
Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is one of the leading causes of nosocomial infections. Severe infections, such as pneumonia or bacteraemia, are associated with high mortality rates and are often difficult to treat, as the repertoire of useful anti-pseudomonal agents is limited (some β -lactams, fluoroquinolones and aminoglycosides, and the polymyxins as last-resort drugs); moreover, *P. aeruginosa* exhibits remarkable ability to acquire resistance to these agents. Acquired resistance arises by mutation or acquisition of exogenous resistance determinants and can be mediated by several mechanisms (degrading enzymes, reduced permeability, active efflux and target modification). Overall, resistance rates are on the increase, and may be different in different settings, so that surveillance of *P. aeruginosa* susceptibility is essential for the definition of empirical regimens. Multidrug resistance is frequent, and clinical isolates resistant to virtually all anti-pseudomonal agents are increasingly being reported. Monotherapy is usually recommended for uncomplicated urinary tract infections, while combination therapy is normally recommended for severe infections, such as bacteraemia and pneumonia, although, at least in some cases, the advantage of combination therapy remains a matter of debate. Antimicrobial use is a risk factor for *P. aeruginosa* resistance, especially with some agents (fluoroquinolones and carbapenems), and interventions based on antimicrobial rotation and restriction of certain agents can be useful to control the spread of resistance. Similar measures, together with the prudent use of antibiotics and compliance with infection control measures, are essential to preserve the efficacy of the currently available anti-pseudomonal agents, in view of the dearth, in the near future, of new options against multidrug-resistant *P. aeruginosa* strains.

Keywords *Pseudomonas aeruginosa*, antibiotic resistance, treatment, control, review

Clin Microbiol Infect 2005; 11 (Suppl. 4): 17–32

INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative, aerobic, Gram-negative rod that normally lives in moist environments. It has minimal nutrition requirements while being able to use several organic compounds for growth. This metabolic versatility contributes to a broad ecological adaptability and distribution, and reflects a genome of larger size and complexity compared with that of many other bacterial species [1,2].

PSEUDOMONAS AERUGINOSA AS A PATHOGEN: AN OVERVIEW

Occasionally, *P. aeruginosa* can colonise human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonisation by *P. aeruginosa* in healthy subjects is usually low, but higher colonisation rates can be encountered following hospitalisation, especially amongst subjects treated with broad-spectrum antimicrobial agents. Colonisation is common in the respiratory tract of mechanically ventilated patients, in the gastrointestinal tract of patients receiving anticancer chemotherapy, and on the skin of burn patients. Also, sinks, mops, disinfectant solutions, respiratory equipment, food mixers and other moist environments can act as reservoirs of *P. aeruginosa* in the hospital setting [3,4].

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P. aeruginosa is typically an opportunistic pathogen that seldom causes disease in healthy subjects. Normally, for an infection to occur, some disruption of the physical barriers (skin or mucous membranes), or by-passing of them (e.g., by urinary catheters, endotracheal tubes or other invasive devices), and/or an underlying dysfunction of the immune defence mechanisms, such as neutropenia, is necessary. As a consequence, *P. aeruginosa* is mostly a nosocomial pathogen. According to data from the Centers for Disease Control and Prevention National Nosocomial Infection Surveillance System, in the USA, *P. aeruginosa* was the second most common cause of nosocomial pneumonia, the third most common cause of nosocomial urinary tract infections, and the seventh most common cause of nosocomial bacteraemia [5]. In Europe, *P. aeruginosa* was found to be the third most common isolate from nosocomial infections in intensive care units (ICUs) [6].

Overall, community-acquired infections by *P. aeruginosa* are uncommon. The most frequent are: (i) folliculitis and infections of the ear canal, mostly acquired after bathing in contaminated waters; (ii) cheratitis, usually associated with the use of a contact lens contaminated during lens care; (iii) malignant otitis externa with involvement of the underlying tissues and possibly of the temporal bone and basilar skull, primarily seen in diabetics and the elderly; (iv) osteomyelitis of the calcaneus in children, e.g., following puncture wounds through sneakers whose inner pad is contaminated by *P. aeruginosa*; and (v) endocarditis in intravenous drug users, resulting from the injection of contaminated drug solutions [4,7–11]. The latter is the most severe community-acquired *P. aeruginosa* infection, often requiring valve replacement, and is associated with high mortality rates [12]. *P. aeruginosa* is also an uncommon cause of community-acquired pneumonia, which may occur in subjects (usually middle-aged and with a history of smoking) exposed to contaminated aerosolised water. In these cases, patients rarely receive appropriate empirical chemotherapy and mortality can be high [13].

Nosocomial infections caused by *P. aeruginosa* most frequently involve the respiratory tract, the urinary tract and wounds. *P. aeruginosa* is amongst the leading causes of nosocomial pneumonia, especially in mechanically ventilated patients. Overall, these patients have a much higher probability of developing nosocomial

pneumonia, with *P. aeruginosa* being the most frequent cause. Mortality rates ranging from 40% to more than 60% have been reported in bacteraemic nosocomial pneumonia and in ventilator-associated pneumonia [14–16]. Nosocomial urinary tract infections caused by *P. aeruginosa* are usually related to catheterisation or other invasive procedures, and may be complicated by bacteraemia [4]. According to surveillance data, *P. aeruginosa* was the third and fifth most common cause of hospital-acquired urinary tract infections in the USA and Europe, respectively [17,18]. Wound infections are particularly serious in burn patients, where they are often complicated by bacteraemia [19]. *P. aeruginosa* bacteraemia and septic shock are primarily observed in immunocompromised patients, and are associated with high mortality rates (from one-third to almost two-thirds of cases) [20–23]. All situations associated with severe neutropenia and mucosal ulcerations, such as haematological malignancies, cancer chemotherapy and organ transplantation, create a significant risk for the development of *P. aeruginosa* bacteraemia [4,24–27]. Other predisposing factors include diabetes mellitus, immunoglobulin deficiency states, severe burns, steroid therapy, surgery and the use of invasive devices [4]. In cancer patients, *P. aeruginosa* can be responsible for up to 30% of culture-proven cases of bacteraemia, with mortality rates ranging from 5% to 50% [28]. In a recent surveillance study on nosocomial bloodstream isolates carried out in the Americas, *P. aeruginosa* was found to be the third most common pathogen [29]. *P. aeruginosa* can also cause peritonitis in patients on chronic ambulatory peritoneal dialysis [30].

P. aeruginosa is a major pathogen in cystic fibrosis patients, most of whom, sooner or later, develop respiratory tract infection with *P. aeruginosa* which typically exhibits a mucoid phenotype. The abnormal airway epithelia of these patients allow long-term colonisation by *P. aeruginosa* and, once infected, they rarely, if ever, clear the microorganism, which, in turn, plays a critical role in the progression of lung disease [4,31].

Finally, *P. aeruginosa* can be an important cause of morbidity and mortality in both paediatric and adult patients with acquired immunodeficiency syndrome with very low CD4 counts [32–34], a condition that altogether has become less frequent since the introduction of highly active anti-retroviral regimens.

The virulence mechanisms of *P. aeruginosa* are complex and only partially understood. Adherence mediated by pili and other adhesins appears to be important for the colonisation of mucous membranes and other surfaces [35–37], while the production of a mucoid exopolysaccharide matrix that surrounds the cells and anchors them to each other and to the environment is important for growth as a biofilm, in which the bacterial cells are protected from the host innate and immune defences and are overall less susceptible to antibiotics [38–41]. A role for tissue damage and invasion has been recognised for a number of products secreted by *P. aeruginosa*, including elastase, alkaline protease, cytotoxin, phospholipase C and rhamnolipid [42–46]. Finally, local and systemic toxicity is most probably related to endotoxin release, and to the production of exotoxin A (an extracellular enzyme that inhibits mammalian protein synthesis) and exoenzyme S (which can ADP-ribosylate several GTP-binding proteins) [47–51]. The production of exoenzymes and other virulence factors is controlled by a quorum-sensing regulatory mechanism that leads to a coordinate expression of the corresponding genes only when the microbial cell density exceeds certain values. This mechanism allows the production of virulence factors only when there is a reasonable chance that the infection may overcome the host defences, and thus reduces the possibility of immunisation against these products which may be detrimental to the bacterium [52]. Indeed, functional quorum-sensing systems are important for *P. aeruginosa* virulence, although quorum-sensing mutants are not avirulent, revealing that pathogenesis is also regulated by other factors [53–55].

The laboratory diagnosis of infection is usually simple, as *P. aeruginosa* grows on most routine culture media and is easily identified by commercial identification systems for Gram-negative pathogens [56]. Recovery of *P. aeruginosa* from normally sterile sites is always considered to be clinically significant, while recovery from non-sterile sites is only considered to be significant when associated with a typical clinical syndrome (e.g., otitis externa). In the case of intubated patients, it is particularly important to discriminate between colonisation and infection as, in the latter case, the mortality rates are high and aggressive antimicrobial chemotherapy is required. In these cases, the presence of large

amounts of Gram-negative bacilli and polymorphonuclear leucocytes in Gram-stained secretions obtained by endotracheal suction is suggestive of a diagnosis of nosocomial pneumonia [57].

THE ANTI-PSEUDOMONAL DRUGS AND ACQUIRED ANTIMICROBIAL RESISTANCE IN *P. AERUGINOSA*

P. aeruginosa is intrinsically resistant to many antimicrobial agents, including most β -lactams, the older quinolones, chloramphenicol, tetracycline, macrolides, trimethoprim-sulfamethoxazole and rifampin.

The AmpC chromosomal β -lactamase, the production of which is inducible, contributes to the intrinsic resistance of this species to ampicillin and to most cepheems, which act as inducers and are degraded by this enzyme [58]. An overall low permeability of the outer membrane and the presence of a number of active multidrug efflux systems also contribute to the intrinsic resistance or reduced susceptibility of this species to several antimicrobial agents [59,60]. For instance, the three-component MexA–MexB–OprM, MexC–MexD–OprJ, MexE–MexF–OprN and MexX–MexY–OprM systems can extrude a wide variety of antimicrobial agents (including quinolones, tetracycline, chloramphenicol, macrolides, sulphonamides, trimethoprim, aminoglycosides and most β -lactams) and, with their basal activity, provide an important defence against these drugs, as demonstrated by the increased susceptibility to the various agents exhibited by knock-out mutants for these systems [61–64]. The aminoglycosides can also be actively extruded, in *P. aeruginosa*, by the MexX–MexY linker-pump module in combination with different outer membrane channels (OpmG and OpmI) [65].

As a consequence, the repertoire of antimicrobial agents that can be used against *P. aeruginosa* infections is relatively limited. The most important anti-pseudomonal agents include some β -lactams (ticarcillin, ureidopenicillins, piperacillin, cefoperazone, ceftazidime, cefepime, aztreonam, imipenem and meropenem), aminoglycosides (gentamicin, tobramycin, netilmicin and amikacin) and fluoroquinolones (of which ciprofloxacin remains the most active compound) [66,67]. Polymyxins (polymyxin B and colistin) are also active but, due to their higher toxicity, are usually considered only for multidrug-resistant (MDR)

strains that are resistant to the other agents [68]. Concerning the β -lactam- β -lactamase inhibitor combinations, piperacillin-tazobactam is preferable to ticarcillin-clavulanate for the treatment of *P. aeruginosa* infections because of the more favourable pharmacokinetics of tazobactam, the superior anti-pseudomonal activity of piperacillin, and the fact that, unlike tazobactam, clavulanate usually induces the production of the *P. aeruginosa* AmpC enzyme and could antagonise the antimicrobial activity of ticarcillin [69,70].

Acquired resistance to any of the anti-pseudomonal agents is possible and has been described.

Resistance to anti-pseudomonal β -lactams is common and can result from one or more of several different mechanisms (Table 1). Mutations leading to the increased production of the AmpC β -lactamase can occur at frequencies of 10^{-7} – 10^{-9} , and may result in decreased susceptibility to overt resistance (depending on the amount of enzyme, mutant phenotype and β -lactam compound) to those compounds that are normally active as they do not induce AmpC production,

but that are not entirely stable to the enzyme (penicillins, cepheims and monobactams) [58]. Three different mutant phenotypes have been described, including a moderate-level constitutive production, a high-level constitutive production and a moderate-level basal with hyperinducible production [71,72]. The mutational events leading to the deregulation of AmpC production in *P. aeruginosa* have been identified only in part [73,74], but they appear to be substantially different from those usually responsible for the same phenomenon in Enterobacteriaceae. Moreover, in *P. aeruginosa*, multiple mutational pathways could be responsible for these phenotypes [74], which would explain the phenotype heterogeneity and notable tendency to segregate similar mutants. Mutations leading to a decreased amount of the OprD porin can occur at relatively high frequency (10^{-7}) and result in resistance to imipenem and reduced susceptibility to meropenem [75,76]. Loss of OprD production can be due to deletions, substitutions or insertions that cause inactivation of the *oprD* gene [77,78], but OprD production can

| Resistance mechanism | Affected anti-pseudomonal agents |
|--|---|
| <i>Acquired by mutation</i> | |
| AmpC derepression | Penicillins, cepheims, monobactams ^a |
| OprD loss | Carbapenems |
| Up-regulation of efflux pumps | |
| MexA–MexB–OprM | All β -lactams except imipenem Fluoroquinolones |
| MexC–MexD–OprJ | Some β -lactams (cefoperazone, cefpirome, cefepime, meropenem) Fluoroquinolones |
| MexE–MexF–OprN | Fluoroquinolones (Carbapenems) ^b |
| MexX–MexY–OprM | Some β -lactams (cefoperazone cefpirome, cefepime, meropenem) Fluoroquinolones Aminoglycosides |
| GyrA and/or ParC modification | Fluoroquinolones |
| Membrane changes | Polymyxins, aminoglycosides |
| <i>Acquired following transfer of exogenous resistance genes</i> | |
| β -Lactamases | |
| Narrow-spectrum molecular class A (e.g., PSE-1, PSE-4, TEM-1) and class D (e.g., OXA-3) enzymes | Penicillins, cefoperazone |
| Extended-spectrum molecular class A (e.g., PER-1, VEB-1, GES-1, GES-2, TEM-42, SHV-5) and class D (e.g., OXA-11, OXA-14, OXA-18, OXA-28) enzymes | Penicillins, cepheims, monobactams ^c |
| Molecular class B metallo-enzymes (IMP-, VIM-, SPM- and GIM-type enzymes) | All β -lactams except monobactams |
| Aminoglycoside-modifying enzymes ^d | |
| AAC(3)-I | Gentamicin |
| AAC(3)-II | Gentamicin, tobramycin, netilmicin |
| AAC(6')-I | Tobramycin, netilmicin, amikacin |
| AAC(6')-II | Gentamicin, tobramycin, netilmicin |
| ANT(2')-I | Gentamicin, tobramycin |

Table 1. Acquired resistance mechanisms to anti-pseudomonal agents

^aThe effect of AmpC derepression on susceptibility is lower with some compounds that are poorly hydrolysed by the enzyme (e.g., carbenicillin and cefepime).

^bIn this case, decreased susceptibility to carbapenems is dependent on the concomitant OprD decrease observed in these mutants.

^cSubstrate profiles can be somewhat different for different enzymes. For instance, PER-1 exhibits poor activity against piperacillin. GES-2 also exhibits a modest carbapenemase activity that can confer resistance to imipenem when associated with permeability defects.

^dOther modification enzymes can also be found, although rarely, in *Pseudomonas aeruginosa*.

also decrease following regulatory mutations that cause both the down-regulation of OprD and the up-regulation of the MexE–MexF–OprN efflux system [62,79]. In the former case, only susceptibility to imipenem is affected, while, in the latter case, susceptibility to quinolones is also decreased [62]. Mutations leading to the up-regulation of efflux systems (MexA–MexB–OprM, MexC–MexD–OprJ, MexE–MexF–OprN and MexX–MexY–OprM) can variably result in decreased susceptibility to full resistance (depending on the system, the level of up-regulation and the compound) to anti-pseudomonal β -lactams. Up-regulation of the MexA–MexB–OprM system, which is usually caused by mutations that inactivate the *mexR* regulatory gene [80–82], decreases susceptibility to virtually all anti-pseudomonal β -lactams, except imipenem [64,80,82], while up-regulation of the MexC–MexD–OprJ and MexX–MexY–OprM systems only affects susceptibility to some cepheims (ceftazidime, ceftazidime, ceftazidime, ceftazidime) and to meropenem [64]. On the other hand, the MexE–MexF–OprN system does not efflux β -lactams, but the up-regulation of this system indirectly affects carbapenem susceptibility due to concomitant down-regulation of OprD [62,79]. Finally, the acquisition of secondary β -lactamase genes by horizontal transfer can be responsible for acquired β -lactam resistance, the spectrum of which reflects the substrate specificity of the acquired enzyme. From this point of view, the acquired β -lactamases found in *P. aeruginosa* can belong to three different groups: (i) narrow-spectrum active site-serine enzymes of molecular classes A and D (e.g., PSE-1, PSE-4 and some OXA-type enzymes) that efficiently degrade the anti-pseudomonal penicillins and cefoperazone, but have no significant activity against the other anti-pseudomonal cepheims, monobactams or carbapenems [58,83,84]; (ii) extended-spectrum active site-serine enzymes of molecular classes A and D (e.g., PER-1, VEB-1, GES-1, GES-2, various OXA-type enzymes and, although rarely, also TEM- and SHV-type extended-spectrum variants) that, in addition to penicillins, can also degrade the anti-pseudomonal cepheims and monobactams but not carbapenems [58,83,85–87] (the GES-2 enzyme also has a modest carbapenemase activity that can confer resistance to imipenem when associated with impermeability-mediated resistance mechanisms [88]); and (iii) metallo-enzymes of molecular class B (e.g., the enzymes of

the IMP, VIM, SPM and GIM type) that efficiently degrade virtually all the anti-pseudomonal β -lactams except monobactams [89–93]. Members of the last two groups are the most worrisome from the clinical standpoint due to their broad substrate profiles; and although, in most cases, isolates producing these are still found sporadically or may cause small outbreaks, similar enzymes are progressively disseminating in the clinical setting and are currently included amongst the emerging β -lactamases of increasing clinical importance [90,94–96]. In some cases, multiple acquired β -lactamases can be found in clinical isolates, broadening the spectrum of β -lactam resistance. In particular, the simultaneous presence of an extended-spectrum β -lactamase and a metallo- β -lactamase can result in a phenotype of resistance to all the anti-pseudomonal β -lactams [97].

Acquired resistance to aminoglycosides can be due to the production of aminoglycoside-modifying enzymes encoded by horizontally acquired resistance determinants, or by mutations that reduce aminoglycoside accumulation in the bacterial cell [98] (Table 1). The most prevalent aminoglycoside-modifying enzymes found in *P. aeruginosa* are the acetyl-transferases AAC(6′)-II (resistance to gentamicin, tobramycin and netilmicin), AAC(3)-I (resistance to gentamicin), AAC(3)-II (resistance to gentamicin, tobramycin and netilmicin) and AAC(6′)-I (resistance to tobramycin, netilmicin and amikacin), and the adenylyl-transferase ANT(2′)-I (resistance to gentamicin and tobramycin) [99,100]. Reduced aminoglycoside uptake could be due to mutations causing lipopolysaccharide changes [101] or up-regulation of efflux systems based on the MexX–MexY linker-pump module [64,65]. Unlike resistance mediated by modifying enzymes, the spectrum of which can be variable depending on the nature of the enzyme, resistance mediated by efflux systems tends to be broad spectrum [64,65].

Acquired resistance to fluoroquinolones can be due either to mutations that cause the up-regulation of efflux systems, including MexA–MexB–OprM, MexC–MexD–OprJ, MexE–MexF–OprN and MexX–MexY–OprM [60 and references therein], or to mutations of the topoisomerase targets (*gyrA* and also *parC*) [102,103] (Table 1). Both mechanisms are responsible for cross-resistance to all fluoroquinolones, and none of the new fluoroquinolones retains activity against cipro-

floxacin-resistant isolates [104]. Efflux mutants also typically exhibit a decreased susceptibility to other drugs, while only quinolone susceptibility is affected in target mutants. Efflux and target mutations can cooperate to increase the resistance level [105]. The multiple mutational events that can be responsible for quinolone resistance account for the relatively high frequency at which these drugs can select for resistant isolates.

Finally, acquired resistance to polymyxins has been occasionally described in *P. aeruginosa* isolates from cystic fibrosis patients treated for long periods with the nebulised drug, and seems to be related to mutations causing changes in the outer membrane structure [106].

Different mechanisms can cooperate to increase the resistance level to certain antimicrobial agents. For instance, loss of OprD and up-regulation of the MexAB–OprM efflux system can cooperate to increase the level of resistance to meropenem [76], while the loss of OprD and constitutive high-level production of the AmpC enzyme can cooperate to increase the level of resistance to imipenem, as the enzyme has a very modest activity against imipenem that can become significant when the permeability rate is reduced [107].

Mutational resistance can emerge during antimicrobial chemotherapy and result in therapeutic failures. This phenomenon is associated with an increase in morbidity and mortality, length of hospital stay and total hospital costs [108]. Emergence of resistance during therapy can be more frequent with some antimicrobial agents. For instance, a 17% rate of resistance emerging during imipenem treatment of *P. aeruginosa* infections has been reported [109], and controlled studies comparing imipenem with ceftazidime, piperacillin–tazobactam or ciprofloxacin, for the treatment of *P. aeruginosa* pneumonia, showed that imipenem was less effective than comparators because of the ease with which *P. aeruginosa* becomes resistant to this carbapenem during therapy [110–112].

Although the simultaneous emergence of multiple mutants is highly unlikely, sequential emergence is possible and is facilitated by the fact that infections caused by a strain resistant to certain antibiotics are thus treated with a different antibiotic [106]. Moreover, in some cases, a single mutational event can compromise multiple drugs with different mechanisms (e.g., mutations leading to the up-regulation of efflux systems that

cause resistance to both fluoroquinolones and β -lactams). On the other hand, acquired resistance genes are often carried on genetic elements (such as plasmids, transposons or integrons) in which several resistance determinants are clustered, so that an MDR phenotype can be acquired in a single step upon acquisition of the element [113–116]. All of this accounts for the remarkable potential of *P. aeruginosa* to evolve rapidly towards multidrug resistance.

ANTIMICROBIAL SUSCEPTIBILITY AND RESISTANCE TRENDS

Surveillance of the antimicrobial susceptibility of *P. aeruginosa* clinical isolates has been monitored in various large-scale multicentric longitudinal studies, e.g., SENTRY antimicrobial surveillance programme (http://www.ewi.med.uu.nl/enare/sentry_antimicrobial_resistance_about.html), MYSTIC programme (<http://www.mystic-info.com/media/default.asp>) and Intensive Care Antimicrobial Resistance Epidemiology (ICARE) project [117], as well as in a large number of smaller scale surveys [118–123, and references therein]. Surveillance of *P. aeruginosa* susceptibility is particularly important because of the large number of cases in which antimicrobial chemotherapy must be initiated empirically, and to the higher failure rates when the pathogen proves to be resistant to the agents prescribed empirically [124].

All surveillance studies reveal that there is no single drug active against 100% of *P. aeruginosa* clinical isolates (polymyxins are usually not tested). According to recent data from the largest multicentric surveillance study [125], amikacin, piperacillin–tazobactam and carbapenems remain the most active drugs worldwide, while ticarcillin and aztreonam show the lowest activities (Table 2). Susceptibility rates indicate significant geographical differences: overall, the highest rates are observed in North America and the Asia-Pacific region, while the lowest rates are observed in Latin America, with Europe being in an intermediate position (Table 2). In the case of some drugs, regional differences slightly overrule this general pattern: for instance, the fluoroquinolones retain a significantly better activity in the Asia-Pacific region compared with North America, whereas aztreonam retains a better activity in Europe in comparison with other areas (Table 2). Differences in susceptibility rates can also be

Table 2. Antimicrobial susceptibility rates (%) of *Pseudomonas aeruginosa* clinical isolates from different areas. Data are from the SENTRY antimicrobial surveillance programme [125]

| Agent | Europe | North America | Latin America | Asia-Pacific |
|-------------------------|--------|---------------|---------------|--------------|
| Amikacin | 86 | 95 | 73 | 95 |
| Tobramycin | 76 | 92 | 66 | 90 |
| Gentamicin | 72 | 84 | 62 | 84 |
| Piperacillin | 80 | 87 | 68 | 86 |
| Piperacillin-tazobactam | 86 | 90 | 77 | 89 |
| Ticarcillin | 74 | 78 | 58 | 76 |
| Ticarcillin-clavulanate | 78 | 78 | 59 | 75 |
| Ceftazidime | 80 | 80 | 66 | 79 |
| Cefepime | 80 | 83 | 67 | 83 |
| Aztreonam | 73 | 66 | 49 | 67 |
| Imipenem | 82 | 87 | 76 | 88 |
| Meropenem | 85 | 91 | 80 | 90 |
| Ciprofloxacin | 72 | 78 | 63 | 85 |

observed on a smaller scale among different countries [118–122,126]. When comparing data from surveillance studies, however, it should also be considered that, in some cases, different breakpoints are used, which partially accounts for some differences.

In ICUs, where *P. aeruginosa* is one of the leading causes of severe nosocomial infections, susceptibility rates tend to be lower than in general wards for some β -lactams (carbapenems, ceftazidime, ticarcillin-clavulanate) and, in Europe, also for ciprofloxacin and gentamicin, while remarkable differences are not observed with other drugs, such as piperacillin-tazobactam, cefepime and amikacin [123,127]. A comparative analysis of recent data concerning ICU isolates confirmed that, overall, higher resistance rates are observed in Europe compared with the USA; it also revealed a great diversity of resistance rates for different drugs in different European settings and at different times (Table 3). Similar findings

probably reflect the variability in drug prescription policies, as well as the circulation of different *P. aeruginosa* strains in different settings, and underscore the notion that data from local surveillance programmes are essential in determining the most appropriate guidelines for empirical regimens in individual hospital settings.

Concerning resistance trends, data from large-scale surveillance studies indicate, overall, an increasing trend during the past few years, although with notable differences for different drugs and geographical areas. This trend is particularly evident for fluoroquinolones, for which resistance appears to be increasing at a higher rate than for other antimicrobial agents in the USA, Europe and Latin America. Concerning aminoglycosides, a trend of increasing resistance is evident in Europe and in Latin America, but not in the USA. Concerning β -lactams, a trend of increasing resistance to all anti-pseudomonal β -lactams is evident in Europe, but is less marked in Latin America (where ceftazidime and cefepime appear to be spared) and for North America (where increases in the resistance rates for various β -lactams tend to be marginal and sometimes unconfirmed [122,123,129,130]).

Multidrug resistance can be relatively common amongst nosocomial isolates of *P. aeruginosa*, which represent the large majority of clinical isolates. In the SENTRY surveillance programme, the rates of multidrug resistance (defined as being resistant to piperacillin, ceftazidime, imipenem and gentamicin) were found to reflect geographical differences, being higher in Latin America (c. 8%), lower in Europe (c. 5%) and even lower in North America and the Asia-Pacific region (< 2%) [130]. Higher multidrug resistance rates are observed in ICUs [123], probably because of the tendency for MDR strains to cause outbreaks in

Table 3. Antimicrobial resistance rates (%) of *Pseudomonas aeruginosa* clinical isolates from intensive care units (ICUs). Numbers are the rates of intermediately resistant and resistant isolates. Data were derived from Karlowsky *et al.* [123] and Hanberger *et al.* [128]

| Agent | Various EU countries (1990–97) ^a | Europe (1997–98) | Europe (1999) | USA (1997–99) | USA (1998–2001) |
|-------------------------|---|------------------|---------------|---------------|-----------------|
| Amikacin | 4–37 | 13 | 30 | 3 | 7 |
| Gentamicin | 7–70 | 26 | 44 | 15 | 23 |
| Piperacillin-tazobactam | 5–53 | 15 | 36 | 12 | 9 |
| Ceftazidime | 11–57 | 19 | 29 | 24 | 17 |
| Cefepime | 3–57 | 17 | 32 | 19 | 19 |
| Imipenem | 10–52 | 22 | 38 | 15 | 20 |
| Meropenem | n.a. ^b | 16 | 34 | n.a. | 21 |
| Ciprofloxacin | 8–56 | 27 | 40 | 20 | 24 |

^aIncluding Belgium (1990 and 1994–95), Holland (1990), Germany (1990), France (1991 and 1994–95), Portugal (1994–95), Spain (1994–95), Sweden (1994–95 and 1997) and Turkey (1997).

^bn.a., data not available.

such settings. An increasing trend has also been reported for multidrug resistance rates in recent years [106,123]. For instance, the prevalence of MDR isolates (resistant to at least three of six drugs, including amikacin, gentamicin, ciprofloxacin, piperacillin, ceftazidime and imipenem) has increased from 13% in 1997 to 21% in 2000, according to surveillance data from the USA [106].

Isolates that exhibit resistance to virtually all available anti-pseudomonal agents (polymyxins are rarely tested in the clinical laboratory) are increasingly being reported [97,106,130–134]. The appearance of similar 'panresistant' isolates is one of the most worrisome developments in the context of microbial drug resistance, recreating conditions typical of the pre-antibiotic era, and has resulted in the search for anti-pseudomonal agents with alternative mechanisms of action and in the use of polymyxins despite their toxicity [68,135,136].

Continuous surveillance of susceptibility data in nosocomial institutions is of paramount importance, not only for the determination of guidelines for empirical regimens, but also for prompt enforcement of infection control measures. The appearance of multiple *P. aeruginosa* isolates with an unusual susceptibility pattern should immediately alert those responsible for the infection control system to the possibility of a nosocomial outbreak due to an MDR strain, and should thus lead to specific control measures [137].

TREATMENT OF SEVERE INFECTIONS CAUSED BY MDR *P. AERUGINOSA*

In-vitro susceptibility data are essential support for the selection of antimicrobial chemotherapy for *P. aeruginosa* infections, because of the frequency and variability of acquired resistance shown by clinical isolates. Susceptibility testing is well standardised for most anti-pseudomonal agents, but there are no recommended break-points for susceptibility testing of polymyxin B or colistin [56]. MIC determination is preferable in the case of these drugs because the correlation with disk diffusion testing is relatively poor [138].

As *P. aeruginosa* can be a lethal pathogen and the precocity of chemotherapy is critical to the outcome of the infection, empirical regimens adequate for *P. aeruginosa* coverage should always

be initiated prior to receipt of the results of cultures and susceptibility testing when infection by this species is suspected. For the selection of empirical regimens, several aspects should be considered, including: (i) the nature and source (nosocomial vs. community-acquired) of the infection; (ii) information concerning the epidemiology of resistance phenotypes in the individual setting; (iii) pharmacokinetic parameters; (iv) underlying risk factors (e.g., length of hospitalisation, ICU admissions, previous antimicrobial chemotherapy) and diseases; and (v) hospital prescription policies.

Antibiotic monotherapy is usually recommended for urinary tract infections caused by *P. aeruginosa*, with the exception of upper tract infections complicated by abscess formation, or for infections in neutropenic patients, or whenever there is a suspicion of bacteraemia. On the other hand, combination chemotherapy with at least two different anti-pseudomonal agents is normally recommended for the treatment of severe *P. aeruginosa* infections, such as endocarditis, nosocomial pneumonia and bacteraemia [4]. The rationale for combination chemotherapy is essentially to reduce the chances of selection of resistant mutants during therapy, as well as to exploit the potential synergistic activity of some agents. The preferred combination remains aminoglycosides and β -lactams, as synergism between these drugs has been demonstrated by in-vitro studies [139–141], and results of several clinical studies point to the superiority of similar regimens as opposed to monotherapy for the treatment of *P. aeruginosa* bacteraemia, especially in neutropenic patients [20,142–144]. However, some clinical studies have cast doubt on the actual superiority of combination chemotherapy, at least in some cases [22,68,145,146]. These apparently conflicting results probably reflect the complexity of variables that come into play in similar studies. It has also been argued that the absence of demonstrated superiority of combination therapy over monotherapy demonstrated in some studies may be due to the fact that the combinations were not synergistic against the pathogen [147]. Indeed, the superiority of combinations that exhibit synergistic activity *in vitro* as opposed to non-synergistic combinations has been reported in some studies [143,148,149]. Whilst waiting for the results of large prospective randomised trials comparing monotherapy and combination drug

therapy for serious nosocomial *P. aeruginosa* infections, most physicians continue to prefer combination therapy for treatment; combination chemotherapy, although not showing an unequivocally superior effect to monotherapy, has never been shown to be inferior [4,150].

Concerning the empirical treatment of febrile neutropenic patients, where *P. aeruginosa* can be a causative agent, therapeutic regimens, as well as the use of single vs. combination chemotherapy, have been a matter of continued debate. Several studies point to a comparable efficacy of monotherapy with broad-spectrum β -lactams compared with the combination of these drugs and an aminoglycoside [151–157]. However, they also underscore the importance of a careful consideration of local epidemiological data in selection of the therapeutic regimen [155,157]. Indeed, when surveillance data reveal a notable level of resistance in *P. aeruginosa*, empirical regimens for *P. aeruginosa* coverage might require the initial use of two or more agents [130]. Some authors still recommend that, when *P. aeruginosa* bacteraemia is strongly suspected on the basis of clinical setting or local epidemiology, combination therapy should be instituted with an aminoglycoside and a β -lactam with dependable anti-pseudomonal activity, and, if *P. aeruginosa* bacteraemia is subsequently documented, a similar combination regimen should be initiated even if the patient has already responded to monotherapy [4].

In cystic fibrosis patients, early aggressive combination chemotherapy is currently recommended for initial colonisation episodes, to delay as long as possible the onset of chronic *P. aeruginosa* infection, whilst maintenance chemotherapy based on the administration of anti-pseudomonal agents at regular intervals can significantly improve the survival of these patients once they have developed chronic *P. aeruginosa* infections [158–160].

FACTORS INFLUENCING ACQUIRED RESISTANCE

Several factors indicate that the emergence and spread of drug-resistant *P. aeruginosa* can be related to the overuse of antimicrobial agents, although the risk appears to differ with different agents.

A strong association between use and resistance has been documented for carbapenems. A significant increase in the incidence of imipenem-

resistant *P. aeruginosa* was observed at a large New York medical centre following an intervention that restricted the use of cephalosporins (in order to control the spread of ceftazidime-resistant *Klebsiella pneumoniae*) and caused, at the same time, an increased imipenem consumption [161]. A strong association between imipenem use and resistance in *P. aeruginosa* was also observed in a 3-year survey of antimicrobial consumption and resistance carried out in a German hospital [162]. In that study, imipenem consumption was found to be significantly associated with imipenem resistance rates and also with ceftazidime and piperacillin–tazobactam resistance rates from the same month and from the following month. By contrast, no correlation was observed between the consumption of ceftazidime or piperacillin–tazobactam and resistance to the same drugs or to imipenem. Finally, in a cohort study comparing the relative risks for the emergence of resistant *P. aeruginosa* in patients treated with different anti-pseudomonal agents, imipenem was found to be associated with a significantly higher overall risk of emergence of resistance, and exhibited the highest risk of emergence of resistance to itself. On the other hand, ceftazidime had the lowest overall risk for the emergence of resistance and showed no significant association with the emergence of resistance to itself. Piperacillin and ciprofloxacin showed a low overall risk for emergence of resistance, but were distinctly associated with the emergence of resistance to themselves [108].

Several case–control studies carried out amongst hospitalised patients point to the role of antimicrobial agents, in addition to other variables (such as ICU stay and length of hospitalisation), as risk factors for drug-resistant *P. aeruginosa*. In particular, following multivariable analysis, fluoroquinolones were identified as risk factors for piperacillin-resistant *P. aeruginosa* in ventilator-associated pneumonia [163], while imipenem, piperacillin–tazobactam and aminoglycosides were identified as risk factors for imipenem-resistant *P. aeruginosa* [164]; the same drugs plus extended-spectrum cephalosporins were identified as risk factors for piperacillin–tazobactam-resistant *P. aeruginosa* [165].

The effect of antimicrobial restriction on *P. aeruginosa* resistance has been investigated in a recently published study carried out in a large teaching hospital [166]. In that study, the inter-

vention caused a remarkable reduction in the use of ceftazidime and a consistent reduction in the use of imipenem, and was associated with a significant decrease in the resistance rates to these two drugs. Interestingly, a significant decrease in resistance rates was also observed for piperacillin and aztreonam, notwithstanding minimal changes in the overall use of piperacillin and piperacillin-tazobactam and a consistent increase in the use of aztreonam. In another prospective study, carried out in a medical ICU in another large teaching hospital, ceftazidime and ciprofloxacin restriction, in combination with an antimicrobial rotation strategy, was shown to be effective in reducing the overall incidence of ventilator-associated pneumonia and the resistance rates of *P. aeruginosa* to various antimicrobial agents, including extended-spectrum cephalosporins, aminoglycosides and fluoroquinolones [167].

Formulary interventions aimed at the prudent use of certain drugs may therefore be beneficial for the control of the antimicrobial resistance of *P. aeruginosa* in hospital settings, similar to the observations made for other resistant pathogens [168,169]. There is also a need to underscore the importance of strict compliance with recommended infection control practices to limit the spread of MDR *P. aeruginosa* clones within the hospital environment [170,171].

CONCLUSION

P. aeruginosa remains one of the most important and difficult to treat nosocomial pathogens. MDR strains are increasingly being reported and, in these cases, the choice of therapy often becomes very limited, especially when looking for antimicrobial combinations to treat severe infections. An additional matter of concern is represented by the fact that no new antimicrobial agents, active against MDR strains of *P. aeruginosa*, are in advanced stages of development as therapeutic options. The development of clinafloxacin, a fluoroquinolone that is slightly more active than ciprofloxacin against *P. aeruginosa* [172], has been suspended. Inhibitors of multidrug efflux systems [173] and new β -lactamase inhibitors that are highly active against AmpC and/or the metalloenzymes [174,175] are the subject of intensive investigation, and could become valuable tools to rescue the activity of fluoroquinolones and β -lactams against resistant strains, as well as to

broaden the repertoire of useful anti-pseudomonal drugs. Also promising is the development of new antibacterial peptides that disrupt the bacterial lipopolysaccharide, and may be especially useful for topical application against MDR isolates [176–178].

While the medical community awaits the development of new drugs, MDR *P. aeruginosa* strains are likely to represent an increasing threat, and every effort should be made to preserve as long as possible, or to restore, the efficacy of currently available agents.

ACKNOWLEDGEMENTS

The work on *P. aeruginosa* epidemiology and resistance mechanisms in the authors' laboratory is supported by grant no. HPRN-CT-2002-00264 from the European Union (Metallo- β -lactamases as model Zn enzymes Project) and by grant no. 2001068755-003 from Ministero dell' Istruzione, dell' Università e della Ricerca.

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