ABSTRACT

Pseudomonas aeruginosa is a major cause of nosocomial infections. This organism shows a remarkable capacity to resist antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets. Worryingly, these mechanisms are often present simultaneously, thereby conferring multiresistant phenotypes. Susceptibility testing is therefore crucial in clinical practice. Empirical treatment usually involves combination therapy, selected on the basis of known local epidemiology (usually a β-lactam plus an aminoglycoside or a fluoroquinolone). However, therapy should be simplified as soon as possible, based on susceptibility data and the patient’s clinical evolution. Alternative drugs (e.g., colistin) have proven useful against multiresistant strains, but innovative therapeutic options for the future remain scarce, while attempts to develop vaccines have been unsuccessful to date. Among broad-spectrum antibiotics in development, ceftobiprole, sitafloxacin and doripenem show interesting in-vitro activity, although the first two molecules have been evaluated in clinics only against Gram-positive organisms. Doripenem has received a fast track designation from the US Food and Drug Administration for the treatment of nosocomial pneumonia. Pump inhibitors are undergoing phase I trials in cystic fibrosis patients. Therefore, selecting appropriate antibiotics and optimising their use on the basis of pharmacodynamic concepts currently remains the best way of coping with pseudomonal infections.

Keywords  Antibiotic therapy, cystic fibrosis, nosocomial infections, Pseudomonas aeruginosa, resistance, therapeutic options

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strain, achieved in 2000, has provided a great deal of useful information, concerning not only its pathogenicity, but also its potential for resistance [2]. With 5570 open reading frames, the \( P. \) aeruginosa genome is among the largest genomes in the prokaryotic world, and encodes an unusually high proportion of proteins involved in regulation, transport and virulence functions, which may explain the high versatility and adaptive capacity of this species. In addition, 0.3% of the total genes code for proteins involved in antimicrobial resistance. The genome is also highly flexible, with 10% of genes organised in ‘pathogenicity islands’, comprising variable genes coding for virulence factors, and with the ability to easily acquire large mobile genetic elements (integrons) encoding resistance genes [3–5]. The large size and the complexity of this genome is probably the basis for the capacity of \( P. \) aeruginosa to not only thrive in diverse environments and to infect a large variety of body sites, but also to resist (intrinsically or after acquisition of the necessary genes) a large number of antimicrobial agents.

**CLINICAL MANIFESTATIONS**

Most \( P. \) aeruginosa strains involved in infections are both invasive and toxigenic, as a result of the production of surface virulence factors (allowing bacterial attachment, colonisation and invasion) and secreted virulence factors (which damage tissues or trigger the production of cytokines), respectively [3]. The combination of virulence determinants expressed by each strain tends to determine the specific syndromes accompanying an infection. However, in the clinic, it is often difficult to distinguish between simple colonisation and infection, and no diagnostic tool is available to assess the virulence potential of a given isolate.

\( P. \) aeruginosa infects healthy tissues rarely, but, when defences are compromised, it can infect virtually all tissues. This explains why most infections are nosocomial [6]. Table 1 lists the main pathologies caused by \( P. \) aeruginosa. These infections should be considered as severe, and even life-threatening in specific situations, with the highest rates of mortality recorded for cases of bacteraemia in neutropenic patients (30–50%) [7] and cases of nosocomial pneumonia (45–70%) [8,9]. \( P. \) aeruginosa is well-adapted to the respiratory tract environment, especially in patients with chronic obstructive bronchopulmonary disease, who are immunocompromised, or who are hospitalised in intensive care units [10–12]. Accordingly, \( P. \) aeruginosa is the predominant cause of nosocomial pneumonia in ventilated patients [13] and of lung infection in patients with cystic fibrosis [14]. It also causes chronic colonisation of the airways of patients suffering from bronchiectasis, chronic obstructive bronchopulmonary disease or cystic fibrosis [15]. In neutropenic cancer patients undergoing chemotherapy, bacteraemia with \( P. \) aeruginosa is a common complication [16]. Bacteraemia and septicaemia can also occur in patients with immunodeficiency related to AIDS, diabetes mellitus or severe burns [17–19]. Most of these infections are acquired in hospitals and nursing homes [20]. \( P. \) aeruginosa is also the third leading cause (12%) of hospital-acquired urinary tract infections [21]. These infections can occur via ascending or descending routes and are usually secondary to urinary tract catheterisation, instrumentation or surgery [22]. \( P. \) aeruginosa is the predominant causal agent of ‘swimmer’s ear’

| Table 1. Main pathologies caused by \( Pseudomonas \) \( aeruginosa \), grouped according to the infection site (adapted from [1]) |
|-----------------|-----------------|-----------------|
| Infection site | Specific pathologies | Occurrence (at risk population) |
| Respiratory tract | Acute pneumonia | Frequent (hospital; ICU) |
| | Chronic lower respiratory tract infections | (Cystic fibrosis) |
| Blood | Bacteraemia and septicaemia | Frequent |
| | Acute infections | Relatively frequent |
| | Chronic infections | (complication resulting from the presence of foreign bodies) |
| Ear | Otitis externa (‘swimmer’s ear’) | Frequent |
| | Malignant external otitis | |
| | Chronic suppurative otitis media | |
| Skin and soft-tissue infections | Dermatitis | Relatively frequent (Trauma) |
| | Wound infections | |
| | Burn wound sepsis | |
| | Ecthyma gangrenosum | |
| | Pyoderma | |
| | Folliculitis | |
| | Unmanageable forms of acne vulgaris | |
| Eye | Keratitis (corneal ulcer) | Rare (secondary to trauma) |
| | Endophthalmitis | |
| | Neonatal opthalmia | |
| Central nervous system | Meningitis | Rare (secondary to neurosurgery or trauma) |
| | Brain abscess | |
| Heart | Endocarditis | Rare (drug addicts) |
| | Stenocardial pyarthrosis | |
| Bone and joint infections | Vertebral osteomyelitis | |
| | Syphymosis pubis infection | |
| | Osteochondritis of the foot | |
| | Chronic contiguous osteomyelitis | |
| Gastrointestinal tract | Necrotising enterocolitis | Rare |
| | Peri-rectal infections | |

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(a form of external otitis) [23] and of malignant otitis in diabetic patients [24]. Although less frequent than other organisms, *P. aeruginosa* can also be the cause of devastating ophthalmic infections (e.g., bacterial keratitis in individuals with contact lenses [25], or neonatal ophthalmia), meningitis and brain abscesses (spreading from contiguous structures such as the inner ear or paranasal sinus, or subsequent to trauma, surgery or invasive diagnostic procedures [26]), and endocarditis in intravenous drug users [27,28]. Skin and bone infections are rare, but can occur after puncture wounds [1]. *P. aeruginosa* rarely causes true infections of the digestive tract (although peri-rectal infections, typical gastroenteritis and necrotising enterocolitis have been reported), but colonisation by *P. aeruginosa* favours the development of invasive infections in patients at risk [29].

**ANTIBIOTIC RESISTANCE**

*P. aeruginosa* is intrinsically resistant to several antibiotics because of the low permeability of its outer-membrane, the constitutive expression of various efflux pumps, and the production of antibiotic-inactivating enzymes (e.g., cephalosporinases) [30]. Furthermore, it also has a remarkable capacity to develop or acquire new mechanisms of resistance to antibiotics. This may be related to the large size and the versatility of its genome, and to its distribution in aquatic habitats, which could constitute a reservoir for bacteria carrying other resistance genes [31]. Infections caused by resistant strains are a matter of concern in many hospitals worldwide, since they are associated with a three-fold higher rate of mortality, a nine-fold higher rate of secondary bacteraemia, a two-fold increase in the length of hospital stay, and a considerable increase in healthcare costs [32].

Table 2 summarises the main resistance mechanisms that have been described in clinical isolates for the three main classes of current anti-pseudomonal agents (*β*-lactams, aminoglycosides and fluoroquinolones). These mechanisms are often present simultaneously, conferring multidrug resistance to many strains [33,34].

Reduced outer-membrane permeability caused, for example, by qualitative or quantitative alterations of the OprD porin (the uptake pathway for hydrophilic carbapenems such as imipenem

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Table 2. Main mechanisms of resistance to antimicrobial agents used for the treatment of *Pseudomonas aeruginosa* infection [35,36,65]

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Permeability alterations</th>
<th>Antibiotic-inactivating enzymes</th>
<th>Target modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active efflux</td>
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<tr>
<td><em>β</em>-Lactams</td>
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<tr>
<td>Cephalosporines</td>
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<tr>
<td>Over-expressing enzymes</td>
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<tr>
<td>OprD loss</td>
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<tr>
<td>MexAB MexCD MexEF MexXY MexVW</td>
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<tr>
<td>Aminoglycoside-modifying enzymes</td>
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<tr>
<td>Mutations in topoisomerases</td>
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<tr>
<td>Ribosomal methylation</td>
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<tr>
<td>AAC(3)-I</td>
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<td>AAC(6¢)-I</td>
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<td>AAC(6¢)-II</td>
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<td>ANT(2¢)-I</td>
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<th>Antibiotics</th>
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<tbody>
<tr>
<td><em>β</em>-Lactams</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillins</td>
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<td>+</td>
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<tr>
<td>Cephalosporins</td>
<td>+</td>
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<tr>
<td>Aminoglycosides</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>

AMR, antibiotic; GEN, gentamicin; NET, netilmicin; TOB, tobramycin.
and, to some extent, meropenem [35]), have been associated with an increase in drug efflux, a mechanism that confers cross-resistance to many unrelated antibiotic classes [36]. The major efflux systems involved in *P. aeruginosa* resistance belong to the Hydrophobic/Amphiphilic Efflux-1 (HAE1) family, a subclass of the Resistance Nodulation Division (RND) transporter superfamily, which are energised by the proton-motive force. These transporters function in conjunction with a ‘membrane fusion protein’ and an ‘outer-membrane factor’ to allow the efflux of drug molecules across both membranes of the Gram-negative bacterial cell envelope in a single energy-coupled step [37]. Twelve of these putative tripartite assemblies have been identified to date, based on sequence homologies [2], among which seven have already been shown to transport antibiotics [36].

Some of these systems are expressed at a basal level in wild-type strains (MexAB–OprM), and participate in the intrinsic resistance of *P. aeruginosa*. Others are induced markedly in response to antibiotic pressure, but are expressed at a low level (MexXY–OprM) or not at all (MexCD–OprJ and MexEF–OprN) in the absence of antibiotic [37].

Exposure to a single antibiotic may select for mutants with increased pump production that show cross-resistance to all the antibiotics that are substrates of the derepressed pump. Quinolones, which are substrates of all Mex efflux pumps [38], appear to be particularly prone to select for cross-resistance to aminoglycosides or β-lactams (see Table 2 for substrate specificities of efflux pumps). Importantly, the OprD porin and the MexEF–OprN pumps are under the control of common regulators acting in opposite ways, so that increased expression of this pump [39] will also cause resistance to antibiotics that are not effluxed, but require the porin for entry (Table 2).

Efflux is usually considered to confer a low-to-moderate level of resistance [40], but it plays a major role in clinical isolates for at least three reasons. First, it severely narrows the choice of active antibiotics (e.g., the over-expression of MexXY–OprM in clinical isolates confers resistance not only to aminoglycosides, but also to cefepime and fluoroquinolones [41]). Second, it can cooperate with other mechanisms (e.g., mutations in quinolone targets or production of β-lactamases) to confer higher levels of resistance [42–44]. Third, it can favour the emergence of target mutations [45] by lowering the intra-bacterial antibiotic concentrations.

Enzymic inactivation of antibiotics has been described for β-lactams and aminoglycosides. Among β-lactamas, extended-spectrum β-lactamas (ESBLs) and carbapenemases (mainly metallo-β-lactamas) have spread widely in recent years. ESBLs usually confer resistance to all β-lactams except carbapenems (although certain types, such as the GES-2 enzyme, are able to hydrolyse carbapenems [46]). These enzymes have, to date, been found in a limited number of geographical areas, suggesting that certain of these β-lactamase genes may occur in specific ecosystems [46]. However, new enzymes are described regularly [47,48], and the proportion of ESBL-producing strains is increasing globally [49,50]. ESBLs inhibited by clavulanic acid are reported mostly in Enterobacteriaceae, although BEL-1 has been reported only in *P. aeruginosa* and CTX-M enzymes have been reported only in Enterobacteriaceae. The PER-1 ESBL remains mostly confined to *P. aeruginosa* from Turkey and southeast Asia. Carbapenem-hydrolysing metallo-β-lactamas inactivate all subclasses of β-lactams except monobactams. These carbapenemases are reported most frequently in Asia [51], but outbreaks have also been described in Europe in recent years [52–54]. These enzymes belong to the IMP and VIM (mostly VIM-2) classes, or less frequently, to the SIM, GIM or SPM classes. Importantly, the genes encoding IMP-like and VIM-like carbapenemases are located in integrons containing other resistance genes (e.g., aminoglycoside-inactivating enzymes) [51,55,56], so that these isolates will show co-resistance phenotypes. Enzymes inactivating aminoglycosides are present worldwide, and are detected in up to 20% of clinical isolates in Europe and Latin America [57]. Acting on specific substituents of the aminoglycoside molecule, they do not necessarily confer cross-resistance to all aminoglycosides. Thus, amikacin, which is a poor substrate for these enzymes, usually demonstrates better activity against *P. aeruginosa* than do other aminoglycosides [58].

Target mutation is a well-known mechanism of resistance to fluoroquinolones. Whereas fluoroquinolones differ in their affinities for their target enzymes (topoisomerase IV and DNA gyrase [59,60]), the gyrase is the primary target in
P. aeruginosa, making mutations at this level (gyrA) the first step in resistance [61]. Among the fluoroquinolones available currently, ciprofloxacin has the highest affinity for GyrA, and its inhibitory potency is reduced c. 16-fold in gyrA mutants. Other quinolones suffer a similar reduction of activity, which almost always increases the MIC to above the susceptibility breakpoint. Target modification (methylation of 16S rRNA) has also been shown to confer resistance to aminoglycosides [62]. This resistance mechanism could have spread to P. aeruginosa from aminoglycoside-producing Gram-positive organisms [63,64].

Fig. 1 shows the evolution of the susceptibility patterns of P. aeruginosa for nine major antibiotics used currently in clinical practice. This analysis is based on European data collected as part of the ‘Meropenem Yearly Susceptibility Test Information Collection’ (MYSTIC) surveillance study (http://www.mystic-data.org/) and the susceptibility breakpoints proposed by the European Committee for Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org). On average, there is 60% susceptibility to all drugs except meropenem (80% susceptibility) and amikacin (c. 100% susceptibility). A modest trend towards decreased resistance was observed for some drugs during the last decade if the cumulative MIC distributions are considered (causing a decrease in the MIC50), but this is insufficient to modify the percentage of strains falling below the EUCAST clinical susceptibility breakpoints. Perhaps more importantly, the frequencies of multi-drug-resistant P. aeruginosa (defined as showing resistance to at least three main classes of anti-pseudomonal agents (β-lactams, carbapenems, aminoglycosides and fluoroquinolones)) [21] are increasing worldwide, reaching frequencies of up to 20% in intensive care units in the USA and >30% in Asia [11,21,33,65,66]. These isolates combine several mechanisms of resistance, often present on mobile genetic elements, and are usually associated with severe adverse clinical outcomes [21,48,67]. This is also true for isolates producing ESBLs or carbapenemases [49,68].

Control measures to limit the spread of highly resistant clones appear to be essential. At the clinical level, these should include the strict implementation of infection control measures (improvement of hand hygiene) aimed at controlling and preventing cross-transmission among patients, within and across units/wards, and even among hospitals, and the strict isolation and restriction of transfer of infected or colonised patients with multiresistant P. aeruginosa isolates [46]. At the laboratory level, in-vitro studies, including quantitative data (MIC determinations), should be performed on a regular basis to follow the resistance patterns of the clones present in a particular hospital. This knowledge is essential in order to choose the most appropriate antibiotics for empirical treatment. Studies aimed at deciphering the modes of transmission of these clones would also be of interest when formulating rational strategies for better control of their spread.

At the therapeutic level, improvement of antibiotic use is a highly efficient strategy for decreasing rates of resistance [65]. Two lines of action should probably be followed. First, interventions aiming at reducing antibiotic use in general, and at restricting the administration of certain specific drugs, are beneficial [69,70]. Indeed, there is a strong correlation between antibiotic consumption and resistance rates for P. aeruginosa [71], as for many other pathogenic bacteria. Antibiotic rotation (to avoid continuous exposure to the same drugs) has also been proposed, but no data support its benefit for resistance control to date. Second, optimisation of antibiotic dosage regimens, based on the pharmacokinetic/pharmacodynamic properties of the drugs used, is now considered to be essential for appropriate treatment of pseudomonal infections [72]. Table 3 shows an application of these principles to the main anti-pseudomonal agents for which data concerning optimisation are available. The pharmacokinetic/pharmacodynamic breakpoints proposed are largely in agreement with those suggested by EUCAST (Fig. 1).

**DIAGNOSIS**

Based on the wide diversity of P. aeruginosa infections, the frequent spread of epidemic isolates in hospitals, and the high level of drug resistance in this species, diagnostic procedures should aim not only to identify the pathogen, but also to determine its susceptibility to antibiotics. Isolation and identification of P. aeruginosa cultures is easy and is based on classical microbiological growth, cultural and phenotypic characteristics [73]. It is of note that P. aeruginosa can lead to false-positive results in immunological
Fig. 1. Temporal evolution of the MIC distributions of nine antibiotics for clinical isolates of *Pseudomonas aeruginosa* between 1997 and 2005. MIC data were extracted from the MYSTIC database (http://www.mystic-data.org/), but were limited to European countries (including Bulgaria, Croatia, the Czech Republic, Greece, Israel, Italy, Malta, Poland, Portugal, Romania, Russia, Slovenia, Spain, Switzerland and Turkey). The susceptibility breakpoints (S, susceptible; I, intermediately-susceptible; R, resistant) are those proposed by EUCAST (http://www.eucast.org); note that no EUCAST breakpoint has been established to date for piperacillin–tazobactam. The inset tables for each antibiotic give the MIC$_{50s}$ and the percentage of strains with an MIC of less than or equal to the fully-susceptible breakpoint.
### Table 3. Tentative pharmacodynamic breakpoints for anti-pseudomonal agents, based on pharmokinetic/pharmacodynamic (PK/PD) criteria of efficacy and on pharmacokinetic data for conventional dosages, in comparison with the EUCAST breakpoints

<table>
<thead>
<tr>
<th>Drug</th>
<th>PK/PD parameter predictive of breakpoint efficacy (mg/L)</th>
<th>Usual clinical dosage for serious infections (mg/L)</th>
<th>Relevant pharmacokinetic parameter(s)</th>
<th>PD</th>
<th>EUCAST*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>Time &gt; MIC = 40% (static effect) to 100% (max. efficacy) [72]</td>
<td>4.5 g qid [97]</td>
<td>(c_{max} = c. 225 \text{ mg/L}, \text{ half-life } c. 1 \text{ h} [193])</td>
<td>3.5</td>
<td>b</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td></td>
<td>2 g tid [193]</td>
<td>(c_{max} = c. 170 \text{ mg/L}, \text{ half-life } c. 2 \text{ h} [193])</td>
<td>10-40</td>
<td>8/8</td>
</tr>
<tr>
<td>Ceftepime</td>
<td></td>
<td>2 g tid [193]</td>
<td>(c_{max} = c. 160 \text{ mg/L}, \text{ half-life } c. 2 \text{ h} [193])</td>
<td>10-40</td>
<td>8/8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Time &gt; MIC = 22% (static effect) to 100% (max. efficacy) [35]</td>
<td>1 g tid [193]</td>
<td>(c_{max} = c. 60 \text{ mg/L}, \text{ half-life } c. 1 \text{ h} [193])</td>
<td>0.2-15</td>
<td>2/8</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td>1 g tid [193]</td>
<td>(c_{max} = c. 60 \text{ mg/L}, \text{ half-life } c. 1 \text{ h} [193])</td>
<td>0.2-15</td>
<td>2/8</td>
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<tr>
<td>Aminoglycosides</td>
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<tr>
<td>Gentamicin</td>
<td>(c_{max}/\text{MIC} \geq 8 [194])</td>
<td>5 mg/kg [193]</td>
<td>(c_{max} = c. 18 \text{ mg/L} [193])</td>
<td>1.5</td>
<td>4/4</td>
</tr>
<tr>
<td>Tobramycin</td>
<td></td>
<td>7 mg/kg [97]</td>
<td>(c_{max} = c. 25 \text{ mg/L} [193])</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td>5 mg/kg [193]</td>
<td>(c_{max} = c. 25 \text{ mg/L} [193])</td>
<td>3</td>
<td></td>
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<tr>
<td>Fluoroquinolones</td>
<td></td>
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<tr>
<td>AUC/MIC &gt; 100</td>
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<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>120 mg [97]</td>
<td>(c_{max} = c. 100 \text{ mg/L} [193])</td>
<td>12.5</td>
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<tr>
<td>Levofloxacin</td>
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<td></td>
<td></td>
<td>400 mg bid [195]</td>
<td>(AUC = 30 \text{ mg.h/L} [193])</td>
<td>0.3</td>
<td>0.5/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 mg bid [195]</td>
<td>(AUC = 90 \text{ mg.h/L} [193])</td>
<td>1</td>
<td>1/2</td>
</tr>
</tbody>
</table>

*Values are shown as susceptible/resistant (susceptible, antimicrobial activity associated with a high likelihood of therapeutic success; resistant, antimicrobial activity associated with a high likelihood of therapeutic failure).

*Breakpoint not yet defined.

b, the dose indicated is administered twice in 24 h at 12-h intervals; tid, the dose indicated is administered three times in 24 h at 8-h intervals; qid, the dose indicated is administered four times in 24 h at 6-h intervals.

Tests for the detection of Helicobacter pylori [74]. Although phenotypic methods are sufficient to identify the pathogen in most clinical samples, molecular typing methods are often necessary, not only to trace epidemic strains and to detect outbreaks or cross-transmission in the hospital setting [75], but also to characterise long-term colonising isolates with atypical phenotypes, as have been observed in cystic fibrosis patients [76]. Highly discriminatory techniques, refined over the past decade, include pulsed-field gel electrophoresis [77,78], chromosomal restriction fragment length polymorphism analysis [79], random amplified polymorphic DNA analysis [80,81], multilocus sequence typing [82,83], and arbitrarily primed PCR fingerprinting [84]. These techniques are generally available in specialised sentinel laboratories.

P. aeruginosa can be isolated on selective media such as cetrimide agar [85]. However, the frequent occurrence of P. aeruginosa as a colonising organism means that mere isolation of the bacterium from a biological sample does not in itself constitute proof of the involvement of P. aeruginosa in an infectious process. Specific investigations, e.g., X-rays and other imaging techniques, are therefore needed to confirm infections in deep organs.

A key point in laboratory tests for P. aeruginosa involves determining its susceptibility to antibiotics and identification of its resistance mechanisms. Routine procedures include diffusion methods (disk-diffusion and Etests), and dilution methods on solid or liquid media (agar, macro- and microdilutions, and automated systems [86]). However, these methodologies currently lack standardisation, as illustrated by a comparison of existing recommendations from the French Comité de l’Antibiogramme of the Société Française de Microbiologie (CA-SFM; http://www.sfm.asso.fr/), the British Society of Antimicrobial Chemotherapy (BSAC; http://bsac.test.tmg.co.uk/) and the CLSI (http://www.clsi.org/). Moreover, results are influenced markedly by several experimental factors. These include the initial inoculum size (which should be a MacFarland 0.5 standard, i.e., \(1.5 \times 10^8 \text{ CFU/mL}\)), the culture medium and its pH (acidic pH reduces the activity of numerous antibiotics, e.g., aminoglycosides), the concentration of ions (divalent...
CURRENT THERAPEUTIC OPTIONS

Antimicrobial therapy

Guidelines for the specific management of *P. aeruginosa* infections in patients with artificial ventilation [94] and neutropenia [95] have been proposed by a joint task force of the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA). However, the general principles of these guidelines can be applied to other infections [11,32,96–98] and can be summarised as follows (see Fig. 2 and Table 3 for antibiotic doses). First, any suspicion of pseudomonal infection should require bacteriological documentation, including the antibiotic susceptibility profile. Indeed, reliance on empirical treatment entirely is no longer reasonable in a world of increasing multidrug resistance. Second, therapy should be initiated as soon as clinical samples have been collected, using the best available knowledge to cover the suspected pathogens. Early therapy is associated with better outcome [99]. Initial therapy will depend on the patient’s risk-factors and the local epidemiology, but will usually include an anti-pseudomonal β-lactam (penicillin, cephalosporin or carbapenem) associated with either an aminoglycoside or a fluoroquinolone (preferably ciprofloxacin [60]). Third, treatment de-escalation and/or fine-tuning of the therapy must be mandatory once laboratory data are available. This is critical to limit antibiotic pressure and, hence, the selection of resistance, which frequently occurs during therapy and may result in a negative clinical outcome [96,100,101]. Finally, the patient’s condition should be re-evaluated on a regular basis, with appropriate measurements [13,102,103], to decide whether antibiotics should be continued.

In all cases, dosages should be adapted to meet pharmacodynamic criteria of efficacy (Table 3) [104]. Antibiotics with time-dependent activities, e.g., β-lactams, should be administered frequently (e.g., thrice-daily) or in continuous infusion. However, although the limited clinical data comparing the efficacy of these two modes of administration for β-lactams point towards equivalence [105], continuous infusion offers the advantages of increasing the probability of achieving the pharmacodynamic target [106] while limiting nursing workload (however, note that there are hardly any data concerning the clinical effectiveness of continuous infusion for treatment...
Table 4. Tentative standard antibiogram for detecting antibiotic resistance mechanisms of *Pseudomonas aeruginosa*\(^a\)

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\(^a\)Based on data from http://bsac.test.tmg.co.uk/ and [6,57,85,91,196–198]. The limit of 16 antibiotics is meant to allow determination with only one plate, which seems reasonable to implement in most routine laboratories. Antibiotics have been selected on the basis of their clinical interest and/or their usefulness for identifying specific resistance mechanisms. This selection may need to be amended in the light of the local epidemiology of resistance and of drug availability.

\(^b\)Resistance to imipenem in MexEF–OprN overproducers is mediated by the concomitant downregulation of the expression of OprD porin.
of *P. aeruginosa* infections). Stability of the antibiotic over time under the conditions of administration, and compatibility with any other drugs in the perfusion solution, need to be checked carefully [107]. For aminoglycosides, which have concentration-dependent activity, once-daily administration maximises peak concentrations, which allows optimal efficacy and may minimise toxicity [108–110]. For fluoroquinolones, the total daily dosage is probably most critical, as well as a clear understanding that, with current doses, MICs >0.5 mg/L tend to markedly increase the risk of failure and the emergence of resistance [111,112].

Controversial questions remain regarding the management of *P. aeruginosa* infections: (i) the need to maintain antibiotic combinations [113], and (ii) the optimal length of antibiotherapy [114]. Recent studies and meta-analyses of infections caused by non-multiresistant organisms have failed to find a benefit of antibiotic combinations if treatment is based on susceptibility data, whether for sepsis [115], or for ventilator-associated pneumonia [116]. Yet the outcome is poor when aminoglycosides are used for monotherapy rather than in combination with β-lactams [117]. Regarding treatment duration, the trend is also to shorten the period of antibiotic administration. An 8-day period of treatment does not worsen the outcome of patients suffering from ventilator-associated pneumonia [118], but saves money, reduces ecological pressure, and diminishes side-effects [98]. However, as a slightly higher percentage of recurrence of pulmonary infection has been observed, close surveillance of these patients should be maintained after an antibiotic is discontinued.

The situation is much more complex when confronting multidrug-resistant isolates, for which the activity of at least three major antibiotic classes is compromised [21]. It remains to be established whether particular antimicrobial agents better select for such multidrug-resistant strains. As no evidence-based guidelines are available, the antibiotic selection should be adapted on a case-by-case basis, taking into account the susceptibility testing results (preferably the MIC data). In such cases, combinations of several agents are usually recommended [96]. Among the various therapeutic alternatives, colistin has received renewed interest [119]. This molecule, discovered in the early 1950s, was abandoned because of a high incidence of nephrotoxicity [120]. The mode of action of colistin (disruption of the cytoplasmic membrane [121]) shelters it from cross-resistance from other anti-pseudomonal agents, and is unlikely to lead to the rapid selection of resistance [122,123]. The drug displays a concentration-dependent bactericidal activity [122] and has recently been re-introduced for the management of pulmonary infections in cystic fibrosis patients, either by the intravenous route or in the form of an aerosol [124], with lower rates of toxicity than reported previously [125]. A few studies, most of which are observational case series, have reported a favourable clinical response in various types of infections caused by multidrug-resistant *P. aeruginosa*, including bacteraemia, pneumonia and meningitis [126–129]. In-vitro studies also suggest that an association with rifampicin is synergic [130], but this observation needs to be further assessed in clinical settings [11].

The treatment of lung infections caused by *P. aeruginosa* in cystic fibrosis patients raises very specific additional questions, but also offers new opportunities. In this disease, colonisation by *P. aeruginosa* occurs at an early stage [131], with its prevalence increasing with age. Questions are related mostly to: (i) the opportunity of starting antibiotic treatment aimed at eradication in patients who have only recently been colonised; (ii) treatment in patients who are chronically colonised; and (iii) the selection of antibiotics. According to the European consensus definition [132], a chronic respiratory colonisation corresponds to the presence of *P. aeruginosa* in at least

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**Fig. 2.** General algorithm for the clinical management of *Pseudomonas aeruginosa* infections (based on [97]).
three positive respiratory cultures over a period of 6 months, with an interval of at least 1 month between two cultures, but without direct (inflammation, fever, etc.) or indirect (specific antibody response) signs of infection and tissue damage. At this stage of the disease, and even earlier (intermittent colonisation), various morphotypes, including mucoid colonies, tend to develop, which are refractory to antibiotic action [133,134]. Non-mucoid, sensitive strains are often involved initially [135–137] but, without early intervention, irreversible chronic colonisation, most often by mucoid stains, usually occurs within a few months.

The definition of chronic colonisation is still a matter of debate [138,139], but there is no doubt that this state has a major negative impact on the patient's prognosis, as it is associated with an accelerated decline of respiratory function (forced expiratory volume in 1 s (FEV1)) [140–142], shortened median life-expectancy [142,143], much higher treatment costs [144], and a decreased quality of life. For these reasons, avoiding or postponing chronic colonisation by P. aeruginosa has long been considered to be the major challenge in the care of cystic fibrosis patients [144–146]. In this context, close microbiological monitoring that allows early recognition and treatment of the first isolates of P. aeruginosa, as well as patient segregation in cystic fibrosis centres on the basis of bacteriological status, are regarded as key factors [146–148]. Early treatment often includes inhaled colistin or aminoglycoside and/or oral ciprofloxacin [149–153], but there is no current consensus concerning the optimal eradication regimen for early intervention, and the failure rate of this approach has been estimated to be c. 20% [147,151,154].

Arguments for and against early antibiotic use in such patients have been discussed at length in the literature (e.g., [132,134,138]). Arguments against are related to the subsequent high consumption of antibiotics, with the associated risk of selecting multiresistant organisms in patients who will frequently receive antimicrobial agents [155,156]. Arguments in favour include the easier eradication of non-mucoid morphotypes, which can protect patients from further colonisation for several years [132,134,138]. Although, to date, there is no evidence of decreased mortality or morbidity, or of improved quality of life [157,158], current recommendations encourage the latter strategy, based on its microbiological success [132]. It has been suggested that early prophylactic administration of inhaled antibiotics might be very effective [159,160], but this approach needs to be studied further. In chronically colonised patients, chronic suppressive antibiotic therapy with inhaled antibiotics and oral azithromycin is associated with FEV1 improvement and decreased pulmonary exacerbations. Intravenous treatment might be prescribed only when needed, or also as a routine 3-monthly elective regimen [151]. Higher doses of many antibiotics are often required to achieve effective serum levels in these patients because of differences in the volume of distribution and rate of elimination [161]. Finally, an important factor to be considered is the possibility of offering appropriate antimicrobial treatment to cystic fibrosis patients outside of the hospital, which is essential for their quality of life. Potential opportunities include the development of aerosols for the administration of aminoglycosides and colistin [162–166], the administration of β-lactams by continuous infusion using portable home pumps [167,168], and the possibility of using quinolones by the oral route in this special paediatric population [157,169,170]. However, home treatment could be less effective than hospital treatment, and obviously necessitates close supervision [171,172].

Surgery
Surgical treatment of pseudomonal infections is sometimes necessary in order to remove important collections of bacteria that are poorly accessible to antibiotics and to eliminate damaged tissues. Most surgical applications concern brain abscesses, infections of ears or eyes, bones or joints, the heart, and wounds or burns.

THE FUTURE OF ANTI-PSEUDOMONAL THERAPY
Drugs in the pipeline
In recent years, most research devoted to new antibiotics in the pharmaceutical industry has been orientated towards Gram-positive organisms, e.g., methicillin-resistant Staphylococcus aureus and multiresistant Streptococcus pneumoniae.
This is all the more unfortunate because the β-lactams marketed most recently have either weak (cefeplime, cepirome) or no useful (ertapepline) anti-pseudomonal activity. Thus, although P. aeruginosa infections clearly represent a persistent, as well as an evolving need [173], the prospects for the next few years are quite poor, with most of the upcoming drugs being simply more or less new derivatives of existing families of antimicrobial agents. A broad-spectrum cephalosporin (ceftobiprole), a new carbapenem (doripenem) and a new fluoroquinolone (sitafloxacin) are currently in phase III clinical trials, but have not been examined specifically for their anti-pseudomonal activity. Ceftobiprole was designed specifically for its activity against methicillin-resistant Staphylococcus aureus [174], but its MICs for P. aeruginosa are of the same order of magnitude as those of cefepime (MIC50 and MIC90, 2 and 8 mg/L, respectively [175]). As clinical trials of ceftobiprole do not include patients with pseudomonal infections, its registration for the corresponding indications is unlikely in the near future.

Doripenem, a derivative of meropenem, shows slightly improved activity towards P. aeruginosa [176–178]. Like meropenem, it is subject to efflux by MexAB–OprM [35]. Population pharmacokinetics predict that 500 mg of doripenem administered over 1 h every 8 h would be effective against bacterial strains with a doripenem MIC of <2 mg/L, which is the case for most Pseudomonas isolates tested so far, and that less susceptible strains could be treated with prolonged infusions [179]. Doripenem has now received a ‘fast track’ designation from the US Food and Drug Administration (FDA) for the treatment of nosocomial pneumonia. It is on the FDA list of orphan drugs as ‘designated’ (not yet approved) for ‘treatment of bronchopulmonary infection in patients with cystic fibrosis who are colonised with P. aeruginosa or Burkholderia cepacia’, and has been submitted as a New Drug application to the FDA for the treatment of complicated intra-abdominal and complicated urinary tract infections (December 2006). It is also under clinical investigation for complicated skin and soft-tissue infections, and for complicated urinary tract infections [35].

Sitafloxacin has activity comparable to that of ciprofloxacin towards wild-type strains of P. aeruginosa, but shows lower MICs for gyrA or parC mutants, probably because of a better affinity for the mutated targets [180]. However, ongoing clinical trials are orientated towards Gram-positive infections. A phase II, randomised, open-label, multicentre study demonstrated that sitafloxacin was as safe and as well-tolerated as imipenem for the treatment of pneumonia, including a small (c. 10%) proportion of nosocomial infections [181]. Further studies are needed in this setting. Tigecycline, the only broad-spectrum antibiotic to be marketed recently [182,183], is inactive against P. aeruginosa because of efflux mediated by induction of the MexXY–OprM system [184].

Faced with such a gloomy picture concerning new antibiotic molecules, the development of efflux pump inhibitors seemed at first glance to be an innovative and promising strategy (based on a comparison with the successful development and clinical impact of the inhibitors of β-Lactamases [185]). A large number of interesting molecules, acting on a series of efflux pumps in different bacteria, have been designed [186], but their clinical efficacy has not really been demonstrated to date. The most advanced compounds in the series are broad-spectrum inhibitors of Mex pumps in P. aeruginosa [187,188], with one compound now in phase I of clinical development for use as an aerosol with cystic fibrosis patients (http://www.mpexpharma.com). However, this narrow indication and specific mode of administration shows that systemic bioavailability and toxicity will probably represent major problems for the successful development of efflux pump inhibitors.

**Immunisation and genetic therapy**

A new avenue for preventing chronic pulmonary colonisation in cystic fibrosis patients, while limiting antibiotic use, could involve immunotherapy. Many efforts have been made in this direction [189], but clinical efficacy has, to date, been disappointing, especially for heterologous strains [190]. However, potential candidate immunotherapies are currently being assessed in a phase III clinical trial [191]. Cystic fibrosis patients also benefit from other vaccinations (viruses, Strep. pneumoniae), which contribute to a reduction in both the number of infective episodes and the number of antibiotics used [191].
CONCLUSIONS
At the turn of the third millennium, P. aeruginosa clearly represents one of the most challenging pathogenic bacteria. For microbiologists, the constant evolution of resistance, including the continuing appearance of new resistance mechanisms, and the complexity of multiresistant phenotypes, force the development of appropriate diagnostic tools. For pharmacologists, optimising current antibiotic use is a necessity based on the severity of infections and on resistance issues. Moreover, the development of new therapeutic strategies, including drugs acting on new targets [3], is urgently needed. For infection control practitioners and clinicians, the implementation of prophylactic measures aimed at reducing the risk of nosocomial infection [192], and the use of treatments based on microbiological and pharmacological data [72], should be priorities.

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