 10.1111/j.1469-0691.2006.01456.x

Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology

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ABSTRACT

The increasing trend of carbapenem resistance in *Acinetobacter baumannii* worldwide is a concern since it limits drastically the range of therapeutic alternatives. Metallo-β-lactamases (VIM, IMP, SIM) have been reported worldwide, especially in Asia and western Europe, and confer resistance to all β-lactams except aztreonam. The most widespread β-lactamases with carbapenemase activity in *A. baumannii* are carbapenem-hydrolysing class D β-lactamases (CHDLs) that are mostly specific for this species. These enzymes belong to three unrelated groups of clavulanic acid-resistant β-lactamases, represented by OXA-23, OXA-24 and OXA-58, that can be either plasmid- or chromosomally-encoded. *A. baumannii* also possesses an intrinsic carbapenem-hydrolysing oxacillinase, the expression of which may vary, that may play a role in carbapenem resistance. In addition to β-lactamases, carbapenem resistance in *A. baumannii* may also result from porin or penicillin-binding protein modifications. Several porins, including the 33-kDa CarO protein, that constitute a pore channel for influx of carbapenems, might be involved in carbapenem resistance.

Keywords *Acinetobacter baumannii*, carbapenems, metallo-β-lactamase, oxacillinase, resistance, review

Accepted: 17 February 2006

Clin Microbiol Infect 2006; 12: 826–836

INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that is frequently involved in outbreaks of infection, occurring mostly in intensive care units [1]. *A. baumannii* is mostly a cause of septicaemia, pneumonia and urinary tract infection following hospitalisation of patients with more severe illness. 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]. Carbapenem resistance in this species is now observed increasingly worldwide, and constitutes a sentinel event for emerging antimicrobial resistance [3]. It is considered that resistance against carbapenems is, in itself, sufficient to define an isolate of *A. baumannii* as highly resistant [4]. Several studies focusing on the genetic and biochemical basis of carbapenem resistance in *A. baumannii* have been reported recently, mostly related to β-lactamase production.

Two intrinsic types of β-lactamases can be identified in most, if not all, *A. baumannii* isolates. An AmpC-type cephalosporinase, expressed at a basal level, does not reduce the efficacy of expanded-spectrum cephalosporins [5]; however, introduction of the insertion sequence IS*Aba1* upstream of the bla_{AmpC} gene enhances β-lactamase expression considerably by providing promoter sequences, resulting in resistance to ceftazidime, but not to carbapenems [6–8]. *A. baumannii* also produces a second intrinsic β-lactamase, which is an oxacillinase represented by the OXA-51/69 variants [9,10]. The genes encoding the $bla_{\mathrm{OXA\text{-}51}}$-like β-lactamases are chromosomally located in all of the *A. baumannii* isolates studied to date. OXA-51/69 enzymes share very weak identities with other known oxacillinases. To date, 11 variants of OXA-51 have been identified in isolates from diverse geographical origins [11,12]. Among these variants, the OXA-51 and OXA-69 β-lactamases have been studied in detail to

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elucidate their carbapenemase activities [9,10]. However, it seems that the level of expression of the corresponding genes is quite low in most cases and, even after it has been cloned into high-copy-number plasmids in *Escherichia coli* and *A. baumannii*, OXA-69 has only a marginal impact on susceptibility to all β-lactams, including carbapenems [9]. Thus, in summary, *A. baumannii*, like *Pseudomonas aeruginosa*, produces a naturally occurring AmpC β-lactamase, together with a naturally occurring oxacillinase with carbapenemase properties [13].

Along with these naturally occurring β-lactamases, several acquired β-lactamases have been identified as a source of carbapenem resistance in *A. baumannii*. These enzymes belong either to the class B enzymes defined by Ambler *et al.* [14] (also known as metallo-β-lactamases), or to the class D enzymes (also known as oxacillinases) [15]. Although metallo-β-lactamases (MBLs) are powerful carbapenemases [16], oxacillinases possessing the ability to hydrolyse imipenem (but not always meropenem) are grouped in a particular subgroup of β-lactamases termed carbapenem-hydrolysing oxacillinases (CHDLs) [17]. Both MBLs and CHDLs are resistant to inhibition by clavulanate and tazobactam. MBLs are susceptible *in vitro* to EDTA inhibition, whereas most CHDLs are susceptible to NaCl inhibition, thus providing a means of their laboratory identification. Use of Etest strips containing imipenem with or without EDTA is helpful for identification of MBL production [16]. Whereas MBLs have been identified in a wide variety of Gram-negative species, but only rarely in *A. baumannii*, most acquired CHDLs have been identified only in *A. baumannii*.

Resistance to carbapenems may also be explained by other mechanisms, such as porin loss or modification, as evidenced recently by the CarO protein (see below), and rarely by modification of penicillin-binding proteins (PBPs).

ACQUISITION OF METALLO-β-LACTAMASE GENES

Five groups of acquired MBLs have been identified to date (IMP-like, VIM-like, SIM-1, SPM-1 and GIM-1 enzymes), but only the first three of these groups have been identified in *A. baumannii*. The IMP group consists currently of 19 variants that cluster in seven phylogroups [16]. Six IMP variants belonging to three different phylogroups have been identified in *A. baumannii*, namely IMP-1 in Italy [18], Japan [19–21] and South Korea [22], IMP-2 in Italy [23] and Japan [19], IMP-4 in Hong Kong [24], IMP-5 in Portugal [25], IMP-6 in Brazil [26], and IMP-11 in Japan [16] (Table 1). In addition, IMP-4 has been identified in an *Acinetobacter junii* clinical isolate from Australia [27]. Of note, VIM enzymes have been identified very rarely in *A. baumannii*, being represented only by VIM-2 reported in South Korea [22,28] (Table 1), which is an area where VIM enzymes are also known to be widespread in *P. aeruginosa* and Enterobacteriaceae. SIM-1 has been reported only in *A. baumannii* from South Korea [29], where this determinant might also be widespread (45th Interscience Conference on Antimicrobial Agents and Chemotherapy, abstract C2-107).

The IMP and VIM variants confer a high level of carbapenem resistance in *A. baumannii* isolates, as well as resistance to all β-lactams except aztreonam, because of their strong hydrolytic efficiency against these antibiotics. Isolates producing SIM-1 have imipenem MICs of 8–16 mg/L [29]. The role of MBL production in the carbapenem resistance of isolates producing IMP or VIM enzymes is easy to determine by using the Etest technique [16]. Using this test, comparison of the MICs of imipenem alone or combined with EDTA on an agar plate allows the identification of MBL production in *A. baumannii*. Only cefepime and cefpirome may retain some residual antibacterial activity against MBL producers, as does, to a lesser extent, piperacillin-tazobactam.

Table 1. Acquired carbapenem-hydrolysing β-lactamases identified in *Acinetobacter baumannii*

β-Lactamase	Ambler class	Plasmid or chromosomal	Geographical origin
IMP-1	B	Plasmid	Italy, Japan, South Korea
IMP-2	B	Plasmid	Italy, Japan
IMP 4	B	?	Hong Kong
IMP-5	B	?	Portugal
IMP-6	B	?	Brazil
IMP-11	B	?	Japan
VIM-2	B	Plasmid	South Korea
SIM-1	B	?	South Korea
OXA-23	D	Plasmid	UK, French Polynesia, Brazil, Iraq
OXA-24	D	Chromosomal	Spain
OXA-25	D	Chromosomal	Spain
OXA-26	D	Chromosomal	Spain
OXA-27	D	?	Singapore
OXA-40	D	Chromosomal	France, Spain, Portugal
OXA-58	D	Plasmid or Chromosomal	France, Spain, Italy, Greece, UK, Austria, Romania, Iraq, Argentina, Kuwait

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Analysis of the genetic surroundings of the MBL-encoding genes identified in *A. baumannii* has revealed very similar structures, since the bla_{IMP}, bla_{VIM} or bla_{SIM} genes are embedded in class-1 integron structures. The MBL genes form part of the gene cassettes that are inserted between the 5′-conserved segment (5′-CS) and the 3′-CS, together with other antibiotic resistance gene cassettes, mostly encoding aminoglycoside-modifying enzymes. In addition, the plasmid location of MBL genes explains their spread among *A. baumannii* and *P. aeruginosa* strains in specific areas, e.g., Italy and Korea.

ACQUISITION OF CARBAPENEM-HYDROLYSING OXACILLINASE GENES

Oxacillinases are unusual β-lactamases that form a heterogeneous group with respect to their structural or biochemical properties [15]. These enzymes usually hydrolyse oxacillin more efficiently than benzylpenicillin. In addition, they hydrolyse amoxycillin, methicillin, cephaloridine and, to some extent, cephalothin. Only a few variants hydrolyse expanded-spectrum cephalosporins (observed in *P. aeruginosa*, but never in *A. baumannii*) and, in such cases, the enzymes responsible are often point mutant derivatives of narrow-spectrum enzymes. In contrast, carbapenemase activity seems to be an intrinsic property of some oxacilllinases that are not point-mutant derivatives of known enzymes. The hydrolytic efficiency of CHDLs against carbapenems is much lower (100- to 1000-fold) than that of the MBLs, which is a property that may complicate their recognition. CHDLs of this type are identified frequently in *A. baumannii*.

Identification of a CHDL-encoding gene was first reported in *A. baumannii* in 1995 [30]. This enzyme, originally named ARI-1, was identified in Scotland and was found to be plasmid-encoded [31]. The enzyme was renamed OXA-23 following its genetic and biochemical characterisation, and shares 56% amino-acid identity with OXA-51/69 (Fig. 1). OXA-23 is a representative of a CHDL subgroup that also includes OXA-27 and OXA-49; OXA-27 was identified in a single carbapenem-resistant *A. baumannii* isolate from Singapore [32], and differs from OXA-23 by Thr to Ala and Asn to Lys substitutions at positions DBL 95 and 247 [33], respectively, while OXA-49 was identified in a single *A. baumannii* isolate from China, and differs from OXA-23 by a Lys to Glu substitution at position DBL 178 and an additional Ala residue at position DBL 222 (GenBank no. AAP40270). The bla_{OXA-23} gene has been identified in two carbapenem-resistant clones that spread rapidly in hospitals in the UK during 2003 and 2004 [34], and in another clone identified in a large area of South France (T. Naas, personal communication). In addition, some OXA-23 producers have been identified in Romania [35], Brazil [36], South Korea [37] and French Polynesia

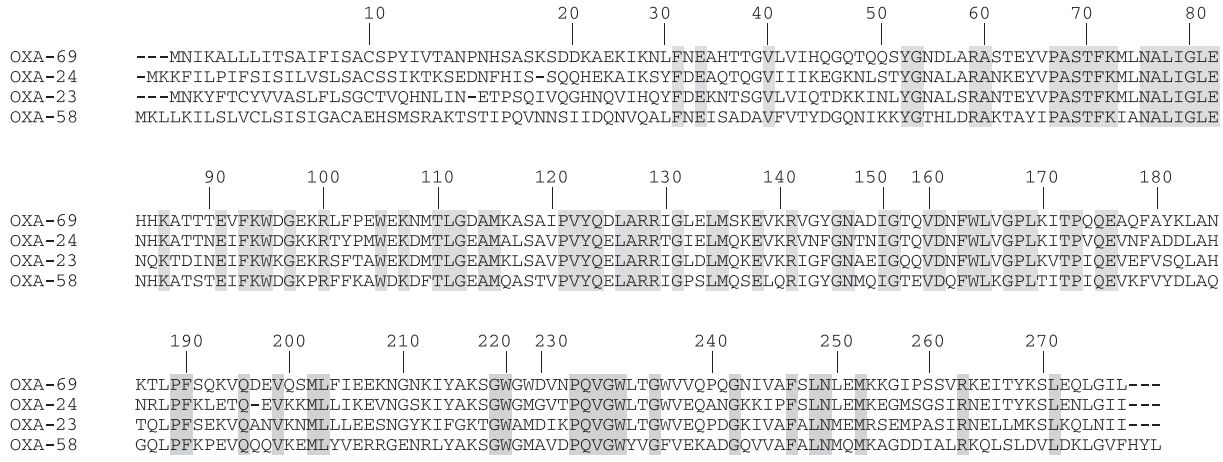

Fig. 1. Alignment of amino-acid sequences of the four main groups of carbapenem-hydrolysing class D β-lactamases (CHDLs) identified in *Acinetobacter baumannii*, including OXA-23, OXA-24 and OXA-58 as representatives of the three groups of acquired CHDLs, and OXA-51 as a representative of the naturally occurring CHDLs. The conserved residues for oxacillinases are shaded. Numbering of β-lactamases is according to Ambler class D β-lactamase numbering DBL [33].

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during outbreak periods [38]. Of note, bla_{OXA-23} has also been identified as being chromosomally located in a *Proteus mirabilis* clinical isolate from France [39].

A second group of CHDLs, comprising OXA-24, OXA-25, OXA-26 and OXA-40 (sharing 63% and 60% amino-acid identity with OXA-51/69 and OXA-23, respectively) has also been identified in *A. baumannii* (Fig. 1). The OXA-24 and OXA-25 variants were identified in carbapenem-resistant *A. baumannii* isolates recovered from Spain, whereas OXA-26 was identified in an isolate from Belgium [32,40]. OXA-40 was identified in a carbapenem-resistant *A. baumannii* isolate recovered in France from a Portuguese patient [41] and has also been shown to be widespread in Spain and Portugal [42–44].

A third group of CHDLs contains OXA-58, which was identified originally in a carbapenem-resistant *A. baumannii* isolate recovered in Toulouse, France [45], following an outbreak in a burns unit [46]. OXA-58 shares 59% amino-acid identity with OXA-51/69, and < 50% with other CHDLs (Fig. 1). A series of epidemiological surveys has identified the bla_{OXA-58} gene in *A. baumannii* clinical isolates from diverse geographical origins including Spain, Turkey, Romania [35], Greece [47], Austria, the UK, Argentina, Kuwait [48] and Italy (A. Carattoli, personal communication). The bla_{OXA-58} gene has also been identified recently in several *A. baumannii* isolates recovered from injured USA military personnel in Iraq (45th Interscience Conference on Infectious Diseases and Antimicrobial Agents, abstract C2-1427). Outbreaks involving multiple carbapenem-resistant clones producing OXA-58 were reported from the intensive care unit of a hospital in Athens, Greece [47], and also from several units of a paediatric hospital in the same city (personal unpublished data), underlining the fact that OXA-58-producers constitute an important threat in Greece. Interestingly, the bla_{OXA-58} gene has also been identified in clinical isolates of a different species, *A. junii*, from Romania and Australia, thereby underlining the potential for dissemination of OXA-23 in the *Acinetobacter* genus [35]. In addition, it is noteworthy that the *A. junii* clinical isolate from Australia co-produced OXA-58 and IMP-4 β-lactamases, both of which possess carbapenemase activity [27].

Little is known concerning the acquisition mechanisms for the CHDL-encoding genes in *A. baumannii*. Whereas the bla_{OXA-23} and bla_{OXA-58} genes have been identified mostly on plasmids, the CHDL genes belonging to the bla_{OXA-24} cluster seem only to be chromosomally-located. Characterisation of the sequences surrounding bla_{OXA-40} in several isolates has not provided evidence for mobilisable elements such as insertion sequences (IS), transposons or integrons, which is in contrast to most oxacillinase genes identified in *P. aeruginosa*, which are integron-borne. It is tempting to speculate that CHDL genes belonging to the OXA-23 and OXA-58 clusters are true acquired resistance genes, whereas genes encoding OXA-24-like enzymes could belong to a subspecies of *A. baumannii* that had acquired this type of gene in the distant past. The reservoir (natural producer) of the different CHDLs mentioned above is unknown, as is the location of the genetic exchange with *A. baumannii*. A single CHDL (OXA-48) [49], found to be plasmid-encoded in a carbapenem-resistant isolate of *Klebsiella pneumoniae* from Turkey, has been shown to have a natural reservoir in *Shewanella oneidensis* [50].

In-silico analyses of GenBank databases and personal observations have indicated that the IS*Aba1* element, belonging to the IS*4* family, is often identified upstream of bla_{OXA-23}, regardless of the geographical origin of the isolate. In addition, another IS element, IS*Aba4*, belonging to the IS*982* family, has been identified recently in *A. baumannii* clinical isolates from France and Algeria (personal unpublished data). The presence of these IS elements upstream of the bla_{OXA-23} gene indicates that they may play a role in bla_{OXA-23} expression by providing promoter sequences, and perhaps they may also have a role in the acquisition process.

Concerning the bla_{OXA-58} gene, genetic investigations performed on several non-clonally-related OXA-58 producers revealed that this gene was bracketed by IS elements that play a role in its expression. In particular, IS*Aba2* (IS*3* family), IS*Aba3* (IS*1* family) and IS*18* (IS*30* family), as well as IS*Abal*, were shown to provide promoter sequences enhancing bla_{OXA-58} expression [51]. However, these IS elements were probably not involved in bla_{OXA-58} acquisition, and it has been demonstrated that repeated 27-bp sequences (named Re27 elements) bracketed a *c*.5.5-kb fragment containing the bla_{OXA-58} gene, indicating that an homologous recombination process may have been at the origin of such an acquisition [51].

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This would represent the first reported mechanism of acquisition of a β-lactamase gene that was unrelated to integrons, transposons or IS elements, but based on recombination events.

Compared with MBLs, the carbapenem resistance level provided by CHDLs in *A. baumannii* is much lower. In particular, hydrolysis of meropenem is not always detectable with these enzymes. Since the exact contribution of CHDLs to the carbapenem resistance of *A. baumannii* clinical isolates was debatable, a study was performed to evaluate this aspect more precisely [52]. The natural plasmids harbouring the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$ genes were electrotransformed into a carbapenem-susceptible *A. baumannii* reference strain, which then demonstrated a significant impact of OXA-23 production on resistance by showing an increased imipenem MIC. However, this effect was lower when OXA-58 was expressed in the same *A. baumannii* reference strain. The same experiment was repeated using an isogenic *A. baumannii* recipient strain that overexpressed its natural AdeABC efflux system. The resulting MICs confirmed the important role of OXA-23 in mediating resistance to carbapenems, but demonstrated a paradoxically lower contribution of OXA-58 (16-fold increase in both imipenem and meropenem MICs) (Fig. 2). Conversely, disruption of the chromosomal $bla_{\text{OXA-40}}$ gene in a carbapenem-resistant isolate of *A. baumannii* restored susceptibility to imipenem and meropenem, thus demonstrating the significant role of OXA-40 in carbapenem resistance (Fig. 3).

Another interesting feature is the establishment of a link between production of some of the naturally occurring OXA-51/69-like oxacillinases and carbapenem resistance in *A. baumannii*. Despite the relatively weak ability of these enzymes to hydrolyse carbapenems, it has been shown that these oxacillinases may sometimes be overexpressed, resulting in a decreased level of susceptibility to carbapenems (J. Turton, N. Woodford and T. Pitt, personal communication). This observation was correlated with the presence of the IS*Aba1* element upstream of the $bla_{\text{OXA-51/69}}$-like gene. Therefore, as observed for the natural bla_{AmpC} gene of *A. baumannii*, IS*Aba1* might provide promoter sequences that enhance expression of associated genes. These promoter sequences are probably extremely efficient in *A. baumannii*, so that insertion of IS*Aba1* upstream of $bla_{\text{OXA-51}}$-like genes might represent a true mechanism of carbapenem resistance, or at least decreased susceptibility. Further work is needed to establish precisely the effect of OXA-51/69 production on resistance to carbapenems.

PORINS AS A CAUSE OF CARBAPENEM RESISTANCE

Until recently, knowledge concerning proteins responsible for the permeability of β-lactam anti-

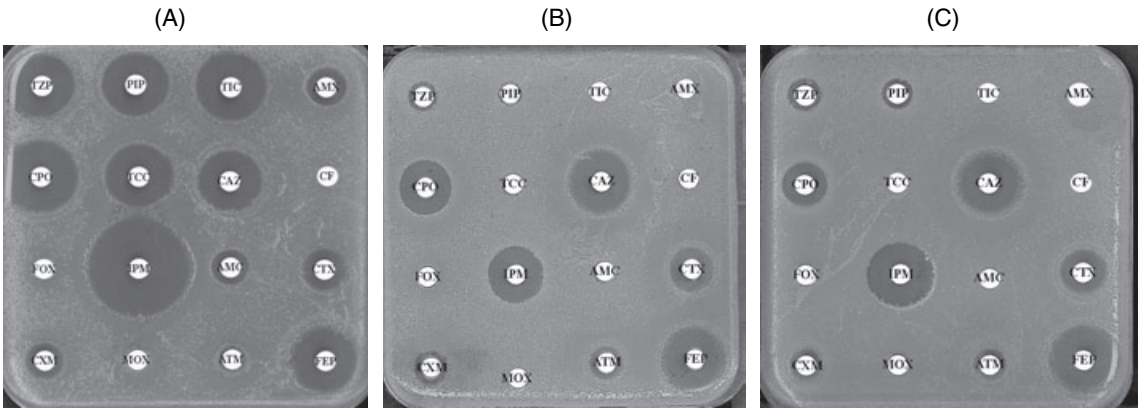

Fig. 2. Contribution of the expression of plasmid-mediated OXA-23 and OXA-58 carbapenem-hydrolysing class D β-lactamase genes in *Acinetobacter baumannii* [52]. (A) *A. baumannii* CIP70.10 reference strain; (B) *A. baumannii* CIP70.10 reference strain possessing a natural plasmid expressing OXA-23; and (C) *A. baumannii* CIP70.10 reference strain possessing a natural plasmid expressing OXA-58. TZP, piperacillin-tazobactam; PIP, piperacillin; TIC, ticarcillin; AMX, amoxycillin; CPO, cefpirome; TCC, ticarcillin-clavulanic acid; CAZ, ceftazidime; CF, cephalothin; FOX, cefoxitin; IPM, imipenem; AMC, amoxycillin-clavulanic acid; CTX, cefotaxime; CXM, cefuroxime; MOX, moxalactam; ATM, aztreonam; FEP, cefepime.

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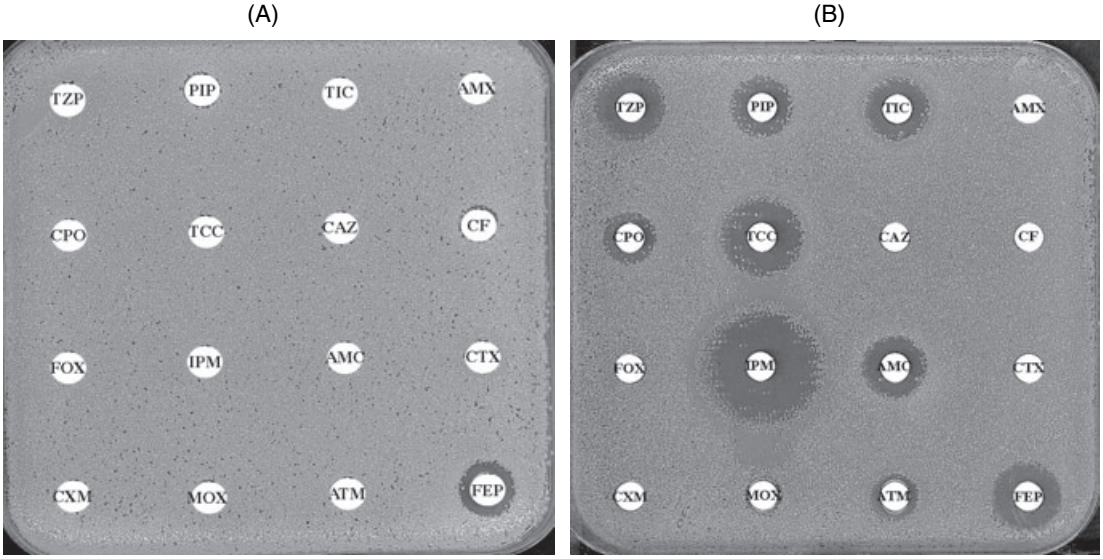

Fig. 3. Contribution of the expression of the chromosomally-encoded carbapenem-hydrolysing class D β-lactamase OXA-40 gene in *Acinetobacter baumannii* [52]. (A) *A. baumannii* CLA producing OXA-40; and (B) *A. baumannii* CLA in which the bla_{OXA-40} gene has been inactivated by homologous recombination. Abbreviations as for Fig. 2.

biotics through the outer-membrane of *A. baumannii* was very limited. By studying the in-vivo selection of carbapenem resistance in *A. baumannii* clinical isolates, Costa *et al.* [53] demonstrated that resistant isolates had acquired two β-lactamases, and that these isolates had also lost a protein of 31–36 kDa.

Recent reports have demonstrated that *A. baumannii* possesses outer-membrane proteins (OMPs) that play a role in carbapenem resistance. In 2002, Limansky *et al.* [54] demonstrated that imipenem resistance was associated with the loss of a 29-kDa OMP in clinical isolates of *A. baumannii* in which no carbapenemase activity had been detected. Similarly, resistance to imipenem and meropenem in multidrug-resistant *A. baumannii* clinical isolates has also been associated with loss of a heat-modifiable 29-kDa OMP, designated CarO [55]. When the chromosomal locus containing the *carO* gene was cloned from clinical isolates and characterised, it was shown that only a single copy of *carO*, present in a single transcriptional unit, was present in the *A. baumannii* genome. The *carO* gene encodes a polypeptide of 247 amino-acid residues, with a typical N-terminal signal sequence and a predicted trans-membrane β-barrel topology. The lack of CarO in different carbapenem-resistant clinical isolates of *A. baumannii* resulted from the disruption of *carO* by distinct insertion elements, supporting the hypothesis that CarO participates in the influx of carbapenem antibiotics in *A. baumannii*. However, another study that focused on a 25/29-kDa OMP band of *A. baumannii*, corresponding to the so-called CarO, demonstrated that the two bands corresponded to two proteins that adopted a typical β-barrel conformation [56]. Only one of these proteins (the CarO protein) displayed pore-forming properties, but no binding site for imipenem was detected in CarO, suggesting a non-specific monomeric channel function rather than a specific function, as suggested previously by Mussi *et al.* [55].

It has also been reported that resistance to carbapenems might be associated with reduced expression of two proteins (22 and 33 kDa) in a multiresistant *A. baumannii* isolate that also produced the CHDL OXA-24 [57], so that both mechanisms, i.e., carbapenemase production and decreased permeability, could be responsible jointly for the high-level carbapenem resistance observed. Another interesting observation was made by del Mar Tomas *et al.* [58], who studied an *A. baumannii* isolate that did not produce any carbapenemase, but exhibited high-level resistance to carbapenems. Analysis of the outer-membrane profile revealed the loss of a 31- to 36-kDa OMP (later defined as 33- to 36-kDa OMP) that, on further characterisation, was found to have an amino-acid sequence and composition

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typical of Gram-negative bacterial porins (i.e., a high glycine content, absence of cysteine residues, a negative charge, an absence of hydrophobic residue stretches, and similarity with trans-membrane β barrels, as well as with bacterial membrane and cell-surface proteins) [58]. By using complementation assays after cloning the corresponding genes on a shuttle plasmid, it was shown that restored production of the OMP compromised the carbapenem resistance.

A further study has revealed that *A. baumannii* possesses an OprD homologue (a porin also called D2, known to be involved in carbapenem resistance in *P. aeruginosa*) [59]. It was demonstrated that the imipenem susceptibility of *A. baumannii* strains producing the OprD-like protein could be modulated by the addition of basic amino-acids. Thus, as observed in *P. aeruginosa*, in which the OprD porin has a major role in imipenem resistance and is also involved in basic amino-acid uptake, carbapenem-susceptible *A. baumannii* isolates express naturally an OprD-like protein of 43 kDa.

OTHER NON-ENZYMIC MECHANISMS AND CARBAPENEM RESISTANCE

Modification of penicillin-binding-proteins (PBPs) as a source of imipenem resistance in *A. baumannii* has been investigated only rarely. Gehrlein *et al.* [60] studied a carbapenem-susceptible isolate and a derived resistant mutant obtained *in vitro*, and showed that the resistant mutant hyper-produced a 24-kDa PBP, but produced six other PBPs at a lower level. Another study has described the existence of 12 PBP patterns among a collection of *A. baumannii* isolates with variable β-lactam resistance profiles [61]. In isolates with imipenem MICs >4 mg/L, the absence of a 73.2-kDa PBP was associated with resistance in conjunction with production of carbapenemases.

An AdeABC efflux system has been identified in *A. baumannii* [62]. This efflux mechanism belongs to the resistance-nodulation-division (RND) family of efflux systems [63], known to accommodate a broad range of structurally unrelated molecules, including most classes of antibiotics. Its role in resistance to aminoglycosides and in decreased susceptibility to chloramphenicol, fluoroquinolones and trimethoprim, as well as cefotaxime, has been demonstrated clearly [62]. However, its effect on carbapenem susceptibility was not initially evaluated. Further studies identified the *adeST* genes, encoding homologues of the sensor kinase/response regulator members of the superfamily of bacterial two-component regulators, upstream of the *adeABC* efflux genes [64]. The corresponding proteins were shown to be implicated in regulating efflux gene expression. By analysing the carbapenem susceptibility levels of strain CIP70.10 and its point mutant derivative BM4547 (in which this regulatory system was inactivated), a two-fold increase in the MICs of imipenem and meropenem was demonstrated when the AdeABC system was expressed constitutively [52]. In addition, when combined with production of a CHDL, a synergic effect was observed that resulted (e.g.) in a carbapenem resistance level with OXA-58 that was not reached with the same enzyme in the wild-type strain background [52]. Consequently, it appears that the AdeABC efflux system may also contribute to carbapenem resistance in *A. baumannii*.

CLINICAL FEATURES OF CARBAPENEM-RESISTANT ISOLATES

Spread of a single *A. baumannii* isolate as a source of a nosocomial outbreak is often linked to contamination of respiratory equipment and transmission via the hands of hospital staff [1,65]. Numerous studies have shown that the hospital environment is a preferential setting in which *A. baumannii* isolates can persist and develop. *A. baumannii* seems to have unique characteristics among nosocomial Gram-negative bacteria that enhance its environmental persistence. The possibility that the community represents a reservoir for *A. baumannii* was evaluated by analysing and comparing isolates recovered from patients in two hospitals in New York, USA, with isolates recovered from the hands of individuals in the community [66]. This study showed clearly that community isolates are distinct from those encountered in hospital settings, whereas hospitals constitute reservoirs for clonal diffusion. In addition, an absence of multidrug resistance was demonstrated in the community isolates, thereby confirming that the battle against increased carbapenem resistance should take place primarily in the hospital.

Another interesting aspect of the relationship between carbapenem resistance and CHDL

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production was demonstrated by the recent finding of a carbapenem-susceptible isolate that was related clonally to a carbapenem-resistant isolate in a hospital environment. Genetic investigations revealed loss of the CHDL-encoding gene in the susceptible isolate in the absence of antibiotic selection pressure [51]. Even if such observations cannot be extended to all of the carbapenem resistance mechanisms described in this review, the possibility that such resistance mechanisms might be reversible clearly strengthens the theory that control of antibiotic consumption may play a significant role in preventing resistance. In addition, it has been shown that some hospitals may face concomitant outbreaks of multidrug-resistant and multidrug-susceptible subclones of *A. baumannii* [67].

Cisneros *et al.* [68] analysed potential risk-factors for the acquisition of imipenem-resistant *A. baumannii* recovered from a series of Spanish hospitals. Among a cohort of 203 patients, 43% were resistant to imipenem, and a wide and multiple clonal distribution was demonstrated, although local spread of particular clones leading to outbreaks was not demonstrated. The major risk-factors identified were a hospital size of > 500 beds, previous antimicrobial treatment, use of a urinary catheter, and surgery. This analysis led to the suggestion that a key element in control should be better differentiation between colonisation and infection in order to limit inappropriate use of antimicrobial agents. Evaluation of risk-factors for colonisation and infection by a carbapenem-resistant isolate in a hospital outbreak context has revealed an independent association with the presence of arterial catheters and the use of imipenem as monotherapy [69], and also an association with pulsative lavage wound treatment [70]. It has been shown that multidrug-resistant *A. baumannii*, defined by carbapenem resistance alone [4], may result from successive contaminations with different strains in a given hospital unit, rather than an increase in resistance by successive steps in a single clone, which makes control more difficult [65].

CONCLUSIONS

The recent identification of the largest antibiotic resistance island known so far (more than 40 resistance genes) [71] in a carbapenem-susceptible and ESBL-producing *A. baumannii* clinical isolate [72] demonstrates the genetic plasticity of *A. baumannii* which enables it to benefit from a variety of resistance mechanisms to easily become more and more resistant when antibiotic pressure is maintained. By chance, although *A. baumannii* is known to have the propensity to develop antibiotic resistance rapidly [1], it seems that in-vitro selection of carbapenem resistance in *A. baumannii* is difficult to achieve. However, the presence of carbapenem-resistant isolates in hospital wards results mostly from the acquisition of carbapenem-hydrolysing β-lactamases. Thus, production of a CHDL seems to constitute the main problem. In the USA, the proportion of *Acinetobacter* isolates resistant or intermediately-resistant to imipenem rose from 6.3% in 1999 to 11.4% in 2001 [73]. The recent evidence of *A. baumannii* infections among patients at military facilities treating injured USA service personnel has reinforced the need for better control [74]. In terms of therapy, some studies have indicated that colistin may be useful for treating infections caused by carbapenem-resistant strains. The debate surrounding the efficacy of this antibiotic remains open, but the resolution of bacteraemia caused by strains of multidrug-resistant *A. baumannii* with a continuous intravenous infusion of colistin has been demonstrated [74,75]. In addition, in-vivo experimental studies have indicated that use of carbapenems could still be appropriate in certain circumstances. For example, in a mouse model of pneumonia, Montero *et al.* [76] demonstrated that the best therapeutic approach involved a combination of imipenem and aminoglycosides in the case of moderate levels of carbapenem resistance, and a combination of imipenem and colistin or tobramycin or rifampicin in the case of high-level imipenem resistance. Combinations of polymyxin B, imipenem and rifampicin have been shown to be synergic *in vitro* [77,78], although there is some debate regarding the fractional inhibitory concentration index score. Tigecycline might also be useful, since tigecycline is known to be effective against some carbapenemase-producing strains and might be a useful alternative to polymyxins [79]. A combination of ampicillin and sulbactam has been shown to be effective against multiresistant isolates for the treatment of life-threatening acinetobacter infections [80]. Nevertheless, as the main problem identified in epidemiological surveys appears to be related to the production of CHDLs,

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it is clear that there is a specific need for a potent class D β-lactamase inhibitor to prevent, at least partially, current therapeutic failures.

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