Antimicrobial Resistance and Clones of *Acinetobacter* Species and *Pseudomonas aeruginosa*

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Antimicrobial resistance in bacteria is problematic in clinical settings and is a growing threat to public health. Multidrug-resistant and pandrug-resistant non-fermenters such as *Acinetobacter* spp. and *Pseudomonas aeruginosa* have recently emerged as a great concern worldwide. Particularly, the prevalence of carbapenem resistance in *Acinetobacter* spp. and *P. aeruginosa* is problematic, and emergence of polymyxin resistance is ominous. In this review, we discuss carbapenem and polymyxin resistance in *Acinetobacter* spp. and *P. aeruginosa* isolates and their major clones.

Key Words: *Acinetobacter*, *Pseudomonas*, Antimicrobial resistance, Carbapenems, Polymyxins

*Acinetobacter* is a Gram-negative coccobacillus, although this morphology is very dependent on its growth phase (1, 2). Originally viewed as a commensal with low virulence in the 1970s, it was often ignored in clinical setting (3). However, *Acinetobacter* spp. have emerged as one of the major causal agents of nosocomial infections associated with significant morbidity and mortality, especially in immunocompromised patients and patients in intensive care units (ICUs) (3). These pathogens are responsible for pneumonia, urinary tract infections (UTIs), skin and soft tissue infections, and bloodstream infections. According to the data from the National Nosocomial Infections Surveillance (NNIS) system, the proportion of *Acinetobacter* species causing ICU pneumonia increased from 1.4% in 1975 to 6.9% in 2003 in the United States. Among *Acinetobacter* species, *A. baumannii* is a representative species involved in hospital-associated infections. However, *A. baumannii* is not easily differentiated from environmental species such as *A. calcoaceticus* and the other two clinically relevant *Acinetobacter* species, *Acinetobacter* genomic species 3 and 13TU, which were recently designated on the basis of phenotypic tests as *A. pittii* and *A. nosocomialis*, respectively (4). They are grouped together and named as *A. calcoaceticus-A. baumannii* (Acb) complex (or *A. baumannii* group). In Korea, another species of Acb complex, *Acinetobacter* genomic species 'close to 13TU', has been identified more frequently than in other countries (5). Recently, the Infectious Diseases Society of America identified *A. baumannii* as one of six particularly problematic pathogens (6).

*Pseudomonas aeruginosa* is also a ubiquitous Gram-negative bacterium present in many diverse environmental settings. The wide metabolic versatility and high intrinsic and acquired resistance to many antimicrobial agents have allowed *P. aeruginosa* to persist in both community and hospital settings (7). It is one of the major organisms responsible for nosocomial infections such as pneumonia, UTIs, surgical site infections, and bloodstream infections (7). Immunosuppressed patients such as those with severe burns, cancer, or acquired immunodeficiency syndrome

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(AIDS) are particularly at risk to *P. aeruginosa* infections. NNIS data from 1986–1998 has identified *P. aeruginosa* as the fifth most frequently isolated nosocomial pathogen (7). *P. aeruginosa* is the second most common cause of healthcare-associated pneumonia including ventilator-associated pneumonia (8). As *P. aeruginosa* as well as *Acinetobacter* spp. cannot ferment glucose and they are closely related phylogenetically, they are frequently classified together as 'non-fermenters'.

**Antimicrobial resistance in *Acinetobacter* spp. and *P. aeruginosa***

Antimicrobial resistance among *Acinetobacter* spp. isolates has increased substantially in recent years (9). The emergence of multidrug-resistant (MDR) *Acinetobacter* spp. isolates has become a serious clinical concern worldwide (3). *A. baumannii* is generally intrinsically resistant to a number of commonly used antimicrobial agents such as aminopenicillins, cephalosporins, and chloramphenicol. In addition, it has shown a remarkable capacity to acquire resistance to broad-spectrum-β-lactams, aminoglycosides, fluoroquinolones, and tetracyclines (10). Such extensive antimicrobial resistance in *A. baumannii* may be due in part to the organism's relatively impermeable outer membrane and its environmental exposure to a large reservoir of resistance genes (11).

Outbreaks by MDR *A. baumannii* isolates have occurred worldwide, and even isolates resistant to most commercially available agents (pandrug resistance, or PDR) are also emerging (3, 12). Of particular concern is resistance to carbapenems such as imipenem and meropenem. Carbapenems are usually recommended as a potent antimicrobial agent against *A. baumannii* infections (13). However, carbapenem resistance in *A. baumannii* is emerging in many parts of the world and the resistance rate has increased to about 30% (14). Thus, few antimicrobial agents can be reliably used for effective therapy against MDR or PDR *Acinetobacter* infections. Although polymyxins such as polymyxin B and colistin have not typically been included in regimens to treat *Acinetobacter* infections since the 1980s because of their neurotoxicity and nephrotoxicity, they are now considered as one of the last resorts against MDR or PDR *Acinetobacter* infections (15, 16). So far, colistin or polymyxin B resistance rates among *Acinetobacter* isolates are very low worldwide (17). However, some investigators have reported the emergence of heteroresistance or resistance to colistin following colistin treatment (18, 19). In addition, high resistance rates against polymyxin B and colistin among *Acinetobacter* isolates from South Korea have been recently reported (20). Even *A. baumannii* isolates showing nonsusceptibilities to all antimicrobials including polymyxins and tigecycline have been found in several countries including South Korea (21, 22).

High mortality in *P. aeruginosa* infections is attributable to the intrinsic resistance to many antimicrobial agents and the development of the MDR phenotype in healthcare settings. The increasing prevalence of MDR among *P. aeruginosa* isolates from ICU patients in the United States - from 4% in 1993 to 14% in 2002 (23) - is noteworthy. As in *A. baumannii* and *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumonia*, carbapenems play a significant role in the treatment of *P. aeruginosa* infections. However, in contrast to *Enterobacteriaceae*, carbapenem resistance is not unusual in *P. aeruginosa*. The rate of imipenem resistance among *P. aeruginosa* isolates has been estimated as 7–23% (7). In South Korea, imipenem-resistant *P. aeruginosa* isolates have increased from 17% in 1997 to 26% in 2009 according to data from the Korean Nationwide Surveillance of Antimicrobial Resistance (KONSAR) program (24).

**Carbapenem resistance in *A. baumannii* and *P. aeruginosa***

Carbapenems such as imipenem and meropenem enter Gram-negative bacteria through outer membrane proteins and acylate the penicillin-binding proteins (PBPs). Carbapenems inhibit the peptidase domain of PBPs and can interfere with peptide cross-linking. As a result, the peptidoglycan is weakened and the cell bursts due to osmotic pressure (25). Carbapenems exhibit an overall broad in
vitro antimicrobial spectrum including Gram-positive and Gram negative bacteria (26, 27). Against Acinetobacter and Pseudomonas infections, carbapenem is the most potent and widely-used agent (10, 28). However, carbapenem resistance in non-fermenting bacteria such as Acinetobacter spp. and P aeruginosa is increasing worldwide and poses a major public health threat. The mechanisms of carbapenem resistance include the production of β-lactamases, efflux pumps, and mutations altering the expression and/or function of porins and PBPs (25).

Genetic and biochemical basis of carbapenem resistance in Acinetobacter spp. have mostly been related to the production of β-lactamases. So far, two intrinsic β-lactamases, AmpC-type cephalosporinase and oxacillinase (OXA-51-like), have been identified in most A. baumannii isolates. However, these intrinsic enzymes are expressed at very low levels and do not enhance the full carbapenem resistance in A. baumannii (29). Instead of these intrinsic β-lactamases, several other acquired β-lactamases have been identified as inducing carbapenem resistance in A. baumannii. These acquired enzymes belong either to the class B enzymes (also known as metallo-β-lactamases, MBLs) or to the class D enzymes (also known as oxacillinases). MBLs such as VIM and IMP confer a high level of carbapenem resistance in A. baumannii isolates, as well as resistance to all β-lactams except aztreonam. However, isolates with SIM-1 can display imipenem minimum inhibitory concentrations (MICs) of 8–16 mg/L. Oxacillinases represented by OXA-23, -24/40, and -58 are able to hydrolyze imipenem, but not always meropenem, and are grouped in a particular subgroup of β-lactamases termed carbapenem-hydrolyzing oxacillinases (CHDLs) (30). Compared with MBLs, the carbapenem resistance level by oxacillinases in A. baumannii is much lower. However, blaOXA-23, blaOXA-58, and blaOXA-58 genes play significant roles in carbapenem resistance (31). In addition, reduced susceptibility to carbapenems has also attributed to the modification of PBPs and porins, or to the up-regulation of the AdeABC efflux system in A. baumannii (29).

So far, MBLs are the major determinants of β-lactamase-mediated resistance to carbapenems in P. aeruginosa. As in A. baumannii, the VIM and IMP enzymes are by far the most common MBLs found in carbapenem-resistant P. aeruginosa isolates (32). While IMP-type MBLs predominate in P. aeruginosa isolates from Asia, VIM-type MBLs are prevalent in Europe (28). However, this distinction is blurred, as both enzymes become disseminated worldwide. In addition to VIM and IMP, GIM-1 has been found in P. aeruginosa isolates from Europe (32). Among the class A β-lactamases (or carbapenemases) such as GES, IMI, KPC, NMC-A, and SME, GES and KPC enzymes have been identified in P. aeruginosa. KPC enzymes showing activity against most β-lactams have primarily been described in Klebsiella pneumoniae, and rarely in P. aeruginosa. For GES, GES-2, and GES-5 have been reported in P. aeruginosa isolates.

Among the five families of efflux pump systems so far described in bacteria, the Resistance Nodulation Division (RND) family is the most significant in the antimicrobial resistance of P. aeruginosa. Of the RND-type efflux pump systems, MexAB-OprM, MexCD-OprJ, and MexXY-OprM contribute to the resistance to carbapenems (7). However, the efflux pump is a minor contributor to carbapenem resistance in P. aeruginosa (28). The most common mechanism of resistance to the carbapenems in P. aeruginosa is loss or alteration of the outer membrane porin protein OprD (35). OprD is the major means for the entry of carbapenems, and inactivation of OprD is the main cause of non-MBL-mediated carbapenem resistance in P. aeruginosa. OprD inactivation frequently operates in conjunction with other mechanisms such as derepressed ampC or MexAB-OprM.

**Polymyxin resistance in Acinetobacter spp. and P. aeruginosa**

Polymyxins (polymyxin B and colistin), which are a group of cyclic decapeptides produced by Bacillus polymyxa, and which have been known since 1949, bind to the anionic bacterial outer membrane, leading to a detergent effect that disrupts membrane integrity (16). They show a high affinity for the lipid moiety of lipopolysaccharide (LPS) and can

preferentially displace Mg$^{2+}$ and Ca$^{2+}$ from cationic binding sites. Colistin was largely replaced by aminoglycosides in the 1970s because of its neurotoxicity and nephrotoxicity (36). However, colistin and polymyxin B are now considered as a therapy of last resort against infections by MDR Gram-negative bacteria, in particular A. baumannii, P. aeruginosa, and K. pneumoniae (36). Colistin resistance rate is now relatively low worldwide, probably due to its low use over the last 50 years. However, colistin (or polymyxin B)-resistant A. baumannii or P. aeruginosa isolates have been identified (20, 37, 38).

In several Gram-negative species, colistin resistance is related to the modification of the lipid A moiety of the LPS outer membrane component. Polymyxin resistance in Salmonella enterica and P. aeruginosa has been linked to the PmrAB and PhoPQ two-component systems, which are involved in modifying the LPS core and lipid A regions with ethanolamine and the addition of aminoarabinose to lipid A (39–41). Recently, mutations in pmrA and pmrB in colistin-resistant derivatives of A. baumannii isolate were identified (42). In addition, it was reported that the complete loss of LPS production may mediate the colistin resistance in A. baumannii (43). Very recently, it was shown that the addition of phosphoethanolamine to lipid A is critical to polymyxin resistance in A. baumannii (44).

In P. aeruginosa, substitution of the LPS lipid A with aminoarabinose contributes to polymyxin resistance (40). This modification is carried out by the products of the araBCADTEF-ugd locus, which is regulated by two-component systems, PmrAB and PhoPQ. It has been reported that mutations in phoQ and pmrB promote the polymyxin B resistance in clinical P. aeruginosa isolates (45, 46). Another two-component system, ParRS, also regulates arnBCADTEF-ugd expression, with a mutation in parR being associated with polymyxin resistance (47). However, the mechanism of polymyxin resistance in A. baumannii and P. aeruginosa is not fully understood.

According to our recent studies on the colistin resistance in A. baumannii and P. aeruginosa, complete correlation among colistin resistance, PmrAB or PhoPQ mutations, and PmrAB or PhoPQ overexpression was not identified (48). Thus, PmrAB or PhoPQ overexpression associated with their amino acid alterations is only partially responsible for colistin resistance.

**MDR clones of A. baumannii and P. aeruginosa**

Based on band pattern typing methods such as amplified fragment length polymorphism (AFLP) and ribotyping, three clones, European clones I, II, and III, have been suggested to be responsible for a majority of hospital outbreaks caused by MDR A. baumannii isolates in European hospitals (49, 50). Recently, it was reported that these European clones have disseminated worldwide, which prompted to be re-designated as Global clones (GCs) or worldwide (WW) lineages I, II, and III (51). Of these, GCs I and II have caused the most outbreaks worldwide. In the multilocus sequence typing (MLST) schemes of Bartual et al. (52), ST92 and its close relatives is the most prevalent clone worldwide including the United States, Europe, and Asia (53–55). Of note, clone ST92 may be responsible for worldwide dissemination of the blaOXA-23 carbapenemase gene in A. baumannii (56, 57).

Also in South Korea, ST92 has been the most frequently identified clone among imipenem-resistant A. baumannii isolates (21). However, it was recently replaced by its single-locus variants, including ST75 and ST138 (5). Although ST75 and ST138 differ from ST92 only in the gpi locus, they showed high resistance rate of carbapenems. Because the gpi locus is as a hot spot of high recombination event (21, 58), a clonal switch of A. baumannii in South Korea is probably due to recombination.

Presumably, P. aeruginosa exhibits a nonclonal epidemic population structure and recombination may be frequent and play a critical role in its evolution (59–61). Thus, a great diversity of STs in MLST has been observed and overlaps between isolates from clinical and environmental sources existed (62). In spite of the nonclonal feature of P. aeruginosa isolates, the emergence, spread, and persistence of a few MDR clones have been observed. One clear example is the MDR O12 clone, a CC/BURST Group (BG) 4. It emerged during the 1980s and includes only clinical
isolates (63). ST111 and ST229 belong to the O12 or BG4 clone (64). CC111, referred as 'Major European MDR clone P12', is also a member of international groups including VIM-2 and VIM-4-producing *P. aeruginosa* isolates (65, 66).

In addition to the O12 clone, the O11 clone is also closely related to epidemic isolates (63). While serotype O12 isolates are a heterogeneous population, serotype O11 isolates often present low diversity (63). The O11 clone is also termed as CC/BURST Group (BG) 11. In particular, CC235, which is the most prevalent clone in nosocomial *P. aeruginosa* isolates, corresponds to this O11 clone. CC235 has been found in many countries such as Austria, Belgium, France, Greece, Hungary, Italy, Japan, Poland, Russia, Serbia, Singapore, Sweden, Turkey, Nigeria, Brazil, and the United States (64, 65). In addition, it was also identified in South Korea (38, 67). In ST235 *P. aeruginosa* isolates, diverse β-lactamases such as VIM, BEL, IMP, OXA, PER, PSE, and SPM have been identified. In a study of *P. aeruginosa* isolates from Mediterranean countries, ST235 was the most common clone and was related to MDR and exoX+/exoU- (61). In particular, the association between ST235 and IMP-6 in South Korea (67, 68) is noteworthy. Because IMP-6 induces high-level meropenem resistance, the combination of the worldwide clone and potent MBL is troubling.

In addition to the two international clones, some CF clones of *P. aeruginosa* such as CC146, CC148, and CC406 have also been reported worldwide since the late 1990s (69). These CF clones often have hypermutable phenotype (i.e., a 'pan-resistant' phenotype), but rarely possess carbapenemases (65). CC277 also has a worldwide distribution and SPM-1-positive isolates have been found in Brazil. CC175 has been identified in many European countries, and VIM-2-producing CC175 isolates have been described in Germany.

The emergence and spread of carbapenem- or polymyxin-resistant *Acinetobacter* spp. and *P. aeruginosa* isolates is a great concern worldwide, especially in South Korea. The understanding of their epidemiology and resistance mechanisms will help to combat the threat posed by antimicrobial resistance.

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