tract
	ST1344*[2] (1)	25-3-6-2-28-1-4	51	OXA-1118*[2]	NDM-1	2019	urine
A. pittii (n=2)	ST119 (1)	36-20-38-16-38-18-20	58	-	NDM-1	2018	respiratory tract
	ST1170 (1)	45-162-138-10-20-18-56	58	-	NDM-1	2018	blood
A. johnsonii (n=2)	ST1346*[2] (2)	205-193-190-96-204-107-178	-	-	NDM-1	2013	blood, surgical site
A. bereziniae (n=1)	ST1345*[2] (1)	198-190-2-2-194-104-173	58	-	NDM-1	2019	surgical site
A. haemolyticus (n=1)	ST1347*[2] (1)	206-191-191-97-205-108-179	58	-	NDM-1	2015	blood
A. junii (n=1)	ST1343*[2] (1)	111-170-183-57-110-56-97	58	-	NDM-1	2013	blood

[1] SLV, single-locus variant; TLV, triple-locus variant.
[2] Newly identified type (ST, OXA type, NDM type) in this study.

sion method, while minimum inhibitory concentrations of meropenem, imipenem and colistin were measured by broth microdilution method.

Identification of carbapenemase genes encoding metallo-β-lactamases (IMP, NDM, VIM) and class D-OXA β-lactamase (OXA-23, -24, -51, -58-like) was performed by multiplex polymerase chain reaction (PCR) using primers previously reported (Queenan and Bush, 2007; Nordmann et al., 2011). Nucleotide sequences of bla_{NDM} and $bla_{\text{OXA-51}}$-like were determined by Sanger sequencing with PCR product, using the primers shown in Table S1, on an automated DNA sequencer (ABI PRISM 3100). Multiple alignments of the sequences and calculation of sequence identity was performed by the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). Sequences of novel types of bla_{NDM} and bla_{OXA} identified were deposited into the GenBank database. Sequence type (ST) was assigned according to the Institute Pasteur multilocus sequence typing scheme (Diancourt et al., 2010). Plasmid-mediated colistin resistance genes, *mcr-1* to *mcr-5* were detected by PCR using primers published previously (Table S1).

Results and discussion

During the study period, 199 clinical isolates of CRA were collected. The isolates were derived from various specimen types/sites: respiratory tract (34%), blood (22%), surgical site (18%), skin (9%), a central venous catheter (10%) and others (7%) (Table S2). We identified 8 species

of *Acinetobacter* (Table 1). Acb complex accounted for 95% of isolates, with *A. baumannii* predominant. These isolates showed high resistance rates (70% to 100%) to most of the antimicrobials tested (Table S3), though all isolates were susceptible to colistin and tigecycline without harboring *mcr* genes.

The most prevalent acquired carbapenemase gene was $bla_{\text{OXA-23}}$-like (49%), followed by $bla_{\text{OXA-24}}$-like (20%), bla_{NDM} (15%) and $bla_{\text{OXA-58}}$-like (9.6%) (Table 1). NDM gene was identified in 29 isolates of 6 *Acinetobacter* species, with *A. baumannii* being the most common (Table 2). NDM-positive isolates had been recovered persistently since 2013, showing higher incidence rates (25% to 28%) in 2018 and 2019 (Table S4). bla_{NDM}-positive isolates were differentiated into 19 STs, among which ST23, a single locus variant of ST10, was the most common. The distribution of these STs to individual provinces is shown in Fig. S1. Although NDM genes were mostly typed as $bla_{\text{NDM-1}}$, a novel $bla_{\text{NDM-42}}$ was identified in an ST79 *A. baumannii* isolate. The deduced amino acid sequence of NDM-42 was different from NDM-1 by only one amino acid (Fig. S2). The intrinsic $bla_{\text{OXA-51-like}}$ genes of NDM-positive *A. baumannii* were discriminated into 10 types, including 2 novel types (OXA-1117, OXA-1118). Sequences of the novel types of NDM and OXA genes were deposited into the GenBank database under the accession numbers shown in Table S5.

Compared with a previous study in Cuba (2010–12) which showed a low incidence of bla_{NDM} (1 in 220 CRA isolates) (Quiñones et al., 2015), in the present study bla_{NDM} was prevalent among CRA (29%) mainly in Acb complex, and distributed also to other species *A. bereziniae, A. haemolyticus, A. johnsonii* and *A. junii*, and there was a relatively lower prevalence of $bla_{\text{OXA-23}}$-like gene. In Cuba, the NDM-type enzyme has been found to be dominant in carbapenem-resistant *Enterobacterales* (2016–21) (Yu et al., 2022b). These findings contrast with the distribution of carbapenemase types among *Acinetobacter* and *Enterobacterales* found in other Latin American countries (García-Betancur et al., 2021; Yu et al., 2022a) and suggest the regional spread of bla_{NDM} over Gram-negative bacteria in Cuba.

In our study, we employed multilocus sequence typing to clarify clonal lineages of *Acinetobacter* because this method was considered the most suitable and feasible for comparison among domestic and global strains to understand their molecular evolution and transmission status. Globally, over 71% of CRAb strains belong to ST2, ST1, ST79 and ST25, among which ST2 is predominant (Hamidian and Nigro, 2019). An endemic situation, regional or inter-regional spread of CRAb were reported mainly in south European and Balkan countries in 2019 (Lötsch et al., 2020), with ST1, ST2 and ST492 being the most common endemic lineages (Kostyanev et al., 2021). Though the prevalence is still low, NDM-producing strains were identified in ST2 and ST492 CRAb, and Serbia was suggested as their potential endemic region (Lukovik et al., 2020). In Latin America, most CRAb belonged to clonal complexes related to ST1, ST2, ST15, ST25 and ST79 (Rodríguez et al., 2016; Levy-Blitchtein et al., 2018; Cerezales et al., 2019), with other distinct lineages prevalent in some countries (Correa et al., 2018; López-Leal et al., 2019). Furthermore, the common carbapenemase produced by *Acinetobacter* was OXA type, with NDM extremely rare (Rodríguez et al., 2018). However, in our study in Cuba, the common STs in Latin America/Europe were less prevalent (only 3 isolates belonged to ST2 or ST79), and the incidence of bla_{NDM} was relatively higher. In contrast, ST23 (single locus variant of ST10) was the most common, along with ST10 and its related types (ST32, ST905, ST921), suggesting the prevalence of ST10-related *A. baumannii* clones in Cuba.

Our study has some limitations. First, it is possible that the clinical isolates collected contain some colonizing bacteria (Bartal et al., 2022). Second, resistance mechanisms other than carbapenemase (Aurilio et al., 2022) were not analyzed. Nevertheless, unique epidemiological features of CRA in Cuba were revealed, i.e., the potential spread of bla_{NDM} over multiple *Acinetobacter* species and various clones, indicating a need for further surveillance in the country.

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Ethical statement

In this study, no human participants were directly involved, so clearance of human ethics was not required. We analyzed isolates that had been routinely cultured from clinical specimens from hospitals and sent to the National Reference Laboratory for Health Care-Associated Infections, Bacteriology-Mycology Department in Pedro Kourí Institute of Tropical Medicine for national surveillance.

Declaration of Competing Interest

The authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2022.08.008.

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