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Molecular Characterization of *Pseudomonas aeruginosa* Isolates Resistant to All Antimicrobial Agents, but Susceptible to Colistin, in Daegu, Korea

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Multi-drug resistant *Pseudomonas aeruginosa* has been implicated in a variety of serious therapeutic problems in clinical environments. Among the 968 *P. aeruginosa* isolates obtained from two hospitals in Daegu, Korea, we acquired 17 isolates that were resistant to all available tested antimicrobial agents, with the exception of colistin (colistin-only sensitive). We characterized the antimicrobial susceptibilities, metallo-β-lactamases, and epidemiological relatedness among the colistin-only sensitive *P. aeruginosa* isolates. All colistin-only sensitive isolates were positive in the modified Hodge test and imipenem-EDTA synergy test, thereby indicating the production of metallo-β-lactamases. 11 isolates from the secondary hospital and six isolates from the tertiary teaching hospital harbored *bla*$_{VIM-2}$ and *bla*$_{IMP-1}$, respectively. The pulsed-field gel electrophoretic analysis of the *Spe*I-digested DNA from *P. aeruginosa* isolates indicated that two different clones of colistin-only sensitive *P. aeruginosa* originated from each hospital, and had spread within the hospital environment. Overall, colistin-only sensitive *P. aeruginosa* was detected in Korea for the first time, but no pan-drug resistant bacteria were identified. Nationwide surveillance is required in order to monitor the emergence of colistin-only sensitive or pan-drug resistant bacteria.

Keywords: antimicrobial resistance, carbapenem, colistin, metallo-β-lactamase, clone

Pseudomonas aeruginosa is one of the principal causes of nosocomial pathogen, particularly among immunocompromised patients. They induce a variety of human infections, including bacteremia, respiratory infections, genitourinary tract infections, and wound infections (Jarvis and Martone, 1992). Nosocomial infections caused by *P. aeruginosa* poses a serious problem in clinical settings due to the high prevalence of infection, particularly in intensive care units and cases of multi-drug resistance (Hancock, 1998; Bush, 2001; Falagas *et al.*, 2005; Rossolini and Mantengoli, 2005; Lagatolla *et al.*, 2006).

Carbapenems, most notably imipenem and meropenem, have been recognized as the most potent β-lactams against multi-drug resistant Gram-negative non-fermenting bacteria, as a consequence of their high affinity for penicillin-binding proteins, stability against extended-spectrum β-lactamases, and marked permeability across outer bacterial membranes (Livermore, 1995; Livermore, 2001). However, the extensive use of carbapenems has facilitated the emergence of carbapenem-resistant bacteria. The production of the β-lactamases responsible for carbapenem hydrolysis (Ambler class B metallo-β-lactamases and class D OXA-type β-lactamases), the reduced uptake of the drugs by absence or low OprD protein expression, and increased efflux pump expression are all basic mechanisms of resistance to carbapenems utilized by *P. aeruginosa* (Rasmussen and Bush, 1997; Poirel and Nordmann, 2006; Ohara *et al.*, 2007). Metallo-β-lactamases hydrolyze virtually all metallo-β-lactams, with the exception of monobactam, and are insensitive to clinically available inhibitors including clavulanic acid, sulbactam, and tazobactam (Rasmussen and Bush, 1997; Poirel *et al.*, 2000; Murphy *et al.*, 2003). IMP and VIM are two common metallo-β-lactamases, and several new types of metallo-β-lactamases--SIM, SPM, and GIM--have recently been added to this group (Poirel *et al.*, 2001; Murphy *et al.*, 2003; Castanheira *et al.*, 2004; Lee *et al.*, 2005). Among the three metallo-β-lactamases detected in Gram-negative non-fermenting bacteria in Korea, IMP-1, VIM-2, and SIM-1, VIM-2 proved to be the most frequently detected metallo-β-lactamase among *P. aeruginosa*, *Acinetobacter* spp., and *Klebsiella pneumoniae*. IMP-1 was identified in *P. aeruginosa* and *Acinetobacter* spp. SIM-1 was only rarely detected in *Acinetobacter* spp. (Oh *et al.*, 2003; Yong *et al.*, 2006).

P. aeruginosa, *Acinetobacter* spp., and *K. pneumoniae* are notorious bacterial species, which are known to readily develop multi-drug resistance to various classes of antimicrobial agents. The extensive use of antimicrobial agents and the evolutionary antimicrobial resistance strategies of bacteria have resulted in the emergence of pan-drug resistant bacteria, i.e., bacteria which evidence resistance against anti-pseudomonal penicillins, cephalosporins, carbapenems, monobactam, aminoglycosides, fluoroquinolone, and polymyxins

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in *P. aeruginosa*. Pan-drug resistant bacterial infections could cause global health problems, in which clinicians may, in the worst case scenario, be left with no rational antimicrobial treatment choice. Currently, pan-drug resistant bacteria are relatively rare in the literature relevant to antimicrobial susceptibilities (Falagas and Bliziotis, 2007).

We have previously described a single clinical isolate of *A. baumannii* that was resistant to all available antimicrobial agents tested, but susceptible to colistin (colistin-only sensitive) in Daegu, Korea (Lee *et al.*, 2007). However, there have, thus far, been no reports regarding the emergence of colistin-only sensitive or pan-drug resistant *P. aeruginosa* in Korea. In a study involving the surveillance of antimicrobial susceptibilities of *P. aeruginosa* isolates from two hospitals in Daegu, Korea, we screened 17 colistin-only sensitive *P. aeruginosa* isolates. As only limited data are currently available, we evaluated the antimicrobial susceptibilities, carbapenem resistance mechanisms, and epidemiological relatedness among the colistin-only sensitive *P. aeruginosa* isolates.

Materials and Methods

Bacterial strains

A total of 968 *P. aeruginosa* isolates, 306 from a secondary hospital and 662 from a tertiary teaching hospital, were collected from two hospitals located in Daegu, Republic of Korea, from January 2004 to November 2006. Only the first isolate from the patients was included. Species identification and routine antibiograms of the isolates were conducted using the Vitek System (bioMerieux, France) in a clinical microbiology laboratory. The antimicrobial agents utilized in routine antimicrobial susceptibility tests included β-lactams (ampicillin and piperacillin), β-lactams and β-lactam inhibitors (ampicillin/sulbactam, ticarcillin/clavulanic acid, and piperacillin/tazobactam), cephalosporins (ceftriaxone, ceftazidime, and cefepime), carbapenems (imipenem), monobactam (aztreonam), aminoglycosides (amikacin, gentamicin, and tobramycin), and fluoroquinolones (ciprofloxacin). Among the 968 isolates *P. aeruginosa*, 17 isolates, 11 from a secondary hospital and 6 from a tertiary teaching hospital, were all found to be resistant to the tested antimicrobial agents. These multi-drug resistant *P. aeruginosa* isolates were investigated further in this study.

Antimicrobial susceptibility test

For the analysis of the minimal inhibitory concentrations (MICs) of antimicrobial agents and the screening of pan-drug resistant *P. aeruginosa*, an antimicrobial susceptibility test was conducted by agar dilution in Mueller-Hinton agar (Difco, USA) in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were utilized as quality control strains. The final concentrations of antimicrobial agents ranged from 0.25 to 128 μg/ml. The antimicrobial agents tested herein included piperacillin, ampicillin-sulbactam, cefotaxime, cefoperazone, ceftazidime, cefepime, imipenem, aztreonam, amikacin, tobramycin, ciprofloxacin, and colistin.

Screening of carbapenemase activity

In order to analyze carbapenemase production, colistin-only sensitive *P. aeruginosa* isolates were screened by a modified Hodge test (Lee *et al.*, 2001). The surface of a Muller-Hinton agar plate was inoculated with an overnight culture suspension of *Escherichia coli* ATCC 25922. An imipenem disk was positioned at the center of the plate, and imipenem-resistant *P. aeruginosa* was streaked heavily from the edge of the disk to the periphery of the plate. The presence of a distorted inhibition zone after overnight incubation was interpreted as a positive result. An imipenem-EDTA double disk synergy test was performed to screen for metallo-β-lactamase production. Overnight cultures of the modified Hodge test-positive strains were inoculated on Muller-Hinton agar plates. The imipenem disk (30 μg) and a blank filter paper disk were placed 15 mm apart from edge to edge, and 1.5 mg of EDTA solution was applied to the blank disk. After overnight incubation, the presence of an enlarged zone of inhibition was interpreted as a positive result, thereby indicating the inactivation of metallo-β-lactamase activity by EDTA.

PCR amplification and sequencing

PCR was conducted in a total volume of 20 μl containing the following: 2 μl of boiled bacterial suspensions, 20 pM of each primer, 250 μM of dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$ and 1.5 U of *Taq* DNA polymerase (Bioneer, Korea). Genes coding for carbapenemases were determined by PCR using primers specific for bla_{IMP-1}; 5'-CAT GGT TTG GTG GTT CTT GT-3' and 5'-ATA ATT TGG CGG ACT TTG GC-3' and bla_{VIM-2}; 5'-ATT GGT CTA TTT GAC CGC GTC-3' and 5'-TGC TAC TCA ACG ACT GAG CG-3' (Jeon *et al.*, 2005). The PCR products were sequenced using an automated sequencer (ABI 3100, Applied Biosystems, USA).

Pulsed-field gel electrophoresis

Genomic DNA was digested with *Spe*I (Boehringer Mannheim) and separated on 1.0% agarose gel using a contour-clamped homogeneous-field apparatus (CHEF DRIII systems, Bio-Rad Laboratories, USA) in a 0.5× TBE buffer (Grundmann *et al.*, 1995). The banding patterns were then analyzed using GelCompar II software (Applied Maths, Belgium).

Results

Antimicrobial susceptibility of P. aeruginosa isolates

We first evaluated the antimicrobial susceptibility data obtained from the clinical microbiological laboratories. Over 50% of the isolates were found to be resistant to ampicillin (95.1%), ampicillin-sulbactam (94.8%), ceftriaxone (77.5%), ticarcillin-clavulanic acid (63.4%), and ciprofloxacin (50.3%). More than 30% of the isolates were resistant to aztreonam (45.8%), ceftazidime (41.3%), piperacillin (38.6%), gentamicin (38.2%), imipenem (36.9%), and cefepime (35.0%). Resistances against tobramycin (27.1%), piperacillin-tazobactam (23.7%), and amikacin (20.0%) were observed in <30% of the isolates. Among the 968 *P. aeruginosa* isolates, 17 were resistant to all tested antimicrobial agents, including β-lactams, cephalosporins, carbapenems, monobactam, fluo-

roquinolones, and aminoglycosides. However, pan-drug resistance of the isolates could not be determined due to the absence of colistin susceptibility in the clinical laboratory data.

We then conducted antimicrobial susceptibility tests of 17 multi-drug resistant *P. aeruginosa* isolates against 12 selected antimicrobial agents, including colistin. The MIC distributions of the *P. aeruginosa* isolates are provided in Table 1. The 17 *P. aeruginosa* isolates proved resistant to all tested anti-

Table 1. MIC distribution of colistin-only sensitive *P. aeruginosa* isolates

Isolate no.	MIC (μg/ml)[a]											
	PIP	SAM	CTX	CEP	CFZ	CFP	IMP	AZT	AMK	TOB	CIP	COL
05FP82	> 128	> 128	> 128	> 128	64	128	> 128	64	> 128	> 128	> 128	1
05FP849	> 128	> 128	> 128	> 128	> 128	128	> 128	128	> 128	> 128	128	1
05FP1063	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	128	1
05FP1066	> 128	> 128	> 128	> 128	64	64	> 128	32	> 128	> 128	128	1
05FP1337	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	128	1
05FP1368	> 128	> 128	> 128	> 128	64	64	> 128	64	> 128	> 128	> 128	1
05FP1563	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	128	1
05FP1639	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	128	1
05FP1686	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	> 128	1
05FP1811	> 128	> 128	> 128	> 128	128	128	64	128	> 128	> 128	32	1
05FP1878	> 128	> 128	> 128	> 128	128	128	> 128	64	> 128	> 128	128	0.5
06KP117	> 128	> 128	> 128	> 128	> 128	> 128	32	64	> 128	> 128	32	1
06KP130	> 128	> 128	> 128	> 128	> 128	> 128	32	32	> 128	> 128	32	1
06KP131	> 128	> 128	> 128	> 128	> 128	> 128	32	64	> 128	> 128	32	1
06KP156	> 128	> 128	> 128	> 128	> 128	> 128	16	32	> 128	> 128	32	2
06KP251	> 128	> 128	> 128	> 128	> 128	> 128	32	64	> 128	> 128	32	1
06KP314	> 128	> 128	> 128	> 128	> 128	> 128	32	64	> 128	> 128	32	1

[a]Abbreviations and resistance criteria: PIP, piperacillin (≥128 μg/ml); SAM, ampicillin-sulbactam (≥32/16 μg/ml); CTX, cefotaxime (≥64 μg/ml); CEP, cefoperazone (≥64 μg/ml); CFZ, ceftazidime (≥32 μg/ml); CFP, cefepime (≥32 μg/ml); IMP, imipenem (≥16 μg/ml); AZT, aztreonam (≥32 μg/ml); AMK, amikacin (≥64 μg/ml); TOB, tobramycin (≥16 μg/ml); CIP, ciprofloxacin (≥4 μg/ml); and COL, colistin (≥4 μg/ml).

Table 2. Characterization of carbapenem resistance and clonal relatedness of colistin-only sensitive *P. aeruginosa* isolates

Isolate no.	Isolation hospital	Carbapenemases			PFGE patterns
		Modified Hodge test	Imipenem-EDTA test	Gene	
05FP82	Secondary	+	+	VIM-2	A2
05FP849	Secondary	+	+	VIM-2	A1
05FP1063	Secondary	+	+	VIM-2	A2
05FP1066	Secondary	+	+	VIM-2	A2
05FP1337	Secondary	+	+	VIM-2	A2
05FP1368	Secondary	+	+	VIM-2	A1
05FP1563	Secondary	+	+	VIM-2	A1
05FP1639	Secondary	+	+	VIM-2	A1
05FP1686	Secondary	+	+	VIM-2	A1
05FP1811	Secondary	+	+	VIM-2	A1
05FP1878	Secondary	+	+	VIM-2	A1
06KP117	Tertiary	+	+	IMP-1	B1
06KP130	Tertiary	+	+	IMP-1	B2
06KP131	Tertiary	+	+	IMP-1	B1
06KP156	Tertiary	+	+	IMP-1	B1
06KP251	Tertiary	+	+	IMP-1	B1
06KP314	Tertiary	+	+	IMP-1	B1

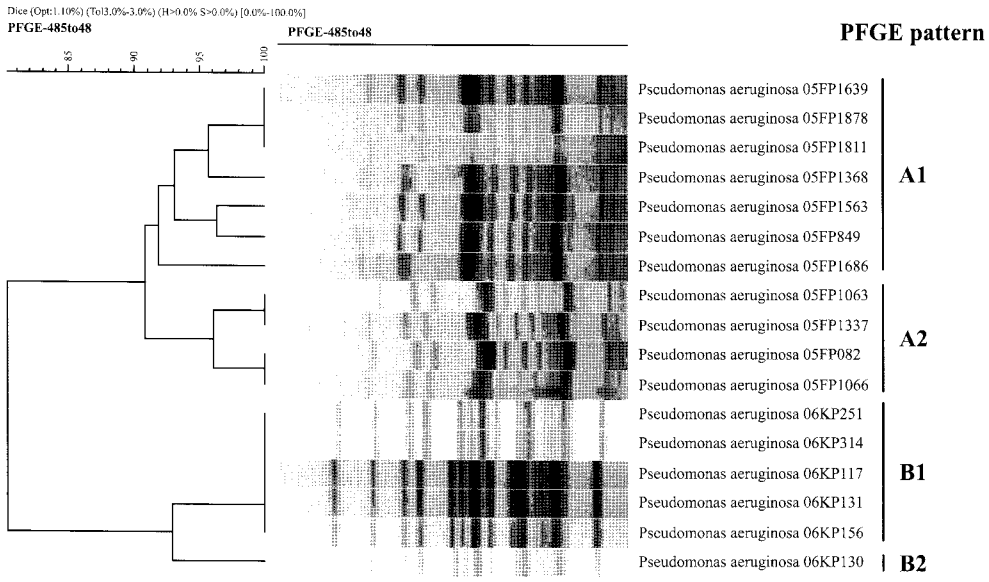

Fig. 1. Clustering of colistin-only sensitive *P. aeruginosa* isolates based on PFGE profiles. The 17 colistin-only sensitive *P. aeruginosa* isolates were compared. The dendrogram is based on cluster analysis by the unweighted-pair group method with average linkages.

microbial agents, but were susceptible to colistin. The MICs of colistin-only sensitive *P. aeruginosa* isolates to piperacillin, ampicillin-sulbactam, cefotaxime, cefoperazone, amikacin and tobramycin were ≥128 μg/ml. The range of MIC to colistin in *P. aeruginosa* was found to be 0.5-2.0 μg/ml. Overall, no pan-drug resistant *P. aeruginosa* isolates were detected, but colistin-only sensitive *P. aeruginosa* isolates emerged in two hospitals located in Daegu, Korea.

Production of metallo-β-lactamases

We evaluated metallo-β-lactamase production in the 17 colistin-only sensitive isolates. All tested *P. aeruginosa* isolates were positive both in the modified Hodge test and imipenem-EDTA double disk synergy test, thereby indicating metallo-β-lactamase production (Table 2). PCR amplification and subsequent sequencing were conducted in order to characterize the metallo-β-lactamases. The 11 *P. aeruginosa* isolates obtained from the secondary hospital harbored bla_{VIM-2} metallo-β-lactamase, whereas the six isolates from the tertiary teaching hospital harbored bla_{IMP-1} β-lactamase (Table 2).

Clustering analysis of PFGE pattern and molecular epidemiology

PFGE was performed in order to determine the clonal relatedness of colistin-only sensitive *P. aeruginosa* isolates. The 17 colistin-only sensitive *P. aeruginosa* isolates were classified into two PFGE groups at a similarity value of 0.82 (Fig. 1). However, 11 bla_{VIM-2}-harboring *P. aeruginosa* isolates obtained from the secondary hospital were subclassified into two PFGE groups (Table 2, Fig. 1) at a similarity value of 0.91. The high degree of similarity of PFGE profiles A1 (n=7) and A2 (n=4) suggests that they originated from identical clones. Among the six colistin-only sensitive *P. aeruginosa* isolates obtained from the tertiary teaching hospital, five of the isolates evidenced identical PFGE patterns. The PFGE pattern of *P. aeruginosa* 06KP130 evidenced three band differences as compared with those of the other five isolates from the tertiary teaching hospital, thereby suggesting that they are genetically closely related (Tenover *et al.*, 1995).

Discussion

In this study, we have evaluated the antimicrobial susceptibilities of *P. aeruginosa* isolates obtained from two hospitals in Daegu, Korea, and have characterized the carbapenem resistance mechanisms and epidemiological relatedness of the colistin-only sensitive isolates. No pan-drug resistant *P. aeruginosa* were detected. However, to the best of our knowledge, the present study is the first to demonstrate the existence of colistin-only sensitive *P. aeruginosa* isolates in Korea. Furthermore, two specific colistin-only sensitive *P. aeruginosa* clones that had originated from each hospital were detected.

We first screened the antimicrobial resistance properties of 968 *P. aeruginosa* isolates to various classes of antimicrobial agents. More than 30% of *P. aeruginosa* isolates proved resistant to the currently utilized antimicrobial agents, which include the anti-pseudomonal β-lactams, the third and fourth generations of cephalosporins, and the fluoroquinolones. Resistance to carbapenems, which constitute the first choice for the treatment of multi-drug resistant *P. aeruginosa* infection, was detected in 37% of *P. aeruginosa* isolates. Amikacin was the most active agent *in vitro*, but the aminoglycosides could not be utilized to treat infected patients as a monotherapy. These results indicate that the choices for antimicrobial therapy for the treatment of multi-drug resistant *P. aeruginosa* infections are currently rather limited. Recently, older antimicrobial agents, such as colistin, were re-utilized to treat multi-drug resistant or carbapenem-resistant Gram-negative

bacteria. The re-emergence of colistin for the treatment of multi-drug resistant bacterial infections contributed to the emergence of pan-drug resistant bacteria against all classes of currently available antimicrobial agents. Thus far, three cases of pan-drug resistant bacterial infections by *P. aeruginosa* and *K. pneumoniae* have been reported (Giamarellos-Bourboulis *et al.*, 2003; Landman *et al.*, 2005; Gales *et al.*, 2006; Falagas and Bliziotis, 2007). In the current study, no pan-drug resistant *P. aeruginosa* isolates were detected, but 17 colistin-only sensitive isolates, 11 from a secondary hospital and 6 from a tertiary teaching hospital, were detected.

Carbapenems have been extensively utilized for the treatment of multi-drug resistant Gram-negative non-fermenting bacterial infections in clinical environments. The extensive use of carbapenems may have facilitated the rapid emergence of resistance and select carbapenem-resistant bacteria in clinical environments. The acquisition of metallo-β-lactamases was shown to be responsible for high levels of carbapenem resistance in many cases. Among the carbapenem-resistant *P. aeruginosa* isolates discovered in Korea, VIM-2 was the most prevalent metallo-β-lactamase, whereas IMP-1 was detected only rarely (Oh *et al.*, 2003; Yong *et al.*, 2006). In the current study, all colistin-only sensitive *P. aeruginosa* isolates were shown to harbor metallo-β-lactamases. The colistin-only sensitive *P. aeruginosa* isolates obtained from the secondary hospital evidenced a high MIC of imipenem and harbored VIM-2 metallo-β-lactamase, whereas the majority of colistin-only sensitive *P. aeruginosa* isolates obtained from the tertiary teaching hospital evidenced a relatively low MIC of imipenem and harbored IMP-1 metallo-β-lactamase. As all of the IMP-1-generating *P. aeruginosa* isolates were collected between February and June of 2006, this is the first reported case of an outbreak of IMP-1-producing *P. aeruginosa* in Korea. Furthermore, the colistin-only sensitive *P. aeruginosa* isolates obtained from two hospitals manifested different PFGE patterns, according to the isolation characteristics of the respective hospitals. These results indicate that *P. aeruginosa* from each hospital acquired different metallo-β-lactamases independently and spread clonally throughout the hospital environments.

In conclusion, two different colistin-only sensitive *P. aeruginosa* clones were detected in hospitals located in Daegu, Korea. Colistin-only sensitive *P. aeruginosa* isolates acquired from two hospitals were shown to possess different carbapenem resistance mechanisms and PFGE patterns, according to the isolation characteristics of the hospitals. This study was the first to demonstrate a specific IMP-1-producing *P. aeruginosa* clone in Korea. Colistin-based combination therapy has been utilized in the treatment of carbapenem-resistant *P. aeruginosa* and *Acinetobacter* infections in Korea. The high frequency of colistin-based therapy may contribute to the emergence of pan-drug resistant bacteria in the near future. Accordingly, nationwide surveillance will be required to monitor the emergence of pan-drug resistant bacteria in Korea in the future.

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References

Bush, K. 2001. New beta-lactamases in Gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin. Infect. Dis.* 32, 1085-1089.

Castanheira, M., M.A. Toleman, R.N. Jones, F.J. Schmidt, and T.R. Walsh. 2004. Molecular characterization of a β-lactamase gene, bla_{GIM-1}, encoding a new subclass of metallo-β-lactamase. *Antimicrob. Agents Chemother.* 48, 4654-4661.

Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M100-S16. Clinical and Laboratory Standards Institute, Wayne, Pa, USA.

Falagas, M.E. and I.A. Bliziotis. 2007. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? *Int. J. Antimicrob. Agents* 29, 630-636.

Falagas, M.E., I.A. Bliziotis, S.K. Kasiakou, G. Samonis, P. Athanassopoulou, and A. Michalopoulos. 2005. Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. *BMC Infect. Dis.* 5, 24-30.

Gales, A.C., R.N. Jones, and H.S. Sader. 2006. 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.* 12, 315-321.

Giamarellos-Bourboulis, E.J., H. Sambatakou, I. Galani, and H. Giamarellou. 2003. *In vitro* interaction of colistin and rifampin on multidrug-resistant *Pseudomonas aeruginosa*. *J. Chemother.* 15, 235-238.

Grundmann, H., C. Schneider, D. Hartung, F.D. Daschner, and T.L. Pitt. 1995. Discriminatory power of three DNA-based typing techniques for *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* 33, 528-534.

Hancock, R.E. 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative Gram-negative bacteria. *Clin. Infect. Dis.* 27 (Suppl. 1), S93-S99.

Jarvis, W.R. and W.J. Martone. 1992. Predominant pathogens in hospital infections. *J. Antimicrob. Chemother.* 29 (Suppl. A), 19-24.

Jeon, B.C., S.H. Jeong, I.K. Bae, S.B. Kwon, K. Lee, D. Young, J.H. Lee, J.S. Song, and S.H. Lee. 2005. Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing OXA-23 β-lactamase in Korea. *J. Clin. Microbiol.* 43, 2241-2245.

Lagatolla, C., E. Edalucci, L. Dolzani, M.L. Riccio, F. De Luca, E. Medessi, G.M. Rossolini, and E.A. Tonin. 2006. Molecular evolution of metallo-β-lactamase-producing *Pseudomonas aeruginosa* in a nosocomial setting of high-level endemicity. *J. Clin. Microbiol.* 44, 2348-2353.

Landman, D., S. Bratu, M. Alam, and J. Quale. 2005. Citywide emergence of *Pseudomonas aeruginosa* strains with reduced susceptibility to polymyxin B. *J. Antimicrob. Chemother.* 55, 954-957.

Lee, J.H., C.H. Choi, H.Y. Kang, J.Y. Lee, J. Kim, Y.C. Lee, S.Y. Seol, D.T. Cho, K.W. Kim, D.Y. Song, and J.C. Lee. 2007. Differences in phenotypic and genotypic traits against antimicrobial agents between *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU. *J. Antimicrob. Chemother.* 59, 633-639.

Lee, K., Y. Chong, H.B. Shin, Y.A. Kim, D. Yong, and J.H. Yum. 2001. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin. Microbiol. Infect.* 7, 88-91.

Lee, K., J.H. Yum, D. Yong, H.M. Lee, H.D. Kim, J.D. Docquier, G.M. Rossolini, and Y. Chong. 2005. Novel acquired metallo-β-lactamase gene, bla_{SIM-1}, in a class 1 integron from *Acine-*

tobacter baumannii clinical isolates from Korea. *Antimicrob. Agents Chemother.* 49, 4485-4491.

Livermore, D.M. 1995. β-Lactamase in laboratory and clinical resistance. *Clin. Microb. Rew.* 8, 557-584.

Livermore, D.M. 2001. Of *Pseudomonas*, porins, pumps and carbapenems. *J. Antimicrob. Chemother.* 47, 247-250.

Murphy, T.A., A.M. Simm, M.A. Toleman, R.N. Jones, and T.R. Walsh. 2003. Biochemical characterization of the acquired metallo-β-lactamase SPM-1 from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 47, 582-587.

Oh, E.J., S. Lee, Y.J. Park, J.J. Park, K. Park, S.I. Kim, M.W. Kang, and B.K. Kim. 2003. Prevalence of metallo-β-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a Korean University Hospital and comparison of screening methods for detecting metallo-β-lactamase. *J. Microbiol. Methods* 54, 411-418.

Ohara, M., S. Kouda, M. Onodera, Y. Fujiue, M. Sasaki, T. Kohara, S. Kashiyama, S. Hayashida, M. Kadono, H. Komatsuzawa, N. Gotoh, T. Usui, H. Itaha, M. Kuwabara, T. Yokoyama, and M. Sugai. 2007. Molecular characterization of imipenem-resistant *Pseudomonas aeruginosa* in Hiroshima, Japan. *Microbiol. Immunol.* 51, 271-277.

Poirel, L., T. Lambert, S. Turkoglu, E. Ronco, J. Gaillard, and P. Nordmann. 2001. Characterization of class 1 integrons from *Pseudomonas aeruginosa* that contain the bla_{VIM-2} carbapenem-hydrolyzing β-lactamase gene and of two novel aminoglycoside resistance gene cassettes. *Antimicrob. Agents Chemother.* 45, 546-552.

Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.D. Cavallo, and P. Nordmann. 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob. Agents Chemother.* 44, 891-897.

Poirel, L. and P. Nordmann. 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin. Microbiol. Infect.* 12, 826-836.

Rasmussen, B.A. and K. Bush. 1997. Carbapenem-hydrolyzing β-lactamases. *Antimicrob. Agents Chemother.* 41, 223-232.

Rossolini, G.M. and E. Mantengoli. 2005. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clin. Microbiol. Infect.* 11 (Suppl. 4), 17-32.

Tenover, F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* 33, 2233-2239.

Yong, D., Y.S. Choi, K.H. Roh, C.K. Kim, Y.H. Park, J.H. Yum, K. Lee, and Y. Chong. 2006. Increasing prevalence and diversity of metallo-β-lactamases in *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* from Korea. *Antimicrob. Agents Chemother.* 50, 1884-1886.