



Review

# Mobile genetic elements related to carbapenem resistance in *Acinetobacter baumannii*



Mariana Pagano<sup>a,c</sup>, Andreza Francisco Martins<sup>b,c,\*</sup>, Afonso Luis Barth<sup>a,c</sup>

<sup>a</sup> Universidade Federal do Rio Grande do Sul (UFRGS), Faculdade de Farmácia, Programa de Pós-Graduação em Ciências Farmacêuticas, Porto Alegre, Brazil

<sup>b</sup> Universidade Federal do Rio Grande do Sul (UFRGS), Instituto de Ciências Básicas da Saúde, Porto Alegre, Brazil

<sup>c</sup> Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil

## ARTICLE INFO

### Article history:

Received 5 November 2014

Accepted 11 February 2016

Available online 4 July 2016

Associate Editor: Jorge Luiz Mello Sampaio

### Keywords:

*Acinetobacter baumannii*  
Antimicrobial resistance  
Mobile genetic elements  
Carbapenem resistance

## ABSTRACT

*Acinetobacter baumannii* is widely recognized as an important pathogen associated with nosocomial infections. The treatment of these infections is often difficult due to the acquisition of resistance genes. *A. baumannii* presents a high genetic plasticity which allows the accumulation of these resistance determinants leading to multidrug resistance. It is highlighted the importance of the horizontal transfer of resistance genes, through mobile genetic elements and its relationship with increased incidence of multidrug resistant *A. baumannii* in hospitals. Considering that resistance to carbapenems is very important from the clinical and epidemiological point of view, the aim of this article is to present an overview of the current knowledge about genetic elements related to carbapenem resistance in *A. baumannii* such as integrons, transposons, resistance islands and insertion sequences.

© 2016 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

The *Acinetobacter baumannii-calcoaceticus* (Abc) complex has emerged as an important nosocomial pathogen. Among the members of this complex, *A. baumannii*, *A. pittii*, and *A. nosocomialis* are the three most common *Acinetobacter* species isolated in clinical settings.<sup>1</sup> *A. baumannii* has been extensively studied due to its association with infections of high mortality

rates. *A. pittii* and *A. nosocomialis* are increasingly identified as causative agents of nosocomial infections.<sup>2</sup>

*A. baumannii* is considered an important nosocomial pathogen, causing a wide range of infections, including ventilator-associated pneumonia, bloodstream infections, urinary tract infections and meningitis. This species is naturally highly resistant to a number of antimicrobials commonly used in the clinical practice, such as first and second generation cephalosporins, aminopenicillins, and chloramphenicol.

\* Corresponding author at: Av. Sarmiento Leite, 500/Prédio 12101, Bairro Farroupilha, Porto Alegre CEP 90050-170, Brazil.

E-mail: [andrezafm20@gmail.com](mailto:andrezafm20@gmail.com) (A.F. Martins).

<http://dx.doi.org/10.1016/j.bjm.2016.06.005>

1517-8382/© 2016 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*A. baumannii* contains an intrinsic AmpC  $\beta$ -lactamase (*bla*<sub>ADC</sub>) and OXA-51 serine-type oxacillinase (*bla*<sub>OXA-51</sub>), which contribute to the natural resistance to  $\beta$ -lactams.<sup>3</sup> Moreover, this organism presents a great capacity to acquire new resistance mechanisms, including those responsible for carbapenem resistance.<sup>4</sup>

Carbapenem resistance in *A. baumannii* involves mainly the carbapenem-hydrolysing class D  $\beta$ -lactamases (CHDLs – Ambler class D) and less frequently, the metallo- $\beta$ -lactamases (MBLs – Ambler class B). Carbapenem resistance may also be caused by other mechanisms such as, production of other carbapenemases, porin modification or loss, or by modification of the penicillin-binding proteins.<sup>1,5</sup>

Several acquired class D OXA-type  $\beta$ -lactamases have been identified as a source of carbapenem resistance in *A. baumannii*. Five main groups of CHDLs have been described in *A. baumannii*, corresponding to OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like and OXA-235-like enzymes.<sup>6</sup> OXA-23-like enzymes are the most widespread in *A. baumannii* worldwide and have been identified in all continents.<sup>6</sup>

In Brazil, OXA-23-like-producing *A. baumannii* is disseminated in many states and it is responsible for high endemic levels of multidrug-resistance.<sup>7,8</sup> The *bla*<sub>OXA-143</sub> gene has thus far been detected only in *A. baumannii* isolates from Brazil and is the second most frequent CHDL encoding gene.<sup>9–11</sup>

The *bla*<sub>OXA-143</sub> gene is frequently found in the Southeast region of Brazil, especially in the state of São Paulo. It is important to note that two new variants of this gene were recently described. The variants *bla*<sub>OXA-235</sub> and *bla*<sub>OXA-231</sub> were described in Minas Gerais and Paraná states, respectively.<sup>12,13</sup> This data demonstrates the detection of these new variants of *bla*<sub>OXA-143</sub> in Brazil is a cause of great concern and shows the potential of these new CHDLs to spread to other Brazilian regions.

Although *bla*<sub>OXA-24/40-like</sub> gene is disseminated in *A. baumannii* in Europe, in Brazil, this gene is still rare, with only a very few reports of a *bla*<sub>OXA-72</sub> (*bla*<sub>OXA-24/40-like</sub> variant) in São Paulo,<sup>9</sup> Recife,<sup>14</sup> Porto Alegre and Curitiba.

Despite MBLs are less commonly identified in *A. baumannii* than the OXA-type carbapenemases, their hydrolytic activities to carbapenems are significantly more potent. Four MBLs have been identified in *A. baumannii*: IMP, VIM, SIM and, more recently, NDM.<sup>15</sup> It is important to note that MBL genes, such as NDM and IMP-1, have been described in *Acinetobacter non-baumannii* species, which demonstrates the capacity of these resistance genes to spread among different *Acinetobacter* species.<sup>16,17</sup>

Most of Ambler class A ESBLs possess activity against penicillins and broad-spectrum cephalosporins. However, specific GES variants have been shown to possess the ability to compromise the efficacy of carbapenems. Among *A. baumannii*, the variants GES-11 and GES-14 possess specific residues enlarging their hydrolysis spectrum (Table 1).<sup>18,19</sup>

The elevated genetic plasticity presented by *A. baumannii* has allowed the accumulation of many resistance determinants, which contributed to the high incidence of *A. baumannii* multiresistant to antibiotics. In this review, we present and discuss the characteristics of the different mobile genetic elements involved in the transfer of resistance determinants in *A. baumannii*.

## AbaR-type genomic resistance islands

Genomic islands containing resistance markers are referred to as resistance islands. Resistance islands have been described mainly in Proteobacteria, including *Shigella flexneri*, *Salmonella enterica*, *Vibrio cholerae*, *Staphylococcus aureus*, and more recently, in *A. baumannii*.<sup>20,21</sup> *A. baumannii* isolates harbor large clusters of horizontally transferred genes conferring resistance to multiple antibiotics and heavy metals, which are integrated at a specific site in a particular ATPase gene.<sup>22</sup>

Fournier et al. described for the first time the *A. baumannii* Resistant Island (AbaR). AbaR is defined as a region which has transposed into a specific position in the chromosome, creating a 5 bp duplication site (ACCGC).<sup>21</sup> The backbone of AbaR is comprised of five open reading frames (ORFs) – *orf1*, *tniA*, *tniB*, *orf2*, *orf3* – which constitute the transposition module, and two other genes encoding to the universal stress protein (*uspA*) and a sulfate permease (*sul*).<sup>21–23</sup>

Several AbaR have already been described containing a variety of resistance genes, including the *bla*<sub>OXA-23-like</sub>, which confers resistance to carbapenems.<sup>24</sup> These resistance islands have been described in *A. baumannii* epidemic strains belonging to the important global clones, European Clone I (EC I) and European Clone II (EC II), known for their increased capacity to spread worldwide.<sup>22</sup>

Several other genomic resistance islands have been fully characterized in *A. baumannii*. The majority were found in strains of EC I, such as, AbaR1, AbaR3, AbaR5, AbaR6, AbaR7, AbaR8, AbaR9, and AbaR10. These AbaRs share a structure represented by a 16.3 kb backbone transposon (Tn6019) interrupted by a large compound transposon that contains a variable-resistance region bounded by directly oriented copies of Tn6018. Exceptions are AbaR6 and AbaR7, each with a large deleted region.<sup>25</sup> Much less is known about AbaRs in EC II. The resistance islands harbored by this clone are integrated at the same site of the ATPase gene as is known for AbaRs in EC I.<sup>25</sup>

AbaR1 is the largest resistance island described to date. This island contains 86 kb and was originally described in the epidemic *A. baumannii* strain AYE belonging to ECI. This strain was responsible for outbreaks in France during 2004.<sup>21</sup> *A. baumannii* AYE strain revealed the presence of a large gene cluster, containing many resistance determinants, inserted into the chromosome.<sup>21</sup>

Of the 45 resistance genes described in AbaR1 resistance island, 25 were associated with resistance to several classes of antibiotics. These include genes that had not been previously described in *Acinetobacter* species such as *strA*, *strB*, *aphA1*, and *aac69* (encoding resistance to aminoglycosides); putative tetracycline-resistance genes *tetA* (tetracycline efflux pump) and *tetR* (repressor protein); *dfrX* (resistance to cotrimoxazole); and the chloramphenicol-resistance gene *cmlA* (chloramphenicol efflux pump). Moreover, Fournier et al. (2006) described the presence of genes in AbaR1 that encode VEB-1 and OXA-10  $\beta$ -lactamases, the aminoglycoside acetyltransferase gene *aac3*, and the aminoglycoside adenylyltransferases *aadA1/DA1/B*; the cotrimoxazole resistance-associated *dfrI*; *cmlA5* and one copy of the chloramphenicol acetyl-transferase *cat*; the rifampin ADP-ribosyltransferase gene *arr-2*; and five

**Table 1 – Characterization of the main mobile genetic elements associated with resistance in *Acinetobacter baumannii*.**

Mobile genetic element	Resistance genes associated	Resistance profile	Geographic region	Reference
ISAba1	<i>bla</i> <sub>OXA-23</sub> <i>bla</i> <sub>OXA-51</sub> <i>bla</i> <sub>OXA-58</sub> <i>bla</i> <sub>AmpC</sub>	β-Lactams including carbapenems	Worldwide disseminated	Villalón et al., 2013 Mugnier et al., 2010 Mugnier et al., 2009
ISAba2	<i>bla</i> <sub>OXA-58</sub> <i>bla</i> <sub>AmpC</sub>	Carbapenems Cephalosporins	France Italy Spain	Villalón et al., 2013 Fernández Cuenca et al., 2012 Marqué et al., 2005
ISAba3	<i>bla</i> <sub>OXA-58</sub>	Carbapenems	China Italy Taiwan Lebanon	Villalón et al., 2013 Donnarumma et al., 2010 Zarrilli et al., 2008 Fu et al., 2014
ISAba4	<i>bla</i> <sub>OXA-23</sub>	Carbapenems	France Belgium	Bogaerts et al., 2008 Corvec et al., 2007
ISAba10	<i>bla</i> <sub>OXA-23</sub>	Carbapenems	Korea	Lee et al., 2011
ISAba125	<i>bla</i> <sub>NDM-1</sub> <i>bla</i> <sub>NDM-2</sub> <i>bla</i> <sub>AmpC</sub> <i>aphA6</i>	Carbapenems Cephalosporins Aminoglycosides	India Switzerland Greece	Bonnin et al., 2012a Kaase et al., 2011 Mishra et al., 2013
IS18	<i>bla</i> <sub>OXA-58</sub>	Carbapenems	Australia Lebanon Turkey	Hamidian et al., 2012 Villalón et al., 2013 Zarrilli et al., 2008 Marqué et al., 2005
Tn2006	<i>bla</i> <sub>OXA-23</sub>	Carbapenems	Spain Tahiti France Turkey Vietnam Romania Lybia Australia	Mugnier et al., 2010 Corvec et al., 2007
Tn2007	<i>bla</i> <sub>OXA-23</sub>	Carbapenems	France Algeria	Corvec et al., 2007
Tn2008	<i>bla</i> <sub>OXA-23</sub>	Carbapenems	United Arab Emirates Bahrain	Mugnier et al., 2010
Int1	<i>bla</i> <sub>GES-11</sub> <i>bla</i> <sub>GES-14</sub> <i>dfrA1</i> <i>sat2</i> <i>aadA1</i> <i>orfX</i> <i>ybfA</i> <i>ybfB</i>	β-Lactams including carbapenems Aminoglycosides	Europe (widespread) Korea Iran Brazil	Bonnin et al., 2011 Nemec et al., 2004 Lee et al., 2005 Japoni-Nejad et al., 2013 Mendes et al., 2007
Int2	<i>dfrA1</i> <i>sat2</i> <i>aadA1</i> <i>orfX</i> <i>ybfA</i> <i>ybfB</i> <i>ybgA</i>	Aminoglycosides	Argentina Chile Brazil	Pagano et al., 2012 Ramirez et al., 2012 Fonseca et al., 2011

copies of the sulfonamide-resistance gene *sulI* encoding dihydropteroate synthetase, a component of class 1 integrons.<sup>21,26</sup>

AbaR2 was described in the epidemic, multidrug-resistant *A. baumannii* strain named ACICU.<sup>27</sup> This strain belongs to ECII and carries the plasmid-mediated *bla*<sub>OXA-58</sub>. *A. baumannii* AYE and ACICU belong to different clonal groups (European clones I and II, respectively), however, the presence of related resistance islands in both lineages suggests that AbaR1 and AbaR2 derived from an island acquired by a common *A. baumannii* ancestor before their divergence into two different clonal lineages.<sup>21,27</sup>

The genomic resistance island variant AbaR3 appears to be an ancestral of several AbaR variants which have arisen from AbaR3 by loss of segments of different lengths that include one or more of the antibiotic resistance genes.<sup>28</sup> AbaR3 contains eight genes associated with antibiotic resistance. Unique sequences in AbaR3 include a *bla*<sub>TEM</sub> gene that is associated with a Tn3 transposon and a small cluster of genes, including two that encode to a DNA topoisomerase and a single-strand binding protein that are similar to proteins from a broad-host-range plasmid.<sup>29</sup> In addition, it is noteworthy that the presence of genes for a plasmid-derived

DNA topoisomerase may contribute to the resistance island mobility.

## Transposable elements

Transposable elements have the ability to move within the bacterial genome, being able to translocate themselves from one site of the genome to other sites. These transpositions are considered one of the major causes of bacterial DNA rearrangements, which in turn can cause changes in gene expression.<sup>30</sup> In *A. baumannii*, transposable elements, such as transposons and insertion sequences have been responsible for the expression and spread of antimicrobial resistance mechanisms.<sup>1</sup>

## Insertion sequences

Bacterial insertion sequences (IS) are the least complex type of transposable elements; they rarely exceed 2 kb in size and may be as small as 0.5 kb. These elements possess an important role in the spread of resistance genes since the presence of two copies of the same IS flanking a resistance gene form a complex structure called composite transposon. Composite transposons are able to mobilize a variety of resistance genes, contributing to antimicrobial resistance dissemination.<sup>31</sup>

Besides their transposition role, some IS have been shown to activate or to increase the expression of neighbor genes. This capacity may be due to the presence of promoter regions in the insertion sequence or by the formation of new promoters after the insertion event.<sup>31</sup>

Some IS elements have an important role in *A. baumannii* antimicrobial resistance. IS*Aba1*, IS*Aba2*, IS*Aba3*, IS*Aba4* and IS18 are commonly associated with the expression of carbapenemases genes in *A. baumannii* (Table 1).<sup>32</sup> Villalón et al. (2013) investigated the presence of these IS elements in 59 multidrug-resistant *A. baumannii* isolates and observed a prevalence of 93.2%, 25.4%, 20.3% and 5.1% for IS*Aba1*, IS*Aba2*, IS*Aba3* and IS18, respectively. IS*Aba4* was not detected in any of the isolates in this study.<sup>32</sup>

It is important to note that IS elements such as IS*Aba1* can contribute to the spread of carbapenemase genes among different *Acinetobacter* species. Poirel et al. (2008) hypothesized that *bla*<sub>OXA-23</sub> was likely mobilized by the IS*Aba1* insertion sequence from *A. radioresistens* to *A. baumannii*.<sup>33</sup> The authors demonstrated that *A. radioresistens* is the progenitor of the *bla*<sub>OXA-23-like</sub> gene, which was mobilized to *A. baumannii* through IS*Aba1* insertions sequence provided by *A. baumannii*. This hypothesis is based on the identification of genes encoding both OXA-23-like and ATPase-like enzymes on the *A. radioresistens* chromosome without the presence of IS*Aba1* elements, that is involved in the mobilization of *bla*<sub>OXA-23</sub> gene.<sup>33</sup>

The IS*Aba1* element belongs to the IS4 family and has 11-bp inverted repeats sequences flanked by 9-bp direct repeats of the target sequence. Although this element is considered exclusive to *A. baumannii*, Segal et al. (2005) identified IS*Aba1* in *Acinetobacter lwoffii* isolates, demonstrating the high mobility of these elements and indicating that transposition events of the IS*Aba1* occur frequently.<sup>34</sup>

IS*Aba1* has been found upstream the *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-51-like</sub>, *bla*<sub>OXA-58-like</sub> and *bla*<sub>AmpC</sub> genes in *A. baumannii*. This IS acts as a promoter sequence which increases the expression of resistance genes. In fact, it was demonstrated that it is necessary the presence of IS*Aba1* upstream *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub> for these genes to confer resistance to carbapenems.<sup>35</sup> Although several authors have demonstrated the relationship between IS*Aba1* upstream *bla*<sub>OXA-51</sub> and carbapenem resistance, this may not be enough to confer resistance, as *A. baumannii* isolates susceptible to carbapenems with the association IS*Aba1*/*bla*<sub>OXA-51</sub> have already been described.<sup>36</sup>

The IS*Aba2*, IS*Aba3* and IS*Aba4* elements have also been identified upstream *bla*<sub>OXA-58-like</sub> and *bla*<sub>OXA-23-like</sub> genes in *A. baumannii* isolates.<sup>37</sup> Giannouli et al. (2009) analyzed the insertion sequences of 24 *A. baumannii* isolates with *bla*<sub>OXA-58</sub> gene and identified the presence IS*Aba2*, IS18 or IS*Aba1* located at the 5' end, while at 3' end all isolates presented the IS*Aba3* element. Of note, the IS elements at 5' end of *bla*<sub>OXA-58</sub> were evidenced in strains of distinct PFGE profiles and ST groups in the same geographical area. It suggests that these elements might have been acquired through horizontal gene transfer and confirms their dissemination capacity among *A. baumannii* isolates.<sup>38</sup>

Corvec et al. (2007) described the first *A. baumannii* isolate harboring an IS*Aba4* element upstream *bla*<sub>OXA-23</sub> gene, in France. Subsequently, it was shown that an isolate from Belgium containing the association of IS*Aba4* and the *bla*<sub>OXA-23</sub> presented the same PFGE profile as the isolate from France. These findings demonstrate the propensity of resistant strains to spread, highlighting the importance of epidemiological surveys to estimate the true prevalence of isolates harboring IS*Aba4*/*bla*<sub>OXA-23</sub>.<sup>39,40</sup>

Lee et al. (2011) identified a novel 1203 bp insertion sequence, named IS*Aba10*. This element was found to be inserted into the IS*Aba1* element upstream *bla*<sub>OXA-23</sub> gene in an *A. baumannii* presenting high minimum inhibitory concentrations (MICs) to carbapenems ( $\geq 32 \mu\text{g/mL}$ ). In addition, isolates without the insertion of this element showed MICs between 8 and 16  $\mu\text{g/mL}$ . The authors suggested that this sequence may increase 2–5-fold the *bla*<sub>OXA-23</sub> gene expression. Based on these results, they suggested that the IS*Aba10* element may play an important role in carbapenem resistance by providing an additional promoter sequence to the *bla*<sub>OXA-23</sub> gene.<sup>41</sup>

IS elements have also been associated to metallo- $\beta$ -lactamases such as *bla*<sub>NDM</sub>, which have been increasingly reported in *Acinetobacter baumannii* and in other *Acinetobacter* species such as *A. johnsonii*, *A. pittii*, *A. junii* and *A. lwoffii*.<sup>16,42–44</sup> The *bla*<sub>NDM</sub> can be located either on the plasmid or chromosome in *Acinetobacter* species.<sup>44</sup> However, it was evidenced that the spread of the *bla*<sub>NDM</sub> gene was not associated with clonal dissemination, but horizontal spread of the genetic structure.<sup>42</sup>

Several studies reported that *bla*<sub>NDM</sub> gene is located between two copies of the IS*Aba125* element, forming a composite transposon named Tn125. IS*Aba125* element provides the -35 sequence of the hybrid promoter responsible for the expression of the *bla*<sub>NDM</sub> gene.<sup>45</sup> Curiously, this IS element has been originally identified from an *A. baumannii* isolate

without any association with the *bla*<sub>NDM</sub> gene. By contrast, this IS has been identified in *Enterobacteriaceae* and *P. aeruginosa* as a remnant of the Tn125 and has never been identified alone in these species. This observation suggests that *A. baumannii* is a likely reservoir of IS<sub>Aba125</sub>. Findings like these highlight that even though *A. baumannii* is usually recognized as a final acceptor for resistance genes, it may acquire several resistance determinants and then transfer them to *Enterobacteriaceae* and *Pseudomonas* spp.

Recently, a study demonstrated that Tn125 has been disrupted by IS26 in *A. baumannii* NDM-producing isolates from India. This new rearrangement has resulted in *bla*<sub>NDM-1</sub> being within an IS26 composite transposon, which might potentially mobilize *bla*<sub>NDM-1</sub> and contribute to the spread of the carbapenemase gene.<sup>46</sup>

Robledo et al. (2010) described the first report of *bla*<sub>KPC</sub> gene in *A. baumannii* isolates from Puerto Rico. In that study, four variants of *bla*<sub>KPC</sub> were identified: KPC-2, -3, and -4 and a novel variant, KPC-10. The integration of these genes in the *A. baumannii* chromosome was related to a transposition event mediated by the transposase of ISEcp1.<sup>31,47</sup> This element is likely to be responsible for mobilizing numerous *bla*<sub>CTX-M</sub> genes and several other resistance genes such as *qnrB19*, *rmtC*, *bla*<sub>ACC-1</sub> and *bla*<sub>CMY-2g7,16,21</sub>.<sup>48</sup> In addition, it was responsible for the mobilization of *bla*<sub>CTX-M-5</sub> from a narrow range plasmid to the chromosome of *A. baumannii*, event similar to what Martinez et al. observed with *bla*<sub>KPC</sub> gene.<sup>47</sup>

As described above, ISs can cause insertion mutations, genome rearrangements and enhance the spread of resistance and virulence determinants within pathogenic species. Besides being involved in the expression and spread of carbapenemases, IS elements such as IS<sub>Aba1</sub>, IS<sub>Aba10</sub> and IS<sub>Aba825</sub> are involved on the disruption of *carO* gene, which codes for an important outer membrane channel. The absence of this outer membrane protein has been correlated with reduced susceptibility to carbapenems.<sup>41,49,50</sup>

## Transposons

Transposons sequences may vary in size from 3 to 40 kb, in some cases containing dozens of genes. These elements are into two main classes: composite transposons or complex transposons. Composite transposons have resistance genes in its central region; furthermore, these elements are flanked by an insertion sequence (IS) at each end. Complex transposons have a more complicated genetic structure than IS elements or composite transposons. The classic complex transposon is Tn3, which is derived from resistance plasmid R1.<sup>51</sup>

In *A. baumannii*, transposons have been characterized as genetic structures harboring important resistant genes, such as *bla*<sub>OXA-23</sub>. Three transposons have been related to *bla*<sub>OXA-23</sub>: Tn2006, Tn2007 and Tn2008. In Tn2006, the *bla*<sub>OXA-23</sub> gene is flanked by two copies of the insertion sequence IS<sub>Aba1</sub>, which is located in opposite directions. Tn2008 is similar to Tn2006 but lacks the second copy of IS<sub>Aba1</sub>. Finally, in Tn2007 the *bla*<sub>OXA-23</sub> gene is associated with one copy of IS<sub>Aba4</sub> located upstream to this gene.<sup>52</sup> Several studies have demonstrated that Tn2006 is currently the most common determinant of

carbapenem resistance, with a great ability to spread among *A. baumannii* isolates.<sup>53</sup>

## Integrations

These elements are natural cloning and expression systems that incorporate ORFs by site-specific recombination and convert them to functional genes due to the presence of a promoter sequence (Rowe-Magnus et al., 2001). It is now well established that these mobile elements constitute the major vectors of antibiotic multiresistance in Gram-negative and, to a lesser extent, in Gram-positive bacteria.<sup>54</sup>

Five different classes of mobile integrations have been defined to date, based on the sequence of the encoded integrases.<sup>54,55</sup> It is known that three (classes 1, 2 and 3) of these classes have an important role in the dissemination of antimicrobial resistance genes.<sup>55,56</sup> These classes are well described in the literature and are associated to multiresistant phenotypes.<sup>54,55</sup>

Several studies have demonstrated a high prevalence of class 1 integrations in *A. baumannii* isolates in Europe, Asia and United States.<sup>57</sup> Due to its greater spread capacity, class 1 integrations are the main experimental model of integrations. This class is usually associated to functional or non functional transposons derived from Tn402 which may be inserted into larger transposons as Tn21. Class 1 integrations have been associated to a variety of insertion sequences, including IS26, IS1999, IS2000 e IS6100.<sup>58</sup>

Most acquired MBL genes in *A. baumannii* have been found within class 1 integrations, often containing an array of resistance gene cassettes.<sup>1,6</sup> Mendes et al. (2007) described seven *bla*<sub>IMP-1</sub> harboring *Acinetobacter* spp. isolates recovered from Brazilian inpatients. All isolates possessed a class 1 integration, named In86, carrying the same cassette array: *bla*<sub>IMP</sub>, *aac*(6')-31, and *aadA1*, which was plasmid-located in five of the isolates (Mendes et al., 2007). This gene cassette contained a aminoglycoside resistance gene – *aac*(6')-31 – that might be capable of conferring resistance to all clinically available aminoglycosides. This gene was able to disseminate among unrelated *A. baumannii* clinical isolates from a Brazilian hospital (Mendes et al., 2007). Recently, Cayô et al., reported a new structure of class 1 integration, In990, harboring the *bla*<sub>IMP-10</sub> in *A. baumannii* isolates from Brazil. The cassette arrangement of In990 was very similar to that of In86 described by Mendes et al.<sup>59,60</sup>

Class 2 integrations are included in the Tn7 family of transposons, and consist of an integrase gene followed by gene cassettes. Tn7 are identified as a sophisticated mobile genetic element containing a transposition module formed by five transposition genes, *tnsA*, *tnsB*, *tnsC*, *tnsD*, and *tnsE*, rather than the one or two seen in many other transposable elements.<sup>56</sup> Class 3 integrations are less prevalent than class 2 and are also located in transposons.<sup>54</sup>

Despite reports of a higher prevalence of class 1 integrations in *A. baumannii*, studies conducted in Latin American countries such as Chile, Argentina and Brazil demonstrated a greater distribution of class 2 integrations among isolates of *A. baumannii* in these regions.<sup>36,61</sup> Fonseca et al. (2011) demonstrated that all class 2 integrations obtained from Brazilian isolates

were inserted into Tn7 transposon, besides having the gene cassette containing the arrangement of genes *dfrA1* (trimethoprim resistance), *sat2* (streptothricin resistance) and *aadA1* (spectinomycin and streptomycin resistance).<sup>62</sup>

Martins et al. (2015) investigated the association of class 2 integrons and gene cassettes with clonal lineages of *A. baumannii*. They reported the association of class 1 and 2 integrons with CC109/1 (International Clone I) and CC113/79 *A. baumannii* strains, respectively. The authors hypothesized that class 2 integron, predominant in Latin America, may be accounted for the high prevalence of the CC113/79 type. In the same study, a similar prevalence was observed for *A. nosocomialis*.<sup>63</sup>

Class 1 and 2 integrons have been described in *A. baumannii* isolates related to nosocomial infection outbreaks. In a study published by Turton et al. (2005), it was observed that all *A. baumannii* isolates associated with outbreaks contained class 1 integrons, in contrast, none sporadic isolate presented this class of integron.<sup>64</sup>

More than 130 different gene cassettes containing resistance genes have been identified in integrons. Distinct genes are evidenced in gene cassettes, promoting resistance to a variety of antimicrobial classes. Together, these gene cassettes provide resistance to most classes of antibiotics including  $\beta$ -lactams, all aminoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin, quinolones, and antiseptics of the quaternary ammonium-compound family.<sup>65</sup> Besides these genes, several ORFs with unknown function have been identified in gene cassettes.<sup>66</sup>

In *A. baumannii*, gene cassettes have been described containing several genes, such as *aacA4* responsible for resistance to amikacin, netilmicin and tobramycin, the *catB8* gene is an acetyltransferase which encodes resistance to chloramphenicol, *aadA1* is responsible for resistance to streptomycin and spectinomycin, *aac3* responsible for resistance to gentamicin and *bla<sub>OXA-10</sub>* encodes resistance to  $\beta$ -lactams, except carbapenems and extended-spectrum cephalosporins.<sup>21</sup>

## Final remarks

This review highlighted the role of resistance determinants in the capacity of spread in *A. baumannii*. This species shows a considerable ability to acquire foreign DNA such as drug resistance genes, which provide a genetic diversity and overcomes the antibiotic selection pressure. It is important to note that the main carbapenem-resistance mechanism involved in *A. baumannii* (production of oxacillinases) presents a low hydrolytic power when it is not associated with an insertion sequence. Moreover, the capacity of OXA genes to spread is directly related to their association with a composite transposon (Tn2006). These features highlight the importance of investigating the genetic context of these genes in order to define their real clinical significance.

The continuous description of gene cassettes in integrons, mainly those leading to resistance to  $\beta$ -lactams and aminoglycosides, has been of great concern. Furthermore, the number of resistance genes inserted in the same plasmid, even in the same integron, seems to be increasing. This integration of resistance determinants in the same plasmid may facilitate

the persistence in the environment for long periods because of the physical association of integrons with other elements, allowing their continued selection.<sup>57</sup>

In this context, the knowledge about the genetic structure of resistance determinants is very important in order to understand the capacity of resistance genes to spread in *A. baumannii*.

## Conflicts of interest

The authors declare no conflicts of interest.

## REFERENCES

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev*. 2008;21:538–582.
2. Turton JF, Shah J, Ozongwu C, Pike R. Incidence of *Acinetobacter* species other than *A. baumannii* among clinical isolates of *Acinetobacter*: evidence for emerging species. *J Clin Microbiol*. 2010;48:1445–1449.
3. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect*. 2006;12:826–836.
4. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *Int J Antimicrob Agents*. 2012;39:105–114.
5. Gordon NC, Wareham DW. Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance. *Int J Antimicrob Agents*. 2010;35:219–226.
6. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents*. 2015;45:568–585.
7. Pagano M, Barin J, Martins AF, Zavascki AP. High endemic rates of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* isolates caused by the persistence of major clones in hospitals in a Brazilian city 5 years after an outbreak. *Infect Control Hosp Epidemiol*. 2015;36:860–862.
8. Vasconcelos AT, Barth AL, Zavascki AP, et al. The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. *Diagn Microbiol Infect Dis*. 2015;83:382–385.
9. Antonio GS, Neves PR, Medeiros M, Mamizuka EM, Elmor de Araujo MR, Lincopan N. High prevalence of carbapenem-resistant *Acinetobacter baumannii* carrying the *bla<sub>OXA-143</sub>* gene in Brazilian hospitals. *Antimicrob Agents Chemother*. 2011;55:1322–1323.
10. Werneck JS, Picao RC, Carvalhaes CG, Cardoso JP, Gales AC. OXA-72-producing *Acinetobacter baumannii* in Brazil: a case report. *J Antimicrob Chemother*. 2011;66:452–454.
11. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2009;53:5035–5038.
12. Gionco B, Pelayo JS, Venancio EJ, Cayo R, Gales AC, Carrara-Marroni FE. Detection of OXA-231, a new variant of *bla<sub>OXA-143</sub>*, in *Acinetobacter baumannii* from Brazil: a case report. *J Antimicrob Chemother*. 2012;67:2531–2532.
13. Girlich D, Poirel L, Nordmann P. First isolation of the *bla<sub>(OXA-23)</sub>* carbapenemase gene from an environmental *Acinetobacter baumannii* isolate. *Antimicrob Agents Chemother*. 2010;54:578–579.

14. de Sa Cavalcanti FL, Almeida AC, Vilela MA, de Moraes Junior MA, de Moraes MM, Leal-Balbino TC. Emergence of extensively drug-resistant OXA-72-producing *Acinetobacter baumannii* in Recife, Brazil: risk of clonal dissemination? *Diagn Microbiol Infect Dis.* 2013;77:250–251.
15. Abbott I, Cerqueira GM, Bhuiyan S, Peleg AY. Carbapenem resistance in *Acinetobacter baumannii*: laboratory challenges, mechanistic insights and therapeutic strategies. *Expert Rev Anti Infect Ther.* 2013;11:395–409.
16. Pagano M, Poirel L, Martins AF, et al. Emergence of NDM-1-producing *Acinetobacter pittii* in Brazil. *Int J Antimicrob Agents.* 2015;45:444–445.
17. Lu PL, Huang LY, Lian ST, et al. How carbapenem-resistant *Acinetobacter* spp. established in a newly constructed hospital. *Int J Antimicrob Agents.* 2008;31:463–466.
18. Moubareck C, Bremont S, Conroy MC, Courvalin P, Lambert T. GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2009;53:3579–3581.
19. Bogaerts P, Naas T, Garch FE, et al. GES extended-spectrum  $\beta$ -lactamases in *Acinetobacter baumannii* isolates in Belgium. *Antimicrob Agents Chemother.* 2010;54:4872–4878.
20. Schmidt H, Hensel M. Pathogenicity islands in bacterial pathogenesis. *Clin Microbiol Rev.* 2004;17:14–56.
21. Fournier PE, Vallenet D, Barbe V, et al. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. *PLoS Genet.* 2006;2:e7.
22. Post V, White PA, Hall RM. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother.* 2010;65:1162–1170.
23. Seputiene V, Povilonis J, Suziedeliene E. Novel variants of AbaR resistance islands with a common backbone in *Acinetobacter baumannii* isolates of European clone II. *Antimicrob Agents Chemother.* 2012;56:1969–1973.
24. Zhu L, Yan Z, Zhang Z, et al. Complete genome analysis of three *Acinetobacter baumannii* clinical isolates in China for insight into the diversification of drug resistance elements. *PLOS ONE.* 2013;8:e66584.
25. Krizova L, Dijkshoorn L, Nemeč A. Diversity and evolution of AbaR genomic resistance islands in *Acinetobacter baumannii* strains of European clone I. *Antimicrob Agents Chemother.* 2011;55:3201–3206.
26. Bonnin RA, Poirel L, Nordmann P. AbaR-type transposon structures in *Acinetobacter baumannii*. *J Antimicrob Chemother.* 2012;67:234–236.
27. Iacono M, Villa L, Fortini D, et al. Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. *Antimicrob Agents Chemother.* 2008;52:2616–2625.
28. Krizova L, Nemeč A. A 63 kb genomic resistance island found in a multidrug-resistant *Acinetobacter baumannii* isolate of European clone I from 1977. *J Antimicrob Chemother.* 2010;65:1915–1918.
29. Adams MD, Goglin K, Molyneaux N, et al. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J Bacteriol.* 2008;190:8053–8064.
30. Lewin B. Transposons. In: Hall PP, ed. *Genes VIII.* 8th ed. NJ: Upper Saddle River; 2004:467–480.
31. Mahillon J, Chandler M. Insertion sequences. *Microbiol Mol Biol Ver.* 1998;62:725–774.
32. Villalón P, Valdezate S, Medina-Pascual MJ, Carrasco G, Vindel A, Saez-Nieto JA. Epidemiology of the *Acinetobacter*-derived cephalosporinase: carbapenem-hydrolysing oxacillinase and metallo- $\beta$ -lactamase genes, and of common insertion sequences, in epidemic clones of *Acinetobacter baumannii* from Spain. *J Antimicrob Chemother.* 2013;68:550–553.
33. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother.* 2008;52:1252–1256.
34. Segal H, Garny S, Elisha BG. Is IS(Aba-1) customized for *Acinetobacter*? *FEMS Microbiol Lett.* 2005;243:425–429.
35. Turton JF, Ward ME, Woodford N, et al. The role of ISAb1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett.* 2006;258:72–77.
36. Pagano M, Martins AF, Machado AB, Barin J, Barth AL. Carbapenem-susceptible *Acinetobacter baumannii* carrying the ISAb1 upstream blaOXA-51-like gene in Porto Alegre: southern Brazil. *Epidemiol Infect.* 2012;141:1–4.
37. Poirel L, Nordmann P. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene bla(OXA-58) in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2006;50:1442–1448.
38. Giannouli M, Tomasone F, Agodi A, et al. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* strains in intensive care units of multiple Mediterranean hospitals. *J Antimicrob Chemother.* 2009;63:828–830.
39. Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene bla(OXA-23) in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2007;51:1530–1533.
40. Bogaerts P, Cuzon G, Naas T, et al. Carbapenem-resistant *Acinetobacter baumannii* isolates expressing the bla(OXA-23) gene associated with ISAb4 in Belgium. *Antimicrob Agents Chemother.* 2008;52:4205–4206.
41. Lee Y, Kim CK, Lee H, Jeong SH, Yong D, Lee K. A novel insertion sequence: ISAb10, inserted into ISAb1 adjacent to the bla(OXA-23) gene and disrupting the outer membrane protein gene carO in *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 2011;55:361–363.
42. Bonnin RA, Poirel L, Naas T, et al. Dissemination of New Delhi metallo- $\beta$ -lactamase-1-producing *Acinetobacter baumannii* in Europe. *Clin Microbiol Infect.* 2012;18:E362–E365.
43. Pilonnetto M, Arend L, Vespero EC, et al. First report of NDM-1-producing *Acinetobacter baumannii* sequence type 25 in Brazil. *Antimicrob Agents Chemother.* 2014;58:7592–7594.
44. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int.* 2014;2014:249856.
45. Poirel L, Bonnin RA, Nordmann P. Analysis of the resistome of a multidrug-resistant NDM-1-producing *Escherichia coli* strain by high-throughput genome sequencing. *Antimicrob Agents Chemother.* 2011;55:4224–4229.
46. Jones LS, Toleman MA, Weeks JL, Howe RA, Walsh TR, Kumarasamy KK. Plasmid carriage of bla NDM-1 in clinical *Acinetobacter baumannii* isolates from India. *Antimicrob Agents Chemother.* 2014;58:4211–4213.
47. Martínez T, Vázquez GJ, Aquino EE, Martínez I, Robledo IE. ISEcp1-mediated transposition of blaKPC into the chromosome of a clinical isolate of *Acinetobacter baumannii* from Puerto Rico. *J Med Microbiol.* 2014;63:1644–1648.
48. Toleman MA, Walsh TR. Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. *FEMS Microbiol Rev.* 2011;35:912–935.
49. Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. *Antimicrob Agents Chemother.* 2005;49:1432–1440.
50. Ravasi P, Limansky AS, Rodríguez RE, Viale AM, Mussi M.A. ISAb825 a functional insertion sequence modulating genomic plasticity and bla(OXA-58) expression in

- Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2011;55:917–920.
51. Woodford N, Johnson A. *Methods in Molecular Biology: Genomics, Proteomics and Clinical Bacteriology*. vol. 266. Towota, NJ: Humana Press; 2005.
  52. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the bla(OXA-23) carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis*. 2010;16:35–40.
  53. Lee HY, Chang RC, Su LH, et al. Wide spread of Tn2006 in an AbaR4-type resistance island among carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Taiwan. *Int J Antimicrob Agents*. 2012;40:163–167.
  54. Cambay G, Guerout AM, Mazel D. Integrons. *Annu Rev Genet*. 2010;44:141–166.
  55. Gillings MR. Integrons: past, present, and future. *Microbiol Mol Biol Rev*. 2014;78:257–277.
  56. Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol*. 2006;4:608–620.
  57. Lee YT, Huang LY, Chen TL, et al. Gene cassette arrays: antibiotic susceptibilities, and clinical characteristics of *Acinetobacter baumannii* bacteremic strains harboring class 1 integrons. *J Microbiol Immunol Infect*. 2009;42:210–219.
  58. Fluit AC, Schmitz FJ. Resistance integrons and super-integrons. *Clin Microbiol Infect*. 2004;10:272–288.
  59. Mendes RE, Castanheira M, Toleman MA, Sader HS, Jones RN, Walsh TR. Characterization of an integron carrying blaIMP-1 and a new aminoglycoside resistance gene: aac(6')-31, and its dissemination among genetically unrelated clinical isolates in a Brazilian hospital. *Antimicrob Agents Chemother*. 2007;51:2611–2614.
  60. Cayo R, Rodrigues-Costa F, Matos AP, Carvalhaes CG, Jove T, Gales AC. Identification of a new integron harboring bla(IMP-10) in carbapenem-resistant *Acinetobacter baumannii* clinical isolates. *Antimicrob Agents Chemother*. 2015;59:3687–3689.
  61. Gonzalez G, Sossa K, Bello H, Dominguez M, Mella S, Zelman R. Presence of integrons in isolates of different biotypes of *Acinetobacter baumannii* from Chilean hospitals. *FEMS Microbiol Lett*. 1998;161:125–128.
  62. Fonseca EL, Freitas FD, Scheidegger EMD, Jacinto T, Vicente ACP. Class 2 integrons in multidrug-resistant *Acinetobacter baumannii* circulating in different Brazilian geographic regions. *Int J Antimicrob Agents*. 2011;38:95–96.
  63. Martins N, Picao RC, Adams-Sapper S, Riley LW, Moreira BM. Association of class 1 and 2 integrons with multidrug-resistant *Acinetobacter baumannii* international clones and *Acinetobacter nosocomialis* isolates. *Antimicrob Agents Chemother*. 2015;59:698–701.
  64. Turton JF, Kaufmann ME, Glover J, et al. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. *J Clin Microbiol*. 2005;43:3074–3082.
  65. Rowe-Magnus DA, Mazel D. Integrons: natural tools for bacterial genome evolution. *Curr Opin Microbiol*. 2001;4:565–569.
  66. Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol Rev*. 2009;33:757–784.