Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting

Shiri Navon-Venezia, Ronen Ben-Ami and Yehuda Carmeli

Purpose of review

Infections with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are of great concern for hospitalized patients, especially with multidrug-resistant strains. This review focuses on recent data that may help us to understand the emergence, spread, and persistence of antibiotic resistance, and summarizes the optional treatment feasible for these resistant bacteria.

Recent findings

Multidrug-resistant *P. aeruginosa* and *A. baumannii* are increasingly causing nosocomial infections; multidrug-resistant clones are spreading into new geographic areas, and susceptible strains are acquiring resistance genes. New extended-spectrum β-lactamases and carbapenemases are emerging, leading to pan-resistant strains. Current studies focus on the effect of antibiotics on gene expression in *P. aeruginosa* biofilms and their contribution to resistance to therapy. Treatment options for multidrug-resistant *P. aeruginosa* and *A. baumannii* infections are limited in most cases to carbapenems. Sulbactam is a treatment option for pan-resistant *A. baumannii*, and or renewed use of an old drug, colistin, is being entertained for pan-resistant *A. baumannii* and *P. aeruginosa*. Immunotherapy is a promising new modality being explored. Prevention of emergence of resistance through combination therapy and pharmacokinetic strategies are studied.

Summary

The emergence and spread of multidrug-resistant *P. aeruginosa* and *A. baumannii* and their genetic potential to carry and transfer diverse antibiotic resistance determinants pose a major threat in hospitals. The complex interplay of clonal spread, persistence, transfer of resistance elements, and cell–cell interaction contribute to the difficulty in treating infections caused by these multidrug-resistant strains. In the absence of new antibiotic agents, new modalities of treatment should be developed.

Keywords

Acinetobacter baumannii, emergence of resistance, epidemiology, multi-drug resistance, *Pseudomonas aeruginosa*

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Divisions of Epidemiology and Infectious Diseases, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Correspondence to Yehuda Carmeli MD MPH, Division of Epidemiology, Tel Aviv Medical Center, 6 Weizmann St., Tel Aviv 64239, Israel
Tel: +972 3 697 3388; fax: +972 3 697 4966;
e-mail: ycarmeli@bidmc.harvard.edu

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Abbreviations

ESBL	extended spectrum β-lactamase
MBL	metallo-β-lactamase
MDR	multidrug resistant
MPC	mutant prevention concentration

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Introduction

Pseudomonas aeruginosa and *Acinetobacter baumannii* are aerobic Gram-negative bacteria that do not ferment glucose and are ubiquitous in the environment. In hospitals, these nosocomial pathogens affect severely ill patients, and cause a wide spectrum of infections from skin and wound infections to septicemia. The infections they cause are often associated with invasive procedures and devices. Their epidemiology and the clinical syndromes they cause have a number of similarities and differences. These two species are intrinsically resistant to many antibiotic agents and resistance to additional agents is often acquired.

The epidemiology of *P. aeruginosa*

P. aeruginosa is ubiquitous in the environment owing to its ability to grow in nutrient-poor conditions (including distilled water) and extreme temperatures. Moisture plays a critical role in the epidemiology of this pathogen. In one survey from Northern Ireland, *P. aeruginosa* was isolated from 30% of all tested hydrotherapy pools, 72% of Jacuzzis and spas, and 38% of swimming pools [1]. Additional reservoirs of medical importance include whirlpools, wading pools, and contact lens solutions [2]. The water supply in hospitals may be an important source for colonization and infection in susceptible patients [3], more likely from faucets that are contaminated in the process of hand washing, rather than from within the water supply itself.

The relative contribution of endogenous colonization and exogenous acquisition to nosocomial infection by *P. aeruginosa* is a matter of controversy [4]. Epidemiologic studies using DNA fingerprinting methods have demonstrated that the emergence of drug resistance during treatment may occur both by cross-infection with resistant strains and by acquisition of drug resistance by existing strains [5–7,8•]. Risk factors linked to infection with multidrug-resistant (MDR) *P. aeruginosa* include

severity of illness, a bedridden state, invasive devices, and exposure to antibiotics, especially β-lactams and fluoroquinolones [9,10,11•,12].

The epidemiology of *A. baumannii*

The genus *Acinetobacter* is widely distributed in nature as well as in the hospital environment. These widespread organisms are Gram-negative, nonfermenters, and oxidase-negative. *A. baumannii*, a genomic species 2, is closely related to species 1 (*A. calcoaceticus*) and to the unspecified species 3 and 13TU, jointly referred to as the *A. calcoaceticus–A. baumannii* complex [13••]. This bacterium has rarely been isolated from the skin of healthy individuals in England [14], but was commonly detected in healthy residents of Hong Kong [15]. The species most frequently isolated from clinical specimens is *A. baumannii*, which was previously considered an opportunistic pathogen of relatively low virulence, affecting mostly patients in intensive care units [16]. In a recently published national US survey of hospital laboratories [17•], *A. baumannii* infections accounted for only 1.3% of nosocomial bloodstream infections. Reports from various locations around the world [18–22], however, suggest that *A. baumannii* is now frequently isolated, and that it is associated with severe infections and adverse outcomes. In our institution, as well as in other Israeli hospitals, *A. baumannii* nosocomial bacteremia occurs as frequently as *P. aeruginosa* bacteremia. Thus, *A. baumannii* is emerging as an important MDR pathogen spreading in hospitals, and causing severe adverse outcomes. *A. baumannii* seems to be spreading from hospital to hospital, and it has established endemicity in various geographical areas through multiple hospital outbreaks [23]. It has become a leading nosocomial pathogen in many hospitals, second only to *P. aeruginosa* among nonfermenting Gram-negative bacilli. In many cases, the dissemination of multidrug resistance to *A. baumannii* is due to patient-to-patient spread of resistant organisms, rather than to acquisition of a new mechanism of resistance [24]. Procedures which cause splashing, such as suction [25,26] and pulsatile lavage (a high-pressure irrigation treatment used to debride wounds) [27••], may lead to environmental contamination and should be regarded as high-risk activities for which appropriate infection control precautions should be used. Environmental cleaning has been reported as being of great importance in controlling such outbreaks [6,28].

Identified risk factors for the acquisition of *A. baumannii* include long hospital stay, particularly in the intensive care unit, treatment with antibiotics, invasive procedures and devices, and being severely ill. The nosocomial epidemiology of this organism is complex: a review of *Acinetobacter* outbreaks over 20 years [20] proposed that the endemicity and the increasing rate of new antimicrobial resistances in a collection of isolates suggest transmission. In several cases for which outbreak was apparent by using classical epidemiological methods, molecular typing of the organisms suggested a more complex situation wherein multiple genetically unrelated strains caused the increasing rates of infections by this pathogen. To better understand the spread of *A. baumannii*, further long-term detailed epidemiological and molecular studies are required.

Reports on bloodstream infections from military medical facilities for treating service members injured in Afghanistan and the Iraq/Kuwait region [29••], together with similar reports during the Vietnam War, have raised the possibility of environmental contamination of wounds as a potential source. In our institution, community acquisition of *A. baumannii* infections is rare, if it exists at all. Less than 3% of the patients do not have a clear nosocomial source, and *A. baumannii* is first isolated after a mean hospital stay of 17 days [30•]. MDR *A. baumannii* clones may become dominant and persist for many years [31]. Three major epidemic European clones have been identified. Clones I and II were responsible for outbreaks in northwestern European hospitals. Clone I was also recovered from Spain, South Africa, Poland, the Czech Republic and Italy, and clone II was recovered from Spain, Portugal, South Africa, France, the Czech Republic, Greece and Turkey. Clone III was found in France, The Netherlands, Italy and Spain [32]. These data suggest that these are highly fitted clones, which are resistant to antibiotics and are virulent, succeeding in various geographical areas. These strains cause outbreaks that are difficult to control, and establish endemicity in hospitals.

Antibiotic resistance in *P. aeruginosa* and *A. baumannii*

P. aeruginosa and *A. baumannii* are intrinsically resistant to many antibiotic agents and emergence of resistance during therapy occurs frequently in *P. aeruginosa*, but not in *A. baumannii* [33]. Resistance is associated with adverse clinical outcomes [34]. Resistance to β-lactams in *P. aeruginosa* and *A. baumannii* is most commonly associated with production of high levels of naturally produced cephalosporinase (AmpC). Both *P. aeruginosa* and *A. baumannii* may have an MDR phenotype. Two major intrinsic mechanisms confer resistance to multiple antimicrobial drug classes: mutations in outer membrane porins resulting in reduced permeability to antimicrobials, and overexpression of multidrug efflux pumps. MDR strains may, however, arise due to unrelated mechanisms accumulating sequentially in an organism. A several-fold increase in the prevalence of MDR *P. aeruginosa* in the US was recently described: D'Agata [35] observed an increase from 1% to 16% in the prevalence of MDR *P. aeruginosa* during a 9-year period, and Jung *et al.* [36] noted that while only 22% of

P. aeruginosa isolates were resistant to any antipseudomonal agent in 1998, 32% of isolates were resistant to at least three agents by 2002. In contrast, data collected between 2001 and 2003 across the US showed stability in the susceptibility of *P. aeruginosa* to most drugs and only a minor increase in the prevalence of MDR strains, indicating that the trend may have been halted [37•].

Emerging resistance mechanisms in *P. aeruginosa* and *A. baumannii*

Newly acquired enzymes are a recognized source of resistance to β-lactam agents, including carbapenems. New enzymes are being discovered, and others have been detected in various parts of the world. In this section we summarize the recent data on newly recognized enzymes as well as the new data on the dissemination of acquired enzymes (Table 1).

Acquired extended spectrum beta-lactamases

In addition to the increased expression of AmpC β-lactamases, recent reports describe the occurrence of extended spectrum β-lactamase (ESBL) enzymes, which are class A β-lactamases, conferring resistance to expanded-spectrum cephalosporins in *P. aeruginosa* and *A. baumannii* [38]. The presence of ESBLs in these bacteria has important clinical implications because they confer resistance to all penicillins and cephalosporins but are difficult to detect phenotypically, because of the concurrent production of the chromosomal cephalosporinases. This may lead to the false reporting of an organism as being susceptible to certain antipseudomonal penicillins or cephalosporins and, consequently, to inappropriate therapy.

The ESBL enzymes described in *P. aeruginosa* belong to various families: the TEM and SHV types which are common among *Enterobacteriaceae*, the PER type which mostly originates from Turkey [39], the VEB type from Southeast Asia [40] or, more recently, the IBC types and the GES type which have been reported from various parts of the world, including France, Greece, South Africa and Brazil [41]. The GES-2 enzyme [42] is particularly worrisome since along with its ESBL activity, it also has a weak carbapenemase activity, which may partly contribute to decreased susceptibility of *P. aeruginosa* to carbapenems. When these enzymes are combined with an additional mechanism, such as low permeability or efflux, the strains may become carbapenem resistant.

Improved molecular techniques for ESBL detection in *P. aeruginosa* and *A. baumannii* are necessary and critical in order to direct treatment, and minimize the spread of these plasmid-carried enzymes. Establishment of local or regional diagnostic laboratories for the detection of ESBLs in these bacteria may be highly valuable.

Carbapenem resistance

Antipseudomonal (group 2) carbapenems are often agents of last resort. Thus, the emergence of carbapenem resistance in *P. aeruginosa* and *A. baumannii* is of particular concern. Carbapenem resistance is primarily caused by two mechanisms, either reduced intracellular concentration, or by hydrolysis of the drug.

Reduced intracellular carbapenem concentration

The most common mechanism of imipenem resistance in *P. aeruginosa* is the loss of or reduced expression of the outer membrane porin, OprD. Meropenem resistance is associated with both the loss of OprD and the induction of an efflux pump (Mex AB – OprM). These different mechanisms may have implications with respect to cross resistance, combination therapy, and the emergence of resistance. In *A. baumannii* carbapenem resistance is not well defined, and various mechanisms of resistance to carbapenems are likely present: production of carbapenemases, decreased outer-membrane permeability caused by the loss or reduced expression of porins, overexpression of multidrug efflux pumps, and alterations in penicillin binding proteins all may contribute to carbapenem resistance.

Carbapenem hydrolysis

For both organisms, acquired carbapenamases are a growing source of resistance.

Acquired carbapenemases

There has been an increase in the reports on carbapenemases over the last few years [43]. These enzymes, mainly classified as Ambler class B metallo-β-lactamases (MBLs), are zinc dependent and possess a very broad substrate profile, including expanded-spectrum cephalosporins and carbapenems. The three subclasses of clinically relevant, mobile MBLs that have been identified thus far in *P. aeruginosa* are IMP, VIM and SPM-1. A novel fourth subclass of Ambler class B enzyme, GIM-1, was recently identified in isolates that originated from Germany [44•].

MBLs are spreading throughout various parts of the world. In one Italian hospital, 20% of all *P. aeruginosa* isolates and 70% of the carbapenem-resistant isolates reported carried either $bla_{\text{VIM-1}}$ or $bla_{\text{VIM-2}}$ [45]. In Korea, among imipenem-nonsusceptible isolates, 11% of the *Pseudomonas* spp. carried bla_{VIM}, and 15% of the *Acinetobacter* spp. carried either bla_{VIM} or bla_{IMP} [46]. These *bla*MBL genes are located on class 1 integrons residing on mobile plasmids [47,48]. The resistance cassettes carried on these MBL-containing integrons may vary, but this machinery enables resistance to spread horizontally between species in all cases.

Table 1. Emergence and dissemination of antibiotic resistance mechanisms in multidrug-resistant *P. aeruginosa* and *A. baumannii* in 2004

Organism	Resistance mechanism	Novel enzymes	Dissemination of known enzymes
P. aeruginosa	ESBLs		Class A GES-1 in Brazil GES-2 in South Africa
	Carbapenemase	Class B IMP-16 in Brazil GIM-1 in Germany VIM-7 in the US	Class B VIM-2 in Latin America, Japan VIM-4 in Poland IMP-1 in Singapore, China IMP-4 in Australia SPM-1 in Brazil
A. baumannii	ESBLs		Class A OXA-20
	Carbapenemase	Class D OXA-58	Class B OXA-40 in the Iberian peninsula OXA-58 in France

ESBL, extended spectrum β-lactamase. Source: [49–59].

The OXA family is another important source of acquired carbapenamases. An *A. baumannii* clone carrying OXA-40 was shown to spread over the Iberian Peninsula and to persist over many years as a major contributor to carbapenem resistance [49]. A novel carbapenamase OXA-58 has been found in *A. baumannii* isolates from France [50]. Some acquired β-lactamases are shown in Table 1 [49–59].

P. aeruginosa biofilms

The ability of *P. aeruginosa* to form biofilms greatly enhances its ability to adhere to and survive on environmental surfaces, medical devices, and the airways of patients with chronic lung disease, in particular those suffering from cystic fibrosis.

The most striking feature of persistent *P. aeruginosa* infections in cystic fibrosis patients is the selection of mucoid mutants producing the exopolysaccharide alginate. These mutant bacteria grow inside a biofilm and survive because their surrounding matrix protects them from phagocytes and complement activity. *P. aeruginosa* cells in biofilms are often resistant to antibiotics (e.g. aminoglycosides, β-lactam antibiotics, fluoroquinolones) and disinfectants. The exact nature of the increased resistance inside biofilms is unclear but has been attributed to slow growth, penetration barriers, high concentrations of β-lactamases, as well as other factors. *P. aeruginosa* also produces other less well defined biofilms essential in the colonization of in-dwelling devices such as catheters. Recently, the *las* cell-to-cell signaling system has been shown to be involved in the differentiation of *P. aeruginosa* biofilms.

Growth within biofilms gives rise to extensive genetic diversity that, in turn, enhances the potential for resistance against disinfectants, antibiotics and environmental stress [60]. A recently characterized locus within the *P. aeruginosa* PAO1 genome, termed psl, was shown to be responsible for generating the exopolysaccharide matrix required for biofilm formation [61••].

The dynamics of β-lactamase induction and gene expression in cells growing in biofilms differ from the planktonic forms. Peripheral cells respond differently to sub-minimal inhibitory concentration levels of imipenem relative to cells located in the center of the biofilm [62••], in which induction or repression of 34 different genes occurs: they include genes encoding for alginate biosynthesis, leading to the mucoidic nature of the strains colonizing the lungs of cystic fibrosis patients. Although these mucoid strains are less invasive, they attenuate the host proinflammatory responses and thus hinder the effective elimination of the pathogen [63•].

Microbial interaction

Little is known about the interaction between various microorganisms, which are part of the ecosystem leading to infections. In biofilms, interactions between different microorganisms may occur in the presence of increased levels of extracellular metabolites. A fascinating example of such an interaction is the effect of *P. aeruginosa* on the morphology of the polymorphic fungus *Candida albicans*. The virulence of this fungus is linked to its transition from yeast to a filamentous form. *P. aeruginosa* appears to limit the growth of *C. albicans* when both microorganisms coexist within the human host [64]. This inhibition is probably achieved by quorum-sensing molecules excreted by *P. aeruginosa* capable of blocking the induction of filamentation of the fungus, thereby reverting it back to the less virulent yeast cell growth [65••,66]. This interaction can explain the fact that treatment with antibiotics is often followed by an increase in the *C. albicans* population. Another example of microbial interaction was noted between the yeast *Saccharomyces cerevisiae*, *Acinetobacter* spp., and the nematode *Caenorhabditis elegans*. *S. cerevisiae* enhanced the growth of various *Acinetobacter* spp. by the production of ethanol

that was not only utilized by the bacteria as a carbon source, but also increased the resistance of the bacteria to the toxic effects of salt. Furthermore, ethanol-fed *A. baumannii* displayed increased pathogenicity when confronted with *C. elegans* [67]. The ability of *A. baumannii* to utilize ethanol as a carbon source for its maintenance, or even in various cases for its growth, could be problematic in the hospital environment, where ethanol-based solutions are used widely as disinfectants of surfaces and various equipments.

These pioneering studies are providing new insights into the complex interactions among bacteria and antibiotic agents at the site of infection, which may explain the periodic changes in success of specific pathogens.

These inter-species interactions may reflect the complex processes that occur where different species can exist and compete at the same site. Moreover, exploring further the interactions between various coexisting microorganisms is essential for understanding the indirect effect of antibiotic therapy.

Treatment modalities in the era of multidrug resistance

Despite the rising threat of MDR *A. baumannii* and *P. aeruginosa*, no new classes of drugs have been introduced since the advent of imipenem in the early 1980s, and none are expected to appear for commercial use in the near future. New approaches are clearly required to prevent the propagation of drug-resistant mutants.

Antibiotics

The classic measure of antimicrobial potency is the minimal inhibitory concentration for a particular antibiotic with a given pathogen. A new concept, applied thus far to fluoroquinolones, is mutant prevention concentration (MPC). MPC is the concentration threshold of a drug above which an organism would require two simultaneous resistance mutations in order to grow [68,69••]. In theory, MPC data can be applied to construct dosing schemes that would achieve plasma and tissue drug levels sufficient not only to inhibit growth but also to prevent the emergence of resistant mutants. Subpopulations with preexisting first mutations or even resistant subpopulations are, however, likely to be selected even by drug levels above that of MPC, thus the practical role of the MPC concept needs to be further explored.

Drug combinations, most commonly those involving an antipseudomonal β-lactam and either an aminoglycoside or a fluoroquinolone, have long been considered to constitute optimal antibacterial treatment for *P. aeruginosa* infection. Theoretical advantages of combining two drugs with synergistic activity *in vitro* include enhanced clinical efficacy and prevention of the emergence of resistant strains. A recent meta-analysis [69••] found no advantage of combination therapy over monotherapy in terms of mortality or prevention of resistance, and described combination therapy as being associated with more adverse effects, especially nephrotoxicity. This analysis included only a limited number of patients with *P. aeruginosa* infection so that the results are not conclusive. Another meta-analysis [70], in which five of 17 studies evaluated *P. aeruginosa* infection, found a mortality benefit of combination therapy for *P. aeruginosa* (odds ratio 0.50, 95% CI 0.32–0.79) but not for other Gram-negative infections. Theoretical considerations, including activity in lung and abscesses and improved safety profile, suggested that a combination therapy consisting of a β-lactam with a quinolone might be superior to a combination with aminoglycosides. This view is supported by an in-vitro pharmacokinetic model [71]. In addition to the above-mentioned effects of combination therapy, another important aspect of dual therapy is the broadening of the empirical coverage.

As therapeutic options for MDR *P. aeruginosa* and *A. baumannii* diminish, medicine turns to drugs which have been all but phased out. Colistin (polymyxin E) is a cationic peptide with bactericidal activity against Gram-negative organisms, including MDR strains of *P. aeruginosa*. Salvage therapy with colistin was successful in 14 of 23 critically ill patients with MDR *P. aeruginosa* [72]. The addition of rifampicin to colistin was found to be synergistic *in vitro*, and the combination may be an option for difficult to treat infections with MDR *P. aeruginosa* [73].

Sulbactam is now often used for the treatment of MDR *A. baumannii*, usually as ampicillin/sulbactam. For isolates with moderate resistance to imipenem, this is still the most effective therapy, while for high-level resistance, colistin is preferable [74]. Finally, tigecycline is a new agent with promising activity against *A. baumannii* [75].

A novel approach to *P. aeruginosa* infections may be to attack the structure of the bacterial biofilm. Although they possess virtually no antipseudomonal activity *per se*, macrolides have been shown to inhibit the formation of *P. aeruginosa* biofilms [76], a fact that may explain their salutary effects on *P. aeruginosa*-associated chronic lung diseases, such as cystic fibrosis and diffuse panbronchiolitis. Possible mechanisms for such responses include inhibition of quorum sensing [77], and the immunomodulatory effects of macrolides.

Alternative treatments

Development of clinical vaccines may induce protective antibodies for the clearance of *P. aeruginosa* in systemic

and chronic infections [78•]. Successful induction of the production of antipseudomonas antibodies was achieved in a burn patient injected with a recombinant vaccine of OprF and OprI [79••]. In another study [80], combined oral vaccination with *Salmonella* expressing *P. aeruginosa* O antigen, and human monoclonal antibodies specific for mannuronic acid components of alginate, increased the survival of mice in an acute pneumonia model. In a 10-year matched-control retrospective study [81], vaccination of young cystic fibrosis patients with a polyvalent conjugate vaccine reduced the frequency of chronic infection with *P. aeruginosa* and improved the preservation of lung function.

Conclusion

P. aeruginosa and *A. baumannii* are important nosocomial pathogens that are resistant to many antibiotic agents and for which new resistance mechanisms are being continuously identified. Resistant strains are evolving and spreading, thus further limiting treatment options. The prevalence of MDR strains and even of pan-resistant isolates is increasing, while few antibacterial agents are being developed in parallel. The complex epidemiology of these resistant strains needs to be further studied in order to design measures to control their spread. New agents and treatment paradigms are required to provide clinicians with the means to treat these potentially dangerous pathogens.

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