REVIEW ARTICLE

Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models

Michael J. McConnell[1], Luis Actis[2] & Jerónimo Pachón[1]

[1]Unit of Infectious Disease, Microbiology, and Preventive Medicine, Institute of Biomedicine of Sevilla (IBiS), University Hospital Virgen del Rocío/CSIC/University of Sevilla, Sevilla, Spain; and [2]Department of Microbiology, Miami University, Oxford, OH, USA

Correspondence: Michael McConnell, Hospital Universitario Virgen del Rocío, Instituto de Biomedicina de Sevilla, Laboratorio 208, Avenida Manuel Siurot s/n, 41013 Sevilla, Spain. Tel.: +34 955923100; fax: +34 955013292; e-mail: mcconnell.mike75@gmail.com

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Abstract

Acinetobacter baumannii has emerged as a medically important pathogen because of the increasing number of infections produced by this organism over the preceding three decades and the global spread of strains with resistance to multiple antibiotic classes. In spite of its clinical relevance, until recently, there have been few studies addressing the factors that contribute to the pathogenesis of this organism. The availability of complete genome sequences, molecular tools for manipulating the bacterial genome, and animal models of infection have begun to facilitate the identification of factors that play a role in *A. baumannii* persistence and infection. This review summarizes the characteristics of *A. baumannii* that contribute to its pathogenesis, with a focus on motility, adherence, biofilm formation, and iron acquisition. In addition, the virulence factors that have been identified to date, which include the outer membrane protein OmpA, phospholipases, membrane polysaccharide components, penicillin-binding proteins, and outer membrane vesicles, are discussed. Animal models systems that have been developed during the last 15 years for the study of *A. baumannii* infection are overviewed, and the recent use of these models to identify factors involved in virulence and pathogenesis is highlighted.

Introduction

Acinetobacter baumannii has become an increasingly important human pathogen because of the increase in the number of infections caused by this organism and the emergence of multidrug-resistant (MDR) strains. The majority of infections caused by *A. baumannii* are hospital-acquired, most commonly in the intensive care setting in severely ill patients. In addition, *A. baumannii* has emerged as a cause of infections acquired in long-term care facilities, in the community, and in wounded military personnel (Anstey *et al.*, 1992, 2002; Leung *et al.*, 2006; Scott *et al.*, 2007; Schafer & Mangino, 2008; Sebeny *et al.*, 2008; Sengstock *et al.*, 2010). The types of infections produced by this pathogen include, but are not limited to, pneumonia (both hospital and community-acquired), bacteremia, endocarditis, skin and soft tissue infections, urinary tract infections, and meningitis. In most cases, it is thought that infections are acquired after exposure to *A. baumannii* that persists on contaminated hospital equipment or by contact with healthcare personnel that have been exposed to the organism through contact with a colonized patient (Maragakis *et al.*, 2004; Crnich *et al.*, 2005; Dijkshoorn *et al.*, 2007; Asensio *et al.*, 2008; Rodríguez-Baño *et al.*, 2009). However, despite the increasing clinical importance of *A. baumannii* infections, relatively little is known about the factors that contribute to its pathogenesis. Of the studies addressing *A. baumannii* that have been carried out over the preceding decades, the majority either describe the epidemiology, risk factors, and outcomes of infections caused by this bacteria or aimed to optimize antibiotic regimens for the treatment of infections produced by MDR strains. While these studies provide important information regarding the epidemiology and clinical management of *A. baumannii* infections, they do not address the underlying biological basis for the increasing success of this organism as a human pathogen. Fortunately, a number of studies have

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begun to address characteristics of *A. baumannii* that may have contributed to its clinical emergence and to explain how this bacterium produces human disease on a molecular level. These studies have begun to provide important insight into this important human pathogen and may reveal targets for developing novel treatment and prevention strategies.

Human infections caused by *A. baumannii*

Hospital-acquired pneumonia represents the most common clinical manifestation of *A. baumannii* infection. These infections occur most typically in patients receiving mechanical ventilation in the intensive care setting. It is thought that ventilator-associated pneumonia caused by *A. baumannii* results from colonization of the airway via environmental exposure, which is followed by the development of pneumonia (Dijkshoorn *et al.*, 2007). The crude mortality rate of ventilator-associated pneumonia caused by *A. baumannii* has been reported to be between 40% and 70% (Fagon *et al.*, 1996; Garnacho *et al.*, 2003), although the mortality directly attributable to *A. baumannii* infection has been the subject of controversy. Recently, however, a handful of studies and a systematic review have concluded that nosocomial infection with *A. baumannii* is associated with increased attributable mortality (Falagas *et al.*, 2006a; Abbo *et al.*, 2007; Falagas & Rafailidis, 2007; Lee *et al.*, 2007) . Community-acquired pneumonia caused by *A. baumannii*, although much less frequent than nosocomial infection, has also been described (Anstey *et al.*, 1992, 2002; Chen *et al.*, 2001; Leung *et al.*, 2006). Community-acquired pneumonia is associated with mortality rates between 40% and 60% and is often associated with underlying host factors such as alcohol abuse or chronic obstructive pulmonary disease.

Acinetobacter baumannii is also a common cause of bloodstream infections in the intensive care setting (Wisplinghoff *et al.*, 2004). The most common sources of *A. baumannii* bloodstream infections are lower respiratory tract infections and intravascular devices (Seifert *et al.*, 1995; Cisneros *et al.*, 1996; Jang *et al.*, 2009; Jung *et al.*, 2010), although wound infections and urinary tract infections have also been reported as foci of infection (Seifert *et al.*, 1995). Risk factors associated with acquiring *A. baumannii* bloodstream infections include immunosuppression, ventilator use associated with respiratory failure, previous antibiotic therapy, colonization with *A. baumannii*, and invasive procedures (García-Garmendia *et al.*, 1999; Jang *et al.*, 2009; Jung *et al.*, 2010). Inappropriate empirical antibiotic therapy, comorbidities, neutropenia, and the presence of disseminated intravascular coagulation have all been associated with poorer clinical outcomes after the acquisition of *A. baumannii* bloodstream infections (Cisneros *et al.*, 1996; Falagas *et al.*, 2006b; Erbay *et al.*, 2009). Crude mortality rates for *A. baumannii* bloodstream infections have been reported to be between 28% and 43% (Seifert *et al.*, 1995; Wisplinghoff *et al.*, 2004). As with other types of infections caused by this pathogen, the emergence of drug resistance has posed an increasing challenge to clinicians. This point is illustrated by a retrospective study performed in the United Kingdom between 1998 and 2006 in which carbapenem resistance rates rose from 0% in 1998 to 55% in 2006 in *A. baumannii* isolates causing bacteremia (Wareham *et al.*, 2008). In addition to clinical complications, the emergence of drug resistance has also resulted in an additional economic burden on health systems. Lee *et al.* (2007) reported that bacteremia caused by MDR strains required $3758 in additional medical costs and 13.4 additional days of hospitalization per patient compared with bacteremia with non MDR strains in a tertiary care hospital in Taiwan.

Acinetobacter baumannii is an important cause of burn infections, although it can be difficult to differentiate between infection and colonization of burn sites. Because of the high rates of multidrug resistance and the poor penetration of some antibiotics into burn sites, these infections can be extremely challenging for clinicians. Recent studies reporting high incidences of *A. baumannii* infection in burn units have underscored the importance of *A. baumannii* in this patient population (Albrecht *et al.*, 2006; Chim *et al.*, 2007; Keen *et al.*, 2010a, b), although the prevalence of *A. baumannii* burn site infection likely varies considerably depending on institution and geographic location. *Acinetobacter baumannii* has also emerged as an important cause of burn infection in military personnel as evidenced by a recent report characterizing bacterial infections in a military burn unit which identified *A. baumannii* as the most common cause of burn site infection (22%), with 53% of isolates demonstrating multidrug resistance (Keen *et al.*, 2010a). Burn infection can be especially problematic as it can delay wound healing and lead to failure of skin grafts, and wound site colonization can progress to infection of the underlying tissue and subsequent systemic spread of the bacteria (Lyytikainen *et al.*, 1995; Roberts *et al.*, 2001; Trottier *et al.*, 2007). Despite the potentially serious complications that can result from *A. baumannii* burn infection, data regarding clinical outcomes of infected burn patients do not provide a clear picture of the mortality attributable to the pathogen in this patient population. A case–control study of burn patients that acquired *A. baumannii* bloodstream infections demonstrated that infected patients had an overall mortality of 31%, whereas uninfected controls had a mortality of 14% (Wisplinghoff *et al.*,

1999). In contrast, a retrospective cohort study found that although *A. baumannii* was a common cause of burn site infection, it was not an independent risk factor for mortality (Albrecht *et al.*, 2006).

Soft tissue infections caused by *A. baumannii* have emerged as a significant problem in military personnel sustaining war-related trauma in Iraq and Afghanistan (Murray *et al.*, 2006; Johnson *et al.*, 2007; Scott *et al.*, 2007; Sebeny *et al.*, 2008). Like other infections caused by this organism, the treatment of these infections has been complicated by multiresistant strains. Skin and soft tissue infections related to war injury can produce cellulitis and necrotizing fasciitis, which require surgical debridement in addition to antibiotic therapy (Sebeny *et al.*, 2008). To identify the source of infection in military treatment facilities, Scott *et al.* (2007) screened patient skin samples, soil samples, and treatment areas within the facilities for the presence of *A. baumannii.* Their findings demonstrating that *A. baumannii* was present on the skin of only one of 160 patients (0.6%), in only one of 49 soil samples (2%), but in all of the treatment areas suggest that the source of infection is within the treatment facilities. Separate studies assessing skin colonization have reported higher rates (Griffith *et al.*, 2006; Doi *et al.*, 2010), although differences in bacterial identification methodology between studies should be taken into account as they may affect reported colonization rates. In addition to military personnel, skin and soft tissue infections caused by *A. baumannii* were also identified in wounded survivors of the tsunami that occurred in Southeastern Asia in December of 2004 (Garzoni *et al.*, 2005; Maegele *et al.*, 2005). In the nonmilitary setting, *A. baumannii* has been reported to be a cause of surgical site infections in some institutions (Cisneros *et al.*, 1996; Rodríguez-Baño *et al.*, 2004) and an infrequent cause of skin and soft tissue infections in the ICU setting (Sader *et al.*, 2002; Gaynes & Edwards, 2005).

Acinetobacter baumannii is an increasingly important cause of meningitis, with the majority of cases occurring in patients recovering from neurosurgical procedures (Siegman-Igra *et al.*, 1993; Katragkou *et al.*, 2006; Ng *et al.*, 2006; Ho *et al.*, 2007; Huttova *et al.*, 2007; Metan *et al.*, 2007; Paramythiotou *et al.*, 2007; Sacar *et al.*, 2007; Rodríguez Guardado *et al.*, 2008; Krol *et al.*, 2009; Cascio *et al.*, 2010), although rare cases of community-acquired *A. baumannii* meningitis have been reported (Chang *et al.*, 2000; Taziarova *et al.*, 2007; Lowman *et al.*, 2008; Ozaki *et al.*, 2009). Clinical features of *A. baumannii* meningitis are consistent with those of bacterial meningitis caused by other organisms and include fever, altered consciousness, headache, and seizure (Rodríguez Guardado *et al.*, 2008). Mortality rates associated with *A. baumannii* meningitis are difficult to estimate because of a limited number of studies with adequately sized study populations. A retrospective study identified 51 cases of postsurgical *A. baumannii* meningitis in two tertiary care hospitals between 1990 and 2004 (Rodríguez Guardado *et al.*, 2008). These cases represented 10.9% of all meningitis cases at these institutions and had a crude mortality of 33%. A similar study evaluating postsurgical *A. baumannii* meningitis in 28 patients reported a crude mortality of 71% (Metan *et al.*, 2007).

Osteomyelitis caused by *A. baumannii* occurs predominantly in military personnel sustaining war-related trauma and has become as a significant problem in U.S. military operations in Iraq and Afghanistan (Davis *et al.*, 2005; Schafer & Mangino, 2008). A study describing 18 cases of *A. baumannii* osteomyelitis in wounded soldiers in a military tertiary care center reported that all patients required surgical debridement of necrotic bone and that three cases were associated with bacteremia (Davis *et al.*, 2005). Mortality in this cohort was 0%, although it should be noted that the young age of the patients (median age; 26 years) may have contributed to the low mortality rate.

In addition to the above-mentioned infections, *A. baumannii* is an infrequent cause of endocarditis. Individual case reports have described *A. baumannii* endocarditis associated with prosthetic valves (Olut & Erkek, 2005; Menon *et al.*, 2006; Kumar *et al.*, 2008) and intravascular catheters (Bhagan-Bruno *et al.*, 2010).

Antibiotic resistance: contribution of genome plasticity and effect on pathogenesis

The ability of *A. baumannii* to acquire antibiotic resistance mechanisms has allowed this organism to persist in hospital environments and has facilitated the global emergence of MDR strains. Especially alarming are reports describing infections caused by pandrug-resistant strains with resistance to all clinically used antibiotics (Taccone *et al.*, 2006; Valencia *et al.*, 2009). These strains represent a challenge for clinicians treating these infections and necessitate the development of novel strategies for preventing and treating infections caused by this organism. There are a number of reviews that provide comprehensive information on antibiotic resistance mechanisms and clinical aspects of *A. baumannii* infection (Chopra *et al.*, 2008; Peleg *et al.*, 2008a; Vila & Pachón, 2008; Fishbain & Peleg, 2010; Gordon & Wareham, 2010) . The major resistance mechanisms that have been identified in *A. baumannii* for different antibiotic classes are summarized in Table 1.

Recent technical and computational advances have facilitated the global genomic comparative analyses of

Table 1. Major resistance mechanisms found in *Acinetobacter baumannii*

Drug class	Resistance mechanism	Examples
β-lactams	Inactivating enzymes	β-lactamases (AmpC, TEM, VEB*, PER, CTX-M, SHV) Carbapenemases (OXA-23, -40, -51, -58- 143-like, VIM, IMP, NDM-1, -2)
	Decreased outer membrane protein expression	CarO, 33–36 kDa protein, OprD-like protein
	Altered penicillin-binding protein expression	PBP2
	Efflux pumps	AdeABC
Fluoroquinolones	Target modification	Mutations in *gyrA* and *parC*
	Efflux pumps	AdeABC, AdeM
Aminoglycosides	Aminoglycoside modifying enzymes	AAC*, †, ANT, APH*, †
	Efflux pumps	AdeABC, AdeM
	Ribosomal methylation	ArmA
Tetracyclines	Efflux pumps	AdeABC, TetA*, TetB
	Ribosomal protection	TetM
Glycylcyclines	Efflux pumps	AdeABC
Polymyxins (colistin)	Target modification	Mutations in the PmrA/B two-component system (LPS modification), mutations in LPS biosynthesis genes

*Found in AbaR1 from the *A. baumannii* strain AYE (Fournier *et al.*, 2006).
†Found in AbaR2 from the *A. baumannii* strain ACICU (Iacono *et al.*, 2008).

clinical isolates and have shown the remarkable capacity of *A. baumannii* to acquire and rearrange genetic determinants that play a critical role in its pathobiology (see Table 2 for important sequenced strains of *A. baumannii*). The first report describing this type of analysis showed that the MDR phenotype of *A. baumannii* AYE is because of the acquisition of the 86-kb AbaR1 resistance island (Fournier *et al.*, 2006). The acquisition of this island, which includes 45 resistance genes as well as genetic traits coding for DNA mobilization functions (transposases) and is absent in sensitive strains, could be explained by horizontal gene transfer from unrelated sources. Other *A. baumannii* strains such as the European Clone (EC) II strain ACICU harbor the AbaR2 resistance island (Iacono *et al.*, 2008). More recently, the comparative genome-wide analysis of ACICU and three strains belonging to the A, B, and C types determined by pulse-field gel electrophoresis isolated during an outbreak at the National Institutes of Health Clinical Center (Snitkin *et al.*, 2011) confirmed the presence of discrete genomic regions dedicated to antimicrobial resistance. This report also showed that *A. baumannii* has the capacity to adapt to hospital environments not only by horizontally acquiring genetic traits responsible for the evolution of non-MDR ancestors into MDR outbreak strains, but also by rearranging preexisting genes. *Acinetobacter baumannii* strains can shuffle, add, and/or delete genes coding for important virulence factors, particularly those associated with cell-surface products, such as surface proteins and O-antigens and adhesins, and the expression of the functions needed to acquire essential nutrients such as iron (Snitkin *et al.*, 2011). Taken together, these observations indicate that non-MDR strains may serve as a source of antigenic variants that could play a critical role in the diversification and emergence of MDR *A. baumannii* clinical isolates. Such a possibility is supported by a recent report (Imperi *et al.*, 2011) showing that *A. baumannii* has a relatively small-sized core genome and a rather large accessory genome that hosts numerous antibiotic resistance and virulence determinants and is likely acquired by horizontal gene transfer processes.

A small number of studies have characterized the effect of acquisition of antibiotic resistance on the fitness and virulence of *A. baumannii*. A colistin-resistant strain, isolated after growth of a colistin-sensitive strain in sub-inhibitory concentrations of colistin, showed decreased *in vitro* and *in vivo* growth compared with the parental strain (López-Rojas *et al.*, 2011). Additionally, the LD_{50} of the colistin-resistant strain was 10-fold higher than the parental strain in a mouse model of intraperitoneal sepsis. Targeted gene sequencing showed that the colistin-resistant strain had acquired a mutation in the *pmrB* gene, a mechanism that has previously been described to confer resistance to colistin (Adams *et al.*, 2009). A separate study in which the fitness and virulence of an *A. baumannii* strain in which ciprofloxacin resistance was induced by growth in the presence of ciprofloxacin was compared with the parental strain showed similar results (Smani *et al.*, 2012). The ciprofloxacin-resistant derivative induced less cell death, reduced *in vitro* and *in vivo* growth, and reduced mortality in a mouse model of peritoneal sepsis. Taken together, these studies indicate that, at least in some cases, the acquisition of antibiotic resistance in *A. baumannii* comes at a biological cost.

Table 2. Important sequenced strains of *Acinetobacter baumannii* and associated iron uptake systems

Strain/System	$Fe2^+$	Heme*	Heme†	Cluster 1	Cluster 2	Acinetobactin	Cluster 4	Cluster 5
ATCC 19606^T	+	+	–	+	–	+	–	+
ATCC 17978	+	+	–	+	+	+	–	–
AYE	+	+	–	+	–	+	+	
AB0057	+	+	+	+	–	+	–	+
AB307-294	+	+	–	+	–	+	–	+
ACICU	+	+	+	+	–	+	–	+
D1279779	ND	ND	ND	+	–	+	–	+
WM99c	ND	ND	ND	+	–	+	–	+
SDF	ND	ND	+	–	–	–	–	–
8399‡	ND	ND	ND	ND	ND	ND	+	ND
ADP1	ND	ND	ND	+	+	–	–	–

The (+) and (–) symbols represent the presence or absence of a particular system in a particular strain, ND signifies that the presence of this system has not been determined for this strain.

*Predicted heme uptake system that does not include an identifiable gene coding for heme oxygenase activity.

†Predicted heme uptake system that includes an identifiable gene coding for heme oxygenase activity.

‡The complete genome sequence has not been determined for this strain. This table was adapted from data previously reported (Antunes *et al.*, 2011b; Eijkelkamp *et al.*, 2011).

Natural habitats of *A. baumannii*

According to the current taxonomy, the genus *Acinetobacter* includes 27 valid species [J.P. Euzéby taxonomy site (http://www.bacterio.cict.fr/a/acinetobacter.html)], the most recent addition of *Acinetobacter indicus* sp. nov. (Malhotra *et al.*, 2012) and nine provisional species based on DNA–DNA hybridization, all of which encompass strains found in a wide range of ecological niches. However, the most medically relevant species belong to the *A. baumannii* complex, which includes *A. baumannii* and the genomic species 3 and 13TU, which were recently renamed *Acinetobacter pitti* sp. nov. and *Acinetobacter nosocomialis* sp. nov., respectively (Nemec *et al.*, 2011). Because of the widespread presence of *Acinetobacter* in different ecological niches, one of the main misconceptions regarding the natural habitat of *A. baumannii* is its ubiquitous presence in nature and consequent isolation from water, animal, and soil samples. Reports describing these types of isolations should be carefully considered, particularly if they refer to strains that were not identified to the species level according to the current taxonomy using validated methods, which are more accurate than those used some time ago, especially before 1986 when the taxonomy of the genus underwent major revisions (Bouvet & Grimont, 1986). Equally misleading is the concept that *A. baumannii* is a normal component of the human flora. On the basis of the ecology, epidemiology, and antibiotic phenotype of different isolates, Towner proposed the existence of three major *Acinetobacter* populations (Towner, 2009). One of them, which consists mainly of *A. baumannii* and closely related members of the *A. baumannii* complex, is represented by strains isolated from medical environments and equipment, medical personnel, and hospitalized patients. In general, these isolates tend to be resistant to multiple antibiotics, although strains such as the clinical isolates ATCC 19606^T and ATCC 17978, which are sensitive to most antibiotics, clearly belong to this group. The second population is represented by strains that can be found in human and animal skin flora as well as in spoiled food samples. Members of this group include *Acinetobacter johnsonii*, *Acinetobacter lwoffii*, and *Acinetobacter radioresistens*. The last group includes antibiotic-sensitive isolates obtained from environmental sources such as soil and wastewater samples and mainly comprises *Acinetobacter calcoaceticus* and *A. johnsonii*. Although most members of the these last two groups are sensitive to antibiotics, some *A. radioresistens*, *A. johnsonii*, and *A. calcoaceticus* isolates have been found to contain carbapenemase resistance genes (Figueiredo *et al.*, 2011). While this grouping makes sense considering the current understanding of the taxonomy, epidemiology, and ecology of different members of the *Acinetobacter* genus, the fact that strains such as ATCC 19606^T, which is the *A. baumannii*-type strain (Bouvet & Grimont, 1986), and ATCC 17978, which was the first to be fully sequenced (Smith *et al.*, 2007), are sensitive to most if not all antibiotics used in human medicine may, at first glance, contradict the existence of these groups.

The ability of *A. baumannii* to resist desiccation and persist on hospital materials and medical devices (Villegas & Hartstein, 2003) has played a critical role in the emergence of this bacterium as a relevant human pathogen. However, many host, environmental, and bacterial factors affecting the virulence phenotype of *A. baumannii* remain to be identified and characterized. For example, it was

observed that exposure of *A. baumannii* to ethanol enhances not only its growth in media containing ethanol, but also serves as an environmental signal that controls responses to salt tolerance and increased pathogenicity when tested in *Caenorhabditis elegans* (Smith *et al.*, 2004). Further genomic and mutagenesis analysis of the strain ATCC 17978 showed that the enhanced ethanol-mediated virulence response in *C. elegans* worms and *Dictyostelium discoideum* amebae relates to genes located in pathogenicity islands, some of which code for novel gene products (Smith *et al.*, 2007). Interestingly, some of the mutants harbor mutations impairing the expression of ABC transporters, an uncharacterized urease activity, and transcriptional regulators. The latter finding suggests that ethanol could play a global regulatory function, a hypothesis that is supported by the data obtained using global RNA-sequencing (Camarena *et al.*, 2010). This study, which resulted in the identification of 49 ethanol-induced genes coding for metabolic functions, stress responses and virulence functions, suggests that ethanol affects the pathobiology of *A. baumannii*. Such findings could be significant because the presence of ethanol in clinical settings may have an impact as previously reported (Edwards *et al.*, 2007). More recently, it was reported that *A. baumannii* also senses and responds to light, an unexpected observation considering that this is a nonphotosynthetic microorganism (Mussi *et al.*, 2010). This observation led to the hypothesis that the outcome of certain infections, such as surface-exposed wound infections, could depend on the exposure of bacteria to light and temperatures lower than 37 °C.

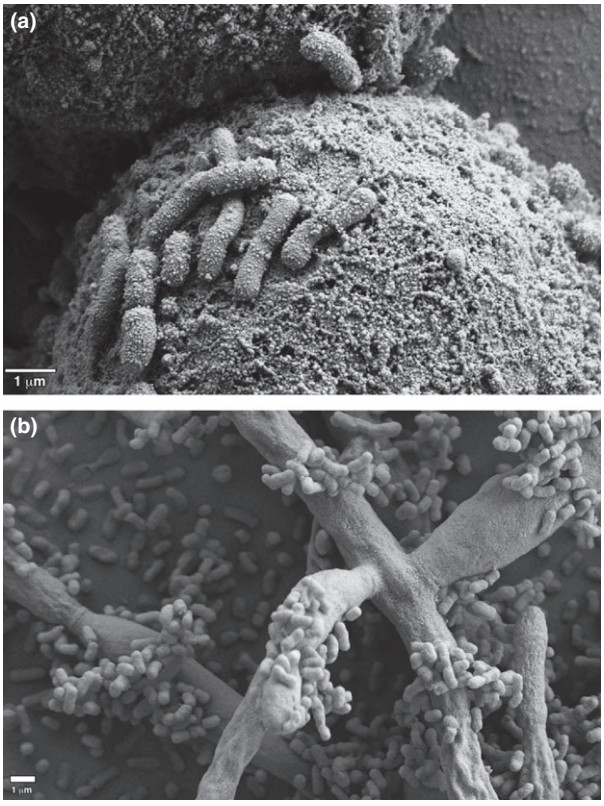

Fig. 1. (a) Scanning electron microscopy of *Acinetobacter baumannii* cells attached to the surface of an A549 human alveolar epithelial cell. (b) Scanning electron microscopy of *Acinetobacter baumannii* cells attached to the surface of *Candida albicans* filaments-. Figure from the laboratory of Luis Actis.

Adherence and biofilm formation appear to contribute to pathogenicity

Acinetobacter baumannii has a remarkable capacity to survive and prosper in hospital environments, most likely due to its ability to interact with different types of surfaces, including abiotic substrata normally found in medical settings, such as furniture, linen, and medical equipment (Neely *et al.*, 1999; Neely, 2000; Villegas & Hartstein, 2003; Borer *et al.*, 2005). Such behavior is in accordance with the described capacity of *A. baumannii* clinical isolates to survive long stretches of time under highly desiccated conditions on abiotic surfaces, a property that is uncommon among other Gram-negative pathogens (Wendt *et al.*, 1997; Jawad *et al.*, 1998). *Acinetobacter baumannii* also adheres to and colonizes indwelling devices such as catheters and respiratory equipment (Villegas & Hartstein, 2003), as well as biotic surfaces such as those of human epithelial cells (Fig. 1a), which may be a target during respiratory infections, or *Candida albicans* filaments (Fig. 1b; Lee *et al.*, 2006). The latter type of interaction could represent the capacity of *A. baumannii* to interact with other components of the human microbial flora under particular environmental conditions and serve as a reservoir, as was shown with *Helicobacter pylori* (Salmanian *et al.*, 2008).

The adherence of *A. baumannii* is variable among clinical isolates as it has been shown that strains belonging to the EC II strain are more adherent than the EC I strain to human bronchial epithelial cells, although no significant differences were observed between outbreak and nonoutbreak strains (Lee *et al.*, 2006).

Generally, the adherence of *A. baumannii* to biotic and abiotic surfaces results in the development of biofilms, which are complex multicellular three-dimensional structures with cells in intimate contact with each other and encased in an extra-cellular matrix that can be comprised of carbohydrates, nucleic acids, proteins, and other macromolecules (Costerton *et al.*, 1999). It is hypothesized that *A. baumannii* persists in medical environments, resists antimicrobials, and causes disease because of its capacity to form biofilms on solid surfaces (Donlan &

Costerton, 2002; Gaddy & Actis, 2009). Some *A. baumannii* clinical isolates form complex biofilm structures on the surface of liquid media, which are known as pellicles (Fig. 2; Martí *et al.*, 2011; McQueary & Actis, 2011). Pellicle formation and biofilm formation on abiotic surfaces are quite variable among *A. baumannii* clinical isolates with no apparent correlation between the nature of different types of substrata and bacterial surface properties (McQueary & Actis, 2011). Furthermore, there are significant variations not only in the amount of biofilm formed on abiotic surfaces but also in the type of cell arrangements formed on these surfaces. Some cell arrangements are simple monolayers of bacteria attached in an organized or random manner while others are complex multilayered structures encased within a biofilm matrix (McQueary & Actis, 2011).

A number of *A. baumannii* gene products have been shown to play a role in biofilm formation and adherence to abiotic surfaces. Initial studies showed that pilus production mediated by the CsuA/BABCDE usher-chaperone assembly system is required for attachment and biofilm formation on abiotic surfaces by the *A. baumannii* ATCC 19606^T strain (Tomaras *et al.*, 2003). This operon seems to be widespread among clinical isolates, an indication that the pili assembled by this system could be a common factor among different clinical isolates. However, the ATCC 19606^T strain has the capacity to produce alternative pili that may participate in the interaction of this pathogen with bronchial epithelial cells (de Breij *et al.*, 2009). Preliminary observations (C.N. McQueary & L.A. Actis, unpublished results) also indicate that *A. baumannii* ATCC 17978 has pili that are different from those described in the ATCC 19606^T strain. The ATCC 17978 pili are long and thin and they tend to bundle; this observation is congruent with the fact that ATCC 17978 cells do not produce CsuA/B, which is considered the pilin subunit of the CsuA/BABCDE-mediated pili, and with the fact that this strain forms much weaker biofilms on plastic when compared with the ATCC 19606^T strain.

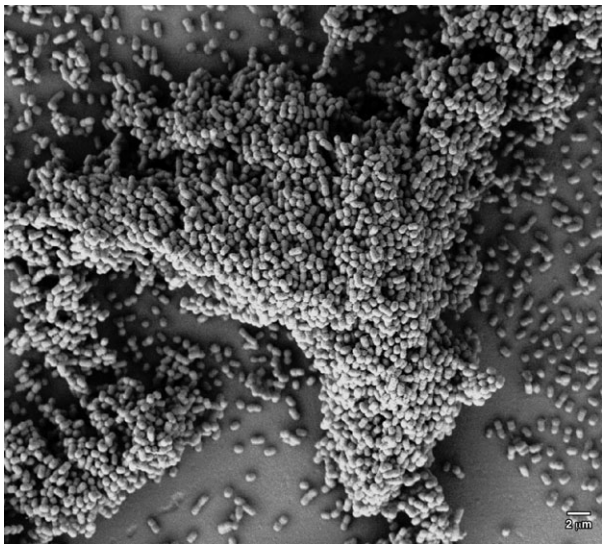

Fig. 2. Scanning electron microscopy of *Acinetobacter baumannii* pellicle collected on the surface of a coverslip. Figure from the laboratory of Luis Actis

In strain 307-0294, mutational loss of a large outer membrane protein, which has high similarity to the staphylococcal biofilm-associated protein (Bap), resulted in a diminishment of the volume and thickness of biofilms formed by this strain on glass (Loehfelm *et al.*, 2008). On the basis of its cellular location and participation in biofilm formation and development, the Bap protein, which is conserved among different clinical isolates, appears to be needed for cell-to-cell interactions that support biofilm development and maturation (Loehfelm *et al.*, 2008). The two latter processes also depend on the capacity of *A. baumannii* clinical isolates to produce and secrete poly-β-1-6-*N*-acetylglucosamine (PNAG), an exopolysaccharide produced by almost all tested strains that is critical for the formation of fully developed biofilms on glass by cells cultured statically (Choi *et al.*, 2009).

In the ATCC 19606^T strain, a two-component regulatory system comprised of a sensor kinase encoded by *bfmS*, and a response regulator encoded by *bfmR* is involved in bacteria–surface interactions (Tomaras *et al.*, 2008). Insertional inactivation of *bfmR* resulted in a loss of expression of the *csuA/BABCDE* operon and the ensuing lack of pili production and biofilm formation on plastic when cells were cultured in rich medium (Tomaras *et al.*, 2008). Inactivation of the *bfmS* sensor kinase gene resulted in a diminishment, but not abolishment of biofilm formation. When the BfmRS system was not expressed, the composition of the culture medium still influenced the interaction of cells with abiotic surfaces (Tomaras *et al.*, 2008). This indicates that BfmR could crosstalk with other sensing components and suggests that multiple and different environmental stimuli could control biofilm formation via the BfmRS regulatory pathway.

In contrast to the ability to adhere to abiotic surfaces, much less is known regarding the *A. baumannii* factors that play a role in adherence to and biofilm formation on biotic surfaces. As mentioned above, this pathogen attaches to human epithelial cells and *C. albicans* filaments, in a process that involves at least the outer membrane protein OmpA. While OmpA could also play a role in biofilm development on plastics, this outer membrane protein is critical for the interaction of the pathogen with human and *Candida* cells when the latter are in a filamentous form (Gaddy *et al.*, 2009). The ATCC 19606^T-*C. albicans* filament interactions are independent of the

pili assembled by the *csu* usher-chaperone system and lead to apoptotic death of the fungal filaments (Fig. 3). These results suggest that there is no direct correlation between biofilm formation on abiotic and biotic surfaces, and that there is wide variation in the cell-surface and cell–cell interactions that result in adherence and biofilm formation by different *A. baumannii* clinical isolates. In spite of this information, the role of pili in bacterial virulence and the pathogenesis of the infections *A. baumannii* causes in humans remains to be confirmed using appropriate derivatives and experimental infection models.

Adherence and biofilm formation are well-orchestrated processes that respond to a wide range of cellular and environmental cues (Stanley & Lazazzera, 2004). For instance, the ability of *A. baumannii* to form biofilms could depend on the presence and expression of antibiotic resistance traits, such as the $bla_{\text{PER-1}}$ gene. A positive correlation was found between the presence and level of expression of this gene and the amount of biofilms formed on plastic and the adhesiveness of bacteria to human epithelial cells (Lee *et al.*, 2008). However, an independent study found that only two of 11 isolates carrying the $bla_{\text{PER-1}}$ gene formed stronger biofilms when compared with isolates lacking this genetic determinant (Rao *et al.*, 2008), which brings to question the relevance of the presence and expression of this gene in biofilm formation by *A. baumannii* isolates. Environmental cues such as temperature and the concentration of extracellular free iron, which are relevant for the interaction of *A. baumannii* with the host, also affect the amount of biofilm formed by this pathogen on abiotic surfaces. *Acinetobacter baumannii* ATCC 19606^{T} formed more biofilm when cultured in LB broth at 30 °C or in M9 minimal medium at 37 °C in the presence of the synthetic iron chelators 2,2′-dipyridyl (DIP) or ethylenediamine-di-(*o*-hydroxyphenyl) acetic acid (EDDHA; Tomaras *et al.*, 2003). However, a global transcriptomic analysis later showed a significant down-regulation of some of the *csu* genes in cells cultured under iron-chelated conditions (Eijkelkamp *et al.*, 2011). This apparent discrepancy might be explained by the fact that these two studies used different strains (ATCC 19606^{T} vs. ATCC 17978). In ATCC 17978 cells, the *csu* operon does not appear to be active and iron-regulated cell products other than pili may play a role in adherence and biofilm formation (McQeary Zimbler and Actis, unpublished observations). The chelating agent EDTA also affects the interaction between clinical *A. baumannii* isolates with biotic and abiotic surfaces, as it has been shown that it significantly reduces bacterial attachment and biofilm formation on human respiratory epithelial cells and plastic surfaces (Lee *et al.*, 2008). The molecular mechanisms by which these chelators produce this effect remain to be elucidated.

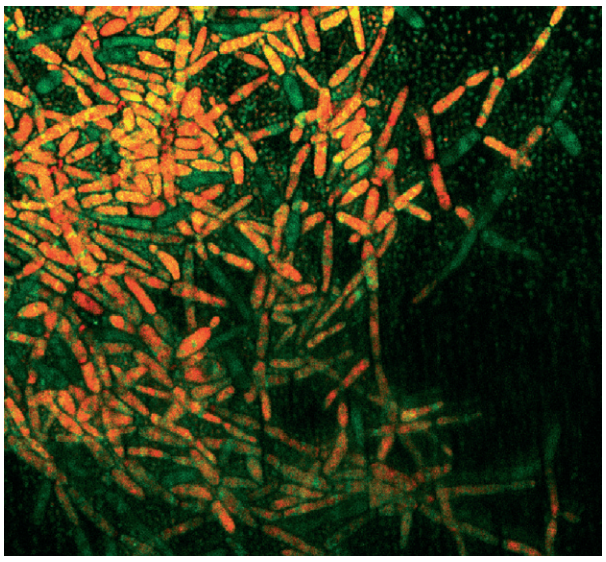

Fig. 3. Laser scanning confocal microscopy of Live/Dead-stained *Acinetobacter baumannii* cells attached to *Candida albicans* filaments. Live and dead fungal filaments are stained green and red, respectively. Live bacterial cells, stained green, attached to the surface of dead fungal filaments appear as yellow co-fluorescence areas. The micrograph was taken at 400 ×. Figure from the laboratory of Luis Actis.

Cell population density is another mechanism by which bacteria control adherence and biofilm formation. Accordingly, environmental and clinical isolates produce quorum sensing signaling molecules (González *et al.*, 2001, 2009). Interestingly, these studies showed that a large proportion of the tested isolates produce one or more quorum sensors that seem to belong to three types of molecules. Although none of these sensors could be assigned to a particular species, the Rf1-type sensor is more frequently found in isolates belonging to the *A. calcoaceticus-baumannii* complex. More detailed studies showed that the *A. baumannii* M2 clinical isolate produces an *N*-acyl-homoserine lactone [i.e. *N*-3-hydroxy-dodecanoyl-homoserine lactone (3-OH-C12-HSL)], the product of the *abaI* autoinducer synthase gene, which is important for the formation of fully developed biofilms on abiotic surfaces (Niu *et al.*, 2008). This autoinducer also plays a role in the ability of this strain to move on semisolid media, as described in the next section. Finally, as mentioned above, the observation that light affects biofilm formation on abiotic surfaces was unexpected considering that *A. baumannii* is a chemotroph not known to conduct photosynthesis (Mussi *et al.*, 2010). This response is mediated by the BlsA photoreceptor protein, which contains a BLUF domain and uses FAD to sense light. The mechanisms by which BlsA transduces the light signal and controls gene expression are not known (Mussi *et al.*, 2010). The *A. baumannii* response

to light seems to have a global effect on the physiology of *A. baumannii*, affecting not only biofilm formation but also motility and virulence. Furthermore, the differential response to illumination is modulated by temperature changes, which result in differential transcription of *blsA* at 28 and 37 °C and hence differentially affect light-controlled phenotypes (Mussi *et al.*, 2010).

In conclusion, biofilm formation and adherence in *A. baumannii* clinical isolates involves a range of bacterial factors and multiple signals or cues. However, the medical relevance of data obtained using *in vitro* models is not clear, considering the lack of correlation between the biofilm phenotype of different clinical isolates and their outbreak, epidemic and antibiotic resistance nature (de Breij *et al.*, 2010). By comparison with other bacterial pathogens, such as *Pseudomonas aeruginosa*, very little is known about the *A. baumannii* products involved in pathogenicity mechanisms and the cellular and environmental signals that control them within the vertebrate host.

Motility on semi-solid surfaces: does it impact on pathogenicity?

Motility in *A. baumannii* is counterintuitive considering that the name of this genus (acinetobacter, nonmotile bacterium) implies the inability of members of this genus to move. However, this phenotype was reported more than 30 years ago when Henrichsen described the influence of the environment on the ability of *A. calcoaceticus* to move on agar plates (Henrichsen, 1975; Henrichsen & Blom, 1975). This issue recently resurfaced because of the observation that *A. baumannii* displays differential motility in response to illumination (Mussi *et al.*, 2010), quorum sensing (Clemmer *et al.*, 2011), and iron chelation (Eijkelkamp *et al.*, 2011). Although the type of motility displayed by *Acinetobacter* strains has not been elucidated unequivocally, current phenotypic and genetic evidence suggests that this pathogen moves on semi-solid surfaces by expressing twitching motility rather than gliding, sliding, swimming, or swarming motility (Barker & Maxted, 1975; Henrichsen, 1984; Eijkelkamp *et al.*, 2011). This possibility is supported by the observation that iron affects motility and the expression of *pil-com* ATCC 17978 genes (Eijkelkamp *et al.*, 2011) that participate in the assembly and function of type IV pili, which are known to be involved in twitching motility (Mattick, 2002). Furthermore, insertional inactivation of *pilT*, which codes for an ATPase activity involved in pilus retraction in other bacteria (Merz *et al.*, 2000; Mattick, 2002), resulted in a significant reduction in the motility of the *A. baumannii* M2 strain (Clemmer *et al.*, 2011). However, a *pilT::Km* derivative of strain M2 was still motile on the surface of 0.35% Eiken agar, suggesting that the strain has type IV pili-independent motility functions. Such a possibility is supported by the reduced motility of M2 transposon insertion derivatives affected in the expression of genes that do not code for type IV pilus assembly and function (Clemmer *et al.*, 2011). Sequence analysis of some of these derivatives showed the potential involvement of a lipopeptide, degradation of peptidoglycan, synthesis of O-antigen, OmpA, and the histidine kinase sensor BfmS, which regulates the expression of the *csuA/BABCDE* operon via the BfmR response regulator (Tomaras *et al.*, 2008).

Most if not all movement displayed by *A. baumannii* occurs on the surface of semi-solid media and diminishes as the concentration of agar or agarose in the medium increases (Clemmer *et al.*, 2011; McQueary & Actis, 2011). Furthermore, different types of agar affect the outcome of the motility response with complex patterns that manifest as either well-defined brunches, with some of them resembling ditching motility already described in *Acinetobacter anitratus* (Barker & Maxted, 1975), or circular cell expansion from the inoculation point with an even pattern of cells on the surface of the agar (Clemmer *et al.*, 2011; McQueary & Actis, 2011). Different strains display different patterns, and not all tested strains move on semi-solid surfaces (Fig. 4; Clemmer *et al.*, 2011; McQueary & Actis, 2011). Currently, we do not know whether motility depends on different motility systems or variations in the capacity of different strains to sense the appropriate environmental cues controlling this complex multicellular process. For instance, the availability of free iron, which is well known for its impact in bacterial human infections (Weinberg, 2009), plays a role in the *pil-com*-dependent motility of *A. baumannii*, as mentioned before (Eijkelkamp *et al.*, 2011). Cell population density is another factor that controls motility as inactivation of the *abaI* gene (specifying the autoinducer 3-OH-C_{12}-HSL) resulted in a drastically reduced motility response, which was corrected by the supplementation of purified autoinducer (Clemmer *et al.*, 2011). Light, particularly blue light, is a third environmental signal that controls *A. baumannii* motility on semi-solid media by mechanisms that remain to be elucidated (Mussi *et al.*, 2010).

In conclusion, *A. baumannii* is capable of moving on the surface of semi-solid media by processes that are mediated, at least in part, by type IV pili-dependent mechanisms, which are affected by environmental and cell signals that also affect bacterial virulence. However, it is unclear whether motility plays a significant role in the virulence of *A. baumannii* and the pathogenesis of the serious infections that it causes in the human host. This is because not all clinical isolates display motility when tested under laboratory conditions (which undoubtedly

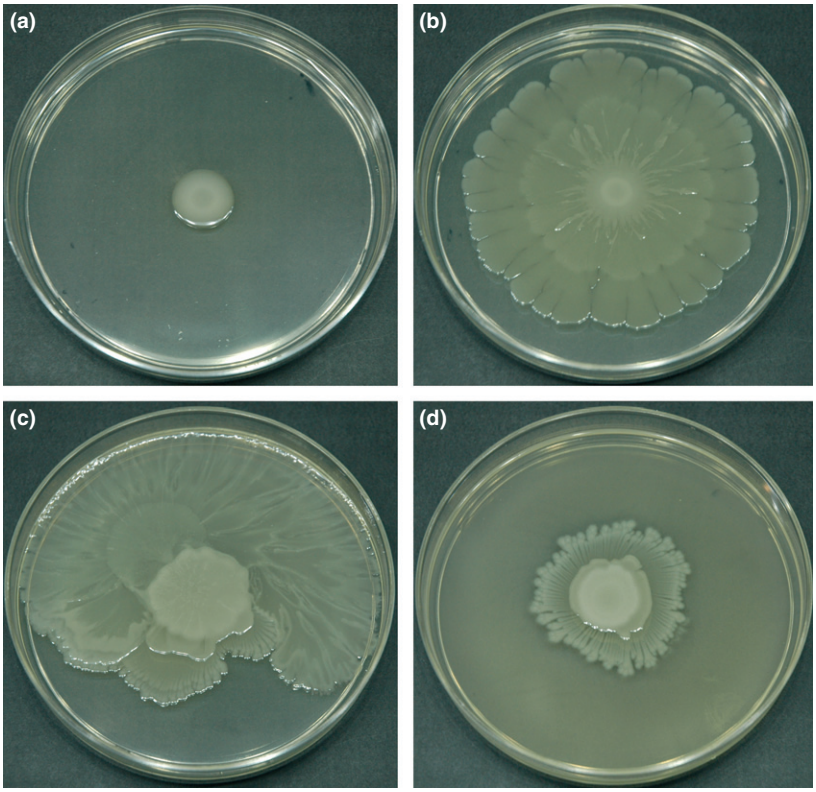

Fig. 4. Surface motility of *Acinetobacter baumannii* strains on semi-solid media. Suspensions of bacteria containing the nonmotile ATCC 19606^T strain (a) and three clonally distinct clinical isolates of *Acinetobacter baumannii* (b–d) were deposited on the center of plates containing 0.4% agar and allowed to grow at 37 °C for 42 h. Figure from the laboratory of Jerónimo Pachón.

do not reflect those encountered by this pathogen in the human host) and because appropriate nonmotile isogenic derivatives have not been tested in relevant animal models.

Iron acquisition from heme and via siderophores

Although iron is abundant in environmental and biological systems, ferric iron is relatively unavailable to cells because of its poor solubility under aerobic conditions and its chelation by low-molecular-weight compounds such as heme and by high-affinity iron-binding proteins such as lactoferrin and transferrin. In response to iron limitation, most aerobic bacteria express high-affinity iron acquisition systems that mainly include the production, export, and uptake of Fe^{3+} chelators known as siderophores. In addition, some bacteria utilize heme or hemoglobin as an iron source, and some are able to remove iron from transferrin or lactoferrin (Crosa *et al.*, 2004; Wandersman & Delepelaire, 2004). *Acinetobacter baumannii* does not bind transferrin (Echenique *et al.*, 1992) and does not carry genetic determinants coding for the proteins involved in the acquisition of iron from transferrin and lactoferrin (Smith *et al.*, 2007). However, the ATCC 19606^T strain uses heme as an iron source (Zimbler *et al.*, 2009). The chromosomal cluster annotated as A1S_1608-A1S_1614 in strain ATCC 17978 seems to be a polycistronic operon that could be involved in the transport of heme from the periplasm into the cytoplasm (Smith *et al.*, 2007). A more recent genomic analysis (Antunes *et al.*, 2011a) showed that different strains can use this compound as an iron source expressing potential heme uptake and utilization systems (Table 2). Taken together, these observations indicate that the *A. baumannii* genome contains genes coding for products devoted to the capture and utilization of heme, a host product that could be available to bacteria at sites where extensive cell and tissue damage are produced by infections such as necrotizing fasciitis (Brachelente *et al.*, 2007; Charnot-Katsikas *et al.*, 2009; Corradino *et al.*, 2010) or in severely injured patients (Peleg *et al.*, 2008a). *Acinetobacter baumannii* may also acquire ferrous iron (Table 2), which would be available under low-oxygen tension conditions, because fully sequenced and annotated genomes show the presence of genes coding for a Feo transport system (Antunes *et al.*, 2011b), the function of which remains to be tested experimentally.

Acinetobacter baumannii is also capable of acquiring ferric ions under iron-limited conditions via siderophores. The best-characterized system is that mediated by the siderophore acinetobactin, which was initially described in the ATCC 19606^T strain and has a molecular structure highly related to anguibactin (Fig. 5), a high-affinity iron chelator produced by the fish pathogen *Vibrio anguillarum* 775 (Yamamoto *et al.*, 1994, 1999; Dorsey *et al.*, 2004; Mihara *et al.*, 2004). The main difference between these two siderophores is that acinetobactin contains an oxazoline ring derived from threonine, while anguibactin has a thiazoline group derived from cysteine. In spite of this difference, these siderophores, which are produced by two unrelated bacterial pathogens found in different environments, are also functionally related because both of them crossfeed derivatives affected in their production (Dorsey *et al.*, 2004). Genetic and functional analyses (Dorsey *et al.*, 2004; Mihara *et al.*, 2004) have shown that a 26.5-kb chromosomal region harbors genes coding for all functions needed for the biosynthesis, transport, and secretion of acinetobactin with the exception of *entA*, which codes for the biosynthetic enzyme 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase. This enzyme is needed for the production of the dihydroxybenzoic acid (DHBA) moiety present in the acinetobactin molecule (Fig. 5). This observation indicates that *A. baumannii* could contain more than one locus involved in siderophore biosynthesis, a possibility that is supported by the recent information made available by genomic analysis of fully sequenced and annotated *A. baumannii* isolates (Table 2; Antunes *et al.*, 2011b; Eijkelkamp *et al.*, 2011). The report by Eijkelkamp *et al.* (2011) describes five gene clusters predicted to be involved in siderophore production and utilization for iron acquisition. The 26.5-kb cluster mentioned above related to acinetobactin, which was named as the acinetobactin cluster, and cluster 1 are present in the genomes of all strains analyzed with the exception of 8399 and the environmental strain *Acinetobacter baylyi* ADP1 (Table 2). Cluster 2 is present only in *A. baumannii* ATCC 17978 and *A. baylyi* ADP1 while cluster 4 seems to be unique to isolate 8399. Cluster 5 is found in all genomes analyzed except that of ATCC 17978 and ADP1 . It is important to note that the SDF strain does not contain any gene cluster related to siderophore-mediated iron acquisition functions.

Taken together, the available experimental and *in silico* observations indicate that *A. baumannii* can acquire iron either by using heme as an iron source or by capturing this metal with acinetobactin and/or one or more additional siderophore-mediated systems. A recent report by Gaddy *et al.* (2012) showed that the acinetobactin-mediated system plays a critical role in the ability of *A. baumannii* ATCC 19606^T to persist and cause cell damage and animal death when tested using human epithelial cells, *Galleria mellonella* caterpillars, and mice infection models, indicating that iron acquisition functions play a critical role in virulence. Whether the other iron acquisition systems listed in Table 2 also play a virulence role or provide an ecological advantage for their persistence in particular ecological niches are interesting possibilities that remain to be examined.

Virulence factors

Compared to other Gram-negative pathogens, relatively few virulence factors have been identified for *A. baumannii*. The recent sequencing of numerous *A. baumannii* complete genomes (Table 2) and the application of methods for manipulating the bacterial genome to generate gene-deficient mutants, together with the use of animal models, have been crucial in the identification of bacterial factors that contribute to pathogenesis (Table 3). These studies have begun to shed light on how this pathogen persists in the environment, interacts with host cells, and causes host cell damage, although there are undoubtedly numerous additional factors that have yet to be identified.

Probably the best-characterized virulence factor of *A. baumannii* identified to date is OmpA. Evidence that *A. baumannii* OmpA contributes to virulence was obtained in a random transposon mutagenesis screen that detected *A. baumannii* mutants that were deficient in inducing apoptosis in a human laryngeal epithelial cell line (Choi *et al.*, 2005). Purified OmpA localized to the mitochondria and induced apoptosis through the release of the proapoptotic molecules cytochrome *c* and apoptosis-inducing factor, suggesting that this may be one pathway by which *A. baumannii* induces damage to human airway cells during infection. The OmpA protein also plays a role in adherence and invasion of epithelial cells may contribute to the dissemination of *A. baumannii* during infection, as the bacterial loads in blood of mice with experimentally induced *A. baumannii* pneumonia were significantly higher in mice infected with a wild-type strain than in mice infected with an equivalent amount of an isogenic *ompA* mutant (Choi *et al.*, 2008). The OmpA protein also contributes to the ability of *A. baumannii* to persist and grow in human serum as it has been shown that OmpA interacts with soluble inhibitors of the alternative complement pathway and allows the bacteria to avoid complement-mediated killing (Kim *et al.*, 2009). However, OmpA is unlikely to be the only factor that contributes to serum resistance as different *A. baumannii* strains, all of which contain putative *ompA* genes, have significantly different capacities for growth and survival in human serum (Antunes *et al.*, 2011a). As mentioned before, in the environment, OmpA may also facilitate the

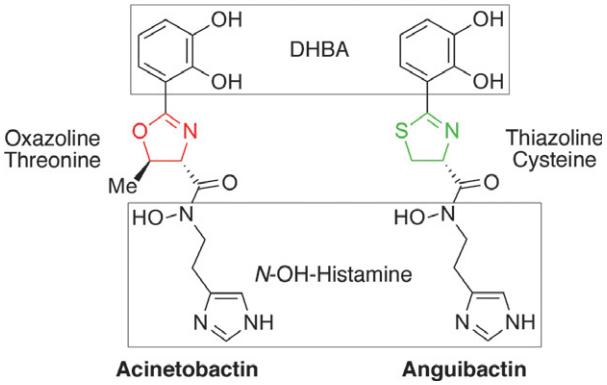

Fig. 5. Molecular structure of the acinetobactin and anguibactin siderophores produced by *Acinetobacter baumannii* and *Vibrio anguillarum*, respectively. The DHBA and *N*-OH-histamine moieties are common to both siderophore molecules. The oxazoline ring, derived from threonine, and the thiazoline ring, derived from cysteine, are unique to acinetobactin and anguibactin, respectively. The DHBA moiety derives from chorismic acid by the action of the EntA, EntB, and EntC enzymes while the *N*-OH-Histamine component is the result of the decarboxylation of histamine (Crosa & Walsh, 2002; Mihara *et al.*, 2004).

persistence and survival of *A. baumannii* by assisting biofilm formation and surface motility (Gaddy *et al.*, 2009; Clemmer *et al.*, 2011). Because of the multiple functions of OmpA in the pathogenesis of *A. baumannii*, it may be an attractive target for the development of novel treatment and prevention strategies.

Acinetobacter baumannii LPS contains a lipid A moiety, the carbohydrate core, and the repetitive O-antigen. The role of LPS in *A. baumannii* pathogenesis was recently investigated using a mutant lacking the LpsB glycotransferase that results in a highly truncated LPS glycoform containing only two carbohydrate residues bound to lipid A (Luke *et al.*, 2010). This mutant showed decreased resistance to human serum and decreased survival in a rat model of soft tissue infection compared with the isogenic parent strain, indicating a role for the surface carbohydrate residues of LPS in pathogenesis. It has also been demonstrated that CD14 and Toll-like receptor 4 play a role in clearing *A. baumannii* from the lung through detection of LPS (Knapp *et al.*, 2006), suggesting that *A. baumannii* LPS activates the innate immune response. Studies further addressing the inflammatory response and the mortality elicited by LPS mutants in animal models would be of interest for further characterizing the contribution of *A. baumannii* LPS to virulence.

The structures of the capsular polysaccharides from two clinical isolates of *A. baumannii* were recently reported revealing a linear aminopolysaccharide consisting of three carbohydrate residues in one strain and a branched pentasaccharide in the other (Fregolino *et al.*, 2011). In addition to LPS, the capsular polysaccharide has also been identified as a pathogenicity factor in *A. baumannii.* Mutants that were deficient for growth in human ascites fluid because of transposon insertion in the *ptk* or *epsA* gene failed to produce a capsule-positive phenotype. These mutants showed decreased growth in both human serum and ascites compared with the wild-type counterpart. Additionally, capsule-deficient strains were completely cleared by 24 h postinfection in a rat model of soft tissue infection, whereas the isogenic parental strain persisted with $> 10^7$ bacteria mL^{-1} of exudative fluid (Russo *et al.*, 2011). Thus, the capsular polysaccharide appears to play an important role in protecting bacteria from the host innate immune response.

Bacterial phospholipases are lipolytic enzymes that catalyze the cleavage of phospholipids. These enzymes are thought to contribute to the pathogenesis of Gram-negative bacteria by aiding in the lysis of host cells, via cleavage of phospholipids present in the host cell membrane, and by degrading phospholipids present at mucosal barriers to facilitate bacterial invasion. Disruption of one of the two phospholipase D genes present in *A. baumannii* genome resulted in reduced survival in serum and a reduced capacity for invading epithelial cells (Jacobs *et al.*, 2010). In addition, in a murine model of pneumonia, mice infected with the phospholipase D-deficient strain had lower bacterial burdens in the heart and liver at 48 h postinfection than mice infected with the parental strain, suggesting that phospholipase D contributes to the ability of *A. baumannii* to disseminate from the lungs. The *A. baumannii* genomes sequenced to date also demonstrate the presence of two putative phospholipase C genes, and culture supernatants from different *A. baumannii* strains contain phospholipase C activity, indicating that these genes are expressed and the resulting proteins are secreted from the bacterial cell (Antunes *et al.*, 2011b). In a separate study, inactivation of one of the phospholipase C genes resulted in a modest decrease in the ability of the mutant strain to induce cell death in an epithelial cell line compared to the parental strain (Camarena *et al.*, 2010).

Penicillin-binding proteins (PBPs) are most commonly associated with binding to and inactivating β-lactam antibiotics. However, PBPs also participate in the final steps of the biosynthesis of the peptidoglycan layer and thus contribute to bacterial cell stability (Sauvage *et al.*, 2008). An *A. baumannii* mutant with a transposon insertion in the *pbpG* gene, which encodes the putative low-molecular-weight penicillin-binding protein PBP7/8, demonstrated reduced growth on ascites plates (Russo *et al.*, 2009). While this mutant grew similarly to its wild-type counterpart in laboratory media, it showed reduced growth in human serum and decreased survival, both in a

Table 3. Identified *Acinetobacter baumannii* virulence factors

Virulence factor (gene)	Proposed role in pathogenesis	Reference(s)
OmpA (*ompA*)	Induction of apoptosis in host cells, adherence and invasion of epithelial cells, biofilm formation, surface motility, serum resistance	Choi *et al.* (2005, 2008), Kim *et al.* (2009), Gaddy *et al.* (2009)
Lipopolysaccharide (*lpsB*)	Evasion of the host immune response, triggering the host inflammatory response	Luke *et al.* (2010)
Capsular polysaccharide (*ptk* and *epsA*)	Evasion of the host immune response, growth in serum	Russo *et al.* (2011)
Phospholipase D (A1S_2989)	Serum resistance, bacterial dissemination, *in vivo* bacterial survival	Jacobs *et al.* (2010)
Penicillin-binding protein 7/8 (*pbpG*)	Peptidoglycan biosynthesis, cellular stability, growth in serum	Russo *et al.* (2009)
Outer membrane vesicles	Delivery of virulence factors to the cytoplasm of host cells, transfer of genetic material between bacterial cells	Jin *et al.* (2011), Rumbo *et al.* (2011)
Acinetobactin-mediated iron acquisition system	Provides iron needed to persist in the host, causes cell apoptosis	Gaddy *et al.* (2012)

rat model of soft tissue infection and a mouse model of pneumonia. Thus, the mutant had reduced fitness in conditions that mimic the environment encountered by the bacterium during the infectious process. Electron microscopy of the mutant and wild-type strains showed a difference in bacterial morphology, supporting the idea that a lack of PBP7/8 may affect cell stability, possibly via effects on the peptidoglycan layer (Russo *et al.*, 2011).

Outer membrane vesicles (OMVs) are vesicles secreted from the outer membrane of various Gram-negative bacteria and consist of outer membrane and periplasmic proteins, phospholipids, and LPS. OMVs have been reported to participate in bacterial virulence by delivering virulence factors to the interior of host cells, to facilitate horizontal gene transfer, and to protect the bacteria from the host immune response (Ellis & Kuehn, 2010). Multiple strains of *A. baumannii* secrete OMVs during growth *in vitro* (Kwon *et al.*, 2009; Jin *et al.*, 2011; McConnell *et al.*, 2011a; Rumbo *et al.*, 2011) . A proteomic analysis of purified *A. baumannii* OMVs identified the OmpA protein as well as putative proteases and a putative hemolysin as potential virulence factors. Moreover, OMVs could deliver OmpA to the interior of eukaryotic cells and induce cell death, exposure of cells to OMVs isolated from an *ompA*-deficient strain did not produce cell death (Jin *et al.*, 2011). Interestingly, OMVs were also able to facilitate the horizontal transfer of the OXA-24 carbapenemase gene, indicating that OMVs may play a role in the spread of antimicrobial resistance in *A. baumannii* (Rumbo *et al.*, 2011).

Animal models of *A. baumannii* infection

As with most bacterial diseases, experimentation in animals is necessary for characterizing both the invading pathogen and the host response. Animal models provide a means for evaluating novel treatments, characterizing the host immune response, and identifying bacterial virulence factors. In the first report describing an animal model for *A. baumannii* infection, a mouse model of acute pneumonia was described (Joly-Guillou *et al.*, 1997). Since this report, the number of animal models used for characterizing *A. baumannii* infection has multiplied, allowing the study of various types of infectious pathologies in a wide range of animal species including rodent, nonrodent, and even nonmammalian models of infection. These studies have begun to yield information about *A. baumannii* biology and its interaction with the host.

A major challenge in the use of animal models to characterize infectious pathologies is to accurately mimic human disease. Although it is unrealistic to expect that infection of laboratory animals, which in many cases are inbred strains, can completely reproduce the human disease process, efforts should be made to simulate these conditions as closely as possible. An important aspect to consider when employing an animal model of *A. baumannii* infection is the selection of the animal strain. In the case of mouse models of *A. baumannii* infection, a number of inbred strains of mice have been employed including C57BL/6, Balb/c, and A/J strains. These strains, although they are likely to give a more homogeneous response to infection, may not accurately reflect the variable response that likely occurs in humans. It is worth noting that a number of outbred strains of mice, such as CD-1 and Swiss Webster mice, have been successfully used in models of *A. baumannii* infection (Joly-Guillou *et al.*, 1997; Braunstein *et al.*, 2004; Crandon *et al.*, 2009; Koomanachai *et al.*, 2009; Song *et al.*, 2009). The animal strain employed can also dramatically affect the outcome of infection. For instance, individual

mouse strains have different susceptibilities to infection with *A. baumannii*, which can result in significant differences in survival, tissue bacterial load, clinical signs, inflammatory cell infiltration, and cytokine response (Joly-Guillou *et al.*, 1997; Qiu *et al.*, 2009a). For these reasons, the effect of the animal strain employed should be carefully considered.

A second important aspect that should be considered is the *A. baumannii* strain that will be employed in an animal model. Although well-defined, sequenced strains of *A. baumannii* are available from the American Type Culture Collection (e.g. ATCC 19606^T and ATCC 17978; see Table 2), their clinical background and epidemiology are unknown. For animal studies, it may be more appropriate to use more recently isolated strains that have known clinical relevance, for example strains belonging to the European clones I–III. The majority of studies performed using animal models have employed uncharacterized clinical isolates for which there are often few epidemiological and microbiological data, making the generalization of results difficult. This point is illustrated by the fact that in mouse models of *A. baumannii* infection, the virulence of individual strains can vary widely. In these models, the use of different strains resulted in significant differences in mortality, tissue bacterial load, histological score, and inflammatory cytokine levels (Eveillard *et al.*, 2010; McConnell *et al.*, 2011b). These findings underscore the importance of strain selection, which would ideally include strains with proven potential to cause infections and spread epidemically. In addition, once a tentative virulence trait has been identified in a reference strain, ideally studies using large collections of well-defined strains should be performed to assess the prevalence of this trait in clinically relevant isolates.

Pneumonia models of infection

Studies employing animal models of *A. baumannii* pneumonia have overwhelmingly employed mice, presumably because of their low cost and ease of handling. The mouse pneumonia model has been used to measure a number of variables related to infection such as mortality, tissue bacterial load, cytokine levels in both bronchoalveolar lavage fluid and serum, inflammatory cell infiltration into the lung, and histological score. In the majority of models developed to date, pneumonia is produced either through direct instillation of a bacterial suspension (typically containing between 10^7 and 10^9 CFU) into the trachea or by intranasal instillation of the inoculum. For intratracheal instillation, a technique developed for inducing pneumonia with *Haemophilus influenzae* has been employed (Esposito & Pennington, 1984). Animals are suspended vertically, and the bacterial suspension is instilled with a microliter syringe using a blunt-tipped needle (Joly-Guillou *et al.*, 1997; Rodríguez-Hernández *et al.*, 2000). Correct positioning of the needle in the trachea is confirmed by localizing the tracheal cartilage with the tip of the needle. After instillation of the bacterial suspension, animals are maintained in a vertical position to facilitate inoculation of the lower airways. For intranasal inoculation, a bacterial suspension is applied to the nares of anesthetized mice and inhaled (Knapp *et al.*, 2006; van Faassen *et al.*, 2007; Crandon *et al.*, 2009; Qiu *et al.*, 2009a, b; Jacobs *et al.*, 2010). While most studies employ intratracheal or intranasal infection, an oral inoculation technique for *A. baumannii* has recently been described in which a bacterial suspension was instilled in the oral cavity and the nares were completely blocked to facilitate inhalation of the inoculum (Koomanachai *et al.*, 2009).

There is no evidence supporting the idea that one inoculation technique (intratracheal vs. intranasal) more accurately reproduces human disease than the other. It could be argued that the intranasal technique may more closely mimic the clinical situation in which a patient's upper airway is colonized before producing pneumonia, and however, there is no literature support for this presumption. In animal models of pneumococcal pneumonia, the intranasal route produces a bronchopneumonia, whereas the intratracheal route results in a lobar pneumonia (Azoulay-Dupuis *et al.*, 1991; Canvin *et al.*, 1995). However, these results have not been confirmed in the case of *A. baumannii* models. In mice, both routes of infection produce an acute pneumonia that is accompanied by an increase in inflammatory cytokine levels in the lungs and histological changes consistent with pneumonia (Joly-Guillou *et al.*, 1997; Knapp *et al.*, 2006; Qiu *et al.*, 2009a; Eveillard *et al.*, 2010). Both intratracheal and intranasal infection have been reported to produce mortality in mice. The bacterial and mouse strains used, as well as whether or not the animals were rendered neutropenic prior to infection, can influence the level of mortality (Joly-Guillou *et al.*, 1997; Braunstein *et al.*, 2004; van Faassen *et al.*, 2007; Crandon *et al.*, 2009; Qiu *et al.*, 2009a; Eveillard *et al.*, 2010).

Because of the low virulence of most *A. baumannii* strains in mice, and for this reason the high infection doses that must be used to establish infection, a number of studies have employed neutropenic mouse models of pneumonia to facilitate infection (Joly-Guillou *et al.*, 1997, 2000; Wolff *et al.*, 1999; Braunstein *et al.*, 2004; Crandon *et al.*, 2009; Koomanachai *et al.*, 2009; Song *et al.*, 2009; Eveillard *et al.*, 2010; Yuan *et al.*, 2010). To induce transient neutropenia, mice are treated with cyclophosphamide, usually in two doses starting 4 days before

infection. The first description of the neutropenic model in C3H/H3N mice reported weight loss in the infected animals, bacterial growth in the lungs, and 85% mortality between days 2 and 3 (Joly-Guillou *et al.*, 1997). Studies employing the neutropenic mouse model have almost exclusively had the objective of characterizing antibiotic efficacy. However, a recent study used this model to compare the virulence of different clinical isolates of *A. baumannii* (Eveillard *et al.*, 2010). The authors report that mortality and tissue bacteria load varied depending on the infecting strain. A caveat is that the neutropenic mouse model may not accurately reproduce some features of infection in non-neutropenic patients, as neutrophils play an important role in the early stages of *A. baumannii* infection in mice (van Faassen *et al.*, 2007).

A commonly used alternative to rendering mice transiently neutropenic is the use of porcine mucin in the inoculum. In these models, the cultured bacteria are mixed with porcine mucin, typically to a final concentration of 5% mucin, before instillation of the inoculum into the lungs (Rodríguez-Hernández *et al.*, 2000, 2001; Montero *et al.*, 2002, 2004; Pachón-Ibañez *et al.*, 2006, 2010; Beceiro *et al.*, 2009; Chiang *et al.*, 2009; Pichardo *et al.*, 2010). Mucin has long been known to increase the virulence of numerous bacterial species in mouse models (Olitzki, 1948). Its use thus allows for the number of bacteria in the inoculum to be reduced dramatically, in some cases by as much as 1000-fold for some bacterial species (Batson *et al.*, 1950). Although the presence of mucin during the establishment of infection has no role in human infection, the use of lower amounts bacteria in the inoculum followed by bacterial growth and the development of pneumonia may more accurately mimic human disease than the rapid instillation of large quantities of bacteria into the lung. Porcine mucin has been used exclusively with the intratracheal route of infection, likely due to the difficulty of administering a viscous solution via the intranasal route.

In a mouse pneumonia model that did not require the induction of neutropenia or the use of porcine mucin, A/J mice were more susceptible to intranasal infection with the ATCC 17961 strain of *A. baumannii* than were C57BL/6 mice (Qiu *et al.*, 2009a, b). An inoculum of 1.5×10^8 bacteria in saline produced 100% mortality by day 3 postinfection. Infection of the A/J strain resulted in reduced early neutrophil infiltration into the lung, lower levels of pro-inflammatory cytokines (IL-1β, MIP-2, and TNF-α), increased bacterial replication, and increased extrapulmonary dissemination compared to C57BL/6 mice (Qiu *et al.*, 2009a).

The mouse pneumonia model has recently been employed for characterizing host factors involved in respiratory infections. The availability of transgenic mice with well-defined genetic changes has facilitated the characterization of these factors to the molecular level. Mice deficient in CD14 and Toll-like receptors 2 and 4 have been used to characterize the role of these molecules in respiratory infections (Knapp *et al.*, 2006). This study concluded that CD14 and Toll-like receptor 4 play a role in clearing *A. baumannii* from the lung through detection of LPS, while Toll-like receptor 2 appears to down-regulate the immune response to *A. baumannii* pulmonary infection. Another study characterizing the role of NADPH phagocyte oxidase in pulmonary infection showed that mice lacking the gene encoding this enzyme had higher postinfection bacterial loads and increased mortality after infection, demonstrating the importance of the neutrophil response in controlling *A. baumannii* infection in mice (Qiu *et al.*, 2009b).

Although the overwhelming majority of animal models of *A. baumannii* pneumonia have employed mice, models using other species have been developed to study the biology of this pathogen during pulmonary infection. A rat pneumonia model previously used to characterize *Escherichia coli* respiratory infection has been adapted for the study of *A. baumannii* (Russo *et al.*, 2008). In this model, Long Evans rats are anesthetized before surgical exposure of the trachea and instillation of the inoculum into the trachea via a needle. Infected rats demonstrated many characteristics that mimic human infection including bacterial growth in the lung, decreased arterial oxygenation (as assessed by PaO_2/FiO_2), increased cytokine levels (TNF-α, IL-1β, and CINC-1) in bronchoalveolar lavage fluid, neutrophil infiltration and mortality. One advantage of the rat model is that, because of their larger size, rats can be infected via direct instillation of the inoculum into the trachea, which may increase the reproducibility of infection by ensuring that the entire inoculum is instilled into the airways. It should also be noted that the rat model described by Russo *et al.* (2008) did not require the use of porcine mucin or the use of animals that had been rendered transiently neutropenic prior to infection. A guinea-pig model of pneumonia was developed to characterize the efficacy of antibiotic combinations in the treatment of MDR *A. baumannii* (Bernabeu-Wittel *et al.*, 2005). This model uses an inoculation technique similar to that described above for inducing pneumonia in rats in that the inoculum is instilled directly into the surgically exposed trachea via a needle. However, in contrast to the rat model, the inoculum contained 5% porcine mucin to increase the virulence of the infecting strain. Histopathological studies of infected lungs demonstrated that infection produced acute inflammation and resulted in neutrophil infiltration into the peribronchial and perivascular spaces. Lung bacterial loads 24 h postinfection in untreated animals receiving an inoculum of approxi-

mately 3.2×10^9 were between 7.9×10^6 and 2.0×10^7 CFU g^{-1} lung tissue, and mortality at this time point was between 83% and 92%, depending on the *A. baumannii* strain used (Bernabeu-Wittel *et al.*, 2005).

Skin and soft tissue infection models

Both mouse (Shankar *et al.*, 2007; Dai *et al.*, 2009; DeLeon *et al.*, 2009; Thomas-Virnig *et al.*, 2009) and rat (Uygur *et al.*, 2009) models of *A. baumannii* burn infection have been developed. In these models, a nonlethal, full-thickness burn is produced on the dorsal surface of the anesthetized animal after shaved skin is either submerged in a heated water bath or exposed to preheated metal blocks. Infection is subsequently initiated either through topical application or subcutaneous injection of a solution containing *A. baumannii* at the burn site. The objective of the studies employing the burn model has exclusively been to evaluate the efficacy of different experimental treatments. For this reason, the most common parameter measured in these studies is the bacterial concentration present at the burn site. In addition to measuring bacterial concentrations at the burn site, some studies have also measured bacterial loads at different anatomical sites (underlying muscle, lung, and blood) and survival (Shankar *et al.*, 2007; Dai *et al.*, 2009; Uygur *et al.*, 2009). Because of the fact that these studies aimed to evaluate treatment efficacy, there are few data available regarding the host response to burn infection by *A. baumannii*.

Animal models for studying soft tissue infection by *A. baumannii* have predominantly employed two distinct techniques for initiating infection. The first technique involves injection of a solution containing *A. baumannii* directly into the thigh muscle. This approach has been used in mice to measure tissue bacteria loads in the muscle after treatment (Dijkshoorn *et al.*, 2004), and in a neutropenic rat model in which survival and bacterial loads in distant organs were measured (Pantopoulou *et al.*, 2007). In the neutropenic rat model, bacteria disseminated to distant organs (lung, liver, and spleen) and infection produced 100% mortality at 2 days in untreated control mice, suggesting that this model results in sepsis secondary to infection of the thigh muscle. A variation on this model is the implantation of filter paper impregnated with a suspension of *A. baumannii* in muscle via a surgical incision (Fetiye *et al.*, 2004). These approaches have been used to evaluate the efficacy of treatments on *A. baumannii* infection, and no data regarding the host response or the bacterial factors involved in the pathogenesis of the infection were reported.

The second approach used for producing soft tissue infection utilizes the formation of a subcutaneous pouch to mimic the formation of an abscess (Russo *et al.*, 2008). In this model, a subcutaneous space is produced on the dorsal surface of an anesthetized rat by injection of air. The space is then filled with 1 mL of an oil mixture, and the rats are maintained for 7 days to allow for the accumulation of exudative fluid which is then inoculated with the desired quantity of bacteria (5×10^6 CFU). It has been reported previously that this model results in neutrophil infiltration, indicating that the response to infection is similar to that seen during natural infection (Dalhoff *et al.*, 1982, 1983). An advantage of this model is that the pouch fluid can be repeatedly sampled at different time points, allowing for continuous measuring of bacterial concentrations and the host response. While both inoculation techniques appear to share some characteristics with human infection, it is not clear how well these models mimic soft tissue infection produced by traumatic injury. Further study characterizing the host response to infection in these models will help to provide insight into this issue.

Animal models of sepsis

Compared to pneumonia and burn and soft tissue infection models, relatively few models of *A. baumannii* sepsis have been developed. In the first model of sepsis, a bacterial suspension is administered intraperitoneally in Balb/c mice (Ko *et al.*, 2004). This approach produced mortality between 87.5% and 100% with an inoculum of approximately 2×10^7 CFU of a clinical isolate. More recently, a similar model has been employed to characterize the efficacy of vaccines for preventing infection by *A. baumannii* (McConnell & Pachón, 2010; McConnell *et al.*, 2011b) and to evaluate the effect of antibiotic resistance on virulence and fitness (López-Rojas *et al.*, 2011) and the role of the acinetobactin-mediated iron uptake system in virulence (Gaddy *et al.*, 2012). In this model, the bacterial inoculum is first combined with porcine mucin to a final concentration of 5% before intraperitoneal instillation to increase the virulence of the infecting strain, allowing for the use of inocula containing fewer bacteria. In this model, different *A. baumannii* strains had widely varying LD_{50} values (up to an 80-fold difference in the strains tested), suggesting that this model can be used to characterize the virulence of different isolates. After intraperitoneal instillation of the inoculum, bacteria rapidly disseminated to distant organs (spleen, kidney, and lung), with bacterial loads in these tissues reaching approximately 1×10^5 CFU g^{-1} of tissue at 1 h postinfection. Serum levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were elevated after infection, consistent with the cytokine storm produced during the development of septic shock. Bacteria continued to reproduce in

distant organs until death occurred between 18 and 48 h, depending on the strain used for infection and the number of bacteria in the inoculum. Importantly, 100% mortality was obtained by 24 h after infection with 5×10^4 CFU of the ATCC 19606^T strain (McConnell *et al.*, 2011b). Because of the high mortality that can be reproducibly achieved with relatively small inocula (compared to mouse pneumonia models), this model is also convenient for the use of vaccine efficacy studies. In a rat model of *A. baumannii* sepsis, the efficacy of various antibiotic regimens has been determined (Cirioni *et al.*, 2009). Similar to the mouse model of sepsis, the inoculum is injected intraperitoneally, however without the addition of porcine mucin. In this study, bacterial loads in the blood, peritoneum, spleen, liver, and mesenteric lymph nodes were measured, in addition to plasma endotoxin and cytokine levels. Infection resulted in 100% mortality 48 h after infection, although it should be noted that a high inoculum was used (2×10^{10} CFU), which may not be physiologic.

The advantages of the animal models of sepsis developed for *A. baumannii* are a facile inoculation procedure, high mortality, and the possibility of measuring tissue bacterial loads in multiple organs. In addition, these models share some important aspects with human sepsis including high serum levels of pro-inflammatory cytokines, bacterial dissemination, and high mortality. However, an obvious difference between these sepsis models and the human disease process is the source of infection. Intraperitoneal instillation of the inoculum produces sepsis that is secondary to intraperitoneal infection, which does not represent a natural route of infection in *A. baumannii* disease. This approach rapidly produces fulminant sepsis in mice and rats, whereas in human disease, the development of *A. baumannii* sepsis is typically more progressive. This aspect may complicate studies aiming to characterize the host response during the early phases of *A. baumannii* sepsis. It is worth noting that a number of the models described above for the study of pneumonia, burn, and soft tissue infections have been shown to result in disseminated infection (Rodríguez-Hernández *et al.*, 2001; Montero *et al.*, 2004; Pantopoulou *et al.*, 2007; Shankar *et al.*, 2007; Dai *et al.*, 2009; Uygur *et al.*, 2009). These models may therefore better mimic clinical scenarios in which sepsis results from dissemination of the infection from a discrete infectious focus.

Other mammalian models of *A. baumannii* infection

Pachón-Ibañez *et al.* (2010) have recently reported the development of a rabbit model of *A. baumannii* meningitis. In this model, anesthetized rabbits are immobilized in a stereotactic frame, and the inoculum is instilled into the intracisternal space. The authors report that features of meningitis in the cerebrospinal fluid developed by 12 h postinoculation. Bacterial loads, lactate dehydrogenase concentrations, and white blood cell counts were quantified in cerebrospinal fluid after obtaining the sample by cisternal puncture. In addition, brain edema was quantified by determining brain weight after desiccation. A major advantage of this model is that the large size of rabbits allows for repeated sampling of the cerebrospinal fluid for monitoring the evolution of meningitis in a single animal.

A rabbit endocarditis model has been described for *A. baumannii* that was developed based on a model initially used for characterizing staphylococcal endocarditis (Perlman & Freedman, 1971; Rodríguez-Hernández *et al.*, 2001, 2004). In this model, an intracardiac catheter is inserted into the left ventricle of New Zealand rabbits 4 days prior to inoculation. For the establishment of infection, mice are inoculated via injection of a suspension containing *A. baumannii* into an ear vein. The authors report that 100% of control rabbits were bacteremic 24 h postinoculation and that *A. baumannii* was isolated from the valves of all infected animals. Valve bacterial loads and valve sterility rates, in addition to quantitative blood cultures, were used to evaluate the efficacy of antibiotic regimens.

There have been two reports describing the development of animal models of *A. baumannii* osteomyelitis. In a mouse model, osteomyelitis can be produced by surgically exposing the tibia and inserting a 0.25-mm stainless steel pin that has been coated with a suspension of *A. baumannii* into the bone metaphysis (Crane *et al.*, 2009). This approach produces a chronic, but nonlethal osteomyelitis. The authors used histology to show that *A. baumannii* resulted in osteoblastic bone formation at the site of infection, in contrast to *Staphylococcus aureus* that produces an osteolytic response. An attempt to produce osteomyelitis in rats has been made by inoculating induced segmental bone defects with *A. baumannii* (Collinet-Adler *et al.*, 2010). The authors report that in this model, the bacteria were cleared without producing a clinically relevant osteomyelitis, although bony lysis and new bone formation were detected in some animals.

Nonmammalian models of *A. baumannii* infection

Mammalian models of *A. baumannii* infection allow characterization of a host response to infection similar to that found in human infections. In addition, because

drug pharmacokinetics and pharmacodynamics can be determined in mammalian species using techniques similar to those used in humans, these models also permit the study of antibiotic regimens for the treatment of *A. baumannii* infection. For these reasons, models based on mammalian species clearly have advantages for study designs that aim to mimic the human disease process. However, recently developed nonmammalian models of infection offer the advantage of lower cost for both purchasing and maintaining the animals, and avoidance of ethical issues that are associated with the use of mammalian species (Table 4). In addition, nonmammalian models, because of the ease of infecting large groups, may be more suitable for high-throughput screening of bacterial mutants.

Galleria mellonella is the larval stage of the greater wax moth, and it has recently been developed for use as model for infection by *A. baumannii*. (Peleg *et al.*, 2009). In this model, caterpillars are inoculated with a suspension containing *A. baumannii* via injection into the caterpillar hemocoel. Postinfection mortality was shown to depend upon the number of bacteria present in the inoculum as well as the active expression of the acinetobactin-mediated iron acquisition system (Gaddy *et al.*, 2012). In addition, infection of the caterpillars with *A. baylyi* and *Acinetobacter lwoffii*, which are thought to be less pathogenic in humans, produced less mortality than infection with an inoculum containing an equal amount of *A. baumannii*. Together, these data may suggest that this model can be used to characterize the virulence of different strains. Treatment of infected caterpillars with antibiotics that had activity against the *A. baumannii* strain *in vitro* increased survival, indicating that this model may also be useful for evaluating antibiotic efficacy.

Caenorhabditis elegans is an unsegmented nematode that feeds on bacteria. Two reports have used *C. elegans* to characterize *A. baumannii*. Smith *et al.* (2007) used an assay in which worms are placed onto lawns of *A. baumannii*, and the proliferation and brood sizes of the worms are measured. With this approach, these authors identified 114 *A. baumannii* mutants that supported growth of the worms on ethanol-containing media, which has been reported to increase bacterial virulence (Smith *et al.*, 2007). A subsequent study demonstrated that *A. baumannii* reduced the ability of *C. albicans* to kill *C. elegans* during co-infection, demonstrating that this model can be used to study eukaryote–prokaryote interactions (Peleg *et al.*, 2008b).

Dictyostelium discoideum is an ameba that is found in soil environments and has recently been used to measure the virulence of *A. baumannii* mutants (Smith *et al.*, 2007). In this model, *D. discoideum* is co-cultured with *A. baumannii* and, because of the slower growth rate of the amebae, *A. baumannii* produces a lawn in which plaques are formed because of the consumption of the bacteria by *D. discoideum*. Bacteria with increased virulence, or bacteria grown under conditions that increase the expression of virulence traits, results in a reduction in the number of plaques formed by *D. discoideum*. Conversely if the virulence of a strain is reduced, for example by the introduction of a mutation, an increase in the number of plaques formed by the ameba is seen. This model was successfully used to identify *A. baumannii* transposon mutants with reduced virulence (Smith *et al.*, 2007).

Table 4. Overview of *Acinetobacter baumannii* infection models

Infection model	Species employed	Variables measured	Study objectives
Pneumonia	Mouse, rat, guinea pig	Drug pharmacokinetics and pharmacodynamics, tissue bacterial loads, survival, cytokine levels, histology	Optimization of antibiotic therapy, characterization of the host response, identification of bacterial virulence factors
Burn and soft tissue infection	Mouse, rat	Bacterial load, survival, abscess formation	Optimization of antibiotic therapy, identification of virulence factors
Sepsis	Mouse, rat	Survival, tissue bacterial load, serum cytokine levels	Compare bacterial fitness between strains, vaccine efficacy, optimization of antibiotic therapy, identification of virulence factors
Meningitis	Rabbit	Drug pharmacokinetics, bacterial load, inflammatory cell infiltration, brain edema	Optimization of antibiotic therapy
Endocarditis	Rabbit	Drug pharmacokinetics and pharmacodynamics, tissue bacterial loads, survival	Optimization of antibiotic therapy
Osteomyelitis	Mouse, rat	Bacterial load, bone morphology	Optimization of antibiotic therapy
Nonmammalian models	*Galleria mellonella, Caenorhabditis elegans, Dictyostelium discoideum*	Survival, melanization, egg count, plaque formation	Identification of virulence factors, interactions with eukaryotic cells

Concluding remarks

With the increasing clinical importance of *A. baumannii* because of a rise in the number of colonizations/infections caused by this organism and the emergence of MDR strains, continued study of this pathogen is required. The use of animal models has already yielded important data regarding the optimization of antibiotic regimens for the treatment of resistant strains and has begun to identify virulence factors that have contributed to the success of *A. baumannii* as a pathogen. With the sequencing of multiple *A. baumannii* genomes and the increasing availability of transgenic animals, it is hoped that these models can be used to continue to identify bacterial and host factors involved in infection at the molecular and cellular levels. These studies will provide insight into the basic biology of this important human pathogen and aid in the identification of targets for the development of novel treatment and prevention strategies.

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