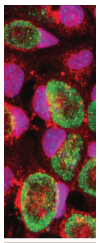

Pathogens and Disease ISSN 2049-632X

MINIREVIEW

Acinetobacter baumannii: evolution of a global pathogen

Luísa C.S. Antunes[1], Paolo Visca[1] & Kevin J. Towner[2]

1 Department of Sciences, Roma Tre University, Rome, Italy
2 Department of Clinical Microbiology, Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Nottingham, UK

This is a timely review of an important human pathogen. The review addresses the literature to date on this organism and provides insights, as well as potential hypotheses for the recent emergence of this human pathogen.

Keywords
comparative genomics; core genome; epidemic clone; multidrug resistance; phylogeny; virulence.

Correspondence: Present address:
Luísa C.S. Antunes, Institut Pasteur, Unité de Biologie Moléculaire du Gène chez les Extrêmophiles, Department of Microbiology, 28 rue du Dr Roux, 75015 Paris, France.
Tel.: +33145688830
fax: +33145688834
e-mail: luisa.antunes@pasteur.fr

Received 26 August 2013; revised 15 December 2013; accepted 15 December 2013. Final version published online 27 January 2014.

doi:10.1111/2049-632X.12125

Editor: David Rasko

Abstract

Acinetobacter baumannii is an opportunistic nosocomial pathogen and one of the six most important multidrug-resistant microorganisms in hospitals worldwide. This human pathogen is responsible for a vast array of infections, of which ventilator-associated pneumonia and bloodstream infections are the most common, and mortality rates can reach 35%. Community-acquired infections have also been reported, but few strains have been recovered from environmental sources and infection reservoirs external to the hospital have not been identified. The majority of *A. baumannii* infections are caused by two main population clones with worldwide distribution. Infection outbreaks are often associated with multidrug resistance, including the recent emergence of strains resistant to all available antibiotics. Nevertheless, *A. baumannii* virulence traits and pathogenic potential have mostly remained elusive. The recent expansion of *A. baumannii* sequenced genomes has permitted the development of large-array phylogenomic and phenotypic analyses, which can offer valuable insights into the evolution and adaptation of *A. baumannii* as a human pathogen. This review summarises these recent advances, with particular focus on *A. baumannii* evolutionary and genomic aspects, and proposes new avenues of research.

Introduction

Gram-negative coccobacilli classified as *Acinetobacter baumannii* are important Gram-negative opportunistic bacterial pathogens that are responsible for 2–10% of all Gram-negative hospital infections (Joly-Guillou, 2005). *Acinetobacter baumannii* is classified by the Infectious Diseases Society of America as one of the six most important multidrug-resistant (MDR) microorganisms in hospitals worldwide (Talbot *et al.*, 2006) The genus *Acinetobacter* has undergone many taxonomic changes over the years, and the species *A. baumannii* was not formally designated until 1986 (Bouvet & Grimont, 1986). This makes earlier accounts of *Acinetobacter* infections in the scientific and medical literature difficult to interpret. Nevertheless, it seems apparent that infections caused by organisms now classified as *A. baumannii* first became a significant problem during the 1970s (Bergogne-Bérézin & Towner, 1996). Since then, *A. baumannii* has gradually increased in importance as a pathogenic organism, mainly, but not exclusively, in the hospital environment. This increase in importance could, at least in part, be related to the fact that the patient mix in hospitals has changed since the early 1970s, with patients in hospitals now being much more vulnerable to *A. baumannii* infections because of their serious underlying conditions and advances in medicine that involve an increased use of highly selective antibiotics, indwelling lines and other invasive devices. Thus, while the aim of this mini-review is to summarise recent advances in knowledge concerning *A. baumannii*, with particular focus on the evolutionary and genomic aspects of *A. baumannii* pathogenicity, it should also be considered that the nature of the host (i.e. human patient) may also play a large part in the outcome of infections caused by *A. baumannii*.

Clinical importance

Acinetobacter baumannii is now recognised as causing a broad range of severe nosocomial infections, including skin and soft tissue infections, wound infections, urinary tract

Pathogens and Disease
FEMS

infections and secondary meningitis (Bergogne-Bérézin & Towner, 1996; Roca *et al.*, 2012; McConnell *et al.*, 2013). However, the most important infections, with the highest mortality rates, are ventilator-associated pneumonia and bloodstream infections (Dijkshoorn *et al.*, 2007). Infections are more common in patients suffering from an underlying disease or who have undergone major surgical procedures. *Acinetobacter baumannii* can easily enter the body through open wounds, intravascular catheters and mechanical ventilators. Infections caused by *A. baumannii* are associated particularly with extended periods of hospitalisation (Peleg *et al.*, 2008), the male gender and older age (Wisplinghoff *et al.*, 1999).

Less commonly, *A. baumannii* can also cause community-acquired infections, including pneumonia (accounting for 85% of reports of community infections caused by *A. baumannii*) and bacteraemia. Other possible community-acquired infections include skin, soft tissue and ocular infections, secondary meningitis and endocarditis (Chang *et al.*, 2000; Falagas *et al.*, 2007). These infections are more common in the male gender and are associated with old age, alcoholism, heavy smoking, diabetes mellitus, chronic obstructive pulmonary disease and renal disease. Community-acquired *A. baumannii* pneumonia is more serious than nosocomial pneumonia, is generally fulminant (death within 8 days of diagnosis) and rates of mortality can be as high as 60% (Falagas & Rafailidis, 2007). Most reports of community-acquired pneumonia have occurred during the summer months in tropical and subtropical climates (Falagas & Rafailidis, 2007).

A relatively recent phenomenon has been the association of *A. baumannii* with infections subsequent to injuries in conflict areas, for example Iraq and Afghanistan (Joly-Guillou, 2005), or following natural disasters such as the Marmara earthquake in Turkey (Centers for Disease Control & Prevention, 2004). There is some evidence that the use of morphine, which might be expected following battlefield or crush injuries, may potentiate *A. baumannii* infections, perhaps as a result of its immunosuppressive effect (Breslow *et al.*, 2011). However, such outbreaks of *A. baumannii* infection may also be a reflection of the extreme pressure that hospital emergency units are under in such situations, leading to breakdowns in infection control precautions and consequent epidemic spread of *A. baumannii* (see below), rather than any direct causal association.

There is some debate over the severity of *A. baumannii* infections, with attributable mortality rates varying, on average, from 8% to 35%, according to strain and type of infection (Falagas & Rafailidis, 2007). Accordingly, different *A. baumannii* strains could be endowed with different pathogenic potential, as suggested by two studies using the mouse model of pneumonia: an MDR strain isolated from the blood culture of a patient with nosocomial pneumonia showed 80% mortality to mice, in contrast to 13% mortality for a strain causing meningitis (Eveillard *et al.*, 2010), while a strain belonging to international clone 1 (see below) and a sporadic isolate were more virulent than a strain belonging to international clone 3 and the type strain (de Breij *et al.*, 2012). Differences in mortality may be attributable to the expression of specific virulence factors and determinants. For example, two strains with the same PFGE profile, where one was a mucoid isolate from cerebrospinal fluid and the other was a nonmucoid isolate from a ventriculo-peritoneal catheter, showed mortality rates of 48% and 19%, respectively, in a mouse model of pneumonia (Eveillard *et al.*, 2010).

Antibiotic resistance

The predominance of certain *A. baumannii* lineages has been related to the MDR phenotype of infecting strains (Diancourt *et al.*, 2010). However, it is currently not clear whether particular epidemic strains (see below) have acquired the MDR phenotype or whether the MDR phenotype is important for individual strains to *become* epidemic. *Acinetobacter baumannii* antimicrobial resistance has progressively increased since the 1970s, at which time the vast majority of strains were sensitive to commonly used antibiotics. By 2007, up to 70% of isolates in certain settings (depending on country, hospital, medical department and clinical sample) were MDR, including resistance to carbapenems, which were once considered to be the mainstay against MDR *A. baumannii* infections (Kempf & Rolain, 2012). Not surprisingly, carbapenem resistance has been observed in isolates from military as well as civilian facilities (Keen *et al.*, 2010; Mera *et al.*, 2010).

Currently, colistin seems to be the most reliably effective drug *in vitro* against MDR *A. baumannii*, but colistin use has been associated with a number of side effects and is not suitable for treating all types of infections (Falagas & Rafailidis, 2009). There are now also reports of colistin resistance worldwide (Cai *et al.*, 2012), resulting in the emergence of strains resistant to all known antibiotics in certain geographical areas (Al-Sweih *et al.*, 2011). Worryingly, resistance appears to be spreading to the community, with reports of carbapenem-resistant *Acinetobacter* spp. in cattle and other animals (Poirel *et al.*, 2012; Seiffert *et al.*, 2013) and in the Seine River in Paris, France (Girlich *et al.*, 2010).

Overall, *A. baumannii* has acquired resistance to a vast array of antimicrobials in recent decades. This capacity is partly dependent on the ability of this bacterium to acquire resistance genes, often by horizontal gene transfer (Adams *et al.*, 2008). Recent studies strongly suggest that acquisition of the MDR phenotype is a determinant factor for the success of *A. baumannii* as a nosocomial pathogen (Imperi *et al.*, 2011).

Individual antimicrobial resistance mechanisms in *A. baumannii* have been described in previous reviews (Peleg *et al.*, 2008; Kempf & Rolain, 2012; Poirel *et al.*, 2011; Cai *et al.*, 2012; Evans *et al.*, 2013).

Epidemiology

Natural habitats

Contrary to other species of the *Acinetobacter* genus, which are frequently isolated from the soil, water and animals

(Towner, 2009), *A. baumannii* is found almost exclusively in the hospital environment, particularly in intensive care units (ICUs). Evidence is accumulating that a similar behaviour can be observed in veterinary medicine with infection and colonisation of seriously ill animals in veterinary clinics and ICUs (Zordan *et al.*, 2011). Although there are reports of occasional isolates of *A. baumannii* from nonhuman sources, such as animals, lice, vegetables, aquaculture and soils (Eveillard *et al.*, 2013), it remains to be assessed if these isolates, which are infrequent, are the result of contamination of the environment from the primary hospital reservoir or are indicative of an alternative natural reservoir of this species.

Population structure

The clinical relevance of *A. baumannii* has dramatically increased since the late 1980s, with the emergence and spread of three predominant clones ('international clonal lineages', ICLs) capable of causing hospital outbreaks worldwide, of which ICL1 and ICL2 are MDR. However, more than 400 MLST sequence types (STs) are currently listed in the *A. baumannii* MLST database (http://pubmlst.org/abaumannii/), and a recent analysis has evidenced the existence of at least six major ICLs distributed through continents worldwide (Karah *et al.*, 2012). These six ICLs include the three major outbreak clones identified originally and demonstrate the worrisome emergence of new epidemic clones. It has been suggested that this emerging population structure is the result of an initial low phylogenetic diversity of *A. baumannii* and subsequent rapid spread following a severe evolutionary bottleneck (Diancourt *et al.*, 2010).

Genome plasticity and evolution of virulence

Since the sequencing of the first *A. baumannii* genome in 2007 (Smith *et al.*, 2007), the field of *Acinetobacter* genomics has seen a rapid increase in the number of sequenced and annotated genomes. As at September 2013, 15 complete and 180 draft chromosomal *A. baumannii* genomes, 31 plasmid and six bacteriophage sequences are available on the NCBI database (http://www.ncbi.nlm.nih.gov), as well as the complete genome sequence of *Acinetobacter baylyi*, 57 draft sequences from other *Acinetobacter* species, four plasmid and five bacteriophage sequences.

This high number of available sequences has enabled comparative genomic analyses focused on resolving *A. baumannii* population structure and phylogenetic relationships and on elucidating clues concerning potential virulence factors and the origin of antimicrobial resistance determinants.

The *A. baumannii* core genome and pan-genome

Comparative genomic approaches have identified a core set of species-specific (Adams *et al.*, 2008; Peleg *et al.*, 2008; Imperi *et al.*, 2011; Farrugia *et al.*, 2013) and genus-specific genes (Vallenet *et al.*, 2008; Chan *et al.*, 2012; Peleg *et al.*, 2012; Zhan *et al.*, 2012; Fondi *et al.*, 2013). The species core genome varies between 1455 and 2688 orthologous coding sequences (CDSs) depending on the number and identity of strains analysed (Adams *et al.*, 2008; Imperi *et al.*, 2011; Peleg *et al.*, 2012; Farrugia *et al.*, 2013). Theoretical predictions of the core genome size of the current clinical strain population, obtained from development plots with an infinite number of genomes, point to a core genome of *c.* 2200 CDSs, corresponding to *c.* 60% of the average strain genome content (Imperi *et al.*, 2011). The functions coded by these genes are mostly related to metabolic and general cellular processes (65%), as well as hypothetical proteins (22%).

In contrast, the *A. baumannii* whole-genome repertoire (the 'pan-genome') is impressively large (over 8800 CDSs for 12 strains) and increases exponentially as new genomes become available (an open pan-genome), which highlights the importance of gene acquisition and loss events in the evolution and adaptation of this human pathogen. The greatest contribution to the species pan-genome is provided by genes that are not shared by all strains (the accessory genome), of which 25–46% of genes are unique to each strain (Adams *et al.*, 2008; Imperi *et al.*, 2011). Compared to the core genome, the accessory genome is enriched in transport and transcription regulation functions (Adams *et al.*, 2008; Imperi *et al.*, 2011). Strain-specific genes mainly encode hypothetical proteins (39%), transposases (36%) and insertion sequences (16%; Imperi *et al.*, 2011).

Acquisition of virulence determinants

Gene functions putatively associated with virulence are mostly found in the core genome of clinical strains, regardless of their grouping as clonal or sporadic isolates or their isolation date. The few exceptions are the genes belonging to the O-antigen biosynthetic cluster, which show very low conservation among genomes (Adams *et al.*, 2008; Kenyon & Hall, 2013). Notably, no single toxin, hydrolytic enzyme or adhesin was detected in the core genomes of ICL1 or ICL2 strains that could account for their clinical prevalence (Imperi *et al.*, 2011). In contrast, CDSs with functions related to adhesion and mobility, iron metabolism, quorum-sensing and two-component systems correspond to up to 8% of the core *A. baumannii* genome (Imperi *et al.*, 2011). This suggests that the acquisition of new virulence traits was likely not a predominant factor in the recent (post-1980s) nosocomial expansion of *A. baumannii* clones.

Thus, other explanations should be considered for the evolution of *A. baumannii* towards pathogenicity. One possibility is that the renowned ability of *A. baumannii* to adapt to the nosocomial environment and to resist adverse environmental challenges is the main factor for pathogenicity in this species. A second possibility is that the conserved set of *A. baumannii* virulence genes is differentially regulated according to strain. In fact, the *A. baumannii* genome contains 59 transcriptional regulators, which is twice the number found in *A. baylyi* ADP1 (Adams *et al.*, 2008); however, no enrichment happened in the core genomes of ICL1 or ICL2 (Imperi *et al.*, 2011). Lastly, polymorphic differences in shared virulence genes (Peleg *et al.*, 2012) or

the combinatory effect of multiple genes (Imperi *et al.*, 2011) may contribute to differences in virulence potential.

Acquisition of resistance determinants

Genes associated with resistance to antimicrobial drugs were found in both the species core and accessory genomes (Imperi *et al.*, 2011). In the accessory genome, antimicrobial resistance genes were found in alien islands and were often flanked by integrases, transposases or insertion sequences (Imperi *et al.*, 2011), suggesting their possible acquisition by horizontal gene transfer from other *Acinetobacter* strains or bacteria that colonise the same environment.

The genome of strain AYE, belonging to ICL1, encodes 52 genes putatively associated with resistance to antimicrobial drugs, of which 45 are encoded by an 86-kb resistance island (AbaR1). This genomic island is also present in other *A. baumannii* strains, but with a much reduced size. The presence of an extraordinary 22 ORFs coding for transposases and insertion sequences is probably responsible for the acquisition of resistance genes in the AYE AbaR1 island, the majority of which are orthologous to CDSs found in *Pseudomonas* spp. (44%), *Salmonella* spp. (34%) and *Escherichia* spp. (17%; Vallenet *et al.*, 2008).

The ICL2 strain ACICU also contains a homolog to AbaR1, but it is much smaller (seven antibiotic resistance coding genes). In this strain, drug resistance is more evenly distributed throughout the genome. For instance, CDSs for drug resistance functions are present in 14 of the 36 *A. baumannii* ACICU alien islands, but correspond on average to $< 5\%$ of the total CDSs of each island (Iacono *et al.*, 2008). The majority of these CDSs have orthologs in *Pseudomonas* spp. (22%) and *Burkholderia* spp. (18%).

Phylogenetic relationships

A phylogenetic analysis of 154 *Acinetobacter* strains belonging to clinically important species other than *A. baumannii*, based on the concatenation of six housekeeping genes (Diancourt *et al.*, 2010), has provided evidence for the monophyletic origin of *A. baumannii*, as well as for the monophyletic origin of the main *Acinetobacter* species of clinical importance (*A. baumannii*, *A. pittii*, *A. nosocomialis*). Together with the less clinically relevant *A. calcoaceticus*, these species comprise the *A. calcoaceticus–baumannii* (Acb) complex.

The monophyletic status of ICLs 1 and 2, the *A. baumannii* species, the Acb complex and the *Acinetobacter* genus have also been confirmed by phylogenomic analysis of 38 *Acinetobacter* species based on 127 core genes of the genus (Chan *et al.*, 2012) and the phylogeny of 136 *Acinetobacter* genomes based on single-nucleotide polymorphism (SNP) analysis (Sahl *et al.*, 2013).

Acinetobacter core genome and pan-genome

The core genome of the genus *Acinetobacter* is much smaller than that of *A. baumannii* and has been estimated at 900–1300 CDSs for 37/38 *Acinetobacter* spp. (25–35% the average number of strain CDSs; Chan *et al.*, 2012; Fondi *et al.*, 2010). This number rises to *c.* 2700 CDSs if only members of the Acb complex are considered (Peleg *et al.*, 2012). Thus, members of the Acb complex may have acquired (or retained) over 1400 CDSs that are absent from nonpathogenic *Acinetobacter* spp.

In contrast, as the core genome of *A. baumannii* has a comparable size to that of the Acb complex, it is plausible that, in large scale, the *A. baumannii* core genome was already represented in the Acb complex ancestral genome and that the evolution of *A. baumannii* was mostly due to the expansion of its accessory genome. In fact, when compared to *A. calcoaceticus*, *A. baumannii* appears to have acquired no more than 170 genes (Peleg *et al.*, 2012). As the majority of *A. baumannii* putative virulence-associated genes are coded in the species core genome and the species core genome seems to be very conserved in the Acb complex, then a comparative analysis of the genomes of Acb complex members could provide important clues concerning the evolution of *A. baumannii* towards pathogenicity. A recent comparative genomics study has demonstrated that very few potential virulence factors are exclusive to *A. baumannii* (Sahl *et al.*, 2013). One of these exceptions is a putative siderophore biosynthesis and uptake cluster, identified with accession numbers ACICU_01672-ACICU_01683 (Antunes *et al.*, 2011b).

Most *A. baumannii* putative virulence factors are conserved or exclusive to the important pathogenic members of the Acb complex (i.e. *A. baumannii*, *A. pittii* and *A. nosocomialis*). The tip adhesion gene *csuE*, which in *A. baumannii* is involved in pilus and biofilm formation, is conserved in all three of these species (Sahl *et al.*, 2013). Moreover, efflux pumps are almost exclusive to the Acb complex, with many of them showing orthology to efflux pumps of other bacterial pathogens, such as *Pseudomonas* spp. The acinetobactin siderophore cluster is conserved only in *A. baumannii* and *A. pittii* and may have been acquired by two independent horizontal gene transfer events, as these two species do not form a monophyletic clade, and in *A. baumannii,* the acinetobactin cluster is found in alien islands with a higher G+C content that are flanked by insertion sequences.

Interestingly, genes coding for a few virulence factors that have been implicated in *A. baumannii* infection (Roca *et al.*, 2012) are also widely distributed in nonpathogenic *Acinetobacter* spp., such as those coding for the two-component BfmRS system, the outer membrane protein OmpA, the cell wall protein PBP 7/8 and phospholipases C and D (NCBI; http://www.ncbi.nlm.nih.gov/RefSeq/). This leaves questions as to whether they are active and what their roles may be in nonpathogenic *Acinetobacter* spp. Notably, several transcriptional regulators have been acquired by *A. baumannii* and by the Acb complex, a few of which are orthologous to transcriptional regulators of other human pathogens (Sahl *et al.*, 2013). Thus, they could be involved in the differential expression of these virulence factors, and their role in *A. baumannii* and Acb complex pathogenicity should not be underestimated.

Evolutionary theories for the emergence and success of *A. baumannii*

The genomic organisation of *A. baumannii*, involving a rather small core genome and a very large accessory genome (Imperi *et al.*, 2011), together with the low level of gene polymorphism in the species and the lack of intraspecies phylogenetic structure (Diancourt *et al.*, 2010), supports an evolutionary model involving two disjointed waves of expansion among the population of *A. baumannii* clinical isolates. The first followed a severe bottleneck in the pre-existing *A. baumannii* population that occurred at some undetermined time in the distant past, while a second wave is now developing through the rapid expansion of a limited number of multiresistant clones that have become highly problematic as nosocomial infectious agents (Diancourt *et al.*, 2010).

The evidence that the core genome of *A. baumannii* is in large part represented in the core genome of the Acb complex and that most *A. baumannii* virulence and resistance factors are conserved in the Acb complex or have orthologs in other nosocomial pathogens indicates that *A. baumannii* may have diverged from the Acb complex at some stage during its evolutionary history. The recent event that limited the genomic diversity of the *A. baumannii* population might have been directly or indirectly connected to the introduction of intensive antibiotic therapy (post-1950s). In that scenario, the recent rapid expansion of very homogenous clonal lineages (post-1980s), whose main difference from nonclonal *A. baumannii* appears to be their antimicrobial resistance, may have resulted from the horizontal acquisition of resistance genes from other nosocomial pathogens.

An alternative hypothesis is that the current breadth of knowledge on nosocomial *A. baumannii* strains does not represent the true diversity of the species. Strains recovered from cases of community-acquired infection show different clinical, epidemiological and phenotypic characteristics (Falagas *et al.*, 2007).

Recently, the genome sequence of a strain responsible for a bacteremic community infection has become available (Farrugia *et al.*, 2013). Strain D1279779 did not belong to any of the two major ICLs, was antibiotic susceptible and, accordingly, lacked many of the *A. baumannii* resistance determinants, including the AbaR resistance island. Possibly for this reason, its genome is *c.* 250 kb smaller and 300 CDSs shorter than the average *A. baumannii* genome. Nevertheless, the D1279779 genome encodes many of the *A. baumannii* virulence-associated factors (e.g. acinetobactin, a capsule, type I and type IV pili, phospholipases C and D). These facts have led to the suggestion that this strain might be a representative of an 'environmental or preantibiotic era' lineage (Farrugia *et al.*, 2013).

In addition, both D1279779 and the clinical strain ATCC 17978 (isolated in 1951 and antibiotic susceptible) use a greater breadth of carbon and nitrogen sources compared to strains belonging to the ICLs. 'Environmental' *A. baumannii* isolates recovered from soil, water and food samples also show extensive genetic diversity compared with clinical *A. baumannii* isolates (P. Visca, unpublished results). Thus, ATCC 17978, D1279779 and other nonclinical, nonclonal *A. baumannii* isolates might represent a wider diversity of the *A. baumannii* population, which did not undergo selection by antimicrobials and consequently did not experience an evolutionary bottleneck. The answer to this question will depend on widening comparative genomic studies on a larger repertoire of 'nonclinical' *A. baumannii* isolates.

Multifactorial and combinatorial virulence

Virulence factors

In recent years, combinatorial approaches involving genomic, phenotypic and infection model analyses have helped in the identification of virulence factors important for *A. baumannii* pathogenicity. So far, outer membrane proteins, hydrolytic enzymes and multifactorial phenotypes have been confirmed as being implicated in *A. baumannii* pathogenicity. The main outer membrane protein OmpA (Choi *et al.*, 2005) is involved in epithelial cell invasion and apoptosis. Phospholipases C and D are responsible for survival in human serum and epithelial cell invasion (Camarena *et al.*, 2010; Jacobs *et al.*, 2010). The siderophore acinetobactin (Gaddy *et al.*, 2012), the cell capsule (Russo *et al.*, 2010) and a penicillin-binding protein 7/8 (Russo *et al.*, 2009) are important for survival in human serum. Growth in serum has also been shown to significantly upregulate iron acquisition systems, genes associated with epithelial cell adherence and DNA uptake, as well as numerous putative antibiotic efflux pumps, leading to increased antibiotic tolerance (Jacobs *et al.*, 2012). In addition, lipopolysaccharide (LPS) is an important cell envelope component, which might play an important role in causing septic shock once *A. baumannii* enters the bloodstream. To date, LPS has received relatively little attention in studies of *A. baumannii* virulence, but it has become evident that most *Acinetobacter* LPS molecules contain an O-polysaccharide chain (O-antigen), which has been reported to influence the pathogenic potential of other Gram-negative bacteria (Pantophlet, 2008). There also appears to be a general O-glycosylation system that appears to be important for biofilm formation and virulence, and the capsular polysaccharide, which is essential for resistance to complement killing (Iwashkiw *et al.*, 2012). Mutations in the initiating glycosyltransferase have been shown to prevent the synthesis of both glycoproteins and capsule, resulting in abnormal biofilm structures and attenuated virulence in mice (Lees-Miller *et al.*, 2013).

The lipid A part of the LPS synthesised by *A. baumannii* likely also plays a similar role in septic shock as that documented for the endotoxin of *Enterobacteriaceae*. Its main pathogenic role is to induce a strong inflammatory response in systemic infection, but to date, there have been no published studies in strains of *A. baumannii* that have specifically addressed this point. Intriguingly, whole cells of clinical *A. baumannii* isolates were found to induce a weaker response, expressed as pro-inflammatory cytokine production by macrophages and epithelial cells, than isolates

belonging to the relatively nonpathogenic species *Acinetobacter junii*, suggesting that *A. baumannii* may survive and persist in the airways of patients and cause disease at least in part by eluding the induction of the host's inflammatory response (de Breij *et al.*, 2010). This is in apparent contrast with a recent study showing that LPS-mediated activation of TLR4 was crucial for *A. baumannii* pathogenicity in a murine model of systemic infection and that TLR4 activation occurred as a consequence of LPS shedding (Lin *et al.*, 2012). Thus, it appears that released (rather than cell-associated) LPS is relevant to *A. baumannii* pathogenicity and that more virulent *A. baumannii* strains shed more LPS during growth than less virulent strains, resulting in enhanced TLR4 activation and host damage (Lin *et al.*, 2012).

Moreover, several other factors contribute to biofilm formation and help sustain cell survival on biotic and abiotic surfaces, such as the Csu pili (Tomaras *et al.*, 2003), the two-component system BfmRS (Tomaras *et al.*, 2008), a quorum-sensing system (Niu *et al.*, 2008), the surface protein Bap (Fattahian *et al.*, 2011), the OmpA protein, the exopolysaccharide poly-β-1,6-N-acetylglucosamine (PNAG; Bentancor *et al.*, 2012b) and the autotransporter Ata (Bentancor *et al.*, 2012a). Additional information on *A. baumannii* virulence factors and mechanisms of host–pathogen interaction can be found in previous reviews (Mortensen & Skaar, 2012; McConnell *et al.*, 2013; Roca *et al.*, 2012).

Nonetheless, no single toxin, hydrolytic enzyme or surface protein has been identified that can unequivocally account for the increased pathogenicity of the ICLs; thus, the reason for the predominance of ICLs 1 and 2 in outbreaks of *A. baumannii* infection is still unclear. The ICLs do not show increased pathogenicity in animal infection models. An ICL1 (AYE), an ICL2 (ACICU) and a non-ICL (ATCC 17978) strain showed comparable lethality in an insect infection model (Antunes *et al.*, 2011a), and a similar result was obtained for a wider collection of strains, including ICL1 and ICL2 clones and the recent emerging genotypes ST25 and ST78 (Giannouli *et al.*, 2013). An ICL1, an ICL2 and a non-ICL strain also showed comparable mortality in a neutropenic mouse infection model (de Breij *et al.*, 2012). Moreover, an ICL1 strain (AYE) was less virulent than a non-ICL strain in a mouse model of pneumonia (Eveillard *et al.*, 2010).

Thus, in summary, no individual factor has been identified that can account for the predominance of the ICLs. ICL2 seems to have become more widely prevalent than ICL1, but their dissemination and colonisation patterns among patients during outbreaks seem to be broadly similar. Each ICL appears able to express a multiplicity of virulence factors and to do so in different combinations. Indeed, ICL1 and ICL2 might have different pathogenic strategies: several studies have reported that ICL1 isolates show higher motility (Antunes *et al.*, 2011a; Eijkelkamp *et al.*, 2011) and higher resistance to desiccation (Antunes *et al.*, 2011a; Giannouli *et al.*, 2013) than ICL2 and non-ICL isolates. Conversely, ICL2 isolates show higher adherence and biofilm formation than ICL1 and non-ICL isolates (Lee *et al.*, 2006; de Breij *et al.*, 2010; Antunes *et al.*, 2011a; Giannouli *et al.*, 2013), as well as higher proteolytic activity (Antunes *et al.*, 2011a). In addition, in agreement with the apparent conservation of virulence-associated genes in the core genome of the species, a nonclinical strain (SDF) showed levels of production of exoenzymes with haemolytic, proteolytic and phospholipase C activities comparable to those of clinical strains, as well as high levels of biofilm formation and an ability to survive in human serum (Antunes *et al.*, 2011a).

Furthermore, members of the genus *Acinetobacter* are able to express virulence-related phenotypes regardless of their status as clinical or nonclinical isolates. Although less toxic and infective in an epithelial cell model, nonclinical *Acinetobacter* spp. showed haemolytic activity comparable to *A. baumannii* and were equally resistance to macrophage phagocytosis (Tayabali *et al.*, 2012). Moreover, *A. baumannii* was less adherent than the less clinically relevant species of the Acb complex and showed similar survival in a murine thigh infection model (Peleg *et al.*, 2012).

Taken together, these results support a multifactorial and combinatorial strategy of *A. baumannii* virulence, a scenario that is reminiscent of *P. aeruginosa* virulence (Lee *et al.*, 2006). This hypothesis is supported by the systematic failure of studies on *A. baumannii* virulence to identify a particular virulence factor unequivocally responsible for the clinical success of *A. baumannii*, including the inability to identify bona fide virulence genes by a transposon inactivation approach (Smith *et al.*, 2007). This perspective highlights the strong adaptive potential of this species and could be the result of the adaptation to different human body sites or different pathogenic strategies (Sahl *et al.*, 2011), as is the case for *E. coli*, *Bacillus* spp., *Legionella* spp. and other pathogenic bacteria (Pallen & Wren, 2007).

In addition, all clinical *A. baumannii* isolates have the ability to grow in iron-depleted media, survive fever-associated temperatures and are more resistant to desiccation, antimicrobials (Antunes *et al.*, 2011a; Giannouli *et al.*, 2013) and heavy metals (P. Visca, unpublished results). These data further suggest that the emergence of the ICLs is probably not directly connected to the acquisition of individual virulence factors, but is the result of their resistance phenotype, a hypothesis that is also supported by genomic comparative analysis (Imperi *et al.*, 2011; Sahl *et al.*, 2013).

Regulation of virulence

Combinatorial approaches to the study of *A. baumannii* virulence have revealed that: (1) ICL strains do not have unique virulence factor expression that is absent from non-ICL clinical strains; thus, at least at present, their clinical predominance cannot be reasonably ascribed to increased virulence potential; (2) clinical strains do not have a set of unique virulence factors that are absent from nonclinical strains; (3) *A. baumannii* does not encode unique virulence factors absent from the genomes of other *Acinetobacter* spp.

Three hypotheses can be considered:

(1) Individual virulence factors may not be important for *A. baumannii* virulence in a human host, despite the fact

that several of them have been found to be important in animal infection models (McConnell *et al.*, 2013).

(2) Alternatively, the same virulence factor might play a different role in different habitats. For instance, the high level of biofilm formation found in ICL2 strains and the nonclinical strain SDF (Antunes *et al.*, 2011a) could reflect multiple functions of biofilm in *A. baumannii*, a strategy that is not uncommon in species with high habitat plasticity (Jensen *et al.*, 2003).

(3) Lastly, the expression of virulence-associated genes could be under different regulation in pathogenic and nonpathogenic species; similar evolutionary profiles are widespread in other bacterial pathogens that have multiple habitats (Pallen & Wren, 2007). Comparative genomic analyses have identified a high percentage of transcription factor regulators in the core genome and pan-genome of *A. baumannii* and pathogenic *Acinetobacter* spp. (Imperi *et al.*, 2011; Sahl *et al.*, 2013). However, the role of gene regulators in controlling *A. baumannii* virulence remains largely unknown. A recent study has reported the identification of a homolog of H-NS, a global negative regulator of virulence in many Gram-negative bacteria, in the genome of clinical strain ATCC 17978 (Eijkelkamp *et al.*, 2013). In this strain, inactivation of the H-NS gene resulted in increased expression of type I pili, the type IV secretion system, the Ata autotransporter and the AbaR resistance island, which had effects on the hydrophobicity, adherence and motility of the strain. In *E. coli* and other bacteria, H-NS is regulated by environmental cues, such as temperature, pH and osmolarity (Fang & Rimsky, 2008). It remains to be assessed what factors determine the activation of H-NS in *A. baumannii*.

Environmental strains

If the expression of particular virulence factors in *A. baumannii* is dependent on strain habitat and evolution, then it is important to investigate the diversity of the nonclinical *A. baumannii* population.

The only nonclinical *A. baumannii* strain that has been well characterised to date, strain SDF, was isolated from the gut of a body louse from a homeless person (La Scola & Raoult, 2004). SDF shows some characteristics that could be linked to its particular adaptation for survival in this host, namely extensive biofilm production, serum resistance and haemolytic activity, as well as the genomic capacity for haem uptake (Antunes *et al.*, 2011b).

SDF could be an example of specialisation to a particular niche or host (Lawrence, 2005). Its genome is smaller than those of clinical strains and contains numerous insertion and prophage sequences, which could indicate a reductive evolution by pseudogenisation and gene loss, and SDF is less resistant to high temperatures, iron starvation and desiccation. Curiously, SDF is prototrophic (Vallenet *et al.*, 2008) and still shares some of the virulence determinants present in clinical strains (Antunes *et al.*, 2011a), suggesting that these determinants have a different function in this strain or are not expressed, perhaps therefore providing an example of a continuing reductive evolutionary process (Pallen & Wren, 2007).

Recently, the genome of a second *Acinetobacter* strain isolated from an haematophage gut, *Acinetobacter* sp. HA, has been sequenced (Malhotra *et al.*, 2012). The genome of this strain is also smaller than the average clinical *A. baumannii* genome (3.1 Mb vs. *c.* 3.9 Mb) and lacks many of the putative *A. baumannii* virulence genes. The species classification of this strain is still uncertain, but its closest neighbours are *A. lwoffii* and *A. baumannii* (Malhotra *et al.*, 2012).

Concluding remarks

The development of large-scale comparative genomic and phenotypic approaches has provided important tools for the study of the evolution of *A. baumannii* as a global pathogen. The virulence potential of *A. baumannii* seems to be a characteristic of this species, as well as other pathogenic members of the Acb complex. The available evidence suggests that *A. baumannii* virulence is probably multifactorial and combinatorial, with the epidemic ICLs 1 and 2 possibly using different combinations of virulence and resistance determinants to optimise adaptability to the human host. However, the success of *A. baumannii* as a human pathogen seems to be intimately linked with the acquisition of antibiotic resistance genes via horizontal gene transfer. It seems that *A. baumannii* is endowed with the ability to adapt to different habitats, but that a reduction in its genetic diversity, possibly following the introduction of antibiotic therapy in the 1950s, led to the evolution and spread of a few highly homogenous clones that were specifically adapted to the nosocomial environment. Nevertheless, several questions and technical shortcomings need to be addressed before a complete picture of the evolution of this important human pathogen can be obtained.

Firstly, the presence of a high number of hypothetical proteins (*c.* 22%) in the species core genome and pan-genome may have obscured genes important for *A. baumannii* pathogenicity, such as toxins, hydrolytic enzymes or cell surface components, that could explain the prevalence of the ICLs.

Secondly, the genomes of pathogenic members of the Acb complex and *A. baumannii* are enriched with transcription factor regulators. Their role in the evolution of *A. baumannii* as an important human pathogen, particularly in the regulation of virulence and the success and adaptation of the international clones, remains to be addressed.

Thirdly, most current studies of *A. baumannii* pathogenicity have been limited to an analysis of chromosome-encoded sequences, although plasmid and phage-encoded CDSs also make an important contribution to the pan-genome of *A. baumannii* (Fondi *et al.*, 2010; Di Nocera *et al.*, 2011). For example, 8 of 85 AB1 phage CDSs show homology in *A. baumannii* genomes (Li *et al.*, 2012), indicating recombination between the chromosomal and phage genomes. In addition, a high degree of sequence similarity was found between plasmid- and chromosome-encoded genes (over 40% of CDSs at 50% sequence identity) for 29 *Acinetobacter* plasmids and 329 plasmid-encoded CDSs (Fondi *et al.*, 2010), thus indicating that considerable

recombination occurs between *Acinetobacter* plasmids and *A. baumannii* chromosomal sequences.

Finally, most studies have focused on the comparative genomics of clinical strains, but it is not known whether the diversity found among clinical *A. baumannii* strains is representative of the whole *A. baumannii* population diversity, particularly considering the fact that *A. baumannii* can be isolated, albeit with low (< 10%) recovery rates, from soil, water, vegetable and animal sources (Eveillard *et al.*, 2013). Thus, further analysis of the phylogenetic diversity of nonclinical *A. baumannii* isolates should help to gain a more complete understanding of the processes leading to the relatively recent evolution of this species as a global pathogen.

Acknowledgements

The authors declare that they have no conflict of interests.

References

Adams MD, Goglin K, Molyneaux N *et al.* (2008) Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J Bacteriol* 190: 8053–8064.

Al-Sweih NA, Al-Hubail MA & Rotimi VO (2011) Emergence of tigecycline and colistin resistance in *Acinetobacter* species isolated from patients in Kuwait hospitals. *J Chemother* 23: 13–16.

Antunes LC, Imperi F, Carattoli A & Visca P (2011a) Deciphering the multifactorial nature of *Acinetobacter baumannii* pathogenicity. *PLoS ONE* 6: e22674.

Antunes LC, Imperi F, Towner KJ & Visca P (2011b) Genome-assisted identification of putative iron-utilization genes in *Acinetobacter baumannii* and their distribution among a genotypically diverse collection of clinical isolates. *Res Microbiol* 162: 279–284.

Bentancor LV, Camacho-Peiro A, Bozkurt-Guzel C, Pier GB & Maira-Litrán T (2012a) Identification of Ata, a multifunctional trimeric autotransporter of *Acinetobacter baumannii*. *J Bacteriol* 194: 3950–3960.

Bentancor LV, O'Malley JM, Bozkurt-Guzel C, Pier GB & Maira-Litrán T (2012b) Poly-N-acetyl-β-(1-6)- glucosamine is a target for protective immunity against *Acinetobacter baumannii* infections. *Infect Immun* 80: 651–656.

Bergogne-Bérézin E & Towner KJ (1996) *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* 9: 148–165.

Bouvet PJM & Grimont PAD (1986) Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Bacteriol* 36: 228–240.

Breslow JM, Monroy MA, Daly JM, Meissler JJ, Gaughan J, Adler MW & Eisenstein TK (2011) Morphine, but not trauma, sensitizes to systemic *Acinetobacter baumannii* infection. *J Neuroimmune Pharmacol* 6: 551–565.

Cai Y, Chai D, Wang R, Liang B & Bai N (2012) Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. *J Antimicrob Chemother* 67: 1607–1615.

Camarena L, Bruno V, Euskirchen G, Poggio S & Snyder M (2010) Molecular mechanisms of ethanol-induced pathogenesis revealed by RNA-sequencing. *PLoS Pathog* 6: e1000834.

Centers for Disease Control and Prevention (2004) *Acinetobacter baumannii* infections among patients at military medical facilities treating injured US service members, 2002–2004. *MMWR* 53: 1063–1066.

Chan JZ, Halachev MR, Loman NJ, Constantinidou C & Pallen MJ (2012) Defining bacterial species in the genomic era: insights from the genus Acinetobacter. *BMC Microbiol* 12: 302.

Chang WN, Lu CH, Huang CR & Chuang YC (2000) Community-acquired Acinetobacter meningitis in adults. *Infection* 28: 395–397.

Choi CH, Lee EY, Lee YC, Park TI, Kim HJ, Hyun SH, Kim SA, Lee SK & Lee JC (2005) Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. *Cell Microbiol* 7: 1127–1138.

de Breij A, Dijkshoorn L, Lagendijk E, van der Meer J, Koster A, Bloemberg G, Wolterbeek R, van den Broek P & Nibbering P (2010) Do biofilm formation and interactions with human cells explain the clinical success of *Acinetobacter baumannii*? *PLoS ONE* 5: e10732.

de Breij A, Eveillard M, Dijkshoorn L, van den Broek PJ, Nibbering PH & Joly-Guillou ML (2012) Differences in *Acinetobacter baumannii* strains and host innate immune response determine morbidity and mortality in experimental pneumonia. *PLoS ONE* 7: e30673.

Di Nocera PP, Rocco F, Giannouli M, Triassi M & Zarrilli R (2011) Genome organization of epidemic *Acinetobacter baumannii* strains. *BMC Microbiol* 11: 224.

Diancourt L, Passet V, Nemec A, Dijkshoorn L & Brisse S (2010) The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* 5: e10034.

Dijkshoorn L, Nemec A & Seifert H (2007) An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 5: 939–951.

Eijkelkamp BA, Stroeher UH, Hassan KA, Papadimitrious MS, Paulsen IT & Brown MH (2011) Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates. *FEMS Microbiol Lett* 323: 44–51.

Eijkelkamp BA, Stroeher UH, Hassan KA, Elbourne LD, Paulsen IT & Brown MH (2013) H-NS plays a role in expression of *Acinetobacter baumannii* virulence features. *Infect Immun* 81: 2574–2583.

Evans BA, Hamouda A & Amyes SG (2013) The rise of carbapenem-resistant *Acinetobacter baumannii*. *Curr Pharm Des* 19: 223–238.

Eveillard M, Soltner C, Kempf M, Saint-André JP, Lemarié C, Randrianarivelo C, Seifert H, Wolff M & Joly-Guillou ML (2010) The virulence variability of different *Acinetobacter baumannii* strains in experimental pneumonia. *J Infect* 60: 154–161.

Eveillard M, Kempf M, Belmonte O, Pailhoriès H & Joly-Guillou ML (2013) Reservoirs of *Acinetobacter baumannii* outside the hospital and potential involvement in emerging human community-acquired infections. *Int J Infect Dis* 17: e802–e805.

Falagas ME & Rafailidis PI (2007) Attributable mortality of *Acinetobacter baumannii*: no longer a controversial issue. *Crit Care* 11: 134.

Falagas ME & Rafailidis PI (2009) Nephrotoxicity of colistin: new insight into an old antibiotic. *Clin Infect Dis* 48: 1729–1731.

Falagas ME, Karveli EA, Kelesidis I & Kelesidis T (2007) Community-acquired Acinetobacter infections. *Eur J Clin Microbiol Infect Dis* 26: 857–868.

Fang FC & Rimsky S (2008) New insights into transcriptional regulation by H-NS. *Curr Opin Microbiol* 11: 113–120.

Farrugia DN, Elbourne LD, Hassan KA, Eijkelkamp BA, Tetu SG, Brown MH, Shah BS, Peleg AY, Mabbutt BC & Paulsen IT (2013)

The complete genome and phenome of a community-acquired *Acinetobacter baumannii*. *PLoS ONE* 8: e58628.

Fattahian Y, Rasooli I, Mousavi Gargari SL, Rahbar MR, Darvish Alipour Astaneh S & Amani J (2011) Protection against *Acinetobacter baumannii* infection via its functional deprivation of biofilm associated protein (Bap). *Microb Pathog* 51: 402–406.

Fondi M, Bacci G, Brilli M, Papaleo MC, Mengoni A, Vaneechoutte M, Dijkshoorn L & Fani R (2010) Exploring the evolutionary dynamics of plasmids: the Acinetobacter pan-plasmidome. *BMC Evol Biol* 10: 59.

Fondi M, Rizzi E, Emiliani G *et al.* (2013) The genome sequence of the hydrocarbon-degrading *Acinetobacter venetianus* VE-C3. *Res Microbiol* 164: 439–449.

Gaddy JA, Arivett BA, McConnell MJ, López-Rojas R, Pachón J & Actis LA (2012) Role of acinetobactin- mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606^T with human lung epithelial cells, *Galleria mellonella* caterpillars, and mice. *Infect Immun* 80: 1015–1024.

Giannouli M, Antunes LC, Marchetti V, Triassi M, Visca P & Zarrilli R (2013) Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78. *BMC Infect Dis* 13: 282.

Girlich D, Poirel L & Nordmann P (2010) First isolation of the blaOXA-23 carbapenemase gene from an environmental *Acinetobacter baumannii* isolate. *Antimicrob Agents Chemother* 54: 578–579.

Iacono M, Villa L, Fortini D *et al.* (2008) Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. *Antimicrob Agents Chemother* 52: 2616–2625.

Imperi F, Antunes LC, Blom J, Villa L, Iacono M, Visca P & Carattoli A (2011) The genomics of *Acinetobacter baumannii*: insights into genome plasticity, antimicrobial resistance and pathogenicity. *IUBMB Life* 63: 1068–1074.

Iwashkiw JA, Seper A, Weber BS *et al.* (2012) Identification of a general O-linked protein glycosylation system in *Acinetobacter baumannii* and its role in virulence and biofilm formation. *PLoS Pathog* 8: e1002758.

Jacobs AC, Hood I, Boyd KL, Olson PD, Morrison JM, Carson S, Sayood K, Iwen PC, Skaar EP & Dunman PM (2010) Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. *Infect Immun* 78: 1952–1962.

Jacobs AC, Sayood K, Olmsted SB, Blanchard CE, Hinrichs S, Russell D & Dunman PM (2012) Characterization of the *Acinetobacter baumannii* growth phase-dependent and serum responsive transcriptomes. *FEMS Immunol Med Microbiol* 64: 403–412.

Jensen GB, Hansen BM, Eilenberg J & Mahillon J (2003) The hidden lifestyles of *Bacillus cereus* and relatives. *Environ Microbiol* 5: 631–640.

Joly-Guillou ML (2005) Clinical impact and pathogenicity of Acinetobacter. *Clin Microbiol Infect* 11: 868–873.

Karah N, Sundsfjord A, Towner K & Samuelsen O (2012) Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. *Drug Resist Updat* 15: 237–247.

Keen EF III, Murray CK, Robinson BJ, Hospenthal DR, Co EM & Aldous WK (2010) Changes in the incidences of multidrug-resistant and extensively drug-resistant organisms isolated in a military medical center. *Infect Control Hosp Epidemiol* 31: 728–732.

Kempf M & Rolain JM (2012) Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *Int J Antimicrob Agents* 39: 105–114.

Kenyon JJ & Hall RM (2013) Variation in the complex carbohydrate biosynthesis loci of *Acinetobacter baumannii* genomes. *PLoS ONE* 16: e62160.

La Scola B & Raoult D (2004) *Acinetobacter baumannii* in human body louse. *Emerg Infect Dis* 10: 1671–1673.

Lawrence JG (2005) Common themes in the genome strategies of pathogens. *Curr Opin Genet Dev* 15: 584–588.

Lee JC, Koerten H, van den Broek P, Beekhuizen H, Wolterbeek R, van den Barselaar M, van der Reijden T, van der Meer J, van de Gevel J & Dijkshoorn L (2006) Adherence of *Acinetobacter baumannii* strains to human bronchial epithelial cells. *Res Microbiol* 157: 360–366.

Lees-Miller RG, Iwashkiw JA, Scott NE, Seper A, Vinogradov E, Schild S & Feldman MF (2013) A common pathway for O-linked protein glycosylation and synthesis of capsule in *Acinetobacter baumannii*. *Mol Microbiol* 89: 816–830.

Li P, Chen B, Song Z *et al.* (2012) Bioinformatic analysis of the *Acinetobacter baumannii* phage AB1 genome. *Gene* 507: 125–134.

Lin L, Tan B, Pantapalangkoor P *et al.* (2012) Inhibition of LpxC protects mice from resistant *Acinetobacter baumannii* by modulating inflammation and enhancing phagocytosis. *MBio* 3: e00312–12.

Malhotra J, Dua A, Saxena A, Sangwan N, Mukherjee U, Pandey N, Rajagopal R, Khurana P, Khurana JP & Lal R (2012) Genome Sequence of *Acinetobacter* sp. Strain HA, Isolated from the Gut of the Polyphagous Insect Pest *Helicoverpa armigera*. *J Bacteriol* 194: 5156.

McConnell MJ, Actis L & Pachón J (2013) *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Rev* 37: 130–155.

Mera RM, Miller LA, Amrine-Madsen H & Sahm DF (2010) *Acinetobacter baumannii* 2002–2008: increase of carbapenem-associated multiclass resistance in the United States. *Microb Drug Resist* 16: 209–215.

Mortensen BL & Skaar EP (2012) Host-microbe interactions that shape the pathogenesis of *Acinetobacter baumannii* infection. *Cell Microbiol* 14: 1336–1344.

Niu C, Clemmer KM, Bonomo RA & Rather PN (2008) Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*. *J Bacteriol* 190: 3386–3392.

Pallen MJ & Wren BW (2007) Bacterial pathogenomics. *Nature* 449: 835–842.

Pantophlet RA (2008) Lipopolysaccharides of *Acinetobacter*. Acinetobacter Molecular Biology (Gerischer U, ed.), pp. 61–98. Caister Academic Press, Norfolk, UK.

Peleg AY, Seifert H & Paterson DL (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 21: 538–582.

Peleg AY, de Breij A, Adams MD *et al.* (2012) The success of *Acinetobacter* species; genetic, metabolic and virulence attributes. *PLoS ONE* 7: e46984.

Poirel L, Bonnin RA & Nordmann P (2011) Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. *IUBMB Life* 63: 1061–1067.

Poirel L, Berçot B, Millemann Y, Bonnin RA, Pannaux G & Nordmann P (2012) Carbapenemase-producing *Acinetobacter* spp. in Cattle, France. *Emerg Infect Dis* 18: 523–525.

Roca I, Espinal P, Vila-Farrés X & Vila J (2012) The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace. *Front Microbiol* 3: 148.

Russo TA, MacDonald U, Beanan JM, Olson R, MacDonald IJ, Sauberan SL, Luke NR, Schultz LW & Umland TC (2009) Penicillin-binding protein 7/8 contributes to the survival of

Acinetobacter baumannii in vitro and *in vivo*. *J Infect Dis* 199: 513–521.

Russo TA, Luke NR, Beanan JM, Olson R, Sauberan SL, MacDonald U, Schultz LW, Umland TC & Campagnari AA (2010) The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. *Infect Immun* 78: 3993–4000.

Sahl JW, Johnson JK, Harris AD, Phillippy AM, Hsiao WW, Thom KA & Rasko DA (2011) Genomic comparison of multi-drug resistant invasive and colonizing *Acinetobacter baumannii* isolated from diverse human body sites reveals genomic plasticity. *BMC Genomics* 12: 291.

Sahl JW, Gillece JD, Schupp JM, Waddell VG, Driebe EM, Engelthaler DM & Keim P (2013) Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in Acinetobacter. *PLoS ONE* 8: e54287.

Seiffert SN, Hilty M, Perreten V & Endimiani A (2013) Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health. *Drug Resist Updat* 16: 22–45.

Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M & Snyder M (2007) New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev* 21: 601–614.

Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG & Antimicrobial Availability Task Force of the Infectious Diseases Society of America (2006) Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis* 42: 657–668.

Tayabali AF, Nguyen KC, Shwed PS, Crosthwait J, Coleman G & Seligy VL (2012) Comparison of the virulence potential of *Acinetobacter* strains from clinical and environmental sources. *PLoS ONE* 7: e37024.

Tomaras AP, Dorsey CW, Edelmann RE & Actis LA (2003) Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology* 149: 3473–3484.

Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA & Actis LA (2008) Characterization of a two-component regulatory system from *Acinetobacter baumannii* that controls biofilm formation and cellular morphology. *Microbiology* 154: 3398–3409.

Towner KJ (2009) Acinetobacter: an old friend, but a new enemy. *J Hosp Infect* 73: 355–363.

Vallenet D, Nordmann P, Barbe V *et al.* (2008) Comparative analysis of Acinetobacters: three genomes for three lifestyles. *PLoS ONE* 3: e1805.

Wisplinghoff H, Perbix W & Seifert H (1999) Risk factors for nosocomial bloodstream infections due to *Acinetobacter baumannii*: a case-control study of adult burn patients. *Clin Infect Dis* 28: 59–66.

Zhan Y, Yan Y, Zhang W, Chen M, Lu W, Ping S & Lin M (2012) Comparative analysis of the complete genome of an *Acinetobacter calcoaceticus* strain adapted to a phenol-polluted environment. *Res Microbiol* 163: 36–43.

Zordan S, Prenger-Berninghoff E, Weiss R, van der Reijden T, van den Broek P, Baljer G & Dijkshoorn L (2011) Multidrug-resistant *Acinetobacter baumannii* in veterinary clinics, Germany. *Emerg Infect Dis* 17: 1751–1754.