High prevalence of \( bla_{\text{OXA-23}} \) in \textit{Acinetobacter} spp. and detection of \( bla_{\text{NDM-1}} \) in \textit{A. soli} in Cuba: report from National Surveillance Program (2010–2012)

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Abstract

As a first national surveillance of \textit{Acinetobacter} in Cuba, a total of 500 \textit{Acinetobacter} spp. isolates recovered from 30 hospitals between 2010 and 2012 were studied. \textit{Acinetobacter baumannii}–\textit{calcoaceticus} complex accounted for 96.4% of all the \textit{Acinetobacter} isolates, while other species were detected at low frequency (\textit{A. junii} 1.6%, \textit{A. lwoffi} 1%, \textit{A. haemolyticus} 0.8%, \textit{A. soli} 0.2%). Resistance rates of isolates were 34–61% to third-generation cephalosporins, 49–50% to \( \beta \)-lactams/inhibitor combinations, 42–47% to aminoglycosides, 42–44% to carbapenems and 55% to ciprofloxacin. However, resistance rates to colistin, doxycycline, tetracycline and rifampin were less than 5%. Among carbapenem-resistant isolates, 75% harboured different \( bla_{\text{OXA}} \) genes (OXA-23, 73%; OXA-24, 18%; OXA-58, 3%). The \( bla_{\text{NDM-1}} \) gene was identified in an \textit{A. soli} strain, of which the species was confirmed by sequence analysis of 16S rRNA gene, \( rpoB \), \( rpoB \)–\( rpoC \) and \( rpoC \)–\( rpoB \) intergenic spacer regions and \( gyrB \). The sequences of \( bla_{\text{NDM-1}} \) and its surrounding genes were identical to those reported for plasmids of \textit{A. baumannii} and \textit{A. lwoffi} strains. This is the first report of \( bla_{\text{NDM-1}} \) in \textit{A. soli}, together with a high prevalence of OXA-23 carbapenemase for carbapenem resistance in \textit{Acinetobacter} spp. in Cuba.

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Introduction

The genus \textit{Acinetobacter} includes opportunistic pathogens capable of causing both community- and health care–associated infections, and it has recently emerged as their major cause because of its propensity to accumulate resistance to multiple antimicrobial drugs [1]. Multidrug-resistant \textit{Acinetobacter baumannii}–\textit{calcoaceticus} complex (\textit{A. baumannii}–\textit{c} complex) isolates are increasingly reported worldwide; it is susceptible to an ‘old’ drug, colistin, which often remains as the only effective therapeutic option. Bacterial isolates showing carbapenem resistance have been increasing as a result of acquisition of carbapenemase belonging to classes A, B and D \( \beta \)-lactamas.

Class B \( \beta \)-lactamas, i.e. metallo-\( \beta \)-lactamas (MBLs), include New Delhi MBL (NDM), a novel MBL first reported in \textit{Klebsiella pneumoniae} and \textit{Escherichia coli} in New Delhi. The emergence and dissemination of NDM-1-producing isolates in both human and environment have been reported in relevant bacteria and many countries, causing a serious threat for antimicrobial therapy [2]. Although \textit{A. baumannii}–\textit{c} complex is the...
most clinically important in the genus Acinetobacter, the spread of carbapenemase genes has occurred also in other Acinetobacter spp. in the last decade [3,4]. Several reports evidenced presence of different types of MBL in A. pittii, A. nosocomialis and A. bereziniae in Korea since 2006 [5]. On the other hand, an A. baylyi strain carrying both blaSIM-1 and blaOXA-23 was reported in China in 2011 [6]. Recently, NDM-1 has been detected in A. soli in Japan, China and Taiwan [7–9], and in other non-baumannii Acinetobacter spp. in China (Acinetobacter junii, A. lwaffii and A. pittii) [10–12], Turkey and Latin American countries (A. pittii) [13–15]. These findings indicate the need for global surveillance of NDM in Acinetobacter spp.

Resistance rates of imipenem and meropenem in A. baumannii in Latin America (except for Cuba) in 2004–2010 were reported as 33.6% and 60.6%, respectively, by a large-scale epidemiological study [16]. The first NDM-1-producing bacterium in Latin America was reported in November 2011 when this enzyme was detected in Klebsiella pneumoniae in Guatemala [17]. After that, the Pan-American Health Organization issued a regional alert to strengthen the Latin American surveillance of carbapenemase producers in Gram-negative rods (http://www2.paho.org/hq/dmdocuments/2010/alertas_epi_2010_02_julio_carbapenemasas.pdf). In Cuba, a surveillance network for Acinetobacter has been established since 2010, connecting different hospitals to forward clinical isolates to the National Institute ‘Pedro Kouri’ in Havana for analysis.

In this study, we described prevalence of Acinetobacter spp. and their phenotype of resistance and genetic characteristics of carbapenem resistance genes obtained from national surveillance data in Cuba during 2010–2012.

Materials and Methods

Bacterial isolates
Clinical isolates of Acinetobacter spp. (only one isolate per patient) from 30 hospitals in ten provinces across the Cuba during 2010–2012 were collected to the National Institute ‘Pedro Kouri,’ and clinical information of individual patients was also obtained. Bacterial identification was performed by conventional microbiological methods and later confirmed by API 20NE strip (bioMérieux, Marcy l’Etroite, France). For identification of a single strain, genetic analysis was used as described below.

Antimicrobial susceptibility testing
Minimum inhibitory concentration (MIC) against 18 antibiotics was measured by Etest (bioMérieux), and susceptibility was judged according to Clinical and Laboratory Standards Institute guidelines [18], except for rifampin, which was based on a standard of the French Microbiology Society (http://www.sfm-microbiologie.org/UserFiles/files/casfm_2010.pdf). All isolates with imipenem MIC ≥16 mg/L were considered potential carbapenemase producers and were selected for testing of MBLs through the imipenem-EDTA double-disc synergy test as well as molecular detection of carbapenem resistance genes. As a carbapenem susceptible reference strain, E. coli strain ATCC 25922 was used.

Genetic analysis
For all the carbapenem-resistant isolates, the presence of blaOXA genes encoding OXA-51-like, OXA-23-like, OXA-24-like and OXA-58-like enzymes, and blaIMP, blaVIM and blaNDM was examined by multiplex PCR with specific primers, as described previously [19,20]. For phenotypically MBL-positive isolates, PCR was performed to detect more metalloenzyme genes blagIMS, blaSIM and blaSPM as described previously [21].

Sequence analysis
For a single strain (CU244) with NDM gene (blaNDM), partial sequences of 16S rRNA gene, rpoB, rpoB-rpoC and rpoL-rpoB intergenic regions, and gyrB were determined for species identification. The PCR products were purified using the Wizard SV Gel and PCR Clean-up System (Promega, WI, USA). Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI PRISM 3130). Sequences of NDM-1 gene and its upstream and downstream regions were determined with PCR products amplified by primers designed based on sequences of A. lwaffii strain WJ10621 plasmid pNDM-BJ01 (GenBank JQ001791). Search for homology with cognate gene sequences was performed using BLAST software (Basic Local Alignment Search Tool, http://blast.ncbi.nlm.nih.gov/). Sequences of the CU244 were deposited in the GenBank database under accession numbers KP347604 to KP347608 (16S rRNA gene, rpoB, rpoB-rpoC and rpoL-rpoB intergenic regions, and gyrB) and KP347609 (blaNDM-1 cluster).

Results

Through the national surveillance program for antimicrobial resistance of Acinetobacter spp. in Cuba for 2-year period (2010–2012), a total of 500 nonduplicated clinical isolates were collected. Original specimens of these isolates were respiratory samples (n = 193, 38.6%), blood (n = 155, 31.1%), skin and soft tissue (n = 66, 13.1%) surgical wounds (n = 32, 6.4%), catheter (n = 14, 2.8%), cerebrospinal fluid (n = 13, 2.6%), sputum (n = 7, 1.4%), lochia (n = 5, 1%) and others (n = 15, 3%). Four hundred
ninety-two isolates (98.4%) were obtained from hospitalized patients admitted to different wards, including intensive care unit (60.2%), neonatology (12%) and surgical (11.4%) wards and other departments (16.4%). The remaining eight isolates (1.6%) were derived from community-acquired infections.

*A. baumannii–c* complex accounted for 96.4% of all the *Acinetobacter* isolates, while frequencies of other species were low (*A. junii* 1.6%, *A. lwof* 1%, *A. haemolyticus* 0.8%, *A. soli* 0.2%). The antimicrobial resistance of *Acinetobacter* spp. isolates is shown in Table 1. Resistance rates were 34–61% to third-generation cephalosporin, 49–50% to β-lactam/inhibitor combinations, 42–47% to aminoglycosides, 42–44% to carbapenems and 55% to ciprofloxacin. The most susceptible antimicrobial drugs were colistin, doxycycline and tetracyclin, showing resistance rates of less than 5%. Multidrug resistance, defined as resistance to three or more antimicrobial agent groups, was detected in 57% of the isolates, and 32% of isolates showed extensive drug resistance (multidrug-resistant isolates plus carbapenem resistance).

Among the 220 meropenem-nonsusceptible *A. baumannii–c* complex isolates, 17% (37 isolates) were revealed to be MBL producers by the disk diffusion test with EDTA. All the *A. baumannii–c* complex organisms were positive for the intrinsic blaOXA-51-like gene. Among carbapenem-resistant isolates, 75% harboured different blaOXA (OXA-23, 76%; OXA-24, 18%; OXA-58, 3%; combination of OXA-23 and OXA-24, 3%). PCR performed with primers specific for IMP- and VIM-type enzyme genes was negative for all the carbapenem-resistant isolates. However, the blaNDM gene was detected in only one isolate (CU244) of non-*A. baumannii–c* complex. Among other MBL-positive isolates, metalloenzymes genes encoding GIM, SIM and SPM were not detected by PCR.

**TABLE 1. Resistance rates of *Acinetobacter* spp. in Cuba and antimicrobial susceptibility (MIC) of *A. soli* strain CU244**

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>Resistance rate (%) of <em>Acinetobacter</em> spp. (n = 500)</th>
<th>MIC (μg/mL) of <em>A. soli</em> strain CU244</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipercillin</td>
<td>54</td>
<td>≥128</td>
</tr>
<tr>
<td>Ticarcillin/clavulanic acid</td>
<td>50</td>
<td>≥128</td>
</tr>
<tr>
<td>Piperacillin–tazobactam</td>
<td>49</td>
<td>≥128/4</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>61</td>
<td>≥32</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>55</td>
<td>≥64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>34</td>
<td>≥64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>42</td>
<td>≥16</td>
</tr>
<tr>
<td>Meropenem</td>
<td>44</td>
<td>≥16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>47</td>
<td>≥2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>42</td>
<td>≥2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>3</td>
<td>0.016</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>4</td>
<td>0.016</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim–sulphamethoxazole</td>
<td>19</td>
<td>1/1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Colistin</td>
<td>1</td>
<td>0.125</td>
</tr>
</tbody>
</table>

**MIC, minimum inhibitory concentration.**

Partial 16S rRNA sequence (670 bp) of CU244 was 100% identical to those of *A. baylyi* and *A. soli* (GenBank accession nos. JQ229812.1 and JX499235.1, respectively). However, partial rpoB gene (365 bp), rpoB–rpoC intergenic spacer (177 bp), rpaL–rpoB intergenic spacer (249 bp) and gyrB gene (686 bp) showed 98–100% identities to those of *A. soli*, which were clearly higher than those to *A. baylyi* (rpoB, 87%; gyrB, 85%). Therefore, CU244 was identified as *A. soli*. The NDM gene of *A. soli* strain CU244 was identified as blaNDM-1 by sequencing analysis. Upstream of the NDM-1 gene, ISAba125 was located, and genes from bleR to groEL reported for *A. lwof* strain WJ10621 were identified downstream of blaNDM-1 (Fig. 1). The sequence of blaNDM-1 and its surrounding regions, determined for CU244, was identical to that of *A. baumannii* strain ZW85-1 plasmid pAbNDM-1 (GenBank NC_019985.2), *A. lwof* strain WJ10621 plasmid pNDM-BJ01 (GenBank JQ001791) and *A. soli* strain M131 in Taiwan (GenBank JX072963.1). The NDM-1-producing CU244 strain was resistant to all β-lactams but showed good susceptibility to other antimicrobial groups (Table 1), and possessed no OXA genes examined. Table 2 shows the clinical information of the patient infected with *A. soli* producing NDM-1 carbapenemase. This patient had risk factors, such as prolonged hospitalization in an intensive care unit, an intravenous catheter and underlying disease.

**Discussion**

*A. baumannii–c* complex is responsible for hospital-acquired infections and has become one of the most important healthcare-associated infections in hospitals. This pathogen is usually associated with multiple antibiotic resistance and few therapeutic options of effective agents, which was also evidenced in the present report from the national surveillance in Cuba. Together with studies in other countries and international studies, it is clear that the frequency of drug-resistant *A. baumannii* strains is increasing worldwide [22]. According to the SENTRY Antimicrobial Surveillance Program in Latin America (2008–2010) for Gram-negative bacilli, imipenem-resistant *Acinetobacter* spp. rates increased from 0–12.6% in the 1997–1999 period to 50.0–84.9% in 2008–2010 in Argentina, Brazil and Chile [23]. In our study, resistance rates to imipenem and meropenem were 42% and 44%, respectively, which was slightly lower than but comparable to those in other Latin American countries, suggesting widespread carbapenem resistance in this region.

The most susceptible antimicrobial drugs to Cuban isolates were colistin sulphate, doxycycline, tetracycline and rifampicin, which were considered to be available for therapy. Over the last decade, the emergence of multidrug resistant Gram-
negative bacteria and the lack of new antimicrobial drugs have led to a revival of polymyxins, especially colistin. This antibiotic has been reinstated as a key therapeutic option for carbapenem-resistant organisms, particularly A. baumannii, P. aeruginosa and carbapenem-producing Enterobacteriaceae. It is very important in countries with limited resources where the tigecycline is not available.

Several mechanisms of carbapenem resistance have been reported in A. baumannii, including carbapenemase activity, loss of outer membrane proteins, penicillin-binding protein modifications and efflux pump activities [1]. The main mechanisms are fundamentally related to the production of acquired carbapenem-hydrolyzing class D β-lactamases (oxacillinases) of phylogenetic subgroups OXA-58, OXA-23, OXA-24/40 and OXA-143 and, less frequently, to the acquisition of carbapenem-hydrolyzing metallo-β-lactamases such as those of type IMP or VIM. In recent studies in Latin America, the presence of OXA-23 (Brazil, Argentina), OXA-24 (Mexico, Argentina) and OXA-58 (Chile, Bolivia) has been documented [23,24], with OXA-23 being the most prevalent (63–87%), in contrast to the low frequencies of the other two oxacillinases [25–27]. A similar prevalence of class D carbapenemases was found in Asia-Pacific countries [28]. Although in the present study a high detection rate of OXA-23 (76%) was observed, it was of note that 18% of A. baumannii isolates was positive for blaOXA-24, suggesting that this oxacillinase may be locally spread in Cuba. In our present study, 17% of carbapenem-resistant A. baumannii–c complex isolates were found to produce MBL, but they were negative for blaOmp, blaVIM, blaGIM, blaSIM and blaVPS by PCR. Although these MBL genes were not identified, it is conceivable that these strains might have genetic variant of the known metalloenzyme gene, which is difficult to be detected by PCR with reported primers, or might have harboured a novel MBL gene.

We reported in this study identification of NDM-1 in a carbapenem-resistant A. soli strain CU244 isolated from a patient hospitalized in an intensive care unit in Cuba. A. soli is a novel species of Acinetobacter isolated from soil in Korea in 2008 [29]. The presence of blaNDM-1 in A. soli was reported in Japan (two strains) [7], China (strain TCM341) [8] and Taiwan (strain M131) [9]. To our knowledge, CU244 is the first A. soli harbouring blaNDM-1 detected outside Asia. By BLAST search, the genetic organization and sequences of NDM-1 gene and its surrounding genes of CU244 were found to be identical to that of strain M131 in Taiwan but distinct from the Japanese and Chinese strains. As is known as a common genetic feature of NDM-1 gene [30], the insertion sequence ISAb125 and bleomycin resistance gene were located upstream and downstream of blaNDM-1 of CU244, respectively, probably as a part of transposon Tn125, which is considered to be a main vehicle for dissemination of blaNDM in A. baumannii [31].

Detection of two genetically identical blaNDM-1 clusters in Cuba and Taiwan in the rare species A. soli suggested rapid expansion of blaNDM-1 from major species (A. baumannii, A. lwoffi) among various Acinetobacter species. To date, only a few reports have been published for non–A. baumannii Acinetobacter spp. expressing NDM in the Americas (A. pittii in Paraguay and Brazil) [14,15]; nosocomial infections caused by non–A. baumannii Acinetobacter spp. such as A. soli are extremely rare, and their associated mortality is low [32]. However, caution regarding acquisition of NDM-1 by non–A. baumannii Acinetobacter spp. may be needed, and the importance of epidemiological surveillance of non–A. baumannii species, including A. soli, should be emphasized.

In summary, the first National Surveillance Program of Acinetobacter spp. in Cuba was conducted, and we reported high prevalence of blaOXA-23 among Acinetobacter spp. and the

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**TABLE 2. Clinical information of a patient infected with Acinetobacter soli strain CU244**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>Holguín, eastern Cuba</td>
</tr>
<tr>
<td>Date of isolation</td>
<td>January 2011</td>
</tr>
<tr>
<td>Ward</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>Patient age</td>
<td>42 years</td>
</tr>
<tr>
<td>Patient sex</td>
<td>M</td>
</tr>
<tr>
<td>Specimen</td>
<td>Surgical wound</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Useful treatments</td>
<td>Colistin and amikacin</td>
</tr>
</tbody>
</table>
presence of blaNDM-1 in A. soli. Our findings indicate a need for continuous surveillance regarding drug resistance and the prevalence of the gene or genes responsible for carbapenem resistance in Acinetobacter.

Conflict of Interest

None declared.

Acknowledgement

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References