 10.1111/j.1469-0691.2007.01681.x

Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium

N. Mesaros[1], P. Nordmann[2], P. Plésiat[3], M. Roussel-Delvallez[4], J. Van Eldere[5], Y. Glupczynski[6], Y. Van Laethem[7], F. Jacobs[8], P. Lebecque[9], A. Malfroot[10], P. M. Tulkens[1] and F. Van Bambeke[1]

[1]Unité de Pharmacologie cellulaire and moléculaire, Université catholique de Louvain, Bruxelles, Belgium, [2]Hôpital de Bicêtre and Université de Paris XI, Paris, [3]Centre Hospitalo-Universitaire Jean Minjoz and Université de Franche-Comté, Besançon, [4]Centre Hospitalier Régional Universitaire (Hôpital Calmette) and Université de Lille 2, Lille, France, [5]Universitair Ziekenhuis Gasthuisberg and Katholieke Universiteit Leuven, Louvain, [6]Cliniques universitaires de Mont-Godinne, Yvoir and Université catholique de Louvain, [7]Centre Hospitalo-Universitaire St-Pierre and Université libre de Bruxelles, [8]Hôpital Erasme and Université libre de Bruxelles, [9]Cliniques universitaires Saint-Luc and Université catholique de Louvain and [10]Academisch Ziekenhuis and Vrije Universiteit Brussel AZ-VUB, Brussels, Belgium

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

Accepted: 24 November 2006

Clin Microbiol Infect 2007; **13**: 560–578

INTRODUCTION

Known for many years to be a cause of serious wound and surgical infections, but often regarded as a secondary or opportunistic invader rather than a cause of primary infection in healthy tissues, *Pseudomonas aeruginosa* has now clearly emerged as a major nosocomial pathogen in immunocompromised and debilitated patients, as well as in cystic fibrosis patients [1]. *P. aeruginosa* has always been considered to be a difficult target for antimicrobial chemotherapy. However, the complete sequencing of a wild-type *P. aeruginosa*

Correspondence author and reprint requests: F. Van Bambeke, Unité de Pharmacologie cellulaire et moléculaire, UCL7370 Avenue Mounier 73, 1200 Brussels, Belgium
E-mail: vanbambeke@facm.ucl.ac.be

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.

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
Urinary tract	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
	Wound infections	(Trauma)
	Burn wound sepsis	
	Ecthyma gangrenosa	(Neutropenic patients)
	Pyoderma	
	Folliculitis	
	Unmanageable forms of acne vulgaris	
Eye	Keratitis (corneal ulcer)	Rare (secondary to trauma)
	Endophthalmitis	
	Neonatal ophthalmia	
Central nervous system	Meningitis	Rare (secondary to neurosurgery or trauma)
	Brain abscess	
Heart	Endocarditis	Rare (drug addicts)
Bone and joint infections	Stenoarticular pyoarthrosis	Rare
	Vertebral osteomyelitis	
	Symphysis pubis infection	
	Osteochondritis of the foot	
	Chronic contiguous osteomyelitis	
Gastrointestinal tract	Necrotising enterocolitis	Rare
	Peri-rectal infections	

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 bronchiestasis, 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'

(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 multiresistance 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

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

	Resistance mechanisms																						
	Permeability alterations							Antibiotic-inactivating enzymes													Target modifications		
		Active efflux						β-Lactamases						Aminoglycoside-modifying enzymes									
Antibiotics	OprD loss	MexAB	MexCD	MexEF	MexXY	MexGH	MexVW	Cephalosporinase over-expression	Restricted-spectrum penicillinases	Extended-spectrum oxacillinases	Extended-spectrum	Metallo-β-lactamases	AAC (3)-I	AAC (3)-II	AAC (6′)-I	AAC (6′)-II	ANT (2″)-I	Mutations in topoisomerases	Ribosomal methylation
β-Lactams																			
Penicillins		+	(+)		(+)			+	+	+	+	+							
Cephalosporins		+			+			+	(+)	(+)	+	+							
Aztreonam		+	+					+	(+)	+	+								
Imipenem	+											+							
Meropenem	(+)	+										+							
Aminoglycosides					+								GEN	GEN, NET, TOB	NET, TOB, AMK	GEN, NET, TOB	GEN, TOB		+
Fluoroquinolones		+	+	+	+	+	+											+	

AMK, amikacin; 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-lactamases, extended-spectrum β-lactamases (ESBLs) and carbapenemases (mainly metallo-β-lactamases) 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 Enterobacteriacae, although BEL-1 has been reported only in *P. aeruginosa* and CTX-M enzymes have been reported only in Enterobacteriaecae. The PER-1 ESBL remains mostly confined to *P. aeruginosa* from Turkey and southeast Asia. Carbapenem-hydrolysing metallo-β-lactamases 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 Antibiotic 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 MIC_{50}), but this is insufficient to modify the percentage of strains falling below the EUCAST clinical susceptibility breakpoints. Perhaps more importantly, the frequencies of multidrug-resistant *P. aeruginosa* (defined as showing resistance to at least three main classes of antipseudomonal 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

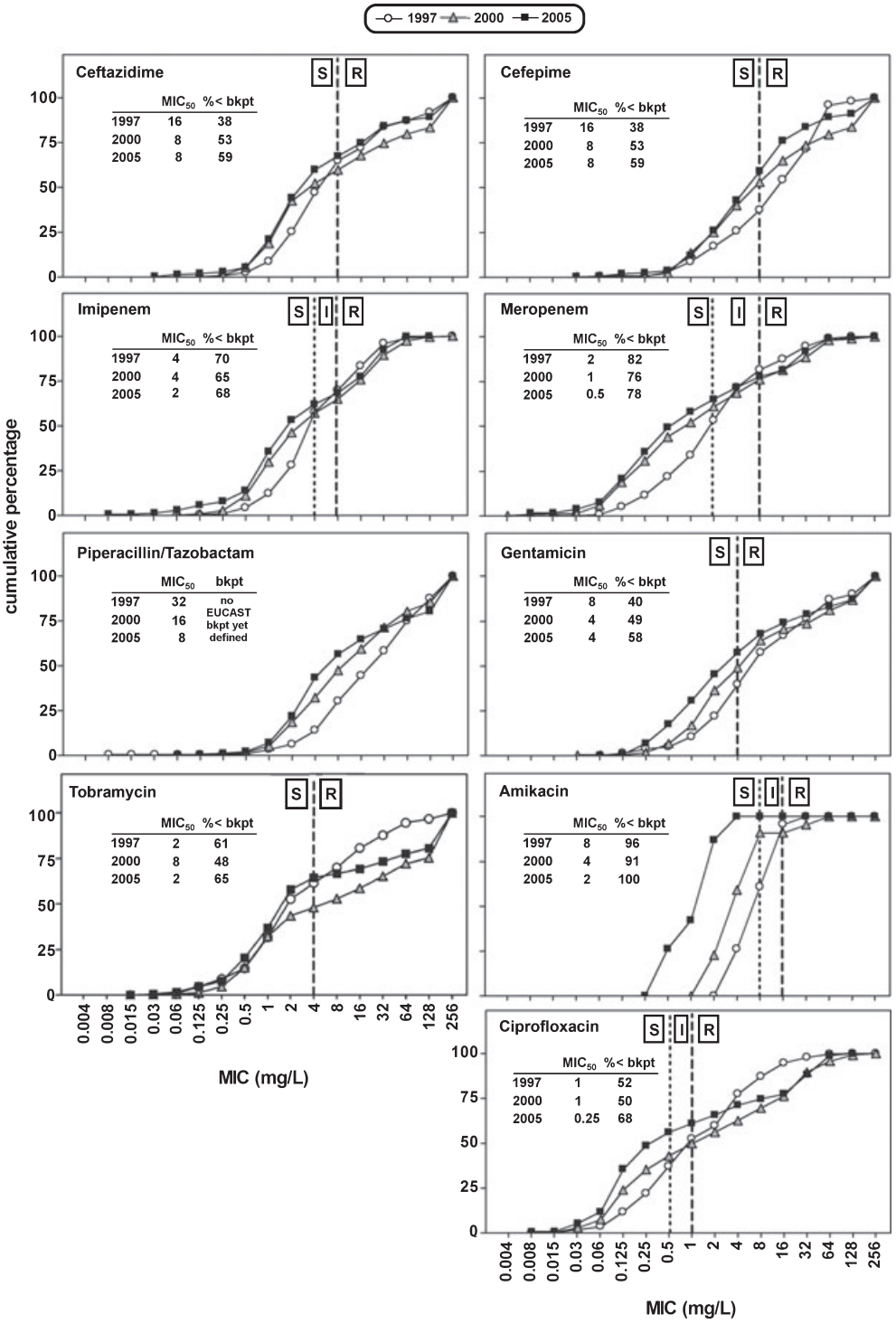

Fig. 1. Temporal evolution of the MIC distributions of nine antibiotics for clinical isolates of *Pseudomonas aeruginosa* between 1997 and 2005. MIC data were extracted from the MYSTIC database (http://www.mystic-data.org/), but were limited to European countries (including Bulgaria, Croatia, the Czech 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_{50}s 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

Drug	PK/PD parameter predictive of breakpoint efficacy (mg/L)	Usual clinical dosage for serious infections (mg/L)	Relevant pharmacokinetic parameter(s)	PD	EUCAST[a]
β-Lactams					
Piperacillin–tazobactam	Time > MIC = 40% (static effect) to 100% (max. efficacy) [72]	4.5 g qid [97]	C_{max} = *c.* 225 mg/L, half-life *c.* 1 h [193]	3.5	[b]
Ceftazidime		2 g tid [193]	C_{max} = *c.* 170 mg/L, half-life *c.* 2 h [193]	10–40	8/8
Cefepime		2 g tid [193]	C_{max} = *c.* 160 mg/L, half-life *c.* 2 h [193]	10–40	8/8
Imipenem	Time > MIC = 22% (static effect) to 100% (max. efficacy) [35]	1 g tid [193]	C_{max} = *c.* 60 mg/L, half-life *c.* 1 h [193]	0.2–15	2/8
Meropenem		1 g tid [193]	C_{max} = *c.* 60 mg/L, half-life *c.* 1 h [193]	0.2–15	2/8
Aminoglycosides	C_{max}/MIC ≥ 8 [194]				
Gentamicin		5 mg/kg [193] 7 mg/kg [97]	C_{max} = *c.* 18 mg/L [193] C_{max} = *c.* 25 mg/L [193]	1.5 3	4/4
Tobramycin		5 mg/kg [193]	C_{max} = *c.* 25 mg/L	3	4/4
Amikacin		15 mg/kg [193] 20 mg/kg [97]	C_{max} = *c.* 77 mg/L [193] C_{max} = *c.* 100 mg/L [193]	9 12.5	8/16
Fluoroquinolones	AUC/MIC > 100 [72,111]				
Ciprofloxacin		400 mg tid [193]	AUC = 30 mg.h/L [193]	0.3	0.5/1
Levofloxacin		500 mg bid [195]	AUC = 90 mg.h/L [195]	1	1/2

[a]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).
[b]Breakpoint not yet defined.
bid, 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., 1.5×10^8 CFU/mL), the culture medium and its pH (acidic pH reduces the activity of numerous antibiotics, e.g., aminoglycosides), the concentration of ions (divalent

cations negatively affect the activity of quinolones and aminoglycosides), the diffusibility of the drug in agar for diffusion methods (very poor for colistin), and the temperature and duration of incubation. Automated methods (VITEK, VITEK 2, MicroScan, PHOENIX, etc.), used routinely in most laboratories, are probably no more reliable, showing poor convergence with microdilution methods (high rates of major errors for piperacillin–tazobactam, and of minor errors for cefepime, aztreonam and carbapenems [87–89]), as these systems monitor the bacterial growth rates optically. Thus, 'false drug susceptibility' may stem from the presence of slowly inducible resistance mechanisms, and 'false resistance' from the use of too large inocula, which reduce the activity of cell-wall-active agents [90].

Considering all these difficulties, the concomitant use of two independent methods would ideally be required for determining *Pseudomonas* susceptibility, and should include determining antibiotic MICs. The selection of antibiotics to be tested is also critical, and should always include good phenotypic markers of resistance mechanisms. Table 4 suggests a tentative antibiogram, based on 16 antibiotics. However, the interpretative reading of susceptibility tests and the recognition of resistance mechanisms based on phenotypic data are extremely difficult in the case of *P. aeruginosa* [91] because of: (i) the frequent occurrence of several resistance mechanisms affecting, partly or totally, the same drugs; (ii) the variable efficacy of these mechanisms in different strains; and (iii) the inappropriateness of the methodologies used to detect low-level resistance. Moreover, antibiograms are established on the basis of the clinical interest of antibiotics rather than on their capacity to distinguish among resistance mechanisms, and the categorisation into susceptible (S), intermediately-susceptible (I) and resistant (R) groups may vary according to the breakpoints considered. The development of genotypic tools to detect emerging resistance mechanisms (as described recently for β-lactamase production and modification in the expression of efflux pumps or porins [92,93]) would be very useful for solving these issues in the near future. Laboratory techniques for detecting most genes coding for ESBLs and carbapenemases are available, and may help in performing extended surveys to detect the spread of novel mechanisms of resistance.

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]

	Resistance mechanisms																
	Permeability alterations					Antibiotic-inactivating enzymes										Target modifications	
		Active efflux				β-Lactamases					Aminoglycoside-modifying enzymes						
Antibiotics	OprD loss	MexAB–OprM	MexCD–OprJ	MexEF–OprN	MexXY–OprM	Cephalosporinase over-expression	Restricted-spectrum penicillinases	Extended-spectrum oxacillinases	Extended-spectrum β-lactamases	Metallo-β-lactamases	AAC (3)-I	AAC (3)-II	AAC (6′)-I	AAC (6′)-II	ANT (2′)-I	Mutations in topoisomerases	Ribosomal methylation
Ticarcillin	S	I/R	S	S	S	I/R	R	R	R	R	S	S	S	S	S	S	S
Ticarcillin–clavulanic acid	S	I/R	S	S	S	I/R	I/R	R	I/R	R	S	S	S	S	S	S	S
Piperacillin	S	S	S	S	S	I/R	R	R	R	I/R	S	S	S	S	S	S	S
Piperacillin–tazobactam	S	S	S	S	S	I/R	I/R	R	I/R	I/R	S	S	S	S	S	S	S
Cefoperazone	S	I/R	S	S	S	R	R	R	R	R	S	S	S	S	S	S	S
Cefotaxime	S	I/R	S	S	S	R	I/R	R	R	R	S	S	S	S	S	S	S
Ceftazidime	S	S	S	S	S	I/R	S	S	R	R	S	S	S	S	S	S	S
Cefepime	S	S	I	S	I	S/I	S	I/R	R	R	S	S	S	S	S	S	S
Aztreonam	S	I/R	S	S	S	I/R	I	S/I/R	R	S/I	S	S	S	S	S	S	S
Imipenem	I/R	S	S	I[b]	S	S	S	S	S	R	S	S	S	S	S	S	S
Meropenem	S/I/R	I	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
Gentamicin	S	S	S	S	I/R	S	S	S	S	S	R	R	S/I	R	R	S	R
Tobramycin	S	S	S	S	I/R	S	S	S	S	S	S	R	R	R	R	S	R
Amikacin	S	S	S	S	I/R	S	S	S	S	S	S	S	R	S	S	S	R
Ciprofloxacin	S	S/I	S/I	S/I	S/I	S	S	S	S	S	S	S	S	S	S	R	S
Colistin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a]Based on data from http://bsac.test.tmg.co.uk/ and [36,57,86,91,196–198]. 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.

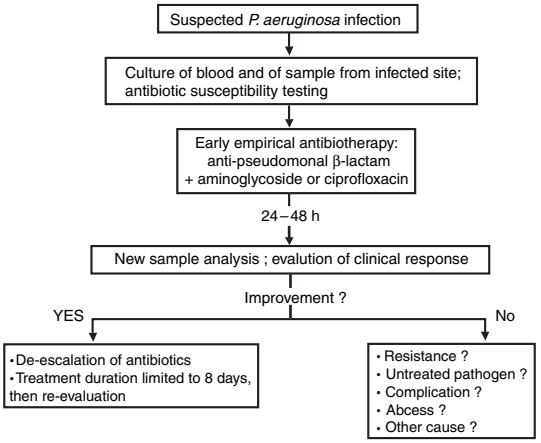

Fig. 2. General algorithm for the clinical management of *Pseudomonas aeruginosa* infections (based on [97]).

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

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 (FEV_1)) [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 FEV_1 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 (cefepime, cefpirome) or no useful (ertapenem) 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 (MIC_{50} and MIC_{90}, 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.

ACKNOWLEDGEMENTS

NM was the recipient of a doctoral fellowship of the Région Bruxelloise/Brusselse Gewest awarded within the context of 'Prospective Research in Brussels Action'. FVB is Maître de Recherches of the Belgian Fonds National de la Recherche Scientifique. We are indebted to AstraZeneca, Belgium, for an unrestricted, educational grant for the organisation of a symposium that resulted in the preparation of this review; however, the material discussed herein has been assembled and prepared exclusively by the authors.

REFERENCES

1. Pier G, Ramphal R. *Pseudomonas aeruginosa*. In: Mandell G, Bennett J, Dolin R, eds, *Principles and practice of infectious diseases*. Philadelphia, PA: Elsevier Churchill Livingstone, 2005; 2587–2615.
2. Stover CK, Pham XQ, Erwin AL *et al*. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature* 2000; **406**: 959–964.
3. Kipnis E, Sawa T, Wiener-Kronish J. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Med Mal Infect* 2006; **36**: 78–91.
4. Woods DE. Comparative genomic analysis of *Pseudomonas aeruginosa* virulence. *Trends Microbiol* 2004; **12**: 437–439.
5. Normark BH, Normark S. Evolution and spread of antibiotic resistance. *J Intern Med* 2002; **252**: 91–106.
6. Morrison AJ, Wenzel RP. Epidemiology of infections due to *Pseudomonas aeruginosa*. *Rev Infect Dis* 1984; **6** (suppl 3): S627–S642.
7. Maschmeyer G, Braveny I. Review of the incidence and prognosis of *Pseudomonas aeruginosa* infections in cancer patients in the 1990s. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 915–925.
8. Crouch Brewer S, Wunderink RG, Jones CB, Leeper KV. Ventilator-associated pneumonia due to *Pseudomonas aeruginosa*. *Chest* 1996; **109**: 1019–1029.
9. Rello J, Rue M, Jubert P *et al*. Survival in patients with nosocomial pneumonia: impact of the severity of illness and the etiologic agent. *Crit Care Med* 1997; **25**: 1862–1867.
10. Crnich CJ, Safdar N, Maki DG. The role of the intensive care unit environment in the pathogenesis and prevention of ventilator-associated pneumonia. *Respir Care* 2005; **50**: 813–836.
11. Ferrara AM. Potentially multidrug-resistant non-fermentative Gram-negative pathogens causing nosocomial pneumonia. *Int J Antimicrob Agents* 2006; **27**: 183–195.
12. Shaw MJ. Ventilator-associated pneumonia. *Curr Opin Pulm Med* 2005; **11**: 236–241.
13. Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2002; **165**: 867–903.
14. Ratjen F. Diagnosing and managing infection in CF. *Paediatr Respir Rev* 2006; **7** (suppl 1): S151–S153.
15. Nicotra MB, Rivera M, Dale AM, Shepherd R, Carter R. Clinical, pathophysiologic, and microbiologic characterization of bronchiectasis in an aging cohort. *Chest* 1995; **108**: 955–961.
16. Krcmery V, Koprnova J, Gogova M, Grey E, Korcova J. *Pseudomonas aeruginosa* bacteraemia in cancer patients. *J Infect* 2006; **52**: 461–463.
17. Marra AR, Bar K, Bearman GM, Wenzel RP, Edmond MB. Systemic inflammatory response syndrome in nosocomial bloodstream infections with *Pseudomonas aeruginosa* and *Enterococcus* species: comparison of elderly and nonelderly patients. *J Am Geriatr Soc* 2006; **54**: 804–808.
18. Sligl W, Taylor G, Brindley PG. Five years of nosocomial Gram-negative bacteremia in a general intensive care unit: epidemiology, antimicrobial susceptibility patterns, and outcomes. *Int J Infect Dis* 2006; **10**: 320–325.
19. Milstone AM, Ruff AJ, Yeamans C, Higman MA. *Pseudomonas aeruginosa* pre-septal cellulitis and bacteremia in a pediatric oncology patient. *Pediatr Blood Cancer* 2005; **45**: 353.
20. Dubois V, Arpin C, Noury P, Andre C, Coulange L, Quentin C. Prolonged outbreak of infection due to TEM-21-producing strains of *Pseudomonas aeruginosa* and enterobacteria in a nursing home. *J Clin Microbiol* 2005; **43**: 4129–4138.
21. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. *Pharmacotherapy* 2005; **25**: 1353–1364.
22. Hamasuna R, Betsunoh H, Sueyoshi T *et al*. Bacteria of preoperative urinary tract infections contaminate the surgical fields and develop surgical site infections in urological operations. *Int J Urol* 2004; **11**: 941–947.
23. Wang MC, Liu CY, Shiao AS, Wang T. Ear problems in swimmers. *J Chin Med Assoc* 2005; **68**: 347–352.
24. Rubin J, Yu VL. Malignant external otitis: insights into pathogenesis, clinical manifestations, diagnosis, and therapy. *Am J Med* 1988; **85**: 391–398.
25. Stern GA. *Pseudomonas* keratitis and contact lens wear: the lens/eye is at fault. *Cornea* 1990; **9** (suppl 1): S36–S38.
26. Wise BL, Mathis JL, Jawetz E. Infections of the central nervous system due to *Pseudomonas aeruginosa*. *J Neurosurg* 1969; **31**: 432–434.

27. Vrochides D, Feng WC, Singh AK. Mycotic ascending aortic pseudoaneurysm secondary to pseudomonas mediastinitis at the aortic cannulation site. *Tex Heart Inst J* 2003; **30**: 322–324.
28. Schmitt TM, Finck SJ, Brumble LM, Lane GE. *Pseudomonas aeruginosa* pseudoaneurysm of the ascending aorta after coronary artery bypass graft surgery. *Tex Heart Inst J* 2003; **30**: 137–139.
29. Marshall JC, Christou NV, Meakins JL. The gastrointestinal tract. The 'undrained abscess' of multiple organ failure. *Ann Surg* 1993; **218**: 111–119.
30. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. *Clin Infect Dis* 1998; **27** (suppl 1): S93–S99.
31. Vaisvila R, Morgan RD, Posfai J, Raleigh EA. Discovery and distribution of super-integrons among pseudomonads. *Mol Microbiol* 2001; **42**: 587–601.
32. Giamarellou H. Prescribing guidelines for severe *Pseudomonas* infections. *J Antimicrob Chemother* 2002; **49**: 229–233.
33. McGowan JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. *Am J Med* 2006; **119**: S29–S36.
34. Thomson JM, Bonomo RA. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril! *Curr Opin Microbiol* 2005; **8**: 518–524.
35. Dalhoff A, Janjic N, Echols R. Redefining penems. *Biochem Pharmacol* 2006; **71**: 1085–1095.
36. Poole K. Efflux-mediated multiresistance in Gram-negative bacteria. *Clin Microbiol Infect* 2004; **10**: 12–26.
37. Poole K, Srikumar R. Multidrug efflux in *Pseudomonas aeruginosa*: components, mechanisms and clinical significance. *Curr Top Med Chem* 2001; **1**: 59–71.
38. Kohler T, Michea-Hamzehpour M, Plesiat P, Kahr AL, Pechere JC. Differential selection of multidrug efflux systems by quinolones in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1997; **41**: 2540–2543.
39. Kohler T, Michea-Hamzehpour M, Henze U, Gotoh N, Curty LK, Pechere JC. Characterization of MexE–MexF–OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. *Mol Microbiol* 1997; **23**: 345–354.
40. Lomovskaya O, Lee A, Hoshino K *et al*. Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; **43**: 1340–1346.
41. Hocquet D, Nordmann P, El Garch F, Cabanne L, Plesiat P. Involvement of the MexXY–OprM efflux system in emergence of cefepime resistance in clinical strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006; **50**: 1347–1351.
42. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother* 2006; **50**: 1633–1641.
43. Le Thomas I, Couetdic G, Clermont O, Brahimi N, Plesiat P, Bingen E. In vivo selection of a target/efflux double mutant of *Pseudomonas aeruginosa* by ciprofloxacin therapy. *J Antimicrob Chemother* 2001; **48**: 553–555.
44. Nakajima A, Sugimoto Y, Yoneyama H, Nakae T. High-level fluoroquinolone resistance in *Pseudomonas aeruginosa* due to interplay of the MexAB–OprM efflux pump and the DNA gyrase mutation. *Microbiol Immunol* 2002; **46**: 391–395.
45. Niga T, Ito H, Oyamada Y *et al*. Cooperation between alteration of DNA gyrase genes and over-expression of MexB and MexX confers high-level fluoroquinolone resistance in *Pseudomonas aeruginosa* strains isolated from a patient who received a liver transplant followed by treatment with fluoroquinolones. *Microbiol Immunol* 2005; **49**: 443–446.
46. Weldhagen GF, Poirel L, Nordmann P. Ambler class A extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. *Antimicrob Agents Chemother* 2003; **47**: 2385–2392.
47. Poirel L, Brinas L, Verlinde A, Ide L, Nordmann P. BEL-1, a novel clavulanic acid-inhibited extended-spectrum beta-lactamase, and the class 1 integron In120 in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 3743–3748.
48. Wang CY, Jerng JS, Cheng KY *et al*. Pandrug-resistant *Pseudomonas aeruginosa* among hospitalised patients: clinical features, risk-factors and outcomes. *Clin Microbiol Infect* 2006; **12**: 63–68.
49. Chayakulkeeree M, Junsriwong P, Keerasuntonpong A, Tribuddharat C, Thamlikitkul V. Epidemiology of extended-spectrum beta-lactamase producing gram-negative bacilli at Siriraj Hospital, Thailand, 2003. *SE Asian J Trop Med Pub Hlth* 2005; **36**: 1503–1509.
50. Masterton RG, Turner PJ. Trends in antimicrobial susceptibility in UK centres: the MYSTIC Programme 1997–2002). *Int J Antimicrob Agents* 2006; **27**: 69–72.
51. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; **18**: 306–325.
52. Aubron C, Poirel L, Fortineau N, Nicolas P, Collet L, Nordmann P. Nosocomial spread of *Pseudomonas aeruginosa* isolates expressing the metallo-beta-lactamase VIM-2 in a hematology unit of a French hospital. *Microb Drug Resist* 2005; **11**: 254–259.
53. Cardoso O, Leitao R, Figueiredo A, Sousa JC, Duarte A, Peixe LV. Metallo-beta-lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* from Portugal. *Microb Drug Resist* 2002; **8**: 93–97.
54. Giakkoupi P, Petrikkos G, Tzouvelekis LS, Tsonas S, Legakis NJ, Vatopoulos AC. Spread of integron-associated VIM-type metallo-beta-lactamase genes among imipenem-nonsusceptible *Pseudomonas aeruginosa* strains in Greek hospitals. *J Clin Microbiol* 2003; **41**: 822–825.
55. Walsh TR. The emergence and implications of metallo-beta-lactamases in Gram-negative bacteria. *Clin Microbiol Infect* 2005; **11** (suppl 6): 2–9.
56. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect* 2002; **8**: 321–331.
57. Poole K. Aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 479–487.
58. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. *Antimicrob Agents Chemother* 1999; **43**: 727–737.
59. Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis* 2000; **31** (suppl 2): S24–S28.
60. Van Bambeke F, Michot JM, Van Eldere J, Tulkens PM. Quinolones in 2005: an update. *Clin Microbiol Infect* 2005; **11**: 256–280.

61. Mouneimne H, Robert J, Jarlier V, Cambau E. Type II topoisomerase mutations in ciprofloxacin-resistant strains of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; **43**: 62–66.
62. Yokoyama K, Doi Y, Yamane K *et al.* Acquisition of 16S rRNA methylase gene in *Pseudomonas aeruginosa*. *Lancet* 2003; **362**: 1888–1893.
63. Thompson J, Skeggs PA, Cundliffe E. Methylation of 16S ribosomal RNA and resistance to the aminoglycoside antibiotics gentamicin and kanamycin determined by DNA from the gentamicin-producer, *Micromonospora purpurea*. *Mol Gen Genet* 1985; **201**: 168–173.
64. Liou GF, Yoshizawa S, Courvalin P, Galimand M. Aminoglycoside resistance by ArmA-mediated ribosomal 16S methylation in human bacterial pathogens. *J Mol Biol* 2006; **359**: 358–364.
65. Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clin Microbiol Infect* 2005; **11** (suppl 4): 17–32.
66. Navon-Venezia S, Ben Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. *Curr Opin Infect Dis* 2005; **18**: 306–313.
67. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Antimicrob Agents Chemother* 2006; **50**: 43–48.
68. Poirel L, Weldhagen GF, De Champs C, Nordmann P. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended-spectrum beta-lactamase GES-2 in South Africa. *J Antimicrob Chemother* 2002; **49**: 561–565.
69. Gruson D, Hilbert G, Vargas F *et al*. Strategy of antibiotic rotation: long-term effect on incidence and susceptibilities of Gram-negative bacilli responsible for ventilator-associated pneumonia. *Crit Care Med* 2003; **31**: 1908–1914.
70. Regal RE, DePestel DD, Van den Bussche HL. The effect of an antimicrobial restriction program on *Pseudomonas aeruginosa* resistance to beta-lactams in a large teaching hospital. *Pharmacotherapy* 2003; **23**: 618–624.
71. Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. *J Hosp Infect* 2006; **64**: 7–15.
72. Burgess DS. Use of pharmacokinetics and pharmacodynamics to optimize antimicrobial treatment of *Pseudomonas aeruginosa* infections. *Clin Infect Dis* 2005; **40** (suppl 2): S99–S104.
73. Acharya A, Paterson D. Pseudomonas aeruginosa. In: Yu V, Weber R, Raoult D, eds, *Antimicrobial therapy and vaccines*. New York: Apple Trees Productions, LLC, 2002; 549–592.
74. Dominguez-Bello MG, Reyes N, Teppa-Garran A, Romero R. Interference of *Pseudomonas* strains in the identification of *Helicobacter pylori*. *J Clin Microbiol* 2000; **38**: 937.
75. Blanc DS. The use of molecular typing for epidemiological surveillance and investigation of endemic nosocomial infections. *Infect Genet Evol* 2004; **4**: 193–197.
76. Speert DP. Molecular epidemiology of *Pseudomonas aeruginosa*. *Front Biosci* 2002; **7**: e354–e361.
77. Grothues D, von der Koopmann UHH, Tummler B. Genome fingerprinting of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. *J Clin Microbiol* 1988; **26**: 1973–1977.
78. Struelens MJ, Schwam V, Deplano A, Baran D. Genome macrorestriction analysis of diversity and variability of *Pseudomonas aeruginosa* strains infecting cystic fibrosis patients. *J Clin Microbiol* 1993; **31**: 2320–2326.
79. Wolz C, Kiosz G, Ogle JW *et al*. *Pseudomonas aeruginosa* cross-colonization and persistence in patients with cystic fibrosis. Use of a DNA probe. *Epidemiol Infect* 1989; **102**: 205–214.
80. Speert DP, Campbell ME, Henry DA *et al*. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. *Am J Respir Crit Care Med* 2002; **166**: 988–993.
81. Sazakli E, Leotsinidis M, Vantarakis A, Papapetropoulou M. Comparative typing of *Pseudomonas* species isolated from the aquatic environment in Greece by SDS-PAGE and RAPD analysis. *J Appl Microbiol* 2005; **99**: 1191–1203.
82. Vernez I, Hauser P, Bernasconi MV, Blanc DS. Population genetic analysis of *Pseudomonas aeruginosa* using multilocus sequence typing. *FEMS Immunol Med Microbiol* 2005; **43**: 29–35.
83. Curran B, Jonas D, Grundmann H, Pitt T, Dowson CG. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. *J Clin Microbiol* 2004; **42**: 5644–5649.
84. Kersulyte D, Struelens MJ, Deplano A, Berg DE. Comparison of arbitrarily primed PCR and macrorestriction pulsed-field gel electrophoresis typing of *Pseudomonas aeruginosa* strains from cystic fibrosis patients. *J Clin Microbiol* 1995; **33**: 2216–2219.
85. Kodaka H, Iwata M, Yumoto S, Kashitani F. Evaluation of a new agar medium containing cetrimide, kanamycin and nalidixic acid for isolation and enhancement of pigment production of *Pseudomonas aeruginosa* in clinical samples. *J Basic Microbiol* 2003; **43**: 407–413.
86. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. *Clin Infect Dis* 2006; **42** (suppl 4): S153–S163.
87. Sader HS, Fritsche TR, Jones RN. Accuracy of three automated systems (MicroScan WalkAway, VITEK, and VITEK 2) for susceptibility testing of *Pseudomonas aeruginosa* against five broad-spectrum beta-lactam agents. *J Clin Microbiol* 2006; **44**: 1101–1104.
88. Joyanes P, del Carmen CM, Martinez-Martinez L, Perea EJ. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. *J Clin Microbiol* 2001; **39**: 3247–3253.
89. Saegeman V, Huynen P, Colaert J, Melin P, Verhaegen J. Susceptibility testing of *Pseudomonas aeruginosa* by the Vitek 2 system: a comparison with Etest results. *Acta Clin Belg* 2005; **60**: 3–9.
90. Doern GV, Brueggemann AB, Perla R *et al.* Multicenter laboratory evaluation of the bioMerieux Vitek antimicrobial susceptibility testing system with 11 antimicrobial agents versus members of the family Enterobacteriaceae and *Pseudomonas aeruginosa*. *J Clin Microbiol* 1997; **35**: 2115–2119.
91. Vedel G. Simple method to determine β-lactam resistance phenotypes in *Pseudomonas aeruginosa* using the disc agar diffusion test. *J Antimicrob Chemother* 2005; **56**: 657–664.
92. Dumas JL, Van Delden C, Perron K, Kohler T. Analysis of antibiotic resistance gene expression in *Pseudomonas*

aeruginosa by quantitative real-time-PCR. *FEMS Microbiol Lett* 2006; **254**: 217–225.
93. Yan JJ, Hsueh PR, Lu JJ, Chang FY, Ko WC, Wu JJ. Characterization of acquired β-lactamases and their genetic support in multidrug-resistant *Pseudomonas aeruginosa* isolates in Taiwan: the prevalence of unusual integrons. *J Antimicrob Chemother* 2006; **58**: 530–536.
94. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005; **171**: 388–416.
95. Hughes WT, Armstrong D, Bodey GP *et al.* 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis* 1997; **25**: 551–573.
96. Eggimann P, Revelly JP. Should antibiotic combinations be used to treat ventilator-associated pneumonia? *Semin Respir Crit Care Med* 2006; **27**: 68–81.
97. Craven DE, Palladino R, McQuillen DP. Healthcare-associated pneumonia in adults: management principles to improve outcomes. *Infect Dis Clin North Am* 2004; **18**: 939–962.
98. Rello J, Diaz E, Rodriguez A. Advances in the management of pneumonia in the intensive care unit: review of current thinking. *Clin Microbiol Infect* 2005; **11** (suppl 5): 30–38.
99. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. *Chest* 2002; **122**: 262–268.
100. Carmeli Y, Troillet N, Eliopoulos GM, Samore MH. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999; **43**: 1379–1382.
101. Carmeli Y, Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. *Arch Intern Med* 1999; **159**: 1127–1132.
102. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic 'blind' bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1991; **143**: 1121–1129.
103. Singh N, Rogers P, Atwood CW, Wagener MM, Yu VL. Short-course empiric antibiotic therapy for patients with pulmonary infiltrates in the intensive care unit. A proposed solution for indiscriminate antibiotic prescription. *Am J Respir Crit Care Med* 2000; **162**: 505–511.
104. DeRyke CA, Lee SY, Kuti JL, Nicolau DP. Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: impact on the development of resistance. *Drugs* 2006; **66**: 1–14.
105. Benko AS, Cappelletty DM, Kruse JA, Rybak MJ. Continuous infusion versus intermittent administration of ceftazidime in critically ill patients with suspected gram-negative infections. *Antimicrob Agents Chemother* 1996; **40**: 691–695.
106. Tam VH, Louie A, Lomaestro BM, Drusano GL. Integration of population pharmacokinetics, a pharmacodynamic target, and microbiologic surveillance data to generate a rational empiric dosing strategy for cefepime against *Pseudomonas aeruginosa*. *Pharmacotherapy* 2003; **23**: 291–295.
107. Viaene E, Chanteux H, Servais H, Mingeot-Leclercq MP, Tulkens PM. Comparative stability studies of antipseudomonal beta-lactams for potential administration through portable elastomeric pumps (home therapy for cystic fibrosis patients) and motor-operated syringes (intensive care units). *Antimicrob Agents Chemother* 2002; **46**: 2327–2332.
108. Fayed DF, Dahmash NS, al Zeer AH, Shibl AM, Huraib SO, Abu-Aisha H. Efficacy and safety of once-daily amikacin in combination with ceftazidime in critically ill adults with severe gram-negative infections. *J Chemother* 1996; **8**: 457–464.
109. Barza M, Ioannidis JP, Cappelleri JC, Lau J. Single or multiple daily doses of aminoglycosides: a meta-analysis. *BMJ* 1996; **312**: 338–345.
110. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother* 1999; **43**: 1003–1012.
111. Thomas JK, Forrest A, Bhavnani SM *et al.* Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. *Antimicrob Agents Chemother* 1998; **42**: 521–527.
112. Hyatt JM, Schentag JJ. Pharmacodynamic modeling of risk factors for ciprofloxacin resistance in *Pseudomonas aeruginosa*. *Infect Control Hosp Epidemiol* 2000; **21**: S9–S11.
113. Kiem S, Schentag JJ. Relationship of minimal inhibitory concentration and bactericidal activity to efficacy of antibiotics for treatment of ventilator-associated pneumonia. *Semin Respir Crit Care Med* 2006; **27**: 51–67.
114. Mehta RM, Niederman MS. Nosocomial pneumonia in the intensive care unit: controversies and dilemmas. *J Intens Care Med* 2003; **18**: 175–188.
115. Paul M, Benuri-Silbiger I, Soares-Weiser K, Leibovici L. Beta lactam monotherapy versus beta lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomised trials. *BMJ* 2004; **328**: 668.
116. Cunha BA. Ventilator-associated pneumonia: monotherapy is optimal if chosen wisely. *Crit Care* 2006; **10**: 141.
117. Bodey GP, Jadeja L, Elting L. *Pseudomonas* bacteremia. Retrospective analysis of 410 episodes. *Arch Intern Med* 1985; **145**: 1621–1629.
118. Chastre J, Wolff M, Fagon JY *et al.* Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. *JAMA* 2003; **290**: 2588–2598.
119. Li J, Nation RL, Turnidge JD *et al.* Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 2006; **6**: 589–601.
120. Falagas ME, Kasiakou SK, Tsiodras S, Michalopoulos A. The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature. *Clin Med Res* 2006; **4**: 138–146.
121. Newton B. The properties and mode of action of polymyxins. *Bacteriol Rev* 1956; **20**: 14–27.
122. Li J, Turnidge J, Milne R, Nation RL, Coulthard K. In vitro pharmacodynamic properties of colistin and colistin methanesulfonate against *Pseudomonas aeruginosa* isolates

from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2001; **45**: 781–785.
123. Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *J Clin Microbiol* 2001; **39**: 183–190.
124. Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005; **40**: 1333–1341.
125. Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10**: R27.
126. Michalopoulos AS, Tsiodras S, Rellos K, Mentzelopoulos S, Falagas ME. Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: the renaissance of an old antibiotic. *Clin Microbiol Infect* 2005; **11**: 115–121.
127. Micol JB, de Botton S, Guieze R *et al.* An 18-case outbreak of drug-resistant *Pseudomonas aeruginosa* bacteriemia in hematology patients. *Haematologica* 2006; **91**: 1134–1138.
128. Schina M, Spyridi E, Daoudakis M, Mertzanos E, Korfias S. Successful treatment of multidrug-resistant *Pseudomonas aeruginosa* meningitis with intravenous and intrathecal colistin. *Int J Infect Dis* 2006; **10**: 178–179.
129. Kwa AL, Loh C, Low JG, Kurup A, Tam VH. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2005; **41**: 754–757.
130. Giamarellos-Bourboulis EJ, Sambatakou H, Galani I, Giamarellou H. In vitro interaction of colistin and rifampin on multidrug-resistant *Pseudomonas aeruginosa*. *J Chemother* 2003; **15**: 235–238.
131. West SE, Zeng L, Lee BL *et al.* Respiratory infections with *Pseudomonas aeruginosa* in children with cystic fibrosis: early detection by serology and assessment of risk factors. *JAMA* 2002; **287**: 2958–2967.
132. Doring G, Conway SP, Heijerman HG *et al.* Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur Respir J* 2000; **16**: 749–767.
133. Starner TD, McCray PB. Pathogenesis of early lung disease in cystic fibrosis: a window of opportunity to eradicate bacteria. *Ann Intern Med* 2005; **143**: 816–822.
134. Canton R, Cobos N, de Gracia J *et al.* Antimicrobial therapy for pulmonary pathogenic colonisation and infection by *Pseudomonas aeruginosa* in cystic fibrosis patients. *Clin Microbiol Infect* 2005; **11**: 690–703.
135. Burns JL, Gibson RL, McNamara S *et al.* Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J Infect Dis* 2001; **183**: 444–452.
136. Nixon GM, Armstrong DS, Carzino R *et al.* Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001; **138**: 699–704.
137. Rosenfeld M, Gibson RL, McNamara S *et al.* Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatr Pulmonol* 2001; **32**: 356–366.
138. Doring G, Hoiby N. Early intervention and prevention of lung disease in cystic fibrosis: a European consensus. *J Cyst Fibros* 2004; **3**: 67–91.
139. Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2003; **2**: 29–34.
140. Pamukcu A, Bush A, Buchdahl R. Effects of *Pseudomonas aeruginosa* colonization on lung function and anthropometric variables in children with cystic fibrosis. *Pediatr Pulmonol* 1995; **19**: 10–15.
141. Kosorok MR, Zeng L, West SE *et al.* Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* 2001; **32**: 277–287.
142. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol* 2002; **34**: 91–100.
143. Anonymous. *Patient registry 1996 annual data report*. Bethesda, MD: Cystic Fibrosis Foundation, 1997.
144. Baumann U, Stocklossa C, Greiner W, der Schulenburg JM, von der Hardt H. Cost of care and clinical condition in paediatric cystic fibrosis patients. *J Cyst Fibros* 2003; **2**: 84–90.
145. Anonymous. *Report of the UK Cystic Fibrosis Trust Antibiotic Group*. Bromley: Cystic Fibrosis Trust, 2002.
146. Koch C. Early infection and progression of cystic fibrosis lung disease. *Pediatr Pulmonol* 2002; **34**: 232–236.
147. Lee TW, Brownlee KG, Denton M, Littlewood JM, Conway SP. Reduction in prevalence of chronic *Pseudomonas aeruginosa* infection at a regional pediatric cystic fibrosis center. *Pediatr Pulmonol* 2004; **37**: 104–110.
148. Frederiksen B, Koch C, Hoiby N. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974–1995). *Pediatr Pulmonol* 1999; **28**: 159–166.
149. Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early *Pseudomonas* colonisation in cystic fibrosis. *Lancet* 1985; **i**: 865.
150. Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. *Lancet* 1991; **338**: 725–726.
151. Frederiksen B, Koch C, Hoiby N. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol* 1997; **23**: 330–335.
152. Wiesemann HG, Steinkamp G, Ratjen F *et al.* Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Pediatr Pulmonol* 1998; **25**: 88–92.
153. Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 2001; **358**: 983–984.
154. Taccetti G, Repetto T, Procopio E, Farina S, Campana S. Early *Pseudomonas aeruginosa* colonisation in cystic fibrosis patients. *Lancet* 2002; **359**: 625–626.
155. Ciofu O, Giwercman B, Pedersen SS, Hoiby N. Development of antibiotic resistance in *Pseudomonas aeruginosa* during two decades of antipseudomonal treatment at the Danish CF Center. *APMIS* 1994; **102**: 674–680.

156. Zach MS. Lung disease in cystic fibrosis—an updated concept. *Pediatr Pulmonol* 1990; **8**: 188–202.
157. Wood DM, Smyth AR. Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. *Cochrane Database Syst Rev* 2006; CD004197.
158. Marchetti F, Giglio L, Candusso M, Faraguna D, Assael BM. Early antibiotic treatment of *Pseudomonas aeruginosa* colonisation in cystic fibrosis: a critical review of the literature. *Eur J Clin Pharmacol* 2004; **60**: 67–74.
159. Heinzl B, Eber E, Oberwaldner B, Haas G, Zach MS. Effects of inhaled gentamicin prophylaxis on acquisition of *Pseudomonas aeruginosa* in children with cystic fibrosis: a pilot study. *Pediatr Pulmonol* 2002; **33**: 32–37.
160. Lebecque P, Leal T, Zylberberg K, Reychler G, Bossuyt X, Godding V. Towards zero prevalence of chronic *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *J Cyst Fibros* 2006; **5**: 237–244.
161. de Groot R, Smith AL. Antibiotic pharmacokinetics in cystic fibrosis. Differences and clinical significance. *Clin Pharmacokinet* 1987; **13**: 228–253.
162. Adeboyeku D, Scott S, Hodson ME. Open follow-up study of tobramycin nebuliser solution and colistin in patients with cystic fibrosis. *J Cyst Fibros* 2006; **5**: 261–263.
163. Klepser ME. Role of nebulized antibiotics for the treatment of respiratory infections. *Curr Opin Infect Dis* 2004; **17**: 109–112.
164. Pai VB, Nahata MC. Efficacy and safety of aerosolized tobramycin in cystic fibrosis. *Pediatr Pulmonol* 2001; **32**: 314–327.
165. Ryan G, Mukhopadhyay S, Singh M. Nebulised anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2003; CD001021.
166. Mubareka S, Rubinstein E. Aerosolized colistin for the treatment of nosocomial pneumonia due to multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Crit Care* 2005; **9**: 29–30.
167. Rappaz I, Decosterd LA, Bille J, Pilet M, Belaz N, Roulet M. Continuous infusion of ceftazidime with a portable pump is as effective as thrice-a-day bolus in cystic fibrosis children. *Eur J Pediatr* 2000; **159**: 919–925.
168. Vinks AA, Brimicombe RW, Heijerman HG, Bakker W. Continuous infusion of ceftazidime in cystic fibrosis patients during home treatment: clinical outcome, microbiology and pharmacokinetics. *J Antimicrob Chemother* 1997; **40**: 125–133.
169. Alghasham AA, Nahata MC. Clinical use of fluoroquinolones in children. *Ann Pharmacother* 2000; **34**: 347–359.
170. Redmond A, Sweeney L, MacFarland M, Mitchell M, Daggett S, Kubin R. Oral ciprofloxacin in the treatment of *Pseudomonas* exacerbations of paediatric cystic fibrosis: clinical efficacy and safety evaluation using magnetic resonance image scanning. *J Int Med Res* 1998; **26**: 304–312.
171. Bosworth DG, Nielson DW. Effectiveness of home versus hospital care in the routine treatment of cystic fibrosis. *Pediatr Pulmonol* 1997; **24**: 42–47.
172. Thornton J, Elliott R, Tully MP, Dodd M, Webb AK. Long term clinical outcome of home and hospital intravenous antibiotic treatment in adults with cystic fibrosis. *Thorax* 2004; **59**: 242–246.
173. Rice LB. Unmet medical needs in antibacterial therapy. *Biochem Pharmacol* 2006; **71**: 991–995.
174. Livermore DM. Can beta-lactams be re-engineered to beat MRSA? *Clin Microbiol Infect* 2006; **12** (suppl 2): 11–16.
175. Issa NC, Rouse MS, Piper KE, Wilson WR, Steckelberg JM, Patel R. In vitro activity of BAL9141 against clinical isolates of gram-negative bacteria. *Diagn Microbiol Infect Dis* 2004; **48**: 73–75.
176. Chen Y, Garber E, Zhao Q *et al.* In vitro activity of doripenem (S-4661) against multidrug-resistant gram-negative bacilli isolated from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2005; **49**: 2510–2511.
177. Mushtaq S, Ge Y, Livermore DM. Doripenem versus *Pseudomonas aeruginosa* in vitro: activity against characterized isolates, mutants, and transconjugants and resistance selection potential. *Antimicrob Agents Chemother* 2004; **48**: 3086–3092.
178. Traczewski MM, Brown SD. In vitro activity of doripenem against *Pseudomonas aeruginosa* and *Burkholderia cepacia* isolates from both cystic fibrosis and non-cystic fibrosis patients. *Antimicrob Agents Chemother* 2006; **50**: 819–821.
179. Bhavnani SM, Hammel JP, Cirincione BB, Wikler MA, Ambrose PG. Use of pharmacokinetic-pharmacodynamic target attainment analyses to support phase 2 and 3 dosing strategies for doripenem. *Antimicrob Agents Chemother* 2005; **49**: 3944–3947.
180. Kitamura A, Hoshino K, Kimura Y, Hayakawa I, Sato K. Contribution of the C-8 substituent of DU-6859a, a new potent fluoroquinolone, to its activity against DNA gyrase mutants of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1995; **39**: 1467–1471.
181. Feldman C, White H, O'Grady J, Flitcroft A, Briggs A, Richards G. An open, randomised, multi-centre study comparing the safety and efficacy of sitafloxacin and imipenem/cilastatin in the intravenous treatment of hospitalised patients with pneumonia. *Int J Antimicrob Agents* 2001; **17**: 177–188.
182. Zhanel GG, Karlowsky JA, Rubinstein E, Hoban DJ. Tigecycline: a novel glycylcycline antibiotic. *Expert Rev Anti Infect Ther* 2006; **4**: 9–25.
183. Livermore DM. Tigecycline: what is it, and where should it be used? *J Antimicrob Chemother* 2005; **56**: 611–614.
184. Dean CR, Visalli MA, Projan SJ, Sum PE, Bradford PA. Efflux-mediated resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. *Antimicrob Agents Chemother* 2003; **47**: 972–978.
185. Lynch AS. Efflux systems in bacterial pathogens: an opportunity for therapeutic intervention? An industry view. *Biochem Pharmacol* 2006; **71**: 949–956.
186. Van Bambeke F, Pages J, Lee VJ. Inhibitors of bacterial efflux pumps as adjuvants in antibiotic treatments and diagnostic tools for detection of resistance by efflux. *Rec Patents Antiinfect Drug Discov* 2006; **1**: 157–175.
187. Nakayama K, Ishida Y, Ohtsuka M *et al.* MexAB–OprM specific efflux pump inhibitors in *Pseudomonas aeruginosa*. Part 2: achieving activity in vivo through the use of alternative scaffolds. *Bioorg Med Chem Lett* 2003; **13**: 4205–4208.
188. Lomovskaya O, Warren MS, Lee A *et al.* Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrob Agents Chemother* 2001; **45**: 105–116.

189. Holder IA. *Pseudomonas* immunotherapy: a historical overview. *Vaccine* 2004; **22**: 831–839.
190. Sedlak-Weinstein E, Cripps AW, Kyd JM, Foxwell AR. *Pseudomonas aeruginosa*: the potential to immunise against infection. *Expert Opin Biol Ther* 2005; **5**: 967–982.
191. Malfroot A, Adam G, Ciofu O *et al.* Immunisation in the current management of cystic fibrosis patients. *J Cyst Fibros* 2005; **4**: 77–87.
192. Craven DE. Preventing ventilator-associated pneumonia in adults: sowing seeds of change. *Chest* 2006; **130**: 251–260.
193. Amsden G. Tables of antimicrobial agents pharmacology. In: Mandell G, Bennett J, Dolin R, eds, *Principles and practice of infectious diseases*. Philadelphia, PA: Elsevier, 2005; 634–700.
194. Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in gram-negative pneumonia. *Am J Med* 1984; **77**: 657–662.
195. Conte JE, Golden JA, McIver M, Zurlinden E. Intrapulmonary pharmacokinetics and pharmacodynamics of high-dose levofloxacin in healthy volunteer subjects. *Int J Antimicrob Agents* 2006; **28**: 114–121.
196. Livermore DM. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; **8**: 557–584.
197. Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB–OprM, MexCD–OprJ, and MexXY–OprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000; **44**: 3322–3327.
198. Maseda H, Yoneyama H, Nakae T. Assignment of the substrate-selective subunits of the MexEF–OprN multidrug efflux pump of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000; **44**: 658–664.