Acinetobacter Baumannii: A Multidrug Resistant Pathogen in Healthcare Facilities

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ABSTRACT

Acinetobacter baumannii (A. baumannii) is an opportunistic pathogen that has been implicated in various infections that mainly affect critically ill patients in Intensive Care Units (ICUs). Hospital-acquired Acinetobacter spp. Infections include ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, urinary tract infections, secondary meningitis and bloodstream infections. Nosocomial infections that are caused by other Acinetobacter species, such as A. johnsonii, A. junii, A. lwoffii and A. radioresistens are rare and are mainly restricted to catheter-related bloodstream infections. Prevalence of Multidrug resistant (MDR) A. baumannii in healthcare facilities has been common in recent years. The resistance of A. baumannii to antimicrobial agents is mediated by all of the major resistance mechanisms that are known to occur in bacteria, including modification of target sites, enzymatic inactivation, active efflux and decreased influx of drugs. So, it is of an urgent need to emphasis the use of new effective antimicrobial agents to overcome the medical and economic losses caused by MDR A. baumannii.

Key words: A. baumannii, enzymatic, inactivation, active, efflux

GENERAL CHARACTERISTICS

Acinetobacters are usually cocobacillary or coccocal in appearance. Rod-shaped forms, also, occur. They grow well on most types of media used to culture specimens from patients. Acinetobacter spp. are glucose-non-fermentative, non-fastidious, strictly aerobic Gram-negative coccobacilli, usually occurring in diploid formation, or in chains of variable length. They are non-motile, catalase positive and oxidase negative (Van Loovern et al., 2004). A. baumannii is a Gram-negative, non-lactose fermenting organism that is increasingly recognized as a major pathogen causing nosocomial infections. The organism is characterized by its tendency to acquire resistance to multiple classes of antimicrobials (Adams-Haduch et al., 2008), and it is the most commonly isolated species. Acinetobacters, often, are commensals but occasionally cause nosocomial infection. A. baumannii has been isolated from blood, sputum, skin and urine, usually in device-associated infections. Acinetobacter encountered in nosocomial pneumonia often originates in the water of room vaporizers while in patients with acinetobacter bacteremia, intravenous catheters are almost always the source of infection. In patients with burns or with immune deficiencies, Acinetobacter acts as an opportunistic pathogen and can produce sepsis (Brooks et al., 1998).

CLINICAL IMPACT OF ACINETOBACTER INFECTIONS

The risk factors that predispose individuals to the acquisition of and infection with A. baumannii are similar to those that have been identified for other multidrug resistant (MDR) organisms. These include: host factors such as major surgery, major trauma (in particular burn trauma) and prematurity in newborns; exposure-related factors such as a previous stay in an ICU, the length of stay in a hospital or ICU, residence in a unit in which A. baumannii is endemic and exposure to contaminated medical equipment; and factors that are related to medical treatment such as mechanical ventilation, the presence of indwelling devices (such as intravascular catheters, urinary catheters and drainage tubes), the number of invasive procedures that are performed and previous antimicrobial therapy (Garcia-Garmendia et al., 2001). Risk factors that are specific for a particular setting have,
also, been identified such as the hydrotherapy that is used to treat burn patients and the pulsatile lavage treatment that is used for wound debridement (Maragakis et al., 2004 and Wisplinghoff et al., 1999).

The most frequent clinical manifestations of nosocomial A. baumannii infection are ventilator-associated pneumonia and bloodstream infection, both of which are associated with considerable morbidity and mortality, which can be as high as 52% (Cisneros et al., 1996 and Seiffert et al., 1995). Bacteriemic A. baumannii pneumonia has a particularly poor prognosis (Seiffert et al., 1995).

A characteristic clinical manifestation is cerebrospinal shunt-related meningitis caused by A. baumannii in patients who have had neurosurgery (Siegman-Igra et al., 1993). Wound infections have been reported mainly in patients who have severe burns or trauma, e.g. soldiers who have been injured during military operations (Davis et al., 2005 and Wisplinghoff et al., 1999). Urinary-tract infections related to indwelling urinary-tract catheters usually run a more benign clinical course and are more frequent in rehabilitation centers than in ICUs (Wise and Tosolini, 1990).

Antimicrobial Resistance of A. baumannii

The resistance of A. baumannii to antimicrobial agents is mediated by all of the major resistance mechanisms that are known to occur in bacteria, including modification of target sites, enzymatic inactivation, active efflux and decreased influx of drugs. β-lactamases are the most diverse group of enzymes that are associated with resistance, and more than 50 different enzymes, have been identified so far in A. baumannii. Aminoglycoside resistance has been attributed to at least nine distinct modifying enzymes, which can be found in different combinations in some strains (Doi et al., 2004 and Seward et al., 1998). Specific point mutations in the genes that encode DNA gyrase and topoisomerase IV have been correlated with resistance to fluoroquinolones (Vila et al., 1997), and resistance to tetracyclines has been associated with genes that encode tetracycline-specific efflux pumps (Huys et al., 2005).

Most genes that encode inactivating enzymes and specific efflux pumps are present only in some strains and are associated with genetic elements such as transposons, integrons or plasmids, which suggests they were acquired by horizontal transfer (Lenie et al., 2007). Chromosomal Acinetobacter-derived cephalosporinase (ADC)-type β-lactamases can be upregulated as a consequence of the upstream insertion of a sequence called Insertion Sequence A. baumannii a1 (ISAba1), which provides an efficient promoter (Heritier et al., 2006). This insertion sequence is wider spread in A. baumannii and is thought to serve as a “moving switch” to turn on those genes with which it is juxtaposed (Livermore and Woolford, 2006). ISAba1 is, also, thought to have a key role in some carbapenem-resistant strains by enhancing the expression of the intrinsic OXA-51-like carbapenemases (Turton et al., 2006b). Another chromosomal system that is typical of A. baumannii is the AdeABC efflux system (Magnot et al., 2001). Upregulation of AdeABC is so far the only mechanism that has been proven to decrease susceptibility to multiple antimicrobial classes in A. baumannii.

The diversity of the determinants that confer resistance of A. baumannii to a particular group of antibiotics can be best illustrated by the mechanisms that are associated with carbapenem-resistance (Poirel and Nordmann, 2006). These include metallo-β-lactamases (VIM-, IMP- and SIM-types), which have been reported worldwide and confer resistance to all β-lactams with the exception of monobactams. Nevertheless, the most widespread carbapenemases in A. baumannii are class D β-lactamases. In addition to the intrinsic OXA-51-like enzymes, three unrelated groups of these carbapenem-hydrolyzing oxacillinas have been distinguished, which are represented by OXA-23, -24 and -58, respectively. Reduced susceptibility to carbapenem 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 (Bou et al., 2000).

The complete genomes of MDR and susceptible strains of A. baumannii have been compared. It was found that, the MDR strain contained an 86-kilobase (Kb) genomic region, termed a resistance island (the largest identified in bacteria to date), in which 45 putative resistance genes were clustered, but the susceptible strain exhibited a 20-kilobase genomic island at the homologous location that comprised genes that encode transposases but no resistance genes. It is conceivable that such a genomic structure could serve as a ‘hot spot’ that facilitates the horizontal acquisition of resistance genes and has had a crucial role in the development of A. baumannii multidrug resistance (Fournier et al., 2006).

β-Lactamases

β-lactamases are antibiotic modifying enzymes produced by microorganisms to protect themselves from the effect of the β-lactams. β-lactam antibiotics are commonly inactivated by these enzymes. The group of β-lactamases identified so far in A. baumannii includes more than 50 different enzymes, or their allelic forms (Lenie et al., 2007), and according to their nucleotide sequences, they can be classified in four groups, named class A to class D β-lactamases. Class A, C, and D have a serine at their active site while the class B enzymes have four zinc atoms at the active site (Fluit et al., 2001). Some of these enzymes are intrinsically found in A. baumannii while others have been acquired through natural transformation or by plasmid conjugation (Table 1). Currently, transposons and integrons are very important in the dissemination of these genetic resistance mechanisms (Perez et al., 2007). Different mechanisms of antimicrobial resistance are shown in Figure 1.

Ambler Class A β-lactamases

This group of enzymes includes narrow and Extended-Spectrum β-lactamases (ESBLs; Table 1). Narrow-spectrum enzymes are mainly active against benzylpenicillins. TEM-1 and TEM-2 are active against
aminopenicillins, and β-lactamases active on carbencillins (CARB-5) like carboxypenicillins (Lenie et al., 2007). The ESBLs not only inactivate benzylpenicillins but also some cephalosporins and monobactams (Fluit et al., 2001). Recently, a TEM-92 extended spectrum β-lactamase was detected in an Italian hospital and structural analysis showed that it was associated with a Tn3-like transposon (Endimiani et al., 2007). Finally, CTX-M-2 (Active on cefotaxime) hydrolyzes cefotaxime and ceftriaxone and has been isolated from an epidemic strain in a neurosurgical ward in Japan and in Bolivia.

**Ambler Class B β-lactamases**

Class B metallo-β-lactamases (MBLs) are characterized by the presence of a metal ion in the active site (usually Zinc) and can inactivate penicillins, cephalosporins and carbapenems. In A. baumannii, three groups of acquired MBLs have been identified (Table 1); IMP-like, VIM-like and SIM-1 (Poirel and Nordmann, 2006 and Fluit et al., 2001). IMP MBLs is a group of enzymes mostly detected as part of a class 1 integron. At present, six representatives of this group have been identified: IMP-1, IMP-2, IMP-4 (also identified in A. junii clinical isolates), IMP-5, IMP-6 and IMP-11. VIM and SIM MBLs are two groups of enzymes that are found in A. baumannii. To date, only the VIM-2 and SIM-1 have been reported from Korea (Perez et al., 2007 and Poirel and Nordmann, 2006).

MBLs are most commonly found within integrons, which are special genetic structures that facilitate the acquisition and expression (via a common promoter) of resistance determinants. Most acquired MBL genes in A. baumannii have been found within class 1 integrons, often containing an array of resistance gene cassettes, especially those encoding aminoglycoside-modifying enzymes (Tsakris et al., 2006, Lee et al., 2005, Zarrilli et al., 2004, Houang et al., 2003a and Riccio et al., 2000).

**Ambler Class C β-lactamases**

Class C β-lactamases are chromosomally encoded cephalosporins and carbapenems. In A. baumannii (Table 1). These enzymes hydrolyze penicillins and cephalosporins, but not cepfepime or carbapenems. Phylogenetic analysis suggests that the genes encoding for these enzymes (bla genes) have a common ancestor, and it has been proposed to classify them in a distinct family of β-lactamases: the Acinetobacter-derived cephalosporinases (ADCs), which are intrinsic to A. baumannii (Lenie et al., 2007). To date, 28 blaADC genes have been found in A. baumannii (Perez et al., 2007). Although these genes are normally expressed at low level, their function can be upregulated with the presence of an efficient promoter upstream of the gene (Lenie et al., 2007 and Mahillon and Chandler, 1998).

**Ambler Class D β-lactamases**

The most prevalent carbapenemases in Acinetobacter spp. are the carbapenem-hydrolysing class D β-lactamases (CHDLs); which are divided into four phylogenetic subgroups (Table 1). Subgroup 1 (OXA-23-like) contains the OXA-23, -27 and -49 β-lactamases, and is distributed in Europe and Australia. They are plasmid or chromosomally-encoded, and are associated with the insertion sequences (IS) ISAba1 and ISAba4. Subgroup 2 (OXA-24-like) is composed of OXA-24, -25, -26 and -40. It shares 60% amino acid identity with respect to the subgroup 1, was found in Spain and Belgium and are chromosomally or plasmid-encoded. Subgroup 3 includes the OXA-51 variants, which are intrinsic to A. baumannii. It shares 56% and 63% amino acid identity with subgroups 1 and 2, respectively. Subgroup 3 is globally distributed as they are naturally occurring in A. baumannii, are chromosomal-encoded and are associated with ISAba1. The presence of the insertion sequence ISAba1 upstream of this gene is thought to enhance the expression of the intrinsic OXA-51 carbapenemases. Finally, subgroup 4 holds the recently characterized OXA-58 enzymes that shares 59% amino acid identity with OXA-51 and less than 50% with subgroups 1 and 2. Subgroup 4 was found in France and Spain, are plasmid or chromosomally-encoded and are associated with ISAba1, ISAba2, ISAba3 and IS18 (Peleg et al., 2008, Lenie et al., 2007 and Poirel and Nordmann, 2006).

blaOXA-23 and blaOXA-40 appeared to produce higher Minimum Inhibitory Concentrations (MICs) of imipenem than did blaOXA-58, and all blaOXA genes produced higher MICs of imipenem in the presence of an over-expressed AdeABC efflux pump. The impact of IS elements for carbapenem resistance due to oxacillinases in A. baumannii has only recently been appreciated (Corvec et al., 2007, Turton et al., 2006b and Poirel et al., 2005). These elements provide two main functions. First, they encode a transposase and, therefore, are mobile. Second, they can contain promoter regions that lead to over-expression of downstream resistance determinants. Interestingly, certain IS elements, especially ISAba1, appear relatively unique to A. baumannii (Segal et al., 2005). IS elements are, also, important for the expression of resistance to other antibiotics in A. baumannii (Rui et al., 2007, Ruzin et al., 2007, Poirel et al., 2005b and 2003 and Ribera et al., 2003).

**Multidrug Efflux System**

Efflux Pumps As A Mechanism of Antibiotics Resistance: Bacteria can resist the action of antibiotics through several mechanisms. The transporting systems are one of them (in bacteria efflux pumps). Transporters are present in all organisms; including eukaryotic cells (Van Bambeke et al., 2003a) and could drive various compounds such as physiological substrates, non-antibiotic substrates and antibiotics (different chemical classes) inside (influx) or outside (efflux) the cells (Takeda et al., 1999 and Urakami et al., 1998). Bidirectional transporters have, also, been found and these can take various roles. Bacterial efflux pumps that are involved in clinically relevant resistance to antimicrobial agents, have a role in bacterial pathogenicity e.g. in the colonization and the survival of bacteria in the host (Buckley et al., 2006 and Jerse et al., 2003).
Acinetobacter has an outer membrane and a cytoplasmic membrane between which β-lactamases reside. Penicillin-binding proteins (PBPs), located at the level of the cytoplasmic membrane, constitute the final target of β-lactam antibiotics. To bind to these targets, antibiotics must traverse the outer membrane through porin channels into the periplasmic space. Once in the periplasmic space, β-lactam antibiotics bind to PBPs or are actively expelled from bacterial structure through efflux pumps. Acinetobacter can harbor integrons and transposons, genetic elements on the bacterial chromosome or on plasmids, that can carry multiple cassettes with resistant genes.

Table (1): β-lactamases identified in A. baumannii (Lenie et al., 2007)

<table>
<thead>
<tr>
<th>β-lactamase</th>
<th>Class</th>
<th>Target drug</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM-1</td>
<td>A</td>
<td>Aminopenicillins</td>
<td>Plasmid</td>
</tr>
<tr>
<td>SCO-1</td>
<td>A</td>
<td>Penicillins</td>
<td>Plasmid</td>
</tr>
<tr>
<td>CARB-5</td>
<td>A</td>
<td>Carboxypenicillins</td>
<td>Plasmid or chromosomal</td>
</tr>
<tr>
<td>TEM-92, TEM-116, SHV-12</td>
<td>A</td>
<td>Benzylpenicillins, Cephalosporins, monobactams</td>
<td>Plasmid or chromosomal</td>
</tr>
<tr>
<td>CTX-M-2</td>
<td>A</td>
<td>Cefotaxime, ceftriaxone</td>
<td>Plasmid</td>
</tr>
<tr>
<td>IMP</td>
<td>B</td>
<td>Carbapenems</td>
<td>Class 1 integron</td>
</tr>
<tr>
<td>SIM-1, VIM-2</td>
<td>B</td>
<td>Carbapenems</td>
<td>Class 1 integron</td>
</tr>
<tr>
<td>ADC</td>
<td>C</td>
<td>Cephalosporins</td>
<td>Chromosomal (intrinsic to A. baumannii)</td>
</tr>
<tr>
<td>OXA-23-like</td>
<td>D</td>
<td>Carbapenems</td>
<td>Plasmid</td>
</tr>
<tr>
<td>OXA-24-like</td>
<td>D</td>
<td>Carbapenems</td>
<td>Chromosomal, plasmid</td>
</tr>
<tr>
<td>OXA-51-like</td>
<td>D</td>
<td>Carbapenems</td>
<td>Chromosomal (intrinsic to A. baumannii)</td>
</tr>
<tr>
<td>OXA-58-like</td>
<td>D</td>
<td>Carbapenems</td>
<td>Plasmid or chromosomal</td>
</tr>
</tbody>
</table>
There are five families of efflux-pump proteins that are associated with MDR in bacteria: (1) the multidrug and toxic compound extrusion (MATE) family (Brown et al., 1999), (2) the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, (3) the small multidrug resistance (SMR) family, (4) the major facilitator superfamily (MFS) and (5) the resistance-nodulation-cell division (RND) family. This classification is based on several bases including amino acid sequence homology (Piddok, 2006b), the energy source that the pump uses, the number of components that the pump has (single or multiple), the number of transmembrane-spanning regions and the types of substrate that the pump exports (Piddok, 2006a). Drug efflux pumps are found in Gram-negative and Gram-positive bacteria, but resistance of this type in Gram-negative bacteria is a more complex problem due to the molecular architecture of the cell envelope. Drug resistance in many cases is attributable to synergy between reduced drug intake; mainly due to low outer membrane permeability (Nikaido, 2003) and active drug export (via efflux pumps).

**Resistance Nodulation Cell Division (RND) Superfamily**

Gram-negative bacteria are protected by an outer membrane, therefore efflux transporters of the RND family are organized as three-component systems and are similar in various species. The best studied members of this group are the AcrAB-TolC system of E. coli (Ma et al., 1995) and the MexAB-OprM system of P. aeruginosa. These efflux pumps comprise the following: a transporter (efflux) protein (e.g., AcrB), which is located in the inner (cytoplasmic) membrane; a periplasmic accessory protein (also known as a membrane fusion protein-MFP; e.g., AcrA); and an outer membrane protein (OMP) channel (e.g., TolC), which is located in the outer membranes of Gram-negative bacteria (Eswaran et al., 2004, Koronakis et al., 2004).

Many RND-type systems were described in Gram-negative bacteria, amongst which multidrug-resistant clinical pathogens constitute particularly a problematic group. In some species e.g., P. aeruginosa, there are a few systems described, which can be active at the same time. Clinical isolates of P. aeruginosa can express (over-express) two efflux pumps simultaneously MexAB-OprM and MexXY or MexAB-OprM and MexEF-OprN (Llanes et al., 2004). In A. baumannii, AdeB is the multidrug transporter protein, AdeA is the MFP and AdeC is the OMP. The efflux transporter (AdeB) captures its substrates either from within the phospholipid bilayer of the inner membrane or the cytoplasm (Aires and Nikaido, 2005) and then transports them into the extracellular medium via the OMP (AdeC). The periplasmatic protein AdeA mediates in the cooperation between AdeB and AdeC components.

Figure (2) demonstrates the scheme of the structure of the AdeABC. This system was shown to be responsible for decreased susceptibility to a broad spectrum of antimicrobials. Aminoglycosides, tetracyclines, erythromycin, chloramphenicol, trimethoprim, fluoroquinolones, some β-lactams, ethidium bromide (Vila et al., 2007 and Fournier et al., 2006), and also recently tigecycline, were found to be substrates for AdeABC.

The secondary structure of RND-type efflux proteins was proposed to consist of 12 transmembrane segments (TMS), with two long loops between TMS 1 and 2 and TMS 7 and 8 (Tseng et al., 1999). The trimeric form of the OMP generates a continuous, solvent-accessible channel-like structure that spans both the outer membrane and the periplasmic space. MFP could be involved in either the bringing of the inner and outer membranes closer or the stabilization of the OMP structure (Zgurskaya and Nikaido, 2000). The efflux pump system AdeABC, member of RND family, was identified in a multidrug-resistant A. baumannii strain in 2001 (Magnet et al., 2001). This transporter is the main efflux system in A. baumannii. A lot of pumps from this family are widespread amongst Gram-negative bacteria and are associated with multidrug resistance to antibiotics in important pathogens.

**Genetic Organization, Regulation, Expression And Overexpression of AdeABC Genes System And Function**

The genes that encode AdeABC efflux pump are located on the bacterial chromosome. Typically, the genes are organized as an operon - the structural genes adeA, adeB and adeC are contiguous and directly oriented. The gene encoding the periplasmic accessory protein is located adjacent to the gene encoding transporter protein, which is located adjacent to the OMP. There are two regulatory genes, adeS and adeR, which products are closely related to proteins of two component regulatory system. These genes are transcribed in the opposite direction and are localized upstream from adeA (Figure 3). Two-component system are signal transduction pathways in bacteria that respond to environmental conditions; pump is dependent on substrate (Marchand et al., 2004).

The protein AdeR (regulator) consisted of 228 amino acids is typical transcriptional regulator and protein AdeS (sensor kinase) is shorter and demonstrate activity of bacterial histidine kinase, that work together to regulate target gene expression in response to stimuli. The sensor protein monitors the environmental conditions and activates or inactivates the response regulator protein which controls the expression of the efflux pump (Magnet et al., 2001). Experiments performed by Marchand et al suggest that the adeS gene appears to be essential for expression of the adeABC operon. To assess the role of adeRS, the adeR and adeS genes of A. baumannii BM4454 were disrupted by insertion of a suicide plasmid following homologous recombination. Inactivation of these genes restored the sensitivity to aminoglycosides and to other substrates for the pump in resulting mutants (Marchand et al., 2004).

In contrast, examination of the contribution of the adeC gene to multidrug resistance showed that mutants still had been multidrug resistant and the AdeC protein was not essential. The fact that AdeC is not required for resistance suggests that AdeAB can utilize another outer membrane constituent. The AdeK OMP associated with the AdeJK RND efflux pump could be candidate. Some efflux gene clusters RND proteins do not encode an OMP.
(Mine et al., 1999), but can mobilize other outer membrane channels for creating functional three-component pump. The genetic organizations of the genes encoding these efflux systems are, also, similar among different species (Koronakis et al., 2000).

In the RND family in Acinetobacter spp., other pumps are being enumerated such as AdeDE, AdeIJK and AdeXYZ. The adeB sequence was not detected in any species other than A. baumannii genomic DNA groups, suggesting that adeB is an active efflux system specific to A. baumannii. The adeB gene is present exclusively in A. baumannii, whereas adeE and adeY are present most frequently in A.r lwoffii (Chu et al., 2006). Efflux pump AdeIJK has been identified in A. baumannii. The AdeDE has only the MFP gene adeD and the RND transporter gene adeE clustered together. The OMP for AdeDE has not been identified. AdeABC differs from AdeDE by protecting the host from cefotaxime, whilst AdeDE increases the host resistance to ceftazidime and rifampicin (Chau et al., 2004).


Figure (3): Schematic organization of the ade gene cluster in A. baumannii (Marchand et al., 2004).

REFERENCES


