Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities

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Abstract

*Acinetobacter baumannii* is an opportunistic gram-negative pathogen with increasing relevance in a variety of nosocomial infections especially among intensive-care-unit (ICU) patients. Carbapenems have been widely used to treat serious multidrug-resistant *A. baumannii* infections; however, incidences of carbapenem-resistant *A. baumannii* are rising in several parts of the world and large and sustained outbreaks caused by such bacteria have been described. Carbapenem-resistant *A. baumannii* epidemics are sustained by clusters of highly similar strains that successfully spread among different cities and countries; their resistance phenotype is mainly due to the acquisition of carbapenem-hydrolyzing class D β-lactamase (CHDL) genes flanked by insertion sequence (IS) elements. Multi-facility outbreaks can be also sustained by inter-hospital transfer of colonized patients. Here, we review the global epidemiology of carbapenem-resistant *A. baumannii*, with the emphasis on the molecular epidemiology and genetic characterization of carbapenem resistance in epidemic strains.

Key words: *Acinetobacter baumannii*, nosocomial outbreaks, genotyping, carbapenemases

Introduction

*Acinetobacter* spp. are glucose-non fermentative gram-negative coccobacilli that have emerged in recent years as a cause of healthcare-associated infections [1,2]. Considered to be commensals of low-grade pathogenicity, i.e. opportunistic microorganisms, *Acinetobacter* were frequently ignored in the 1970s whenever isolated from clinical specimens [1]. The genus *Acinetobacter* currently contains up to 32 described named and unnamed (genomic) species [1]. *Acinetobacter baumannii*, genomic species 3 and 13TU, three of the most clinically relevant species, are genetically and phenotypically very similar to an environmental species, *A. calcoaceticus*, and are therefore grouped together into the so-called *A. calcoaceticus-A. baumannii* (Acb) complex [1]. Because phenotypic identification of *Acinetobacter* isolates to the species level has proven to be insufficient, several genotypic methods have been developed for genomic species identification, that include amplified 16S rRNA gene restriction analysis (ARDRA), high-resolution fingerprint analysis by amplified fragment length polymorphism (AFLP), or sequence analysis of the 16S-23S rRNA gene spacer region [1,3,4]. However, genotypic methods for species identification are often unavailable in developing countries, where *Acinetobacter* are frequently isolated but identified only at genus level. The species that is most commonly involved in hospital infections is *A. baumannii*, which causes a variety of health-care associated infections, comprising bacteremia, urinary tract infection, surgical-site infection, and nosocomial and ventilator-associated pneumonia, especially in intensive-care-unit (ICU) patients [1,2,5-7]. The rates of recovery of *A. baumannii* from natural environments and its incidence in the community are low, while its rate of carriage by hospitalized patients is high and its occurrence in the hospital setting is frequent [1]. *A. baumannii* has simple growth requirements and can survive in dry conditions. This might contribute to the fitness of *A. baumannii* in the hospital environment, which represents the main reservoir of the bacterium [1].
Carbapenem resistance mechanisms in *A. baumannii*

Resistance to antimicrobial agents may be the main advantage of *A. baumannii* in the nosocomial environment. Multidrug-resistant isolates of *A. baumannii* have been reported increasingly during the last decade, probably as a consequence of extensive use of antimicrobial agents in western countries [2,8]. Also, as recently demonstrated by a retrospective, matched cohort study, patients with infection by multidrug-resistant *Acinetobacter* show higher mortality rate and length of hospitalization than patients with infection by susceptible *Acinetobacter* [5].

Mounting evidence indicates that *A. baumannii* possesses a broad range of mechanisms of resistance to all existing antibiotic classes as well as a prodigious capacity to acquire new determinants of resistance [1,2] Genome sequence analysis of six *A. baumannii* clinical strains has shown the presence of a resistance island with a variable composition of resistance genes interspersed with transposons, integrons, and other mobile genetic elements in three of them [9-11]. Also, plasmids carrying resistance genes and/or resistance determinants involved in horizontal gene transfer have been described in several *A. baumannii* strains [12-19].

The broad-spectrum β-lactam antibiotics, carbapenems, were introduced by 1985 and have been for years the most important agents for the treatment of infections caused by multidrug-resistant *A. baumannii*. Carbapenem resistance in *Acinetobacter* is now observed increasingly worldwide, and constitutes a sentinel event for emerging antimicrobial resistance [2,12]. Carbapenem-resistant isolates of *A. baumannii* are usually resistant to all classes of antimicrobials, and show intermediate resistance to rifampin, while usually retaining susceptibility to tigecycline and colistin [2,12,20]. Resistance against carbapenems is, in itself, considered sufficient to define an isolate of *A. baumannii* as highly resistant [12]. The resistance of *A. baumannii* to carbapenems can be mediated by one of the resistance mechanisms that are known to occur in bacteria, including enzymatic inactivation, active efflux of drugs, and modification of target sites (Table 1). The production of carbapenem-hydrolyzing beta-lactamases is the most common mechanism responsible for carbapenem resistance in *A. baumannii*. Several carbapenem-hydrolyzing β-lactamases have been identified so far in *A. baumannii*. These include metallo-β-lactamases (VIM-, IMP- and SIM-types), which have been sporadically reported in some parts of the world and have been associated with class 1 integrons [2,7,12]. Nevertheless, the most widespread carbapenemases in *A. baumannii* are class D β-lactamases. Three main acquired carbapenem-hydrolysing class D oxacillinase (CHDL) gene clusters have been identified either in the chromosome or in plasmids of *A. baumannii* strains, represented by the *bla*<sub>OXA-23</sub>-, *bla*<sub>OXA-24/40</sub>-, and *bla*<sub>OXA-51</sub>-like genes [12]. Different insertion sequence (IS) elements at the 5' and/or the 3' end of *bla*<sub>OXA-23</sub>- and *bla*<sub>OXA-51</sub>-like genes, such as ISAba1, ISaba2, ISaba3, or IS18, have been demonstrated to regulate their expression [12,13,15-17]. Also, it has been recently demonstrated that the ISAba1 element is capable of transposition in *E. coli* and of mobilizing an antibiotic resistance gene [18]. In addition to these CHDL genes, the chromosomal *bla*<sub>OXA-51</sub>-like gene, intrinsic to *A. baumannii* species, has been demonstrated to confer carbapenem resistance when an ISAba1 element is inserted upstream of the gene [19]. Reduced susceptibility to carbapenems has also been associated with the modification of penicillin-binding proteins and porins or with upregulation of the AdeABC efflux system, and it has been suggested that the interplay of different mechanisms might result in high-level carbapenem resistance in *A. baumannii* (Table 1) [21-23].

Global epidemiology of carbapenem-resistant *Acinetobacter baumannii*

Carbapenem resistance in *A. baumannii* is now an emerging issue worldwide [2]. Surveillance studies indicate that the percentage of carbapenem-resistant isolates gradually increased over the last ten years in Europe, North America, and Latin America [2]. Numerous outbreaks of carbapenem-resistant *A. baumannii* were reported from hospitals in Northern Europe (Spain, Portugal, France, the United Kingdom (UK), the Netherlands, Czech Republic, Poland) [1,2,24-29], Southern Europe and the Middle East (Bulgaria, Greece, Italy, Turkey, Lebanon, Israel, Iran, Iraq and United Arab Emirates) [2,6,8,10,12,14,16,17,30-35], North America and Latin America (Argentina, Brazil, Chile and Colombia) [2,36,37], Tunisia and South Africa [38,39], China, Taiwan, Singapore, Hong Kong, Japan, South Korea [2,40,41], and Australia [42] and from areas as remote as French Polynesia [43]. In the majority of cases, one or two epidemic strains were detected in a given hospital. Transmission of such strains was
Table 1. Carbapenem resistance mechanisms in A. baumannii.

<table>
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<tr>
<th>Mechanism or responsible structure</th>
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<tr>
<td>IMP-1, -2, -4, -5, -6, -11</td>
<td>Class B metallo beta-lactamases. Class 1 integron-associated genes.</td>
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<tr>
<td>VIM-2, SIM-1</td>
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<td>OXA-23 cluster</td>
<td>Class D beta-lactamases. Chromosomal or plasmid genes flanked by IS elements.</td>
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<td>OXA-24/40 cluster</td>
<td>Class D beta-lactamases. Chromosomal or plasmid genes.</td>
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<td>OXA-58 cluster</td>
<td>Class D beta-lactamases. Plasmid or chromosomal genes flanked by IS elements.</td>
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<tr>
<td>OXA-51 cluster</td>
<td>Chromosomal class D beta-lactamase intrinsic to A. baumannii. Confers carbapenem resistances if IS elements are inserted upstream of the gene</td>
<td>2,19</td>
</tr>
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**Changes in outer-membrane proteins (OMPs)**

| | |
| CarO | 26 kDa OMP implicated in drug influx | 21 |
| 33 to 36-kDa OMP | Other OMPs associated with carbapenem resistance | 2,12 |
| OprD-like OMP | | |

**Target alteration**

| | |
| Altered penicillin-binding proteins | Reduced PBP-2 expression | 22 |

observed between hospitals in the same city and also on a national scale [1,2,6,12,24-27,29,30,3,8,42,44-46] and a direct epidemiological link was established in several cases [6,25,28,29,38,42,44-46]. The inter-hospital transfer of colonised patients was demonstrated during multifacility outbreaks that occurred in the Netherlands [25], Italy [6], South Africa [38], and Tunisia [39]. The international transfer of patients colonised by carbapenem-resistant A. baumannii was also reported [28, 29, 42]. More recently, several cases of United Kingdom and US military and nonmilitary personnel returning from operations in Iraq and Afghanistan and harbouring infections caused by carbapenem-resistant A. baumannii were reported [44-46] (Figure 1).

Outbreaks caused by carbapenem-resistant A. baumannii have also been observed in developing countries such as Morocco, Thailand, India, and Indonesia [47,41]. Furthermore, infections caused by Acinetobacter spp. without specifying whether they are caused by carbapenem-resistant strains have been reported in Africa (Lagos, Nigeria) and several Asian countries including Nepal [48-50].

Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii

Genotypic characterization of carbapenem-resistant A. baumannii strains showed the occurrence of blaOXA-23*, blaOXA-24/40*, or blaOXA-58-like genes in multiple isolates from the same hospital or among different hospitals worldwide [2,12,13,33,34,43,52]. blaOXA-23 was mostly detected in isolates from Asian countries [41], but was also reported in South America [36,37] and Europe [12,17,31,51]; blaOXA-58 was frequently found in Europe [6,7,10,14,30,33,16,30-35,51]. blaOXA-24/40 was mostly found in the Iberian peninsula and Asia, but also detected in Iran, Belgium, Czech Republic and the United States of America (USA) [2,6,27,41,44,51,52] (Figure 1). Molecular epidemiology of A. baumannii strains responsible for outbreaks that occurred in several European hospitals revealed clusters of highly similar strains, which were defined as European clones I and II [1,2] and corresponded to sequence type (ST) groups 2 and 1, respectively, identified by sequence-based typing [53]. A recent study on a collection of 96 carbapenem-resistant A. baumannii strains collected in 17 European countries assigned 85% of them to sequence type (ST) groups 1 and 2 by multiple PCRs [51]. The prevalence of carbapenem-resistant epidemic A. baumannii strains belonging to ST group 1 was also demonstrated in Italy and Greece [30,33] along with the spread of a prevalent clone isolated with identical pulsed field gel electrophoresis (PFGE) profiles in two hospitals in Naples, Italy, and in three hospitals in three distinct Greek cities [33]. The circulation of distinct carbapenem-resistant A. baumannii strains observed in a hospital in the Netherlands was also highlighted by the prevalence of sequence type 16, 30, 33, and 35 [51].
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Figure 1

Geographic distribution and genetic characterization of carbapenem-resistant A. baumannii. Countries reporting carbapenem-resistant A. baumannii outbreaks producing OXA-23-, OXA-24/40-, and/or OXA-58-type enzymes are indicated by yellow, blue, and red colours, respectively. Countries reporting carbapenem-resistant A. baumannii outbreaks in which the OXA-type enzyme has not been identified are indicated by green colour. Green arrows indicate hospital transfer of colonized/infected patients by carbapenem-resistant A. baumannii between different countries.

baumannii genotypes belonging to ST group 2 in Greece and in Lebanon, and to two novel ST groups 4 and 5 in different Greek and Turkish cities, was also shown in the same study [33]. The blaOXA-58 gene flanked by IS elements was present in all carbapenem-resistant genotypes analyzed from hospitals in Greece, Italy, Lebanon, and Turkey [7,16,33] (Figure 1). Of note, each of the IS elements flanking the 5’ end of blaOXA-58 occurred in strains of distinct ST groups and PFGE profiles isolated in the same geographic region. Thus, ISAbab2 element was detected in Greece and Italy, IS18 in Lebanon and Turkey, and ISAbal in Turkey and Italy, suggesting that they might have been acquired through horizontal gene transfer [33]. In further support of this hypothesis, plasmid-borne blaOXA-58 has been found in the majority of carbapenem-resistant A. baumannii strains isolated in Europe [6,7,10,13,14,16,33]. The spread of carbapenem-resistant A. baumannii carrying the blaOXA-58 gene might have also been contributed by international transfer of colonised patients, as recently demonstrated from Greece to Belgium [28], Greece to Australia [42], and Iraq to USA military services [44] (Figure 1).

Conclusions

Outbreaks of carbapenem-resistant A. baumannii are increasingly reported in several parts of the world that also include developing countries. They are sustained by clusters of highly similar strains that successfully spread among different cities and countries and are selected because of the acquisition of CHDLs genes flanked by IS elements. Multi-facility A. baumannii outbreaks can be also sustained by inter-hospital transfer of colonized patients. This emphasizes the need to adopt surveillance and infection control programmes to prevent colonisation and infection by multidrug-resistant A. baumannii in the hospital setting. These programmes would include the study of global epidemiology of multidrug-resistant A. baumannii using molecular typing of bacterial isolates and characterization of antibiotic resistance in order to control the spread of A. baumannii infections over a wide geographic region.

Acknowledgments

Work performed in the authors’ laboratories is supported in part by a grant from Agenzia Italiana del Farmaco (AIFA2007 contract no. FARM7X9F8K). Restriction placed on the number of references that could be cited in this review mean that, in many cases, either a single paper or a review is cited. We apologize to those authors whose work has not been cited.

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**Conflict of interest:** No conflict of interest is declared.