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Special Contribution

How Can Antimicrobial Resistance in *Pseudomonas aeruginosa* Be Controlled?

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***Pseudomonas aeruginosa* remains one of the most difficult to treat and to control nosocomial infections. In vitro antimicrobial susceptibility data are required for successful therapy because acquired resistance to such antimicrobials as β-lactams, fluoroquinolones and aminoglycosides is so prevalent in *P. aeruginosa*. Strategies for controlling *P. aeruginosa* infections include early detection of *P. aeruginosa* as the causative pathogen, determination of its antimicrobial susceptibilities, initiation of effective and adequate therapy and strict infection control practice such as hand hygiene and equipment procedures. Once antimicrobial therapy has been initiated against a *P. aeruginosa* infection, its susceptibility to antimicrobials, especially to carbapenems and fluoroquinolones, should be monitored during antimicrobial therapy to detect clonal shifts in resistance and microbial substitutions as early as possible. Continued surveillance of nosocomial infections and monitoring of antimicrobial resistance by the infection control staff plays major roles in preventing nosocomial infections and the spread of antimicrobial resistance. Additional strategies for controlling antimicrobial resistance in *P. aeruginosa* include the development of new methods for rapid detection of antimicrobial resistance and new agents and vaccines against *P. aeruginosa* infections in the laboratories and pharmaceuticals, while preserving the efficacy of currently available antimicrobials for as long as possible in the hospital settings.**

Key words: antimicrobial resistance; antimicrobial therapy; infection control; nosocomial infection; *Pseudomonas aeruginosa*

Pseudomonas aeruginosa as a pathogen

Pseudomonas aeruginosa is a non-fermentative, aerobic, Gram-negative rod that normally lives in moist environments. *P. aeruginosa* is an opportunistic human pathogen and causes pneumonia, urinary tract infections, wound infections and blood stream infections. *P. aeruginosa* has minimal nutritional requirements, which contributes to its broad ecological adaptability and distribution. Water in flower vases, showers and toilets, disinfectant solutions, uncooked vegetables, skin, respiratory equipment and other moist environments can act as reservoirs of *P. aeruginosa* in the hospital settings (Pier et al., 2004; Rossolini and Mantengoli, 2005). Consequently, *P. aeruginosa* is a common nosocomial pathogen and often is the pathogen in cases of ventilator-associated pneumonia, catheter-related urinary tract infections and catheter-related blood stream infections. Community-acquired infections by *P. aeruginosa* are uncommon.

Abbreviations: AAC, aminoglycoside acetyltransferase; ANT, aminoglycoside nucleotidyltransferase; APH, aminoglycoside phosphoryltransferase; MIC, minimum inhibitory concentration; MDRP, multidrug-resistant *Pseudomonas aeruginosa*; OMP, outer membrane protein; QRDR, quinolone resistance-detremining region; RT, reverse transcription; WHO, World Health Organization

Antimicrobial resistance in *P. aeruginosa*

It is difficult to treat *P. aeruginosa* infections because acquired resistance to such antimicrobials as β-lactams, fluoroquinolones and aminoglycosides is common. Clinically it has been shown that *P. aeruginosa* has the capacity to develop resistance rapidly during the course of antimicrobial therapy by several mechanisms (Fish et al., 1995; Hancock, 1998; Carmeli et al., 1999; Le Thomas et al., 2001). It has been also shown that selection of resistance during antimicrobial therapy occurs frequently in *P. aeruginosa* (Harris et al., 1999). Therefore, sequential accumulation of resistance may result in emergence of multidrug resistance in *P. aeruginosa*.

Notably integron-mediated multidrug resistance frequently found in *P. aeruginosa* and is a major clinical problem (Weldhagen, 2004). Integrons are genetic elements that possess the capacity to capture individual antimicrobial resistance genes, including those encoding β-lactamases and aminoglycoside-modifying enzymes, and to promote transcription and expression of these genes. Integrons include a receptor site, *attI*, where the captured genes are integrated, and a recombinase gene, *int*. Widespread integron-mediated resistance poses an increasing threat to the treatment and control of *P. aeruginosa* infections.

Factors influencing the emergence and spread of acquired resistance in *P. aeruginosa* include inadequate use and overuse of antimicrobials (Rossolini and Mantengoli, 2005). Previous reports have shown that use of carbapenems and other antimicrobials increases the risk of emergence of resistant *P. aeruginosa*, although antimicrobial rankings differ between studies (Carmeli et al., 1999; Amari et al., 2001; Harris et al., 2002).

β-Lactam resistance

β-Lactams including carbapenems are commonly used to treat *P. aeruginosa* infections in Japan. The predominant mechanisms conferring β-lactam resistance in clinical *P. aeruginosa* isolates include production of β-lactamases, loss or decreased production of outer membrane proteins (OMPs) and up-regulation of efflux pumps (Szabo et al., 2005).

To date, a number of β-lactamases have been identified in clinical *P. aeruginosa* isolates. Most isolates produce chromosomally encoded AmpC-type β-lactamases (molecular class C) (Ambler, 1980). Hyperproduction of AmpC-type β-lactamases induced by exposure to certain antimicrobials can lead to resistance to penicillins and cephalosporins but not to carbapenems (Livermore, 1987). In *P. aeruginosa*, carbapenem resistance can be conferred by production of metallo-β-lactamases (molecular class B), which hydrolyze all classes of β-lactams (Ambler, 1980). Metallo-β-lactamases are not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Most metallo-β-lactamase genes are located on plasmids and can be transferred to other strains. The first documented instance of transferable metallo-β-lactamase derived from *P. aeruginosa* was found in Japan in 1988 (Watanabe et al., 1991), and transferable metallo-β-lactamases such as IMP- and VIM-types have now been found in Japan, Korea, Europe and USA (Walsh et al., 2005). Additionally *P. aeruginosa* can produce OXA- and PSE-type β-lactamases belonging to molecular class D, resulting primarily in inactivation of penicillins (Bonomo and Szabo, 2006; Naas and Nordmann, 1999). Recently extended-spectrum β-lactamases (ESBLs) derived from molecular class A and D (OXA)-types also have been described (Bonomo and Szabo, 2006).

Mutational impermeability is one of the major mechanisms responsible for carbapenem resistance and arises via mutational loss or decrease of OMPs (Studemeister and Quinn, 1998; Livermore, 2002). OprD is a porin-forming transmembrane channel (D_2 porin), which is accessible to carbapenems but not to other β-lactams. It has been shown that loss or decrease of OprD production or inactivation of OprD results in carbapenem resistance in *P. aeruginosa* but does not confer resistance to other β-lactams (Pirnary et al., 2002; Horii et al., 2003; Muramatsu et al., 2003 and 2005; Wolter et al., 2004; Rossolini and Mantengoli, 2005). It was suggested that carbapenem resistance caused by mutational changes in OprD can emerge during antimicrobial therapy against a *P. aeruginosa* infec-

tion (Horii et al., 2003). Mutations leading to up-regulation of efflux systems such as MexA-MexB-OprM, MexC-MexD-OprJ and MexX-MexY-OprM can variably result in decreased susceptibility to β-lactams and fluoroquinolones (Rossolini and Mantengoli, 2005).

Fluoroquinolone resistance

The major mechanisms of fluoroquinolone resistance in clinical isolates of *P. aeruginosa* include alterations in DNA gyrase and/or topoisomerase IV caused by mutations in the quinolone resistance-determining regions (QRDRs) of GyrA and ParC (Akasaka et al., 2001; Muramatsu et al., 2005). Other reports have suggested that mutations of GyrB are associated with fluoroquinolone resistance (Mouneimnè et al., 1999; Le Thomas et al., 2001). A secondary mechanism, active efflux systems, contributes to reduced susceptibility to fluoroquinolones (Le Thomas et al., 2001; Livermore, 2002). Of the known efflux pumps in *P. aeruginosa*, only MexA-MexB-OprM is expressed constitutively at sufficient levels to result in intrinsic fluoroquinolone resistance (Zhanel et al., 2004).

Aminoglycoside resistance

Mechanisms conferring aminoglycoside resistance in *P. aeruginosa* include enzymatic modification of aminoglycosides, active efflux systems and impermeability (Poole, 2005). Aminoglycosides are inactivated by enzymatic phosphorylation (aminoglycoside phosphoryltransferase [APH]), acetylation (aminoglycoside acetyltransferase [AAC]) and adenylation (aminoglycoside nucleotidyltransferase [ANT]). These modifying enzymes are located on chromosome or plasmids. Individual aminoglycoside-resistant isolates of *P. aeruginosa* carry multiple modifying enzymes, resulting in broad-spectrum aminoglycoside resistance (Poole, 2005).

Detection of antimicrobial resistance in *P. aeruginosa*

Antimicrobial therapy against individual *P. aeruginosa* infections should be based on in vitro antimicrobial susceptibility data generally expressed in terms of minimum inhibitory concentrations (MICs). Techniques for detection of specific antimicrobial resistance alleles, including both clinically available and those limited to research laboratories, are summarized in Table 1.

β-Lactam resistance

Genetic techniques to determine types of β-lactamases include PCR, cloning, DNA probes and nucleotide sequencing, although the nongenetic gold standard remains examination for ability of bacterial crude extracts to hydrolyze β-lactams. Hyperproduction of AmpC-type β-lactamases can be detected by RNA-based techniques such as real-time reverse transcription (RT)-PCR (Quale et al., 2006). Metallo-β-lactamases can be detected easily using microbiological methods (Walsh et al., 2005). Metallo-β-lactamase activity is inhibited by the removal of zinc from the active site. Microbiological laboratories usually apply disk diffusion methods using ceftazidime (substrate) and 2-mercaptoproprionic acid (inhibitor), microdilution methods using imipenem (substrate) and EDTA or 1,10-phenanthroline (inhibitor) and Etest methods using imipenem and EDTA to the examination. Metallo-β-lactamases can also be detected by PCR for the specific bla_{IMP} and bla_{VIM} genes, DNA probes and/or nucleotide sequencing.

Alterations in OprD result from decreased production or inactivation of OprD due to deletions, substitutions or insertions in the *oprD* gene or regulatory mutations (Pirnary et al., 2002; Rossolini and Mantengoli, 2005). The alterations can be detected by SDS-PAGE following the preparation of OMPs, RNA-based techniques or nucleotide sequencing of the *oprD* and relevant genes (Pirnary et al., 2002; Horii et al., 2003; Muramatsu et al., 2003).

Fluoroquinolone resistance

Our understanding of fluoroquinolone resistance is based on nucleotide sequencing of the QRDRs of the *gyrA*, *gyrB* and *parC* genes (Akasaka et al., 2001; Le Thomas et al., 2001; Muramatsu et al., 2005). Additional contributions to fluoroquinolone resistance by efflux systems can be detected by

Table 1. Detection techniques of antimicrobial resistance alleles in *Pseudomonas aeruginosa*

Antimicrobial Resistance mechanism Resistance gene	Gene product	Detection techniques	Reference
β-Lactam			
Production of β-Lactamase			
bla	TEM-, SHV-, OXA-type (class A and D)	PCR, real-time RT-PCR, cloning, DNA probes, nucleotide sequencing, enzymatic analysis	Bauernfeind et al., 1996; Nordmann and Polrel, 2002; Lee et al., 2005; Bonomo and Szabo, 2006; Quale et al., 2006
ampC	AmpC-type (class C)		
bla	IMP-, VIM-type (class B)	PCR, cloning, DNA probes, cloning, nucleotide sequencing, microbiologic methods (disk diffusion and Etest), enzymatic analysis	Lauretti et al., 1999; Franceschini et al., 2000; Shibata et al., 2003; Nordmann and Polrel, 2002; Wash et al., 2002
Loss or decreased OprD production, inactivation of OprD			
oprD	OprD	OMP analysis, real-time RT-PCR, RNA-based techniques, nucleotide sequencing	Pirnary et al., 2002; Horii et al., 2003; Muramatsu et al., 2003; Dumas et al., 2006; Quale et al., 2006
Fluoroquinolone			
Alteration of in DNA gyrase and topoisomerase IV			
gyrA, gyrB, parC	GyrA, GyrB, ParC	Nucleotide sequencing	Akasaka et al., 2001; Le Thomas et al., 2001; Muramatsu et al., 2005
Aminoglycoside			
Enzymatic modification of aminoglycoside			
aac(6')-I, aac(6')-II, aac(3)-I, aac(3)-II, aph(3')-II, ant(2')-I	AAC(6')-I, AAC(6')-II, AAC(3)-I, AAC(3)-II, APH(3')-II, ANT(2')-I	PCR, cloning, DNA probes, nucleotide sequencing	Shaw et al., 1991; Vliegenthart et al., 1991; Mendes et al., 2004
Production of 16S rRNA methylase			
rmtA	RmtA	PCR	Yokoyama et al., 2003
Multidrug			
Up-regulation of efflux systems			
mexA, mexB, oprM	MexA-MexB-OprM	Real-time PCR, nucleotide sequencing, RNA-based techniques, biological assay	Mortimer and Piddock, 1991; Quale et al., 2006
mexC, mexD, oprJ	MexC-MexD-OprJ		
mexX, mexY, oprM	MexX-MexY-OprM		

OMP, outer membrane protein; RT, reverse transcription.

biological assays that measure active intracellular concentrations of fluoroquinolones with and without carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Hirai et al., 1986). Nucleotide sequencing and RNA-based techniques are used to characterize disruption and overexpression of the genes coding for efflux pumps such as MexA-MexB-OprM (Zhanel et al., 2004; Quale et al., 2006).

Aminoglycoside resistance

Although the mechanisms of amionoglycoside resistance are multiplexed, the genes encoding the aminoglycoside-modifying enzymes such as AAC(6')-I, AAC(6')-II, AAC(3)-I, AAC(3)-II, APH(3')-II and ANT(2')-I can be detected by

PCR, DNA probes and/or nucleotide sequencing (Shaw et al., 1991; Vliegenthart et al., 1991; Mendes et al., 2004).

Nosocomial infections caused by antimicrobial-resistant *P. aeruginosa*

Antimicrobial-resistant *P. aeruginosa* are a major cause of nosocomial infections (Table 2). Nosocomial transmission of antimicrobial-resistant *P. aeruginosa* has been associated with endoscopes, tap water and other environmental surfaces (Muscarella, 2004). Contaminated environmental surfaces in the hospital settings can lead to nosocomial transmission via contact with contaminated hands (or gloves) of the healthcare staff, patients themselves or visitors. Of particular concern is the increasing isolation of *P. aeruginosa* from intensive and high care units (Trautmann et al., 2005). The risk factors for nosocomial infections in critically ill patients include length-of-stay and extent of exposure to invasive devices such as mechanical ventilators, urinary bladder catheters and intravenous and intraarterial catheters, as well as inadequate use of antimicrobials (Trilla, 1994; Obritsch, 2005).

Antimicrobial therapy against infections caused by *P. aeruginosa*

The prevalence of antimicrobial resistance in *P. aeruginosa* leads to limitation in efficacious antimicrobial therapies. Acquired resistance that develops during the course of treatment, especially with carbapenems and fluoroquinolones, is another serious problem (Fish et al., 1995; Hancock, 1998; Carmeli et al., 1999; Amari et al., 2001; Le Thomas et al., 2001; Harris et al., 2002). Therefore, in vitro susceptibility data are essential in the choice of antimicrobials: the alternatives include antipseudomonal penicillins (piperacillin and tazobactam/piperacillin), antipseudomonal cephalosporins (cefoperazone, ceftazidime and cefepime), aztreonam, carbapenems, aminoglycosides (tobramycin and amikacin) and fluoroquinolones. In some types of infections such as endocarditis, nosocomial pneumonia and bacteraemia, combination therapy with an antipseudomonal penicillin, an antipseudomonal cephalosporin or aztreonam plus an aminoglycoside is administered. The question of whether combination therapy prevents the emergence of resistance remains highly controversial (Paul et al., 2004; Paterson, 2006).

Carbapenems are widely used in the treatment of *P. aeruginosa* infections, and a strong association between use and resistance has been documented for these antimicrobials (Rossolini and Mantengoli, 2005). In some cases, prior use of a particular antimicrobial predicts development of resistance in *P. aeruginosa* (El Amari et al., 2001). Resistance emerges during antimicrobial therapy in as many as 50% of patients treated for a serious *P. aeruginosa* infection with imipenem. In this context, resistance is most likely attributable to mutational loss of OprD (Livermore, 2002). Cases of clonal shifts in carbapenem resistance resulting in loss or decreased production of OprD during antimicrobial therapies have been reported (Horii et al., 2003). In addition, it was reported that emergence of resistance to both fluoroquinolones and β-lactams such as cefsulodin and aztreonam during monotherapy with ciprofloxacin was caused by amino acid mutations of QRDRs in GyrB plus overexpression of the active efflux system, MexA-MexB-OprM, and required combination therapy with ceftazidime and amikacin (Le Thomas et al., 2001). Accordingly, it is critical to monitor antimicrobial susceptibility, especially to carbapenems and fluoroquinolones, during antimicrobial therapy against a *P. aeruginosa* infection.

Table 2. Recent cases of nosocomial transmission of antimicrobial-resistant *Pseudomonas aeruginosa* in Japan

Case	Year	Number of patients*	Environmental source of bacteria
1†	2000–2001	18 (1)	Urinary catheter
2	2001–2002	30	Urinary catheter
3†	2004	9 (3)	Transesophageal endoscope
4†	2004	11 (2)	Endoscope
5†	2005	6 (5)	Cup for urine examination

*Number of deceased patients is shown in the parenthesis.
†Case was reported from a university hospital.

Multidrug-resistant *P. aeruginosa* (MDRP) in Japan is defined as a strain showing MICs of ≥ 16 mg/L for imipenem, ≥ 4 mg/L for ciprofloxacin and ≥ 32 mg/L for amikacin. Risk factors for MDRP infection include prolonged hospitalization, protracted and broad-spectrum antimicrobial therapy and an immunocompromised state (Obritsch et al, 2005). The frequency of MDRP infections in Japan is reported in the Infectious Diseases Weekly Report (IDWR, see http://idsc.nih.go.jp/kanja/idwr/idwr-j.htm) and varies between hospitals. The intrinsic susceptibility of *P. aeruginosa* is already limited to only several antimicrobials, and the emergence of multidrug resistance compromised most antipseudomonal therapies except colistin and synergistic combinations of antimicrobials (Obritsch et al., 2005). Colistin is a multicomponent polypeptide antimicrobial, comprised mainly of colistin A and B. Colistin became available for clinical use in the 1960s, but is not currently available in Japan. There are no recommended breakpoints for susceptibility testing of colistin for *P. aeruginosa*. Strategies against MDRP infections include combination therapy with cefepime plus amikacin, continuous-infusion meropenem (not applicable in Japan) and parenteral colistin therapy (Obritsch et al., 2005). Concomitantly, strict compliance with recommended infection control practices and isolation procedures is required to prevent the spread of MDRP clones within the hospital settings.

Strategies for controlling infections caused by antimicrobial-resistant *P. aeruginosa*

In 2001, the World Health Organization (WHO) document, "WHO global strategy for containment of antimicrobial resistance", provided a framework of interventions to slow the emergence and reduce the spread of antimicrobial-resistant microorganisms by reducing the disease burden and spread of infection, improving access to appropriate antimicrobials, improving use of antimicrobials, strengthening health care systems and their surveillance capabilities, enforcing regulations and legislation and encouraging the development of appropriate new drugs and vaccines (http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_DRS_2001_2_EN/en/).

In hospital settings, strategies for control of *P. aeruginosa* infections include early detection of *P. aeruginosa* as the causative pathogen, determination of its antimicrobial susceptibilities, effective therapy against the infection with adequate use of antimicrobials and strict infection control practices. Once antimicrobial therapy has been initiated against a *P. aeruginosa* infection, its susceptibility to antimicrobials, especially to carbapenems and fluoroquinolones, should be monitored during antimicrobial therapy to detect clonal shifts in resistance and microbial substitutions as early as possible. Continued surveillance of nosocomial infections and monitoring of antimicrobial resistance by the infection control staff will help prevent nosocomial infections and antimicrobial resistance.

In the future, the development of methods for the rapid detection of antimicrobial resistance, especially to carbapenem and fluoroquinolone, will lead to early detection of clonal shifts in resistance during antimicrobial therapy and identification of resistance alleles associated with nosocomial dissemination of antimicrobial-resistant *P. aeruginosa*. The spread of MDRP represents an increasing threat and efforts should be made to develop new agents and vaccines against *P. aeruginosa* infections in the laboratories and pharmaceuticals, while preserving the efficacy of the currently available antimicrobials for as long as possible in the hospital settings.

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