

Project Title: Genomic Sequencing of a Diversity of US Military *Acinetobacter baumannii* Isolates Based on Pulsed Field Gel Patterns

White Paper Submission Date (MM/DD/YY): 05/13/09 *Acinetobacter baumannii* is an emerging nosocomial pathogen and is an important emerging pathogen in treatment of wounds in US military practice

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***Acinetobacter baumannii* is an emerging nosocomial pathogen and is an important emerging pathogen in treatment of wounds in US military practice**

1. Significance and distribution of *Acinetobacter baumannii*. Nosocomial infections with *Acinetobacter baumannii* in civilian and military health care centers cause a significant level of morbidity and death. The ubiquitous presence of *A. baumannii* and propensity to acquire multiple-antibiotic resistance pose an ever-increasing threat to the civilian population in general and to deploying forces in particular. The impact of this opportunistic pathogen in war-time care was recognized as early as during the Korean War and in every war since.

A member of the genus *Acinetobacter*, *A. baumannii* is a gram-negative, aerobic, non-motile, catalase-positive, and oxidase-negative rod (G+C: 39% to 47%). Thirty-one species of *Acinetobacter* have been identified by DNA hybridization, roughly distributed into 12 groups based on genetic similarities. Four of the 31 species identified (*A. calcoaceticus*, *A. baumannii*, *Acinetobacter* genomic species 3 and 13TU) share a similar phenotype that cannot easily be distinguished by culture and are thus grouped together into the *A. calcoaceticus*-*A. baumannii* complex (Acb). Frequently, the Acb complex is described as *A. baumannii* only, neglecting the other species. However, only *A. calcoaceticus* is an exclusive soil and water organism that is not usually implicated in clinical cases, while the other Acb isolates are more clinically relevant.

Most species of *Acinetobacter* are common part of the normal human skin flora. In the healthy population, *Acinetobacter* species are present in frequency up to 43%, with *A. Iwoffii*, *A. Johnsonnii*, *A. junii* and genomic species 3 among the most common. However, the prevalence of *Acinetobacter* increases to up to 75% among the

hospitalized population. Further, *A. baumannii* poses a unique challenge in hospitals and clinical centers due to a particular hardiness and resistance to extreme environments. The most important nosocomial Acinetobacter species *A. baumannii* is only infrequently found on the skin (less than 3%) and feces (less than 1%) of the non-hospitalized population. In contrast, *A. baumannii* is the most common species isolated from human clinical samples. Nosocomial infections caused by Acinetobacter species other than *A. baumannii* are rare and usually benign. However, *A. baumannii* infections are most associated with ventilator-associated pneumonia, skin or wound infections and blood sepsis. In America and Western Europe, *A. baumannii* is responsible for up to 10% of all gram-negative ICU infections. In addition to high morbidity rates and among trauma patients, *A. baumannii* is directly implicated in significantly longer patient hospital stay and significantly higher costs to the hospital care system.

2. Impact on military medicine. The multi-drug resistance of most clinical isolates of *A. baumannii* has elevated concern for future treatment of combat wounds. In a study of East Timor, Elston *et al.* reported that Acinetobacter was isolated from 86% of patient victims of gun shot injuries. All of the isolated Acinetobacter species were classified as multi-drug resistant (resistant to over 3 drug classes) and 63% of all isolates were resistant to all drug classes except the beta-lactam meropenem and the aminoglycoside amikacin. Many antibiotics have been rendered almost useless by the spread of resistance (95% frequency), including chloramphenicol and the beta-lactams cefamandole and cefoxitin. In a recently conducted retrospective study, *A. baumannii* was cultured from 72% of bronchoalveolar lavage fluid (associated with ventilation) collected from 291 patients in American internal care units. Of these isolates, two were resistant to all drugs tested, 81% were determined to be resistant to the beta-lactam imipenem-cilastatin and one in five was resistant to all antibiotics except colistin (polymyxin B).

Not all *A. baumannii* infections in wounded soldiers are nosocomial in nature. This is especially true in cases of wounded servicemen, who are exposed to soil and animal contamination at time of injury. However, isolates from military cases report similarly high number of *A. baumannii* cases and similarly high percentage of drug resistance. In a retrospective study of wounded during the 2006 East Timor conflict, 19 different species of Acinetobacter were isolated from 86% of hospitalized wounded police officers. All isolates were multi drug resistant (resistant to ≥ 3 drug classes. The number of *A. baumannii* infections in soldiers wounded in Iraq or Afghanistan that are admitted to intensive care units in the Walter Reed Army Medical Center (WRAMC) reaches 30% and involves wound, skin, soft tissue and central venous line infection, pneumonia, ventilator-associated pneumonia, bacteremia and meningitis. Most of these isolates are resistant to various broad-spectrum antibiotics.

Project Objective:

Nosocomial bacterial infection is a formidable public health problem that has become particularly alarming in recent decades as antibiotic resistance has proliferated and multi-drug resistant (MDR) strains of various bacterial pathogens have emerged. Within this milieu, a dramatic increase in the frequency of nosocomial MDR *Acinetobacter baumannii* infections in US hospitals has been reported, resulting in limited therapeutic options since some isolates of *A. baumannii* are resistant to almost all currently available antibacterial agents. Infections with MDR *A. baumannii*-calcoaceticus complex (ABC) and other MDR bacteria have also complicated the care of US combat casualties from recent engagements in Iraq, where the acquisition of MDR appears to be significantly increased compared with past wars. Based on current epidemiological data, specific strains and isolates of MDR ABC may be more closely associated with greater morbidity and mortality than others. Consequently there exists a critical need to rapidly distinguish between different strains of MDR ABC to enhance infection control practices and assist in tracking the proliferation of more virulent strains, and to reveal the underlying molecular basis for virulence differences between strains.

The overall objective of this project is to sequence the genomes of a diverse cross-section of *A. baumannii* isolates, representing both clinical and environmental isolates, collected and genotyped by the WRAIR Division of Bacterial & Rickettsial Diseases. Strains will be selected for genome sequencing based on patterns by Pulsed Field Gel Electrophoresis (PFGE), a standard genotyping method that uses gross genome structure to determine strain relatedness for strain phylogenies and can be used for molecular epidemiology, to make inferences about transmission patterns. Strains that represent major PFGE clusters and isolates with unique PFGE patterns will be chosen for genome sequencing at the JCVI MSC. We also propose that the sequencing of this diverse collection of *A. baumannii* strains be used within this project for the development of a comprehensive species microarray in collaboration with the PFGR at JCVI. Comparative genome hybridization may also be used to inform selection of strains for sequencing and also for the species array effort.

The genome sequencing component of this project is justified because despite the medical importance of this pathogen relatively few complete *A. baumannii* genomes have been made public (six). Thus, the genomic diversity within this species remains to be discovered. In addition, this is an opportunity to exploit a large collection of isolates of this species whose genetic diversity has already been analyzed. Thus, strains can be selected for DNA sequencing to provide a distributed representation of this diversity, including major PFGE clusters as well as apparently unique isolates. This approach will provide a wealth of molecular data for the general scientific community that will serve as

the catalyst for research to elucidate the mechanisms underlying the differences in virulence associated with different strains of MDR ABC. In addition, sequencing of a diversity of strains will also provide an opportunity to retroactively compare PFGE phylogenies with those generated from whole genomes to determine how well PFGE analysis represents total genetic relatedness.

The development of a species microarray from these genomic data is justified because its development and availability will provide the medical community with a new tool that can be used to make much more comprehensive comparisons of the genetic content of *A. baumannii* isolates, allowing for the collection of both molecular epidemiological data and also specific drug resistance gene profiling in a way that is not currently available. The US military is very interested in deploying such a tool for the comprehensive genetic characterization of *A. baumannii* and other MDR isolates for epidemiology and infection control purposes, and it is highly likely that the civilian healthcare world would also find value in a robust species array for *A. baumannii*.

The Division of Bacterial and Rickettsial Diseases is uniquely positioned to conduct this research as we have conducted PFGE analysis of *A. baumannii* isolates from wounded US soldiers in Iraq and casualty care facilities throughout the entire evacuation process as part of a Department of Defense Global Emerging Infections System (DoD-GEIS) funded project. This project includes alternative genetic analysis including microarray approaches. Genome sequencing is not funded in this project, but we could easily produce the genomic DNA preparations for genome sequencing within our current support. We have the capacity to select isolates, culture them and extract gDNA to provide to the JCVI MSC for sequencing. To date we have received and analyzed a diversity of military *A. baumannii* isolates since work commenced in 2003. We now have a collection of over 500 strains with PFGE data for each in our database. Though we have dates and places of isolation for many of these strains, they are not linked to identifiable patient data or human use protocols. In addition to clinical isolates, a number of environmental isolates from different geographic locations are included in our collection. Currently our PFGE analysis indicates that the WRAIR *A. baumannii* collection can be parsed into 22 clusters of strains. Some clusters have significant PFGE diversity within them, while others are more homogenous, containing near-clonal groups of strains. There is also a number of isolates that appear not to fall into these clusters, or appear only distantly related to other strains in the collection. To capture the diversity within this collection, we propose selecting up to three isolates from each PFGE cluster for sequencing, depending on the degree of pattern diversity within a cluster, and then to also select isolates with unique PFGE patterns. Using this approach, 30 to 75 different strains could be selected for sequencing, depending on the density of representation desired. We recommend that at least 50 strains be sequenced to cover the variation within some of the larger PFGE clusters. Both MDR

and susceptible strains should be represented, as well as environmental isolates. The Naval Medical Research Center has done sequencing of three strains that are in our collection. While incomplete, this sequencing will be taken into account; and strains closely related to these three will not be selected for sequencing in this project.

Nature, Availability & Source of Reagents/Samples:

WRAIR has a collection of over 500 *A. baumannii* isolates of which WRAIR will prepare purified genomic DNA from selected strains (30 – 75 strains, depending on agreed-to scope) and deliver to JCVI for sequencing.

Collaborator Role:

At WRAIR, Mikeljon Nikolich will work with Xiao-zhe Huang to select strains for sequencing based on their PFGE database. Dr. Huang will extract gDNA from the selected strains for JCVI. Dr. Patrick McGann of the WRAIR group will also work on the requested JCVI sequencing project on data analysis and microarray development.

NIAID's Genomic Sequencing Center Reagent, Data & Software Release Policy:

<http://www3.niaid.nih.gov/research/resources/mscs/data.htm>

Accept

DNA samples to be stored at JCVI. JCVI sequence data to be deposited in GenBank.

Investigator Signature: ~/please accept as electronic signature/~

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