Are we closing in on an “elusive enemy”? The current status of our battle with Acinetobacter baumannii

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In less than a decade, Acinetobacter baumannii rapidly emerged as an important human pathogen. Around the globe, A. baumannii is increasingly recognized as the quintessential nosocomial “bad bug,” deftly adapted to the modern hospital environment.¹ The distinguishing feature of A. baumannii is its ready capacity to express resistance to a broad range of antimicrobials, forcing clinicians to repeatedly rely on “last-line” agents (i.e., colistin and tigecycline).² The Infectious Diseases Society of America (IDSA) recognized this resistance phenotype as an “unmet medical need” and multidrug-resistant (MDR) A. baumannii is among the pathogens targeted in the call to develop new antibiotics by 2020 (www.idsociety.org). Even more disquieting is the difficulty in grasping the clinical impact of A. baumannii in patients; however, the heavy burden that A. baumannii imposes on healthcare systems is undeniable.

The Evolving Clinical Landscape of Infections Caused by A. baumannii

In tropical or subtropical Australia and regions of the Pacific A. baumannii is a pathogen often recovered in patients with community acquired pneumonia (CAP), skin and soft tissue infection (SSTI), and meningitis.²,³ On the other hand, most US and European physicians are more familiar with A. baumannii infections in intensive care units (ICUs): ventilator associated pneumonia (VAP), wound, and catheter associated bloodstream infection. In survey studies from 75 countries, A. baumannii caused approximately 9% of ICU infections.⁴ Current estimates indicate that critically ill patients with VAP and bloodstream infections caused by A. baumannii experience increased mortality and prolonged lengths of stay in the hospital.⁵

Other alarming patterns are also recognized. Of late, A. baumannii has become an important cause of infection and colonization in patients who require medical attention in long term care facilities (LTCFs) and long-term acute care hospitals (LTACHs).⁶,⁷ Other locations where this pathogen is often recovered are modern military treatment facilities. Many wounded soldiers and civilians from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) were found to be colonized and subsequently infected with A. baumannii.⁸,⁹ Surprisingly, wounded military personnel hospitalized for war-trauma who do not possess significant co-morbidities and experience bloodstream infection caused by A. baumannii rarely die.⁹ As a result, physicians believe that those episodes represent “specimen contamination” or “transient bacteremia” among patients at risk of skin colonization.¹⁰ The notion that A. baumannii is “less virulent” than other Gram-negative bacilli has also been advanced. This opinion lies in stark contrast to the reports of A. baumannii causing devastating necrotizing skin and soft tissue infections¹¹,¹².

Genetic Factors that Permit the Success of A. baumannii

The review of novel therapies for A. baumannii skin infections presented in this issue of Virulence by Mihu and Martinez summarizes innovative treatment approaches. Will the “elusive” nature of A. baumannii defy even the development of these new approaches? Whole genome sequence analysis of A. baumannii has shed light upon several fundamental features of this MDR and versatile pathogen.¹³ Regarding the MDR phenotype, A. baumannii enjoys a variable complement of mobile genetic elements encoding drug-inactivating enzymes and insertion sequences (IS elements), develops a “plastic” genome,¹⁴ and thus becomes resistant to many antimicrobials. In addition to many genes encoding resistance determinants, A. baumannii isolates possess “resistance islands (RIs)” and an “accessory genome.”¹⁵ A first example of the RI is the 86 kb RI described by Fournier et al.¹⁵ Since then ten RIs, also called “AbaR,” are also described and their significance in the evolution of resistance is being defined.¹⁶ Adams et al. found that six closely related clinical isolates of A. baumannii, including four from the same hospital, possessed extensive divergence of the resistance genotype that was linked to differences in antimicrobial susceptibility.¹⁴ Moreover, resistance genes associated with IS elements, plasmids, and chromosomal AbaRs all showed variability. This analysis of a collection that was part of the same PCR/Electrospray Ionization Mass Spectrometry, PCR/ESI-MS, type showed a highly dynamic resistance gene repertoire suggesting rapid evolution of drug resistance. The interplay of RIs, IS elements and plasmids is only now beginning to be understood.

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The Human Host and Virulence

Also relevant are the factors that may define the ability of A. baumannii to live in association with the human; these are clusters of genes that regulate transcription and transport, as well as quorum sensing and biofilm formation. Additionally, there is extensive diversity in genes that contribute to the lipopolysaccharide barrier. Lipopolysaccharide (LPS), a recognized virulence factor that intervenes in multiple aspects of pathogenesis among Gram-negative bacteria, is also present in A. baumannii. Recent studies have characterized the mechanisms of synthesis of LPS in A. baumannii and illustrate its importance for serum resistance in vitro and survival in vivo. Capsular polysaccharide K1 produced by A. baumannii is necessary for its survival and growth in human serum. The regulatory genes of the K1 capsule (ptk and epsA) and their products (a putative protein tyrosine kinase and a putative polysaccharide export outer membrane protein, respectively) stand as natural antivirulence drug targets.

Studies relevant to the antibiotic treatment of A. baumannii implicate the loss of LPS with colistin resistance (through mutations in genes involved in LPS synthesis) and with the two-component system PmrAB. At the same time, certain isolates that are colistin resistant display marked loss of virulence. Proteomic analysis demonstrate that the cost of colistin resistance involves, among others such as chaperones and metabolic enzymes, loss of outer membrane proteins. Resistance to colistin seems to occur by several distinct mechanisms highlighted by the observation that acquisition of colistin resistance in some strains is associated with susceptibility to a broad range of antibiotics, including those that are active against only gram-positive bacteria, while other colistin resistant strains retained resistance to all other relevant antibiotics. This suggests that there may be more than one mechanism for the evolution of this phenotype.

Outer membrane protein A (OmpA) of A. baumannii has multiple important effects in pathogenesis and signal processing, to the point that OmpA (of Escherichia coli) has been termed "a molecular Swiss Army knife." OmpA plays a role in transport and interactions with epithelial cells, induces cytotoxicity and dendritic cell death through mitochondrial targeting. Omps have also been advanced as potential targets for diagnosis of A. baumannii, as they elicit specific IgG, IgM and IgG antibodies. Of tremendous import, an Omp vaccine with multiple surface antigens from the bacterial membrane of A. baumannii elicited protective and therapeutic humoral and cellular responses in a murine sepsis model. How well will this serve as a start to a vaccine remains to be seen.

OmpA of A. baumannii participates in the development of biofilms and attachment to epithelial cells. Biofilm formation on plastic medical devices (i.e., intravascular and intravascular catheters, endotracheal tubes), in combination with the ability to adhere to cells in the host (resulting in colonization) give A. baumannii an advantage in the modern nosocomial environment where invasive devices are all too common and infection control failures facilitate dissemination of pathogens. Recent studies, however, show that biofilm formation and adherence does not differ between A. baumannii and other non-pathogenic Acinetobacter. Thus, these features may contribute but do not solely explain the success of A. baumannii. Nevertheless, quorum sensing signal molecules that have been shown to influence biofilm formation may be important therapeutic targets. The identification of the AbaI autoinducer synthase (and the acyl-homoserine lactone regulated abai gene) may provide the basis for antagonists that inhibit biofilm development in A. baumannii.

The versatility of A. baumannii extends to the growth-dependent regulation of its proteome. Changes in the late stages of growth and stationary phase may allow A. baumannii to withstand otherwise lethal levels of antibiotics and reactive oxygen species and nitrogen intermediates, the killing mechanisms employed by phagocytic cells. A remarkable adaptation that set A. baumannii apart includes the regulation of its pathogenicity by ethanol. The application of RNA sequencing shows that ethanol induces the expression of phospholipase C and influences iron and phosphate transport systems. This correlates with data in animal models that revealed increased virulence in presence of alcohol and tie with the recognized association of alcoholism and severe community acquired infections caused by A. baumannii.

We are left to speculate about the role that the widespread adoption of alcohol hand cleaning in our hospitals has had on the rise of A. baumannii. Conversely, multidrug-resistant A. baumannii isolates from Japan demonstrate reduced susceptibility to chlorhexidine and other disinfectants, an event we don’t usually contemplate and that would have tremendous consequences on how we approach infection control.

A. baumannii grown under iron-limited conditions undergoes upregulation of siderophore biosynthesis gene clusters and down regulation in genes involved in motility. Of note, a novel beta-lactam, the monobactam BAL30072, has a siderophore moiety that can potentially facilitate uptake into the bacterial cell. Proteins related to the molecular targets of beta-lactams, specifically penicillin-binding protein 7/8, have recently been implicated in the pathogenesis of A. baumannii.

**Interactions with the Host: Variations due to Clinical Circumstance**

A. baumannii is often defined as an opportunistic pathogen. Under usual circumstances, the normal host remains impervious to its attack. A. baumannii-host interactions in the lung depend on LPS recognition by sCD14 at the Toll-like receptor (TLR) 2 and 4, which activates the expression of IL-8 and results in neutrophil infiltration and inflammation. Whether such mechanisms result in an insufficient, overwhelming or “just right” inflammatory response defines the disease caused by A. baumannii. Hence, modulation of TLR-dependent pathways presents a potential, yet tricky, target for drug development. Furthermore, pathways of the innate and adaptive immune system undergo changes due to injury (i.e., trauma, surgery) and critical illness that increase susceptibility to subsequent sepsis. Such phenomena may explain the explosive clinical manifestations of A. baumannii, especially in cases of pneumonia and skin infections. A. baumannii is often associated with (usually) Gram-negative co-pathogens in necrotizing skin infections, suggesting a plausible role for synergistic
Researchers in the United States have documented tropical, warm climates, whereas in temperate climates the reservoir is a highly prevalent pathogen and colonizer in *A. baumannii* exclusive to tropical and subtropical regions of Asia-Pacific, this *A. baumannii* serves as an interesting model. Almost severe CAP (associated with shock, respiratory failure and death) and bacterial traits that dictate poor outcomes. In that regard, infection may help to identify the host *A. baumannii* host cell. In experiments performed by Professor G. Bou, OMVs stands as a novel mechanism to deliver virulence factors to the host. In experiments performed by Professor G. Bou, OMVs have also been implicated in the transport of resistance genes.

Further study of the variations in the manifestation of disease caused by *A. baumannii* infection may help to identify the host and bacterial traits that dictate poor outcomes. In that regard, severe CAP (associated with shock, respiratory failure and death) caused by *A. baumannii* serves as an interesting model. Almost exclusive to tropical and subtropical regions of Asia-Pacific, this syndrome presents in the warmer months of the year. Curiously, *A. baumannii* is a highly prevalent pathogen and colonizer in tropical, warm climates, whereas in temperate climates the reservoir of epidemic strains is located in hospitals and long-term care facilities. Researchers in the United States have documented a 17% increase in the monthly rate of *A. baumannii* infection for each 10 degrees F increase in temperature. Even global warming conspires to favor *A. baumannii*!

| Table 1. Important “complex” characteristics of *A. baumannii* |
|---------------------------------------------------------------|
| Multidrug resistance (MDR) phenotype                          |
| Hospital, community, long term care associated infections     |
| Military related pathogen                                      |
| Resistance islands, accessory genome, IS elements             |
| Multiple genes that adapt to the human host (biofilm, transport, quorum sensing) |
| Lipopolysaccharide (LPS); serum and colistin resistance        |
| OmpA                                                          |
| Link to global warming                                        |
| Growth dependant regulation of proteome                       |
| Ethanol induced virulence                                     |
| Siderophores                                                  |
| Outer membrane vesicles as a transport system for resistance and virulence genes |

The evolutionary history of *A. baumannii* is thus closely shaped by antimicrobial selective pressure in the form of advanced cephalosporins and carbapenems used in the hospital setting, as demonstrated by the rapid expansion of a limited number of multidrug-resistant clones designated as European or International clones I-III. Hospital isolates, particularly from outbreaks, usually belong to International clones I or II, which are found everywhere; a third international lineage (clone III) is less common. The global spread of carbapenem-resistant *A. baumannii* is therefore associated with clone II harboring OXA-23, a class D beta-lactamase.

The molecular characterization of *A. baumannii* can also lead us to appreciate global epidemiological trends. Equally important, molecular epidemiological tools applied locally can help us understand the outcomes of infection caused by *A. baumannii*. For instance, among patients with *A. baumannii* from a military treatment facility, those with OXA-23 harboring organisms had less favorable outcomes (in terms of hospital stay). In other analyses, different clone types and genospecies of *A. baumannii* were associated with sites of colonization (respiratory versus skin), different types of infection (bloodstream vs. pneumonia) and biofilm of different characteristics.

While our understanding of the molecular epidemiology and evolution of antimicrobial resistance in *A. baumannii* has advanced, the use of these concepts in the clinical arena is limited. The introduction of rapid and accurate diagnostic platforms to obtain such information, used at the point of care, would bring great rewards to the clinical management of *A. baumannii* infections. For instance, a rapid high-throughput method using electrospray ionization mass spectrometry (PCR/ESI-MS) to carry out base composition analysis of PCR amplification products of highly conserved genes has been applied to identify *A. baumannii*, to obtain genetic fingerprints and trace its molecular epidemiology, and to identify unique DNA changes associated with quinolone resistance. In another example, microarray technology is a powerful tool that can quantify the expression of chromosomal genes and to detect acquired determinants that are expressed. A recent application of a diagnostic microarray to *A. baumannii* enabled the detection of most of the genetic mechanisms responsible for its MDR phenotype. These technologies are evolving. Interest is also keen on the use of methods such as variable-number tandem repeat (VNTR) analysis. Using VNTR analysis, in combination with other typing methods, can inform epidemiological investigations and provide additional characterization of isolates.

Where do we stand with *A. baumannii*? Because of the “complex” nature of *A. baumannii* (Table 1), we conclude that the success of *A. baumannii* as a pathogen is largely driven by the contemporary dynamics of selective pressure driven by broad spectrum antibiotics. *A. baumannii* is evolving to survive exceptionally well in our health care environment. The constellation of all the above factors have resulted in a MDR pathogen that may be having as large an impact on health care as extreme drug-resistant (*XDR*) *Mycobacterium tuberculosis* or drug-resistant Human Immunodeficiency Virus (HIV). We are not yet ready for this battle.

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