Journal of Bacteriology and Virology 2012. Vol. 42, No. 1 p.1 – 8
http://dx.doi.org/10.4167/jbv.2012.42.1.1

Review Article

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

Ji-Young Lee and Kwan Soo Ko*

Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Korea

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 immuncompromised 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 versability 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

Received: November 21, 2011/ Revised: December 26, 2011
Accepted: January 9, 2012
*Corresponding author: Kwan Soo Ko, PhD. Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea.
Phone: +82-31-299-6223, Fax: +82-31-299-6229
e-mail: ksko@skku.edu

(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 chloroamphenicol. 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, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-58}}$, and bla_{OXA40} 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 MLBs 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 Germany (33), and SPM-1 is prevalent in *P. aeruginosa* isolates from Brazil (34). 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). Colisitn 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 $bla_{\text{OXA-23}}$ 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 were 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.

REFERENCES

1) Munoz-Price LS, Weinstein RA. *Acinetobacter* infection. N Engl J Med 2008;358:1271-81.
2) Kappor R. *Acinetobacter* infection. N Engl J Med 2008; 358:2845-6.
3) Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51:3471-84.
4) Nemec A, Krizova L, Maixnerova M, van der Reijden TJ, Deschaght P, Passet V, *et al*. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). Res Microbiol 2011;162:393-404.
5) Park YK, Jung SI, Park KH, Kim DH, Choi JY, Kim SH, *et al*. Changes in antimicrobial susceptibility and major clones of *Acinetobacter calcoaceticus-baumannii* complex isolates from a single hospital in Korea over 7 years. J Med Microbiol 2012;61:71-9.
6) Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline from the antimicrobial availability task force of the Infectious Diseases Society of America. Clin Infect Dis 2006;42:657-68.
7) Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 2009;22:582 -610.
8) Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005;41:848-54.
9) Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, *et al*. Antimicrobial resistance among Gram-negative bacilli as causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol 2007;

45:3352-9.
10) Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol 2007;5:939-51.
11) Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43:S49-56.
12) Jain R, Danziger LH. Multidrug-resistant *Acinetobacter* infections: an emerging challenge to clinicians. Ann Pharmacother 2004;38:1449-59.
13) Gilad J, Carmeli Y. Treatment options for multidrug-resistant *Acinetobacter* species. Drugs 2008;68:165-89.
14) Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother 2008;52:813-21.
15) Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 2005; 40:1333-41.
16) Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, *et al*. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589-601.
17) Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001-2004). Clin Microbiol Infect 2006;12: 315-21.
18) Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in *Acinetobacter* and its association with previous colistin therapy. Antimicrob Agents Chemother 2008;52:351-2.
19) Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, *et al*. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006;50:2946-50.
20) Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, *et al*. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother 2007;60:1163-7.
21) Park YK, Peck KR, Cheong HS, Chung DR, Song JH, Ko KS. Extreme drug resistance in *Acinetobacter baumannii* infections in intensive care units, South Korea. Emerg Infect Dis 2009;15:1325-7.
22) Doi Y, Husain S, Potoski BA, McCurry KR, Paterson DL. Extensively drug-resistant *Acinetobacter baumannii*. Emerg Infect Dis 2009;15:980-2.
23) Obritsch MD, Fish DN, MacLaren R, Jung R. National surveillance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob Agents Chemother 2004;48:4606-10.
24) Lee K, Kim MN, Kim JS, Hong HL, Kang JO, Shin JH, *et al*. Further increases in carbapenem-, amikacin-, and fluoroquinolone-resistant isolates of *Acinetobacter* spp. and *P. aeruginosa* in Korea: KONSAR Study 2009. Yonsei Med J 2011;52:793-802.
25) Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. Antimicrob Agents Chemother 2011;55:4943-60.
26) Lee H, Ko KS, Song JH, Peck KR. Antimicrobial activity of doripenem and other carbapenems against Gram-negative pathogens from Korea. Microb Drug Resist 2011;17:37-45.
27) Baek JY, Ko KS, Kang CI, Song JH, Peck KR. *In vitro* antibacterial activities of doripenem, imipenem, and meropenem against recent *Streptococcus pneumoniae* isolates. Diagn Microbiol Infect Dis 2011;71:297-300.
28) Poole K. *Pseudomonas aeruginosa*: resistance to max. Front Microbiol 2011;2:65.
29) Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-36.
30) Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect 2002;8: 321-31.
31) Héritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49: 3198-202.
32) Gupta V. Metallo beta-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter* species. Expert Opin Investig Drugs 2008;17:131-43.
33) Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a beta-

lactamase gene, bla_{GIM-1}, encoding a new subclass of metallo-beta-lactamase. Antimicrob Agents Chemother 2004;48:4654-61.
34) Picão RC, Poirel L, Gales AC, Nordmann P. Diversity of beta-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. Antimicrob Agents Chemother 2009;53:3908-13.
35) Wang J, Zhou JY, Qu TT, Shen P, Wei ZQ, Yu YS, *et al*. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Chinese hospitals. Int J Antimicrob Agents 2010; 35:486-91.
36) Nation RL, Li J. Colistin in the 21st century. Curr Opin Infect Dis 2009;22:535-43.
37) Antoniadou A, Kontopidou F, Poulakou G, Koratzanis E, Galani I, Papadomichelakis E, *et al*. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother 2007;59:786-90.
38) Lee JY, Song JH, Ko KS. Identification of nonclonal *Pseudomonas aeruginosa* isolates with reduced colistin susceptibility in Korea. Microb Drug Resist 2011;17: 299-304.
39) Gunn JS. The *Salmonella* PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol 2008;16:284-90.
40) Moskowitz SM, Ernst RK, Miller SI. PmrAB, a two-component regulatory system of *Pseudomonas aeruginosa* that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. J Bacteriol 2004;186:575-9.
41) Sun S, Negrea A, Rhen M, Andersson DI. Genetic analysis of colistin resistance in *Salmonella enterica* serovar Typhimurium. Antimicrob Agents Chemother 2009;53:2298-305.
42) Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, *et al*. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother 2009;53:3628-34.
43) Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, *et al*. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 2010;54:4971-7.
44) Arroyo LA, Herrera CM, Fernandez L, Hankins JV, Trent MS, Hancock RE. The *pmrCAB* operon mediates polymyxin resistance in *Acinetobacter baumannii* ATCC 17978 and clinical isolates through phosphoethanolamine modification of lipid A. Antimicrob Agents Chemother 2011;55:3743-51.
45) Abraham N, Kwon DH. A single amino acid substitution in PmrB is associated with polymyxin B resistance in clinical isolate of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 2009;298:249-54.
46) Barrow K, Kwon DH. Alterations in two-component regulatory systems of *phoPQ* and *pmrAB* are associated with polymyxin B resistance in clinical isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2009;53:5150-4.
47) Muller C, Plésiat P, Jeannot K. A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β-lactams in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2011;55:1211-21.
48) Park YK, Choi JY, Shin D, Ko KS. Correlation between overexpression and amino acid substitution of the PmrAB locus and colistin resistance in *Acinetobacter baumannii*. Int J Antimicrob Agents 2011;37:525-30.
49) Dijkshoorn L, Aucken H, Gerner-Smidt P, Janssen P, Kaufmann ME, Garaizar J, *et al*. Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. J Clin Microbiol 1996;34:1519-25.
50) Van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Baauw A, van den Broek P, *et al*. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res Microbiol 2004;155:105-12.
51) Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233-8.
52) Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez-Valera. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4382-90.

53) Adams-Haduch JM, Onuoha EO, Bogdanovich T, Tian GB, Marschall J, Urban CM, *et al*. Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. J Clin Microbiol 2011; 49:3849-54.
54) Park YK, Jung SI, Park KH, Kim DH, Choi JY, Kim SH, *et al*. Changes in antimicrobial susceptibility and major clones of *Acinetobacter calcoaceticus-baumannii* complex isolates from a single hospital in Korea over 7 years. J Med Microbiol 2012;61:71-9.
55) Runnegar N, Sidjabat H, Goh HM, Nimmo GR, Schembri MA, Paterson DL. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a single institution over a 10-year period. J Clin Microbiol 2010;48:4051-6.
56) Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg Infect Dis 2010;16:35 -40.
57) Fu Y, Zhou J, Zhou H, Yang Q, Wei Z, Yu Y, *et al*. Wide dissemination of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* clonal complex 22 in multiple cities of China. J Antimicrob Chemother 2010;65:644-50.
58) Snitkin ES, Zelazny AM, Montero CI, Stock F, Mijares L, Murray PR, *et al*. Genome-wide recombination drives diversification of epidemic strains of *Acinetobacter baumannii*. Proc Natl Acad Sci U S A 2011;108: 13758-63.
59) Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J Clin Microbiol 2004; 42:5644-9.
60) O'Carroll MR, Syrmis MW, Wainwright CE, Greer RM, Mitchell P, Coulter C, *et al*. Clonal strains of *Pseudomonas aeruginosa* in paediatric and adult cystic fibrosis units. Eur Respir J 2004;24:101-6.
61) Maatallah M, Cheriaa J, Backhrouf A, Iversen A, Grundmann H, Do T, *et al*. Population structure of *Pseudomonas aeruginosa* from five Mediterranean countries: evidence for frequent recombination and epidemic occurrence of CC235. PLoS One 2011;6: e25617.
62) Wiehlmann L, Wagner G, Cramer N, Siebert B, Gudowius P, Morales G, *et al*. Population structure of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 2007;104:8101-6.
63) Pirnay JP, Bilocq F, Pot B, Cornelis P, Zizi M, Van Eldere J, *et al*. *Pseudomonas aeruginosa* population structure revisited. PLoS One 2009;4:e7740.
64) Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 2011;35:736-55.
65) Edelstein M. Epidemic clones and dissemination of carbapenem resistance. 2011 ICAAC, p. 173.
66) Samuelsen O, Toleman MA, Sundsfjord A, Rydberg J, Leegaard TM, Walder M, *et al*. Molecular epidemiology of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolates from Norway and Sweden shows import of international clones and local clonal expansion. Antimicrob Agents Chemother 2010;54:346-52.
67) Seok Y, Bae IK, Jeong SH, Kim SH, Lee H, Lee K. Dissemination of IMP-6 metallo-β-lactamase-producing *Pseudomonas aeruginosa* sequence type 235 in Korea. J Antimicrob Chemother 2011;66:2791-6.
68) Lee JY, Lim MH, Heo ST, Ko KS. Repeated isolation of *Pseudomonas aeruginosa* isolates resistant to both polymyxins and carbapenems from one patient. Diagn Microbiol Infect Dis (In press).
69) Cheng K, Smyth RL, Govan JR, Doherty C, Winstanley C, Denning N, *et al*. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. Lancet 1996;348:639-42.