Molecular epidemiology of carbapenem-resistant
Acinetobacter baumannii in South America

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One hundred and twenty-six epidemiologically sequential, unrelated, carbapenem-resistant Acinetobacter baumannii isolates from nine hospitals in six countries of South America were collected between July 2013 and June 2014. Genes coding for Ambler class D and B carbapenemases were sought by PCR. All isolates were typed using the 3-locus sequence typing and blaOXA-51-like sequence-based typing techniques. The blaOXA-23 gene was recovered in all the participating hospitals and in all the isolates of seven of nine medical centres. The blaOXA-72 gene was only recovered in the two medical centres from Guayaquil city, Ecuador. Trilocus sequence typing revealed the presence of sequence groups SG2, SG4 and SG5. blaOXA-51-like sequence-based typing revealed the presence of blaOXA-122, blaOXA-68, blaOXA-69 and blaOXA-64. Our results showed that the population of carbapenem-resistant A. baumannii in South America was principally associated with ST79, ST25 and ST15 (92 %) and harboured the blaOXA-23 gene mainly. CC2 was not detected.

INTRODUCTION

Healthcare-associated infections due to Acinetobacter baumannii are an increasing problem globally. Outbreaks have been intensively documented worldwide and are usually caused by carbapenem-resistant strains (Peleg et al., 2008). The clonal study of hospital strains is very important in terms of understanding the epidemiology of these outbreaks. Different tools have been proposed to investigate the epidemiology of A. baumannii outbreaks. blaOXA-51-like sequence-based typing (SBT) (Pournaras et al., 2014) and 3-locus sequence typing (3-LST) (Turton et al., 2007) represent economic and rapid methodologies, which showed a discriminatory power similar to multilocus sequence typing (MLST).

In Europe and various other regions it was reported that these outbreak strains belonged mainly to three international clones, I, II and III, and that the production of
OXA-23 represented the main mechanism of carbapenem resistance (Karah et al., 2012). In South America (SA), most of the studies were performed in Brazil, Colombia and Argentina and showed epidemiological features different from those in Europe because clones I and II are absent or sporadic (Climaco et al., 2013; Martins et al., 2013; Stietz et al., 2013; Vasconcelos et al., 2015). However, to our knowledge, no international studies have been performed in other countries of SA to evaluate the epidemiology of carbapenem-resistant A. baumannii (CR-Ab) strains. Recently, Labarca et al. (2016) revised the carbapenem-resistant rates in A. baumannii in Latin America. These authors found that the incidence of CR-Ab varied widely across SA countries, from 19% in Bolivia to 81% in Argentina.

The aim of this study was to determine the molecular epidemiology and the molecular mechanisms of carbapenem resistance in isolates of CR-Ab recovered in nine unrelated hospitals from six countries in SA during the years 2013 and 2014.

**METHODS**

A total of 126 CR-Ab sequential and non-duplicated isolates were collected between April 2013 and June 2014 from different clinical specimens from the following hospitals/countries (number of isolates): Hospital de Clínicas José de San Martín, Buenos Aires, Argentina (23); Hospital Nuestra Señora Del Carmen, Mendoza, Argentina (10); Hospital IESS ‘Teodoro Maldonado Carbo’ (27) and Hospital de Infectología ‘José Daniel Rodriguez Maridueña’ (6) Guayaquil, Ecuador; Hospital ‘Dr. Leonardo Guzmán’, Antofagasta, Chile (20); Hospital Materno Infantil ‘San Martín de Porres’ (10) and Hospital ‘Niño Manuel Ascencio Villarreal’ (10), Cochabamba, Bolivia; Cooperatora Asistencial Médica del Este, Colonia, Uruguay (10) and Hospital de Clínicas de Paraguay, Asunción, Paraguay (10). The carbapenem-resistant rate is greater than 90% in the participating hospitals of Argentina, Bolivia and Uruguay, 70% in Paraguay and 40% in Ecuador.

The average distance between the different cities is more than 1000 km, and only Buenos Aires city and Asunción are capital cities.

These isolates were identified at the Hospital de Clínicas José de San Martín using standard biochemical tests, and genospecies was confirmed using matrix-assisted laser desorption and the ionization time-of-flight MS method and detection of blaOXA-51 as previously described by Alvarez-Buylla et al. (2012). The MICs to imipenem and meropenem were determined using the agar dilution method. Interpretations were made according to the Clinical and Laboratory Standards Institute breakpoints (CLSI, 2013). Genes coding for Ambler class B and D (carbapenem-hydrolyzing class D β-lactamase) carbapenemases were sought by PCR using specific primers followed by sequencing (Poirel et al., 2011; Woodford et al., 2006). Repetitive extragenic palindromic DNA sequence-based PCR (rep-PCR) was used for epidemiological analysis (Quelle et al., 2001). Strain delineation was inferred in terms of percentage of banding patterns using the Dice coefficient. Clustering was performed by the unweighted pair-group method using arithmetic average. The cut-off level for PCR-pattern delineation was 80% of similarity. All isolates were typed using the 3-LST protocol developed by the United Kingdom health protection agency, involving two multiplex PCRs and blaOXA-51-like sequence-based typing (Pournaras et al., 2014; Turton et al., 2007). The results from SBT or 3-LST were considered valid if the obtained profiles had been previously assigned to a clone or sequence type (ST) by the MLST technique; otherwise, they were considered indeterminate (Karah et al., 2012; Pournaras et al., 2014; Turton et al., 2007).

**RESULTS AND DISCUSSION**

The carbapenem MIC in all isolates was >4 µg ml⁻¹. The presence of the blaOXA-51 gene was detected in all isolates, whereas none harboured metallo-β-lactamase genes. IMP-type enzymes have previously been detected in Brazil, and more recently NDM-1 has emerged in SA in different Acinetobacter species (Pillonetto et al., 2014; Togmin et al., 2006). Eighteen rep-PCR patterns were identified. Among these, 10 patterns (covering 15 A. baumannii isolates) were considered sporadic. However, 8 rep-PCR patterns, covering 111 isolates, were considered endemic or epidemic strains.

The blaOXA-23 gene was recovered in all participating medical centres and in all isolates from seven of nine centres. The worldwide dissemination of blaOXA-23 is related to the international clone I or II (Mugnier et al., 2010). In SA, this carbapenemase has been commonly associated with ST79 (Grosso et al., 2011). In agreement with previous reports, ST79 blaOXA-23-producing isolates were reported in half of the participating countries, but this carbapenemase was also detected in all STs recovered in this study (ST15, ST25, ST1 and ST79) (Table 1).

The blaOXA-58 gene was been detected in this study as expected, since blaOXA-58 has been progressively replaced by blaOXA-23 in recent years worldwide. In Bolivia and Chile, previous studies of outbreaks by CR-Ab showed the presence of OXA-58 as the main carbapenem-resistant mechanism (Bruno et al., 2013; Opazo et al., 2012; Sevillano et al., 2011).

Table 1. Sequence type, trilocus SBT, blaOXA-51-like SBT, OXA carbapenemase type and origin of A. baumannii isolates

<table>
<thead>
<tr>
<th>Origin of isolation (country)</th>
<th>blaOXA-23 (n)</th>
<th>blaOXA-72 (n)</th>
<th>3-LST group</th>
<th>SBT*</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecuador (33)</td>
<td>28</td>
<td>5</td>
<td>132</td>
<td>15</td>
<td></td>
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<tr>
<td></td>
<td>1</td>
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<td>65</td>
<td>79</td>
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<td>132</td>
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<td></td>
<td>1</td>
<td>4</td>
<td>64</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Paraguay (10)</td>
<td>20</td>
<td>10</td>
<td>4</td>
<td>64</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>20</td>
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<td>64</td>
<td>25</td>
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<tr>
<td></td>
<td>20</td>
<td>4</td>
<td>32</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Argentina (10)</td>
<td>03</td>
<td>4</td>
<td>64</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>(Mendoza)</td>
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<td>4</td>
<td>64</td>
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<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>69</td>
<td>1</td>
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</tr>
<tr>
<td>Argentina (23)</td>
<td>13</td>
<td>4</td>
<td>64</td>
<td>25</td>
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<tr>
<td>(Buenos Aires)</td>
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</table>

ind, indeterminate.
*blaOXA-51-like allele.
2012). The \textit{bla}_{OXA-72} gene was recovered only in the two participating hospitals from Guayaquil city, Ecuador. This carbapenemase often plasmid mediated, has been reported to cause hospital outbreaks in many intensive care units worldwide and recently in North America (Alcantar Curiel et al., 2014; Poirel et al., 2010). In SA, isolates carrying \textit{bla}_{OXA-72} have been reported in Ecuador, and a national surveillance study carried out in Brazil showed that the strains belonged to ST79 (Nuñez-Quezada et al., 2016; Vasconcelos et al., 2015). In our study, the presence of \textit{bla}_{OXA-72} in different STs (ST15 and ST79) and in isolates belonging to ST15 in different rep-PCR patterns may indicate not only clonal dissemination but also plasmid dispersion of the enzyme (Table 1).

MLST is the gold standard technique for epidemiological study. However, SBT and 3-LST data correlated well with MLST with respect to the identification of the major international lineages. In this work, we identified four previously characterized STs and they are as follows: ST15 (39.7 % of isolates) assigned to SG5 and harbouring \textit{bla}_{OXA-132}, ST25 (37.3 % of isolates) assigned to SG4 and harbouring \textit{bla}_{OXA-64}, ST1 (7.9 % of isolates) assigned to SG2 and harbouring \textit{bla}_{OXA-69}, and ST79 (15.1 % of isolates) harbouring \textit{bla}_{OXA-65}. ST79 has not been assigned to any SG to date. The weak performance of 3-LST in isolates from SA has already been communicated (Martins et al., 2013).

Although only limited information is available concerning CR-Ab strains in SA, the predominant circulation of ST79 has been reported in previous studies from Brazil and Argentina (Stietz et al., 2013; Vasconcelos et al., 2015). This clone has also been called WWW5 or Pan-American clone because it was found in North, Central and South America (Higgins et al., 2010). However, in this study ST79 was not the most frequently isolated ST; this ST was only predominant in Paraguay and in Mendoza, Argentina, and it was also detected in sporadic isolates in Ecuador (Table 1).

ST25, also called WWW7, was responsible for epidemics in different European and Asian countries (Karah et al., 2012). In SA, it has previously been detected but only sporadically or in low percentages. ST25 was the ST recovered in most of the countries included in this study (four out of six) and it was the majority ST in three countries (Argentina, Bolivia and Uruguay), which shows a change compared to previous reports (Merkier et al., 2008; Paciel et al., 2011; Rodriguez et al., 2010) (Table 1).

ST15 was predominant in isolates from Ecuador and Chile, but it was not detected in other countries. Isolates belonging to ST15 had previously been reported in Chile (Higgins et al., 2010) (Table 1).

It is known that the international clones I, II and III have low prevalence in the \textit{A. baumannii} population in SA. In this work, clone II was not detected and isolates belonging to clone I were only recovered in Buenos Aires, Argentina. This clone had previously been detected in Argentina and Brazil but in percentages lower than 20 % (Merkier et al., 2008).

In the present study carried out in SA, we could identify similar features compared to other regions regarding CR-Ab epidemiology: (i) a clear predominance of the \textit{bla}_{OXA-23} gene in CR-Ab, (ii) the emergence of the \textit{bla}_{OXA-72} gene in Ecuador, (iii) the spread of isolates belonging to ST25 and finally, and contrary to what was observed worldwide, (iv) the low prevalence of the international clones I, II and III in SA.

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1090


