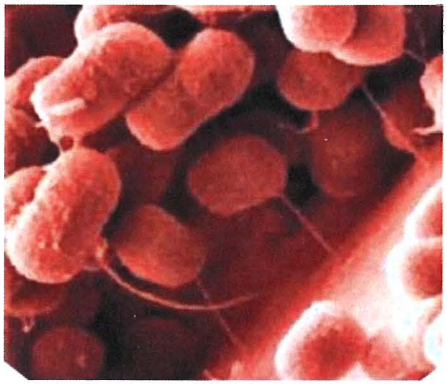
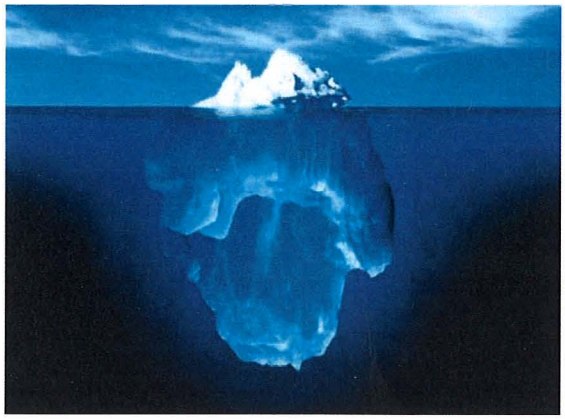
Acinetobacter baumannii:

Is this the Gram (-) MRSA?





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Dr. Hoopman is a faculty member in the Division of Pulmonary and Critical Care Medicine with a secondary appointment in the Department of Microbiology. Dr. Hoopman's research interests center on the study of *Moraxella catarrhalis* and the role it plays in causing human disease, specifically, exacerbations of chronic obstructive pulmonary disease (COPD) in adults and middle ear infections in children. His laboratory focuses upon the elucidation of the bacterial gene products of *M. catarrhalis* involved with the colonization of the living host and the bacterial mechanisms responsible for clinical disease. Recently, work in his laboratory has been initiated to gain a better understanding of the pathogenic mechanisms *Acinetobacter baumannii* utilizes to cause human disease.

This is to acknowledge that Dr. Hoopman has disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Hoopman will be discussing off-label uses in his presentation.

While *Acinetobacter baumannii* may not be the most common pathogen that clinicians associate with hospital-acquired infections, it has demonstrated an astonishing rise from obscurity to a position of prominence in the constant battle against microorganisms. The awareness of *A. baumannii* as a significant nosocomial pathogen has been steadily increasing over the past 15 years. A great deal of this interest has been fueled by the rapid emergence of multidrug resistant (MDR) strains that can result in epidemic outbreaks. Of greater concern are the numerous reports of *A. baumannii* isolates which are resistant to all available antibiotics (1;2). Initially ignored, *A. baumannii* has risen from the designation of a low-grade pathogen, to the status of a significant bacterium that can result in debilitating disease and death. **Several key characteristics and properties of *A. baumannii* have proven vital to its ability to cause disease.** These include: [1] the ability to rapidly acquire antibiotic resistance mechanisms, [2] growth in a diversity of habitats, [3] demonstration of survival despite prolonged periods of desiccation, and [4] the propensity to cause epidemic outbreaks of infection in healthcare facilities amongst the most-vulnerable patients (3).

The threat from Gram-positive pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), has dominated the medical arena for the past two decades. **It has been suggested that the medical establishment is far closer to the end of the antibiotic era with *Acinetobacter* spp. than with any Gram-positive pathogen** (4). With this in mind, the goals of this protocol are to highlight and discuss some of the factors involved with the rise of *A. baumannii* to local, national and international prominence, gain a better understanding of the mechanisms this organism utilizes to cause clinical disease, and describe the treatment modalities available to the practicing clinician.

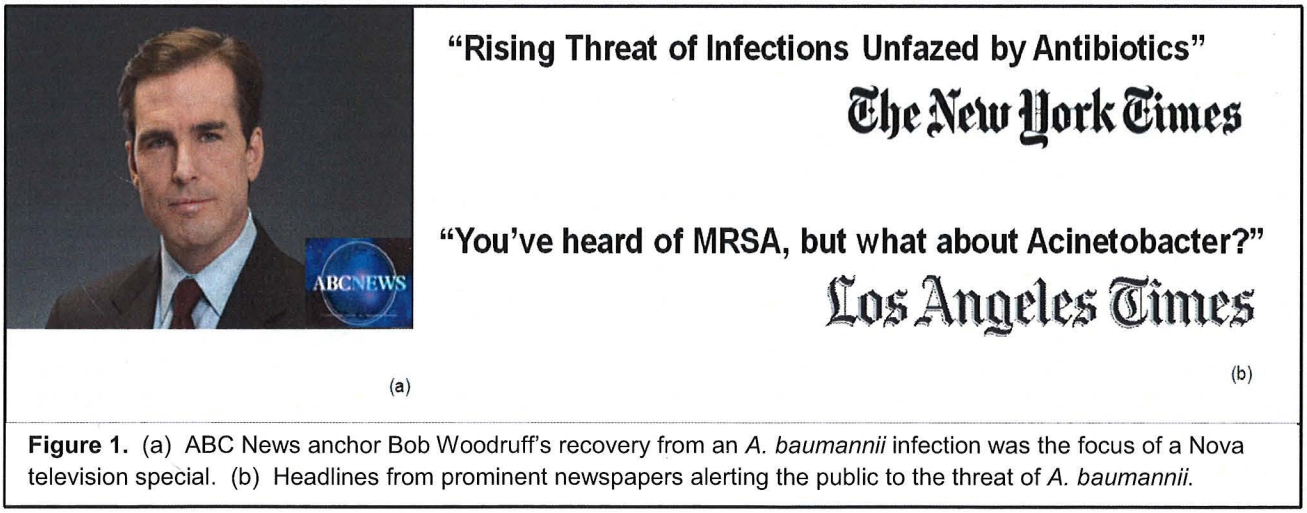

Figure 1. (a) ABC News anchor Bob Woodruff's recovery from an *A. baumannii* infection was the focus of a Nova television special. (b) Headlines from prominent newspapers alerting the public to the threat of *A. baumannii*.

Although *A. baumannii* has made the transition from an emerging to an established pathogen, it remains an obscure and often forgotten pathogen in the minds of clinicians. However, the public has taken notice and the lay press has begun to explore the effect of *A. baumannii* upon the health of Americans. Websites dedicated to 'alerting' the public to the rising health implications of *A. baumannii* infections are easily located with a Google search (www.acinetobacter.org and www.acinetobacterbaumannii.org). When ABC news anchor Bob Woodruff was injured on January 29, 2006 by a roadside bomb outside Bagdad, it was not the blast-related injuries that nearly took his life. Rather, it was the subsequent *A. baumannii* sepsis and ventilator-associated pneumonia that were the focus of the NOVA program detailing his fight for life against this pathogen. Leading newspapers such as the New York Times and the LA Times have recently published articles entitled 'Rising Threat of Infections Unfazed by Antibiotics' and 'You've Heard of MRSA, but what about Acinetobacter?', respectively. Websites, newspaper articles, and real-life stories from journalists have elevated *A.*

baumannii from its status as an obscure and often ignored bacterium to the forefront of the medical arena and the frontpages of our newspapers.

Despite the increased clinical interest in *A. baumannii* and recognition that this organism is responsible for significant morbidity and mortality amongst some the most medically-fragile patients, the bacterial factors that allow this organism to assume a pathogenic role are at best, incompletely understood. A great deal of scientific work and literature has been published about the antibiotic resistance mechanisms *A. baumannii* exploits to avoid control by antimicrobials, but there has been a paucity of research about the virulence factors and bacterial mechanisms this organism utilizes to cause human colonization and infection.

I. Background

A. baumannii began to spread through intensive care units (ICUs) and hospitals in the United States during the 1980s (5). A literature review of *Acinetobacter* outbreaks performed between 1977 and 2000 showed the majority of the reported cases to have occurred during the 1990s (6). The increase in reported cases in the 1990s that has extended into the 21st century is likely a result of several factors. Microbiological laboratories have become more sophisticated and have additional molecular techniques at their disposal to more successfully isolate and accurately identify *Acinetobacter* spp. Additionally, increased knowledge about the organism's pathogenic potential has helped prevent clinicians from dismissing a positive culture result as a possible contaminant. The most concerning factor playing a role in the increase of reported cases of *A. baumannii* is the ability of this organism to rapidly adapt to selective antibiotic pressure and develop resistance to nearly all modern day antibiotics. **As a result, there are simply greater numbers of *A. baumannii* organisms in the modern clinical environment.**

Taxonomy Unfortunately, as is often the case with underappreciated pathogens, *Acinetobacter* organisms are plagued by a confusing taxonomic history. Since its discovery, it has been categorized within multiple different families and received numerous different species' names, including *Moraxella, Herelea, Mima, Achromobacter, and Alcaligenes* (7). There are currently 32 genomic *Acinetobacter* species as determined by DNA-DNA hybridization techniques, of which 17 have been formally named (7). Members of this species include *A. calcoaceticus, A. lwoffii, A. baylyi,* and perhaps the most recognized, *A. baumannii.* The three species of greatest clinical interest from the *Acinetobacter* spp. complex include *A. baumannii, A. calcoaceticus,* and their close relatives, genomic species 3 and 13TU (7-10). Due to difficulty with common laboratory and microbiological procedures used to differentiate between these three species, the vast majority of positive culture results of organisms in this group are simply reported as *A. baumannii* or *A. baumannii – A. calcoaceticus* complex. The overwhelming majority of hospital-acquired infections are due to of members of the *Acinetobacter baumannii-A. calcoaceticus* complex (11).

A. baumannii is a strictly aerobic, non-motile, Gram-negative coccobacillus that grows in pairs or in chains. Its name comes from the Greek adjective 'akinetos' meaning "unable to move" and the Greek noun 'backterion' for "rod" (7). *A. baumannii* is listed as one of the **ESKAPE** pathogens (see Table 1) by the Infectious Diseases Society of America (IDSA). Pathogens in this group include ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumonia,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa,* and ***E****nterobacter* species, and are classified by the IDSA as some of the most challenging pathogens facing clinicians today (12). **According to the IDSA these pathogens cause the majority of US hospital infections, and as a result of multi-drug resistance mechanisms, effectively "escape" from antimicrobials (See Table 1).**

The "ESKAPE" Pathogens
E *nterococcus faecalis*
S taphylococcus aureus
K lebsiella pneumonia
A *cinetobacter baumannii*
P *seudomonas aeruginosa*
E *nterobacter*

Table 1. The Eskape pathogens as described by the IDSA (Boucher, H.W., et. al. *Clin Infect Dis (48)*, 1-12 (2009).

Oftentimes, the therapeutic options available to treat these pathogens are so limited that physicians are being forced to use older, previously abandoned drugs, to treat life-threatening infections. In addition to undesired toxicity issues associated with the use of these older medications, there is often little data available to help guide dosing regimens and duration of therapy (12). While *A. baumannii* is not the most prominent member of ESKAPE list, it has already demonstrated a remarkable ability to adapt itself in ways that are certain to continue to elevate it from its previous status as a mere contaminant to a position of national and international importance (7).

There are several misconceptions permeating the medical literature with regards to *A. baumannii.* These include: [1] *A. baumannii* is ubiquitous in nature, [2] *A. baumannii* can be recovered from soil, water and animals, and [3] *A. baumannii* is a frequent skin and oral colonizer of humans. It is important to note that these three generalizations can be applied to the genus *Acinetobacter* as a whole but that the species most responsible for human disease and nosocomial infection, *A. baumannii*, does not typically demonstrate these particular characteristics. In fact, *A. baumannii* has no known natural habitat outside of the hospital or clinical arena (13).

Natural Habitats of *Acinetobacter* species: The majority of *Acinetobacter* species have been found in various clinical environments and isolated from different patient specimens, however, not all of these isolates are considered to be clinically significant. Many have questioned if there are environmental or community reservoirs for the clinically-significant *Acinetobacter* strains. *Acinetobacter* spp. as a group have been described as ubiquitous organisms in nature and have been recovered from soil, water, plants, animals and humans (13). As mentioned above, the early literature describing *A. baumannii* as a ubiquitous organism in the natural environment must be viewed cautiously given that many of these reports did not differentiate between *Acinetobacter* spp. as a whole and the pathogenic members of the *A. baumannii-A. calcoaceticus* complex.

While the presence of *Acinetobacter* spp. organisms in the natural environment has been demonstrated, a great deal of work has attempted to elucidate the link between nature and the hospital environment. Specifically, research has focused upon mechanisms by which *Acinetobacter* spp. have made the transition from a natural environmental pathogen to one which can colonize, and in many cases, be responsible for epidemic spread in the healthcare environment. Researchers have speculated that a connection between contaminated food sources and hospital-acquired infections may exist. Rigorous examinations attempting to link *A. baumannii* isolated from fruits and vegetables in the hospital to actual patient colonization or infection have not been successful. A 1993 study of meats (fresh and spoiled), fish, vegetables, raw milk and cheese showed that more than half of the *Acinetobacter* isolates were *A. lwoffii* and less than 5% were *A. baumannii* (14). In a similar study from Hong Kong, researchers were able to recover *Acinetobacter* organisms from more than half of the sampled fruits, vegetables, fish and meats. As seen previously, only a small minority (<5%) of the isolates were *A. baumannii* (15). However, 33% of the isolates obtained from the vegetables belonged to the *Acinetobacter baumannii-calcoaceticus* complex, specifically genomic DNA group 3, which has been shown to contain *Acinetobacter* species responsible for clinical infections (15). A 1999 study of fresh fruits and vegetables obtained from local markets and private gardens demonstrated that 17% of the produce samples grew small numbers of *Acinetobacter* spp., with 56% of the isolates belonging to the *Acinetobacter baumannii-calcoaceticus* complex (16). Although there may be a link between fresh produce and *A. baumannii,* studies have not been able to establish this as an indentified source for hospital-acquired infections.

Gastrointestinal (GI) colonization by *A. baumannii* has been reported as a potential source for hospital-acquired infection in susceptible patients. A 1993 report described the GI tract (68% of the cohort) as the initial site of colonization in the majority of the patients found to harbor *A. baumannii* (17). In a 1996 study of intensive care unit (ICU) patients at a 1,000-bed teaching hospital in Spain, fecal colonization by MDR *A. baumannii* was found in 41% of patients studied and more than two thirds of the positive samples were obtained from patients during the first week of their hospital stay, indicating that many of them were already colonized by MDR *A. baumannii* strains at the time of admission (18). In that

study, rectal swabs from 25% of the patients were positive for MDR *A. baumannii* within the first 48 hours. Surprisingly 8 of the patients found to be colonized within the first 48 hours of their admission to the ICU were admitted from the community and did not have a history of prior hospital admissions (18).

The lack of a simultaneous community-based outbreak of MDR *A. baumannii* in the same region of Spain led the investigators to conclude that *A. baumannii* demonstrates an extraordinary level of contagiousness amongst hospitalized ICU patients and possibly has the ability to colonize susceptible, asymptomatic individuals in the community. Finally, there was a significantly greater number of clinical infections due to MDR *A. baumannii* reported for the patients found to have fecal colonization as opposed to patients that did not demonstrate colonization (26% vs. 5%, $p<0.001$) (18). Remaining free of fecal colonization by MDR *A. baumannii* was shown to correlate inversely with the ICU length of stay, with the likelihood of remaining free of MDR *A. baumannii* fecal colonization on the 30th day of an ICU stay at less than 25% (See Figure 2) (18). These findings led the authors to conclude that GI tract colonization could serve as a potentially important epidemiologic reservoir for MDR *A. baumannii* hospital-associated outbreaks (18).

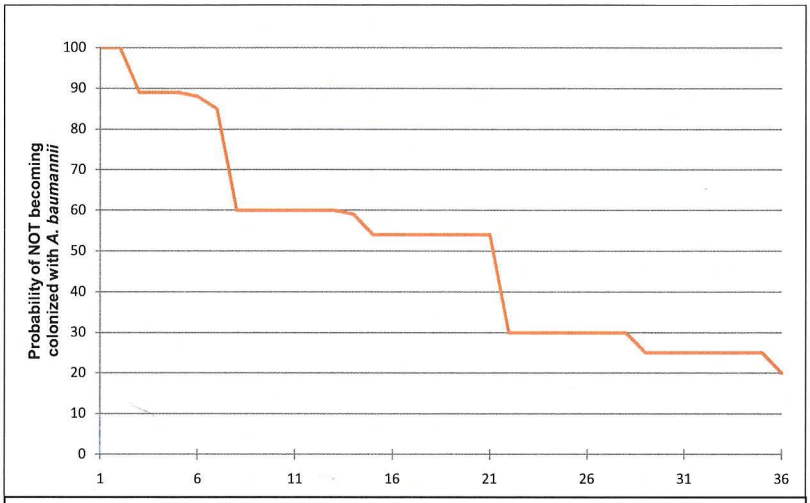

Figure 2. Association between length of ICU stay and probability of **NOT** becoming colonized with *A. baumannii* (adapted from Corbella, et al, CID (23), 319-344 (1996).

The possibility of vector transmission of *A. baumannii* has been raised by a recent study that found 40 different *Acinetobacter* strains on the body lice of homeless individuals in France (19). Additional analysis by the same researchers showed that *A. baumannii* DNA could be detected in 21% of 622 lice specimens collected worldwide (19). These findings led the authors to conclude that an *A. baumannii* epidemic exists in human body lice and may serve as an important source for human *A. baumannii* colonization and infection.

Typically, Gram-negative organisms are not known to be skin colonizers. Despite this, *Acinetobacter* spp. have been isolated from the skin of healthy human volunteers. The majority of the skin isolates are not *A. baumannii*, but rather are other members of the *Acinetobacter* genus (20). A study of skin samples taken from 192 healthy volunteers demonstrated that 40% of the subjects had *Acinetobacter* spp. isolated, with the majority being *Acinetobacter lwoffii*, and only one isolate of *A. baumannii* (21). However, skin colonization by *Acinetobacter* spp. is often more prominent within the hospital environment. *Over* 75% of patients in a German hospital and 42% of the community controls were colonized with *Acinetobacter* spp. The majority of the isolates were, again, *A. lwoffii* and *A. johnsonii* (47% and 21%, respectively). *Acinetobacter baumannii* accounted for less than 2% of the isolates in this study (22). This finding is in contrast to types of isolates that can be obtained during a clinical outbreak in the healthcare setting. Epidemic strains of *A. baumannii* are found at a much higher frequency from patient skin and rectal samples during an outbreak when compared with non-outbreak or community controls (23). Clearly, these data have strengthened the belief that *Acinetobacter* spp. as a whole are ubiquitous in nature and can transition between colonization of soil and water environments to the skin of humans. Unfortunately it has proven difficult to precisely determine the natural reservoir of *A. baumannii* outside of the hospital setting.

At this time there are three major groups of bacteria represented in the genus *Acinetobacter.* These include:

(1) isolates of *A. baumannii* that can colonize and infect at-risk hospitalized patients (typically multidrug resistant)
(2) 'sensitive' isolates that are part of the normal human and animal skin flora as well as fruits and vegetables (non-*A. baumannii* strains)
(3) 'sensitive' isolates found in environmental sources including soil and waste water (non-*A. baumannii* strains)

For the purposes of this discussion, *A. baumannii* and members of the *A. baumannii-A. calcoaceticus* complex will remain the primary focus of the protocol.

Clinical Habitats of *A. baumannii*: The colonization of the hospital environment and the susceptible hospitalized patient by *A. baumannii* is a sentinel event in the pathogenic lifestyle of this organism. **In fact, it is this remarkable ability to survive on hospital surfaces that has helped *A. baumannii* gain a foothold in the clinical arena, propagate epidemic outbreaks, and establish itself as a significant pathogen worldwide.** While many of the mechanisms responsible for this colonization event remain to be elucidated, it is presumed that some of the same bacterial factors that allow this pathogen to survive in the natural environment (soil, plants, water) have been exploited by *A. baumannii* to colonize the types of surfaces found in the hospital, including keyboards, counters, and bedrails (24;25). The list of hospital sources from which *A. baumannii* has been isolated is exhaustive and partially delineated in Table 2 (26).

Sources from which *A. baumannii* has been isolated in the hospital environment		
▪ Hands of staff	▪ Keyboards	▪ Pillows
▪ Ventilator tubing	▪ Service ducts	▪ Bed linens
▪ Gloves	▪ Sinks	▪ Soap dispensers
▪ Bronchoscopes	▪ Humidifiers	

Table 2. Hospital sources from which *A. baumannii* has been isolated (Towner, K.J. *J Clin Infect.* 73, 355-363 (2009).

Survival on Dry Surfaces One of the primary strategies utilized by *A. baumannii* to cause recurrent outbreaks is long-term colonization and persistence on dry hospital surfaces. The vast majority of these surfaces are abiotic and do not possess the types of nutrients that are typically necessary for bacterial survival. The duration of survival of *A. baumannii* after drying on glass cover slips has been shown to be similar to rates of survival demonstrated by *S. aureus* exposed to the same conditions (24). Others have shown that *A. baumannii* can survive on a broad range of inanimate surfaces for prolonged periods of time, including 13 days on formica (27;28), 6 days on dry filter paper (29), and more than 25 days on cotton (30). There have been limited reports of strains of *A. baumannii* persisting on hospital surfaces for up to 3 years (23;25). Interestingly, *A. baumannii* strains have been shown to survive desiccation much better than several of the other *Acinetobacter* strains including *A. johnsonii, A. junii, and A. lwoffii (24;31).* This differential survival ability may provide insight as to why these other *Acinetobacter* spp. have not emerged as significant clinical pathogens and may serve as a target for future study. A highly relevant correlation to modern hospital care is the finding of contamination of computer keyboards by *A. baumannii.* Not surprisingly, those keyboards located in close proximity to patients (i.e. at the bedside) have shown a higher rate of contamination by *A. baumannii* than those located further away from patient care areas (32). This finding has implications for clinical facilities that have transitioned to an electronic medical record and emphasize electronic data entry in close proximity to the patient.

Other sources identified as sites of colonization are logical given their proximity to patients and their utilization for patient-care activities, including: (1) ventilators and tubing, (2) bronchoscopes, (3) bed frames, and (4) blood pressure cuffs. There have even been descriptions of the isolation of *A. baumannii* from the inside of soap dispensers (containing 2% chlorhexidine gluconate) (33). However, other sites that are not in direct contact with patients or patient material can be contaminated with *A. baumannii.* These would include countertops, sinks, and cellular telephones (26). This finding lends support to the idea that members of the healthcare team (doctors, nurses, therapists) likely serve as conduits for the spread of *A. baumannii* among patients in different parts of the hospital and between multiple hospitals within a city or region.

Finally, the survival of *A. baumannii* strains appears to correlate with the environmental or hospital source from which it is isolated. Strains found on wet sources did not survive desiccating conditions as well as those isolated from dry sources. This finding likely indicates that some *A. baumannii* strains are able to utilize and exploit surface-specific survival strategies when confronted with variable environmental conditions. It is assumed that *A. baumannii* must demonstrate differential expression of bacterial gene products in order to survive upon such a broad range of abiotic surfaces (34). **Unfortunately there have been no rigorous studies aimed at elucidating the bacterial gene products responsible for this bacterium's remarkable persistence abilities when faced with desiccating, nutrient poor conditions.**

The ability of *A. baumannii* to survive desiccating conditions and be recovered from abiotic surfaces including plastic, formica, curtains and rubber, serves as an important reminder that successful eradication of epidemic strains from the hospital environment requires extensive and thorough cleaning of all hospital surfaces that have been in contact with an infected patient or with healthcare providers who have cared for colonized or infected patients. In some cases measures beyond standard cleaning and disinfection practices have been implemented. A reported outbreak in a burn unit required changing the curtains twice a week to control the spread of *Acinetobacter (35)* and there are reports of entire ICU facilities being remodeled and/or demolished in order to eradicate an endemic strain of *A. baumannii.*

Airborne Dissemination of *A. baumannii* There have been numerous studies that have described the aerial dissemination of *Acinetobacter* spp. and the role this plays in infection. The earliest description of *Acinetobacter* being spread via airborne mechanisms centered on the investigation of an outbreak of MDR *Acinetobacter anitratus* within an ICU, medical ward, and 3 neurosurgical wards simultaneously. Settle plates were utilized to culture the particulate matter in the environmental air and *A. anitratus* was found on 16 of 82 settle plates (29). Researchers went on to postulate that perhaps the similar size and shape of *Acinetobacter* spp. to *Staphylococcus* spp., in addition to its ability to survive dessicating conditions, may contribute to the aerial spread of this organism. One of the earliest reports of carbepenem-resistant *A. baumannii* in Europe described the persistence of the organism for up to four months on the patient curtains with subsequent airborne spread when the curtains were moved or disturbed (35). Houang and colleagues reported in 2001 that 96% of settle plates from the ICU and 89% of settle plates from the general surgical wards yielded *Acinetobacter* colonies (15). Additional data from this outbreak demonstrated nearly 600 *Acinetobacter*-carrying particles settled per m^2 per hour (15), whereas previous levels had only been noted to be approximately 60 particles per m^2 per hour (36).

Perhaps the strongest evidence of airborne spread of *A. baumannii* originates with a study detailing an outbreak in 3 Dutch hospitals. Two of the hospitals placed their source patients in non-pressurized isolation rooms while in the third hospital, the infectious patient was isolated in a negative pressure room. The settle plates inside and outside the negative pressure room did not grow the outbreak strain while those located in similar locations for the non-pressurized isolation rooms grew the outbreak strain (37). The ability for an endemic strain of *A. baumannii* to spread via airborne routes has significant implications for the isolation of colonized and infected patients as well the types of measures that must be undertaken when cleaning hospital areas housing these patients.

II. *A. baumannii*-Associated Mortality

During the past two decades there has been a belief among clinicians that infections due to *A. baumannii* are not associated with considerable or increased patient mortality (3). However, as more precise and detailed studies utilizing case-controlled cohorts have emerged, it has become clearer that infection with *A. baumannii* does carry an increased mortality risk. Colonization alone by *A. baumannii* has been shown to be associated with an increased mortality rate. A one year, prospective observational survey evaluated the clinical effect of salivary or rectal carriage of *A. baumannii* and/or *Klebsiella pneumoniae* in ICU patients. 33% of the surveyed patients showed carriage of either pathogen and the mortality was significantly higher in the carriage group compared with the non-carriage group (43% vs. 25%, $p<0.001$) (38). While some debate still exists regarding the attributable mortality of *A. baumannii* infections in hospitalized patients, there have been several retrospective and prospective cohort studies that have demonstrated increased mortality rates with ventilator-associated pneumonia (VAP) due to the non-fermentative Gram-negative pathogens as a group. Kolef and colleagues noted that VAP due to non-fermenting Gram-negative rods, such as *A. baumannii*, was an independent risk factor for increased hospital mortality (39). Specifically, this study demonstrated a 65% associated mortality rate when *A. baumannii* was the source organism for the VAP (39).

A recent systematic review of matched cohort and case-control studies that attempted to better understand the attributable mortality of *A. baumannii* infections in ICU patients suggested that infection with *A. baumannii* was associated with increased mortality (40). However this analysis has been faulted due to the inclusion of studies with small sample sizes and an inability to adequately pool the data because of heterogeneity among the trials with regards to site(s) of infection, the population of patients studied, and non-similar matching criteria (40). However, a review by these same authors of four more-recently published case-control studies, has helped to better define the attributable mortality of *A. baumannii* infections and helped to end the controversy surrounding this pathogen's contribution to the mortality rates of infected, critically-ill patients (41). The attributable mortality of an *A. baumannii* infection ranged between 8.4% and 36.5% in this study, a statistically significant increase when compared with case-matched controls (41).

A study of Gram-negative blood stream infections (BSI) in 49 US hospitals showed a similar mortality rate between patients with BSI due to *A. baumannii* (31.5%) and other Gram-negative pathogens (27.9%) (42). This finding led the authors to conclude that *A. baumannii* bacteremias are as severe as other well-described Gram-negative bacteremias and can result in significant mortality. Others have shown that *A. baumannii* bacteremia results in an increased odds ratio of death when compared with bacteremia due to *Klebsiella pneumoniae* (43) or *Pseudomonas aeruginosa* (44) and helps to strengthen the belief that infections with *A. baumannii* are a significant issue facing today's hospitalized patient and should be addressed with the same vigor that bacteremias due to other Gram-negative pathogens receive.

A. baumannii infection has been shown to be associated with a prolonged length of hospitalization and increased morbidity. A retrospective, matched cohort study of patients with *A. baumannii* bacteremia demonstrated a 5-day excess length of mechanical ventilation and ICU stay when compared with critically ill patients without an *Acinetobacter* infection (45). Additional studies have shown that multi-drug resistant *A. baumannii* infection can prolong the ICU stay (46) in addition to the median duration of hospitalization (47). Several studies have shown an increase in mortality when inappropriate initial therapy was utilized for *A. baumannii* infections (48;49). **Importantly, these data highlight the fact that infections with this organism require early and appropriate therapy to help reduce the duration of hospitalization, the transmission to other patients, and mortality rates amongst those patients infected with *A. baumannii*.**

III. Epidemic Spread of *A. baumannii*

Many different studies have examined the patterns of spread of *A. baumannii* in the healthcare arena and there are reports detailing the epidemic spread of MDR *A. baumannii* throughout hospitals and across entire cities, countries, and continents (4;33;50;51). In order to more fully appreciate this organism's potential for epidemic spread it is necessary to examine several of the key outbreaks of *A. baumannii* that have occurred in the recent past.

U.S. Military Experience The outbreak of MDR *A. baumannii* amongst U.S. soldiers garnered a significant amount of press and helped bring this pathogen to the forefront of both the medical and the popular literature. Beginning in 2001, reports began to emerge about a surprising increase in the numbers of wounded U.S. military personnel returning of the theatres of operation in Afghanistan and Iraq who developed MDR *A. baumannii* infections (52). This was not the first time an outbreak of *Acinetobacter* had occurred amongst wounded soldiers. A similar outbreak seen with wounded Marines during the Vietnam War was attributed to the colonization of wounds by environmental sources (soil and plant material) at the time of the blast injury (53). In fact, *Acinetobacter* spp. were the most frequently-recovered Gram-negative organism in the war wounds of U.S. Marines with extremity injuries during the Vietnam War and the second most common cause of Gram-negative bacteremia in this same patient population (54). However, stringent microbiologic testing and taxonomic analysis was not carried out at that time to determine if the isolates belonged to the *A. baumannii-A. calcoaceticus* complex or one of the other described environmental species. However, the more recent outbreak amongst wounded soldiers deployed to the Middle East has been conclusively attributed to *A. baumannii*.

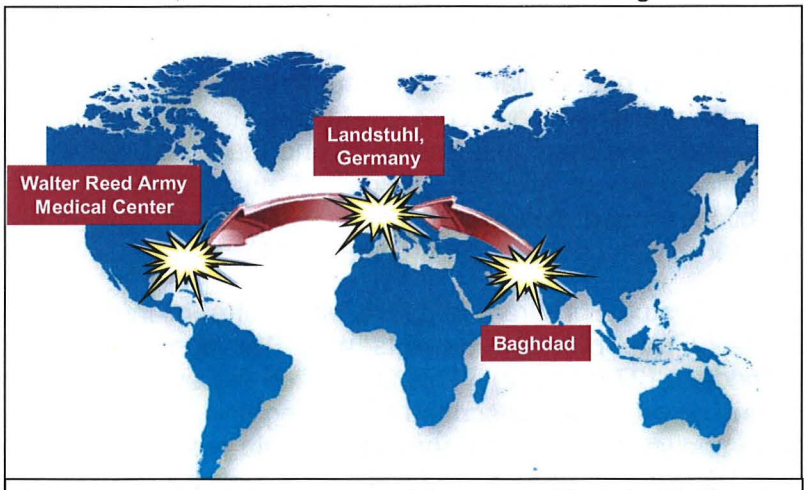

Figure 3. Route of dissemination of *A. baumannii* from the Middle East theatres of operation.

By 2003 physicians at Brooke Army Medical Center (BAMC) in San Antonio, TX were detailing the dramatic rise in the number of soldiers they were treating with wound complications, including osteomyelitis and soft tissue infections, due to *A. baumannii*. A study during that time of 237 active-duty soldiers admitted with the diagnosis of 'injury' showed that 64% of them had been deployed to the Middle East regions of conflict. In that group of injured soldiers, 32% were positive for *A. calcoaceticus-baumannii* complex. Two-thirds of these cases represented infection and the remaining one-third were consistent with colonization (54). In the 14 months prior to the study only 2 active-duty soldiers, of 326 admitted to BAMC, had any evidence of *A. baumannii* infection. This represented a significant increase in the incidence of infection during the study period and alerted physicians to an epidemic outbreak (54). Initially, researchers felt that the source of these infections was pre-injury skin colonization of the soldiers or introduction of the organism from the soil at the time of injury. Extensive epidemiologic studies and field testing of soil samples eventually proved this theory to be incorrect. *A. baumannii* was present in only 1 of 49 (2%) soil samples collected and only 1 of 160 (0.6%) patients that were screened at the time of injury (55). However, *A. baumannii* was isolated from 7 out of 7 battle zone field hospitals utilized for the initial triage and stabilization of wounded soldiers (55). As a result of these findings, it is now accepted that the source of the outbreak of *A. baumannii* among soldiers repatriated from Afghanistan and Iraq was, in fact, contaminated hospital surfaces and patient care equipment in the field hospitals. **The experience with MDR *A. baumannii* infecting soldiers whom had served in the Middle East regions of conflict earned this pathogen the popular nickname 'Iraqibacter.'**

Epidemic Outbreaks in the United States and Europe *A. baumannii* has been shown to be responsible for multifacility outbreaks in both Europe and the United States. A large outbreak of a highly drug-resistant strain of *A. baumannii* occurred across France in 2003-2004. 290 cases of infection and/or colonization of *A. baumannii,* were detected in 53 healthcare facilities throughout the country (56). During the French outbreak, pulsed-field gel electrophoresis was performed upon multiple isolates and showed identical restriction profiles, consistent with a clonal outbreak of this MDR strain of *A. baumannii* (3). This particular strain harbors the ^{bla}VEB-1 extended-spectrum β-lactamase and proved to only be susceptible to imipenem, ticarcillin-clavulanate, and piperacillin-tazobactam. Investigators believed that the transfer of patients between facilities and patient movement within healthcare facilities helped to spread this epidemic strain across France. Similar outbreaks of clonal strains of *A. baumannii* have been reported in Spain, South Africa, Greece, Italy, Turkey, and the Netherlands (51).

Dramatic hospital outbreaks have been reported in various cities and hospital systems across the United States. The ability of a single strain of *A. baumannii* to be the cause of a clinically significant outbreak was recently demonstrated in Chicago and northwestern Indiana (57). This carbapenemase-producing strain has been responsible for infections in at least five hospitals and three long-term care facilities, with more than 200 patients affected. A similar multiple healthcare facility outbreak due to several endemic strains of MDR *A. baumannii* was reported in Brooklyn, NY between July and September 1999 (33;58;59). During this outbreak more than two thirds of the isolates were represented by one strain. Additionally, 53% of the identified isolates were resistant to carbepenem antibiotics and surprisingly, 12% of the identified isolates were resistant to all standard antibiotics (33). Analysis of these isolates demonstrated a significant association ($p=0.004$) between prior cephalosporin usage and subsequent carbapenem resistance (33). As was seen with the European outbreaks of clonal *A. baumannii* infections, once a hospital environment is colonized with an endemic strain it can prove very difficult to eradicate the bacterium. During the Brooklyn, NY outbreak, surveillance cultures were positive for carbepenem-resistant *A. baumannii* at 7 of the 10 participating hospitals with contamination of bed rails, respiratory equipment and soap dispensers (containing 2% chlorhexidine gluconate) (33).

Natural Disasters Devastating earthquakes often serve as unfortunate mechanisms by which the study of infectious diseases and in particular, wound contamination, can be undertaken. On August 17, 1999 a catastrophic earthquake struck the Marmara region of Turkey. With a 7.4 rating on the Richter scale, this disaster reportedly claimed the lives of 17,480 individuals and wounded 43,953 additional people (60). The majority of injuries were due to crush mechanisms and resulted in open, contaminated, and often necrotic wounds. A significant number of the injured residents required amputations, fasciotomies, and long-term hospitalizations.

Several interesting epidemiologic patterns emerged from the analysis of the bacterial infections that occurred as a result of this disaster. A study of patients with acute renal failure (secondary to rhabdomyolysis from crush injuries or hypovolemic shock) showed that the most-commonly isolated bacteria from the blood of patients with sepsis were *Acinetobacter* spp. (60). There were twice as many *Acinetobacter* spp. isolated during septic events than *Pseudomonas* spp. or MRSA (60). Unfortunately the authors did not further speciate the *Acinetobacter* organisms that were recovered. Examination of the microbiological isolates associated with hospital acquired infections (HAI) in a large Turkish hospital that served as a tertiary medical center for those wounded in the earthquake showed that *A. baumannii* was the most-frequently isolated bacterium from wound and blood cultures (61). *A. baumannii* accounted for 31.2% of the HAI pathogens and nearly twice the number of isolates when compared with MRSA, *P. aeruginosa*, or *E. coli*. Prior to the earthquake the prevalence of *A. baumannii* in this Turkish ICU had only been 7.3% of isolates (61).

Following the tsunami catastrophe in Asia on December 24, 2006, facilities in Stockholm, Sweden, which received evacuated patients, reported increased numbers of *Acinetobacter* spp. isolates and the presence of *A. baumannii* isolates that were resistant to all available antibiotics (62). Additionally, data gathered retrospectively from a hospital treating patients injured in the 2005 Pakistan

earthquake which killed more than 82,000 people and injured more than 3.3 million people, showed a significant change in the microbiological isolates from the wounds of hospitalized victims. The incidence of *A. baumannii* wound isolates increased from 5.7% to 15.8%, and 92% of the *A. baumannii* isolates found in the hospitalized patients were MDR (63).

Several factors were felt to be contributing to this rise of *A. baumannii* HAI including: [1] injured and ischemic tissues are more easily colonized as a result of reduced host barrier functions, [2] less than ideal wound care under harsh conditions, and [3] widespread use of prophylactic and therapeutic antibiotics (64). The use of inappropriate empiric antibiotic therapy has been shown to be a risk factor for the spread of highly-virulent forms of *A. baumannii (64)*. In fact, physicians caring for these injured patients saw the rapid emergence of MDR strains of *A. baumannii* after the earthquake and it is generally accepted that this bacterium's ability to rapidly adapt to antibiotic selective pressure is enhanced by the wide-spread (and often non-specific) use of broad spectrum antibiotics after a natural disaster.

The polymicrobial nature of complicated wounds due to injuries sustained during earthquakes or tsunamis may be directly attributing to the acquisition of drug resistance mechanisms. As will be discussed later, *A. baumannii* demonstrates a remarkable ability to acquire the antibiotic resistance mechanisms utilized by other bacterial species. Therefore it is reasonable to conclude that polymicrobial traumatic wounds can facilitate the rapid and efficient transfer of these resistance mechanisms between the different bacterial species. Combine this finding with the often frantic usage of broad-spectrum antibiotics for shortened periods of time or at inadequate dosages and a near "perfect storm" is created for the development of MDR *A. baumannii*. The recent devastating earthquake in Haiti has the potential to serve as a nidus for additional drug-resistant forms of *A. baumannii* due to the high numbers of crush injuries and the usage of antibiotics at less-than optimal doses or for shorter than-desired periods of time due to logistical and financial constraints.

IV. *A. baumannii* Clinical Infections

The colonized to infected patient ratio for *A. baumannii* (10:1 ratio) has been reported to be much higher than that seen with MRSA (2:1 ratio) (5). Therefore, patients that actually demonstrate a clinical infection due to *A. baumannii* likely represent only the 'tip of the colonization iceberg'. **In fact, at the time a clinical diagnosis of *A. baumannii* infection is made it is likely that significant, unobserved dissemination throughout the clinical unit has already been occurring unbeknownst to clinicians and the possibility for an outbreak is high (5).** Stringent infection control measures must be instituted to prevent the spread of the organism to other vulnerable and at-risk patients. Currently, *A. baumannii* is estimated to be responsible for 9-10% of all nosocomial infections with the majority of these infections occurring in the respiratory tract, bloodstream, and open wounds (65).

Common Risk Factors Associated with an *A. baumannii* Infection
ICU admission
Recent surgery
Central vascular catheterization
Tracheostomy
Mechanical ventilation
Enteral feedings
Warmer climates
Treatment with 3rd generation cephalosporins, fluoroquinolones, or carbapenems

Table 3. Common risk factors associated with an *A. baumannii* infection (Munoz-Price LS, et. Al. N Engl J Med, (12), 1271-1281 (2008).

Risk Factors for *A. baumannii* Infection The majority of *A. baumannii* infections occur in hospitalized patients, typically those in the ICU or residents of long-term care facilities (66). Some of the patient-associated risk factors for *A. baumannii* infections are summarized in Table 3. **These risk factors are dominated by three common themes.** First of all, the majority of patients are either extremely ill and in an intensive care unit or are chronically ill and currently receiving medical treatment in a long-term care facility. Additionally, a common association between these infected patients includes the presence of multiple indwelling medical devices such as endotracheal tubes, central venous catheters, and urinary catheters. As has been demonstrated with other hospital pathogens, infection of a susceptible patient by a colonizing bacterium often occurs once a protective physical barrier is traversed or damaged. Finally, many of the patients with *A. baumannii* infections have been previously treated with broad-spectrum antibiotics and are at a much higher risk for developing an infection due to MDR *A. baumannii.*

In addition to these patient-centered risk factors, there have been several studies demonstrating a trend towards increased numbers of *A. baumannii* infections in the warmer months (July through October) (See Figure 4).

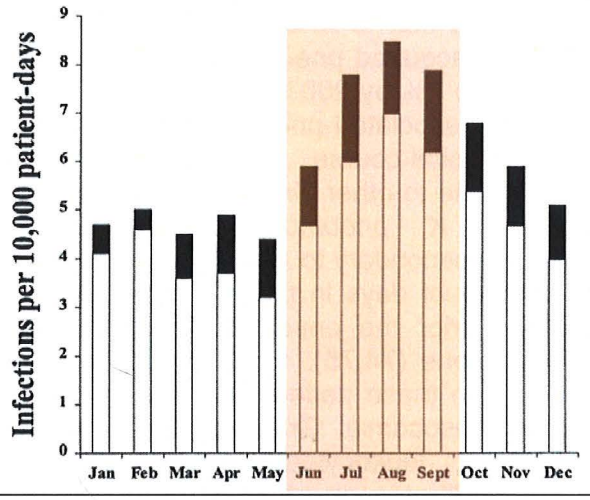

Figure 4. Monthly incidence of *A. baumannii* infections in the intensive care unit, National Noscomial InfectioSurveillance System. Data collected 1987-1996 (McDonald LC, et. al., Clin Infect Dis (29), 1133-1137 (1999).

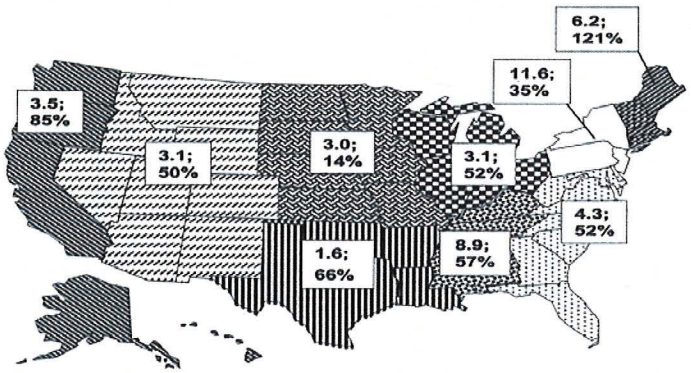

Figure 5. Incidence density of *A. baumannii* infections (per 100,000 patient-days), by region and percentage of increase from period of November-June to that of July-October, National Nosocomial Infection Surveillance System, 1987-1996 . (McDonald LC, et. al., Clin Infect Dis (29), 1133-1137 (1999).

It has been hypothesized that the higher number of infections in warmer months may be the result of increased environmental growth of *Acinetobacter* spp. associated with the warmer temperatures (67). Others have suggested that the warmer weather may promote the formation of *Acinetobacter* spp. biofilms in hospital tap water (68) and thereby serve as a reservoir for future infections in at-risk patients. Finally, investigators have speculated that changes in outdoor humidity (particularly in warmer months) may play a role in the contamination and colonization of air-conditioning and ventilation systems by *Acinetobacter* spp. (67). Studies demonstrating the ability of *Acinetobacter* to be found in droplet form in close proximity to infected patients have served as an argument for the potential spread of this bacterium from contaminated ventilation systems (15;35;36;69;70). **However, there have been no specific studies detailing the mechanisms that are responsible for increased numbers of infections in the warmer months.** Data from the National Nosocomial Infection Surveillance System shows that there was a 66% increase in the number of reported *A. baumannii* infections in Texas during warm months. The increased rates of infection during warm months are not only seen in Southern states. In fact, during the survey period, one of the largest increases (121%

higher) was demonstrated in the upper New England states (Maine, Vermont, New Hampshire (see Figure 5).

The most frequent clinical manifestations of *A. baumannii* infection are ventilator-associated pneumonia (VAP), soft tissue and wound infections, urinary tract infections, and bloodstream infections (BSI) (See Figure 6). The presence of endotracheal and tracheostomy tubes and vascular catheters have been shown to be the most frequent associated sources for bacteremic events (71;72). The clinical manifestations of *A. baumannii* infections are not dramatically different than that seen with other Gram-negative pathogens and typically do not demonstrate any unique features that can easily alert the clinician to the presence of this pathogen. **Rather, clinicians must understand the epidemiology of *A. baumannii* infections and have a high clinical suspicion when caring for at-risk patients.**

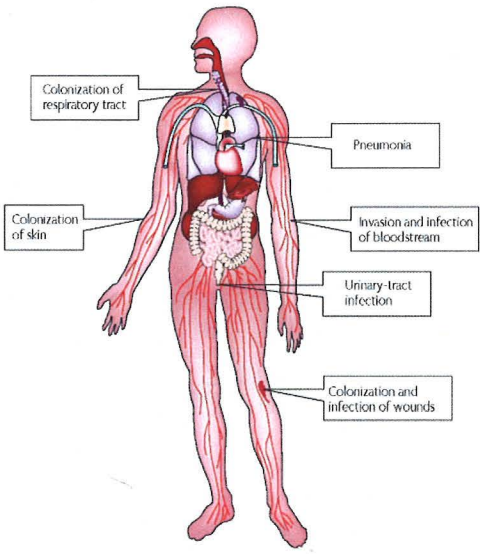

Figure 6. Common Sites of Clinical Infection by *A. baumannii*. Adapted from Dijkshoorn L. *Nat Rev Microbiol*. **5**, 939-951 (2007).

Nosocomial Pneumonia. Large surveillance studies of U.S. hospitals have reported between 5% and 10% of cases of ICU-acquired pneumonia are due to *A. baumannii* (73). Data from the CDC demonstrates that the incidence of ICU-acquired pneumonia due to *A. baumannii* is increasing. In 1986 4% of the ICU-acquired pneumonias were due to *A. baumannii,* with a rise to 7% by 2003 (73). Interestingly, the onset of *A. baumannii*-associated pneumonia tends to occur later in the patient's hospital course. As opposed to hospital-acquired pneumonias due to other Gram-negative pathogens (i.e., *P. aeruginosa, K. pneumonia)*, individuals with nosocomial pneumonia secondary to *A. baumannii* often have spent a greater number of days in the ICU and been on a mechanical ventilator prior the onset of symptoms and/or positive culture specimens (74;75). The clinical signs and symptoms of infection in these patients are similar to those seen with the other nosocomial Gram-negative organisms and consist of fever, leukocytosis, increased sputum production and new infiltrates on chest x-ray examination. Therefore, the finding of Gram-negative rods in the sputum of patient with corresponding symptoms who has been hospitalized over 14 days needs to prompt the clinician to suspect an *A. baumannii* infection and consider initiation of therapy to adequately cover for *A. baumannii* while awaiting identification of the causative organism.

Bloodstream Infections (BSI). A large survey of BSI in the United States (1995-2002) showed that *A. baumannii* was the 10th most common etiologic agent (1.3% of all monomicrobial BSI) (76). However, the crude mortality rate associated with an *A. baumannii* BSI was between 34.0% and 43.4% and was only exceeded by *P. aeruginosa* and *C. albicans (76).* Similar to the pattern seen with nosocomial pneumonia, the onset of a BSI due to *A. baumannii* tended to occur late in the patient's hospital stay. There was a mean of 26 days from the time of admission to onset of infection, ranking *A. baumannii* as the latest-onset BSI in the survey described above (76). As mentioned previously, the typical sources for a BSI include an underlying pneumonia, urinary tract infection (UTI), wound infection, or indwelling vascular catheters (72).

Other Infections. *A. baumannii* has been associated with wound infections in patients with burns (77) and those with injuries due to trauma (i.e., crush injury or battlefield wounds) (10;61), urinary tract infections (typically catheter-associated) (73), postneurosurgical meningitis (usually associated with external ventricular drains) (78), very rarely endocarditis (9;79), and recently, necrotizing fasciitis (80). As the types of sites that can be infected by *A. baumannii* continues to increase and the number of

patients affected by clinically-significant *A. baumannii* infections rises, clinicians are faced with few therapeutic options that can be used to successfully eradicate the pathogen.

V. Virulence Mechanisms Utilized by *A. baumannii*

With the exception of the study of drug resistance mechanisms, very little basic science research has been directed towards the elucidation of the pathogenic and virulence mechanisms utilized by *A. baumannii* to cause human disease. When compared to other common hospital pathogens the disparity becomes even more apparent. A recent search of Pubmed for articles with the term "virulence" in the title yielded only 142 findings for *A. baumannii* as compared with approximately 4,000 articles for MRSA or *P. aeruginosa* (see Figure 7). Equivalent disparities were seen when the search term "pathogenesis" was utilized.

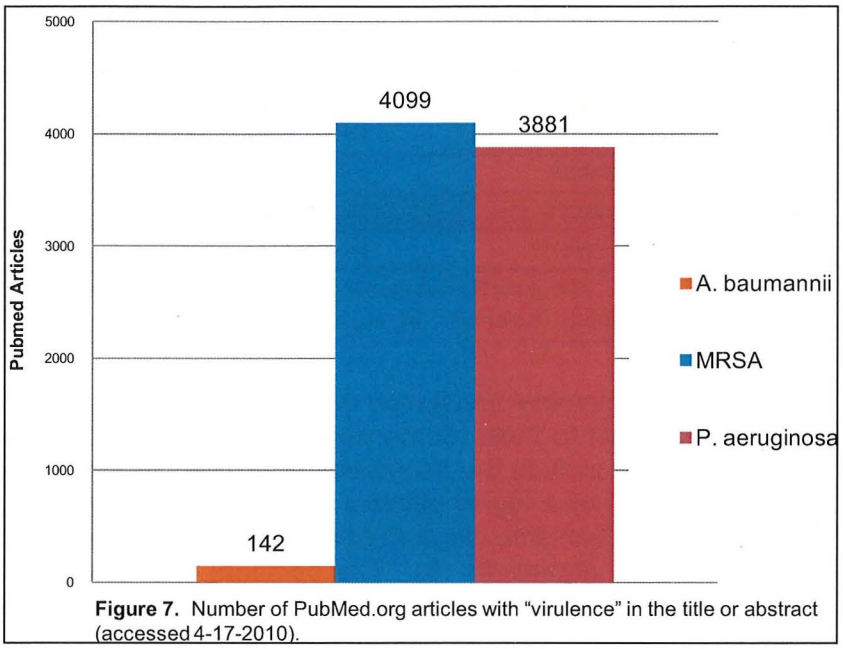

Figure 7. Number of PubMed.org articles with "virulence" in the title or abstract (accessed 4-17-2010).

Some of the virulence elements of *A. baumannii* that have been studied include an outer membrane protein (Omp38) that can cause apoptosis in human epithelial airway cells (81), a novel pilus assembly system involved in biofilm formation (82), a two-component regulatory system that controls biofilm formation (83), a siderophore-mediated iron-acquisition system (84), and a glycosyltransferase involved in lipopolysaccharide synthesis (85). The detailed description of each of these mechanisms is beyond the scope of this protocol. However, given the comparable size of the genome of *A. baumannii* with respect to other known pathogens and its ability to cause similar infectious complications to those seen with MRSA and *P. aeruginosa* it is reasonable to assume that there are a significant number of pathogenic mechanisms that still await discovery and study.

Finally, it is important to note, that with the exception of the work detailing the *A. baumannii* gene products involved with biofilm formation, there have been essentially no molecular studies centered on elucidating the bacterial factors important for this organism's ability to resist desiccation and survive on abiotic surfaces. Such research may prove to be vital to generating better mechanisms to control colonization of the hospital environment and provide new strategies for the eradication of endemic strains from clinical areas.

VI. *A. baumannii* and Drug Resistance

Modern day *A. baumannii* isolates are feared for their ability to demonstrate high-level antibiotic resistance. At the time the species was first recognized as a human pathogen of significance, isolates were quite sensitive to antibiotics. **In fact, testing of these early isolates revealed that 60-70% of strains were susceptible to ampicillin and greater than 90% of isolates were susceptible to gentamycin (86).** Since that time, the rapid development of MDR *A. baumannii* has left many clinicians and researchers scrambling to elucidate the pathogenic and virulence mechanisms this organism utilizes to resist modern antibiotics and cause disease. Many would argue that there are not any other clinically-significant organisms which have developed antibiotic resistance as quickly as *A. baumannii.* As a result

of this organism's ability to acquire and exploit antibiotic resistance mechanisms, clinicians are increasingly having to resort to the use of older, and more toxic antibiotic regimens. It is believed that the expression of these highly-effective antibiotic resistance mechanisms by *A. baumannii* strains is a relatively recent phenomenon that began in the 1970s (87) and has been heavily influenced by the development of broad-spectrum antibiotics, particularly cephalosporins and quinolones.

A recent report by the Centers for Disease Control (CDC) evaluating multidrug resistance among Gram-negative pathogens associated with healthcare-associated infections between 2006-2008 demonstrated that 60% of the *A. baumannii* isolates were resistant to at least 3 classes of antibiotics (including, cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems) (88). This is compared with 3-class antibiotic resistance rates of only 10% for *P. aeruginosa* and 15% for *Klebsiella pneumoniae* during the same time period (88). The discrepancy between *A. baumannii* and these other Gram-negative pathogens was even greater when the resistance to 4 classes of antibiotics was measured (see Table 4).

Organism	3-Class Resistance	4-Class Resistance
P. aeruginosa	676 / 6,489 (10%)	84/3,724 (2%)
A. baumannii	**1,201 / 1,987 (60%)**	**489 / 1,454 (34%)**
K. pneumoniae	679 / 4,527 (15%)	223 / 3,029 (7%)

Table 4. Antiobitic Resistance Rates Reported to the National Healthcare Safety Network (2006-2008). (Kallen AJ, et. al., Infect Control Hosp Epidemiol 2010)

The antimicrobial resistance mechanisms employed by *A. baumannii* are vast and in many cases are of equal effect to those demonstrated by many other significant human pathogens (89;90). These mechanisms can include the acquisition of mobile genetic elements, such as transposons, plasmids, and integrons, as well as a highly efficient ability to undergo natural transformation (3). These highly efficient competence mechanisms allow *A. baumannii* to rapidly acquire genetic elements from a broad range of other pathogenic organisms (8). The bacterial elements responsible for drug resistance function via a broad range of mechanisms including: [1] enzymatic neutralization of antibiotics, [2] alterations in antibiotic target sites in the bacterium, [3] upregulation of multidrug efflux pumps, and [4] altered outer membrane permeability.

A 2007 study of the multidrug-resistant *A. baumannii* strain AYE responsible for the 2003 epidemic outbreak in France, demonstrated a large genetic island containing multiple antibiotic resistance genes. More than 90% of the predicted open reading frames within this resistance island originated from *Pseudomonas* sp., *Salmonella* sp., and *E. coli* sp. (8). In the entire AYE genome, 52 antibiotic resistance genes were identified and 45 (86.5%) of them were found to be located within this resistance island (8). Analysis of a non-MDR strain from the same geographic region of France did not possess any resistance elements (8). This data supports the notion that *A. baumannii* has the ability to easily acquire and exploit genetic elements from numerous other bacterial sources, and that these resistance elements can help elevate *A. baumannii* to epidemic status.

A detailed description of all the antibiotic resistance mechanisms utilized by *A. baumannii* is beyond the scope of this discussion. However it is noteworthy to appreciate several of the broad mechanisms *A. baumannii* has adopted to help ensure its survival in the setting of current modern day antibiotics. In many instances, it has been shown that multiple antibiotic resistance mechanisms work together within the organism to help facilitate resistance and provide a synergistic approach to antimicrobial resistance (91;92).

β-Lactam Resistance

Enzymatic Mechanisms The most prevalent mechanisms for β-lactam resistance in *A. baumannii* involve the degradation of beta lactam antibiotics by β-lactamases (13). These enzymes can be either chromosomally-encoded or expressed on a plasmid. *A. baumannii* also contains inherently-encoded cephalosporinases (serine oxacillinases and metallo-β-lactamases) that provide resistance to extended spectrum cephalosporins (93;94). A great deal of work has focused upon the OXA-type carbapenemase enzymes and their role in resistance to meropenem and imipenem globally (95-97). Metallo-β-lactamases are less-commonly identified in *A. baumannii* than the OXA-type carbapenemases, however, they demonstrate a reported 100- to 1,000-fold greater hydrolytic activity towards carbepenem antibiotics (98). **Given the standard practice of utilizing carbepenem antibiotics as therapeutic agents for *A. baumannii* infections, the development of resistance to this class of antibiotics is considered 'a global sentinel event' (99).** Since the earliest descriptions of carbepenem resistance in the 1980s, four different oxallinase clusters of genes have been described and have been shown to have a far-reaching, world-wide prevalence (13).

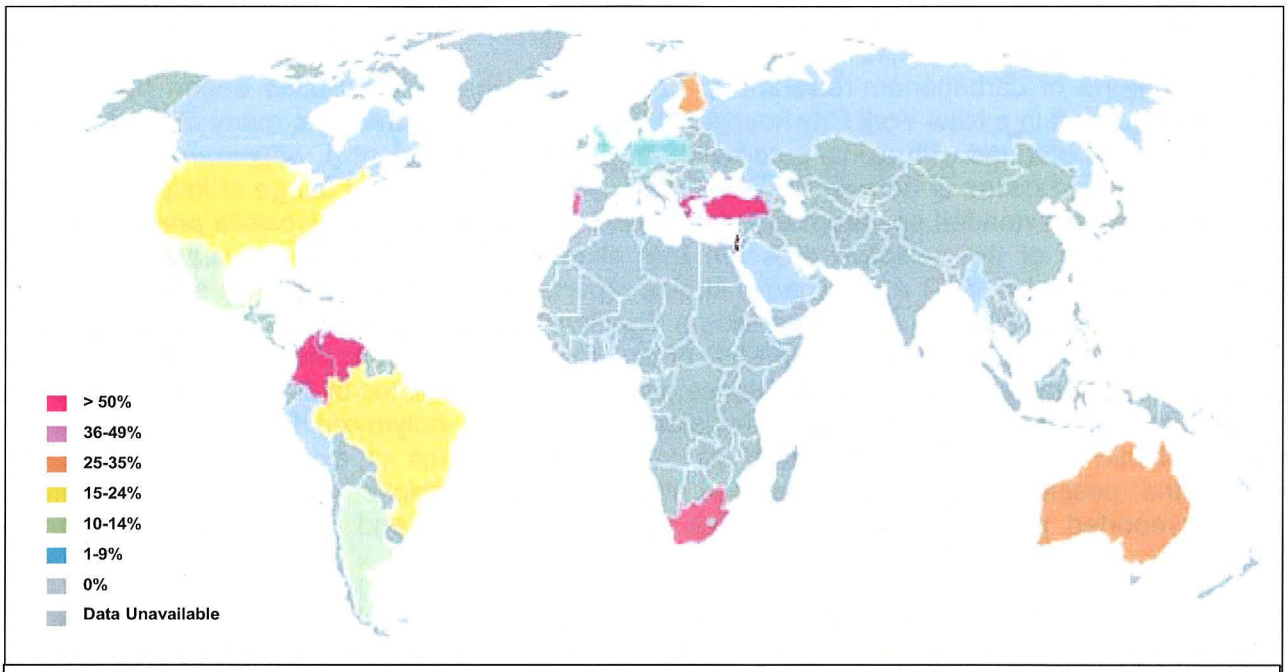

Figure 8. World-wide *A. baumannii* isolates resistant to carbapenem antibiotics. Perez F., et. al., Antimicrob Agent Chemother. (51). 3471-3484 (2007).

Non-enzymatic mechanisms Several different non-enzymatic mechanisms involved in β-lactam resistance have been characterized for MDR *A. baumannii*. These systems involve alterations in outer membrane protein expression (100-102) and increases in the activity of multidrug efflux pumps (103). Changes in outer membrane porin (OMP) expression have been associated with modern-day endemic outbreaks of carbepenem-resistant *A. baumannii* in New York City (91) and Spain (101). The presence of multidrug efflux pumps have been shown to be involved with resistance to β-lactams as well as aminoglycosides and fluoroquinolones by decreasing the concentration of target antibiotics in the periplasmic space (96;104-107). Typically, point mutations in regulatory systems controlling pump expression result in the over-expression of efflux pumps and subsequently, increased resistance (105). Recent studies have shown that naturally-occurring compounds can serve to inactivate the efflux activities of these pumps and may help improve the effectiveness of antibiotics against MDR strains of *A. baumannii (108).*

Resistance to Other Antibiotics: Aminoglycosides, Fluoroquinolones, and Tetracyclines

Numerous enzymatic mechanisms for resistance to aminoglycosides have been described and include the expression of acetyltransferases, nucleotidyltransferases, and phosphotransferases (109). A recently-described mechanism involving 16S rRNA methylation has resulted in strains resistant to such clinically-useful aminoglycosides as gentamycin, tobramycin, and amikacin (110). In a mechanism similar to that utilized to neutralize β-lactams, fluoroquinolones have been shown to be substrates for multidrug efflux pumps. *A. baumannii* has shown a remarkable ability to acquire and exploit resistance mechanisms to one of the newest modified tetracycline antibiotics, tigecycline (90;111). *In vitro* analysis of *A. baumannii* showed that increasing levels of resistance to tigecycline can be seen with multiple rounds of *in vitro* passage of the organism in the presence of this antibiotic (112). This finding confirms that *A. baumannii* can respond very quickly to selective pressure by rapidly modulating its antibiotic resistance mechanisms in response to exposure to new antimicrobial agents. This rapid and highly-effective ability to develop resistance mechanisms creates significant challenges for scientists attempting to develop novel antibiotics to treat *A. baumannii* infections.

Antibiotic Resistance in the United States

Early reports of carbepenem-resistant *A. baumannii* in the United States began to emerge in 1981 after an outbreak in a New York City hospital (113). Around the same time, many other hospitals in New York City were plagued with clonal outbreaks of MDR or pan-resistant *A. baumannii* (113-115). In these earliest reported cases of domestic MDR *A. baumannii*, the increased usage of imipenem to treat an earlier outbreak of extended spectrum beta lactamase (ESBL) producing *Klebsiella pneumoniae* was felt to be the source of the selective pressure that allowed for the MDR *A. baumannii* emergence (105;116). The number of MDR *A. baumannii* strains reported in the U.S. has shown a dramatic rise in recent years. Between 1986 and 2003 there was a significant increase in resistance to amikacin (from 5 to 20% of isolates), ceftazidime (from 25 to 68% of isolates), and imipenem (from 0 to 20% of isolates) (117). More recent surveys of antibiotic resistance amongst clinical isolates of *A. baumannii* have shown a continued rise in the rates of imipenem resistance (118). As polymyxin B emerges as the only available antibiotic agent that can be used as treatment for cases of extremely drug-resistant *A. baumannii*, the possibility of rising resistance rates to this antibiotic have become a concern. Fortunately, reported polymyxin resistance rates remain between 2 and 3% of MDR *A. baumannii* isolates (119).

Parkland Memorial Hospital recently released its susceptibility evaluation of clinical isolates for the last 6 months of 2009 (see Table 5). Fortunately, there were no isolated *A. baumannii* strains that were resistant to colistin. However, it is insightful to note that nearly 35% of the *A. baumannii* isolates were resistant to meropenem and nearly half of the isolates were resistant to the standard antibiotics often utilized at the time a nosocomial infection is suspected (Ticarcillin/Clavulanic Acid, Cefepime, Ciprofloxacin). This resistance pattern and the rising number of *A. baumannii* clinical isolates has significant implications for the clinician when choosing an empiric antibiotic to treat a suspected nosocomial infection.

Antibiotic Agents	Percentage of Resistant Isolates
Ampicillin/Sulbactam	46%
Ticarcillin/Clavulanic Acid	46%
Ceftriaxone	65%
Cefepime	45%
Meropenem	34%
Gentamycin	43%
Ciprofloxacin	42%
Colistin	0%

Table 5. Antibiotic resistance at Parkland Memorial Hospital (July-December 2009).

VII. Infection Control Measures

The ability of *A. baumannii* to persist for prolonged periods of time in the healthcare arena and its ability to cause epidemic outbreaks has forced providers and healthcare facilities to adopt stringent isolation and infection control measures when a multidrug-resistant strain of this pathogen is isolated. Measures designed to control outbreaks of *A. baumannii* need to address the organism's major epidemic modes of transmission and curb the excessive usage of broad-spectrum antibiotics. Identification of a common source for the *A. baumannii* outbreak is the most critical step to help ensure successful infection control. Sources identified as being responsible for persistent outbreaks of *A. baumannii* have included contaminated ventilator equipment (tubing, moisture traps, suctioning devices) (6), the hands of colonized healthcare workers involved with assembly of respiratory circuits after cleaning (120), and secretions from infected patients that can be directly carried from patient to patient by healthcare workers (26). In addition to contact isolation protocols for infected and colonized patients, some institutions have reported successful control of *A. baumannii* after placing patients in negative pressure rooms, closing entire ICUs for extensive cleaning, and in some cases demolishing and remodeling patient care areas.

After the implementation of stringent isolation procedures, aggressive and thorough cleaning of the healthcare environment has served as the next most commonly utilized infection control technique to help control an *A. baumannii* outbreak. The need for stringent and rigorous sanitation of the patient care areas is driven by the ability of *A. baumannii* to survive for weeks or months on abiotic surfaces (wet or dry) and serve as a source for ongoing and future outbreaks (27). A 2007 analysis of more than 1,500 hospital-based epidemics due to a broad range of pathogens showed that closure and extensive cleaning of the patient care areas was required for outbreak control in 22.9% of units affected by *A. baumannii* (121). This is compared with the required closure and cleaning of only 11.7% of the units affected by other pathogens, including many other Gram-negative organisms (121). Despite the isolation of colonized or infected patients and the use of airborne isolation procedures to control *A. baumannii* outbreaks (15;29;35), improvement in hand hygiene habits amongst healthcare workers remains the most cost effective measure to prevent spread of epidemic strains.

Resistance to Disinfectants. A recent study of *A. baumannii* susceptibility to various disinfectants including propanol, triclosan, polyvinylpyrrolidone-iodide, and chlorhexidine demonstrated inhibition of growth with all measured substances when the manufacturer's recommended concentrations and contact times were utilized (122). However, in the second part of the study, researchers showed that contact times of less than 30 seconds or the usage of diluted concentrations of disinfectants resulted in the recovery of a substantial number of viable bacteria (122). The utilization of sanitizing conditions that differed from the manufacturer's recommended guidelines was intended to simulate the day-to-day operational practice often observed in the busy hospital setting. It is therefore not unreasonable to conclude that minor deviations from the manufacturer's recommended concentrations or exposure times may allow for continued colonization of hospital surfaces by *A. baumannii* and increase the risk of epidemic outbreaks.

Additionally, a recent study demonstrated that there was a reduction in the bactericidal effects of disinfectants against *A. baumannii* when organic materials (i.e. albumin) were present (123). The mechanism responsible for this reduced bactericidal activity is not clear at this time. This same group also showed a decreased susceptibility to chlorhexidine when the organism was passaged multiple times in conjunction with the disinfectant (123). Again, these findings highlight this organism's remarkable ability to acquire and exploit survival mechanisms that can be responsible for its persistence in the hospital environment.

VIII. Antibiotic Therapy for *A. baumannii* Infections

As the number of MDR *A. baumannii* isolates resulting in clinical disease continues to rise, the optimal treatment regimens available for these infections become more limited and less clear. *A. baumannii* that is not MDR can be treated with a host of antibiotics that are typically used for Gram-negative infections. These agents would include anti-pseudomonal broad-spectrum penicillins, anti-pseudomonal broad-spectrum cephalosporins, monobactams, aminoglycosides, fluoroquinolones, carbapenems, polymyxins, and sulbactam (26). However, in today's clinical environment the healthcare provider rarely encounters a strain of *A. baumannii* that is uniformly sensitive to these common antibiotics. **Additionally, there are increasing numbers of reports of *A. baumannii* clinical isolates that are resistant to all conventional antimicrobial agents (2;4;33;124).**

Numerous *in vitro* (125;126) and animal model (127;128) studies have been performed to determine the possible antibiotic regimens that are useful against both susceptible and MDR *A. baumannii*. However, the translation of these experimental data into clinical practice can often prove difficult. While there are a host of clinical trials detailing the outcomes of treatment of both susceptible and MDR *A. baumannii* infections they are plagued by inconsistencies with regards to appropriately designed controls and non-random allocation of treatment regimens. Therefore, in many cases, it still remains up to the practicing clinician to choose an initial therapy based upon the local antibiotic susceptibility patterns of *A. baumannii*. It is therefore crucial for the individual healthcare provider to understand these resistance patterns and remain flexible with the choice of antibiotic treatment regimens.

Carbapenems (imipenem, meropenem, doripenem) are now considered to be the mainstay of treatment for *A. baumannii* infections (129). The duration of treatment should be similar to that for infections caused by other Gram-negative bacilli and is dictated by the site of infection and the severity of the illness. Unfortunately, as mentioned previously, the increased usage of this class of antibiotics has resulted in rising numbers of reports of carbepenem-resistant isolates. For example, the United Kingdom reported an increase in carbepenem resistance from <0.5% in 1990 to 24% in 2007 (26). In the United States, a survey by the Centers for Disease Control and Prevention (CDC) showed rates of carbepenem resistance in isolates of *A. baumannii* increased from 9% in 1995 to 40% in 2004 (66;130). In some facilities resistance rates to carbapenems have been reported to be greater than 90% of isolates (131). As a result of the rising rates of resistance to standard antimicrobials, many clinicians have resorted to the usage of older agents, previously abandoned due to concerns about high levels of drug toxicity and significant side effects.

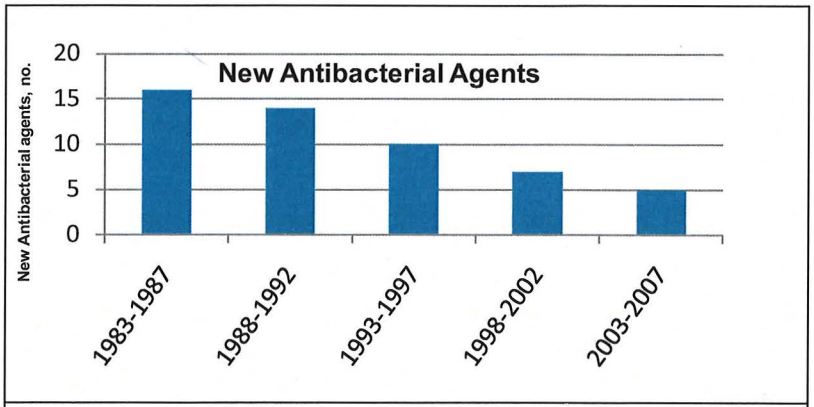

Figure 9. New Antibacterial agents developed between 1983 and 2007. (Adapted from Peleg, AY, et.al, Clin Microbiol Rev (21), 538-582 (2008).

The polymyxins (i.e., colistin) have shown bactericidal activity against *A. baumannii* and the rates of resistance against this agent remain relatively low. Polymyxins disrupt bacterial outer membranes via a detergent action and it is speculated that the use of a polymyxin antibiotic in combination with another antibiotic may enhance killing as a result of better penetration of the bacterial outer membrane (132). Studies have shown that colistin can be used successfully for the treatment of carbepenem-resistant isolates. Additionally, colistin has been utilized to treat a variety of types of infections including ventilator-associated pneumonia (131;133), bloodstream infections (133), and central nervous system infections (134).

In the past, polymyxin use for the treatment of severe infections was abandoned over concerns about increased nephrotoxicity and neurotoxicity. However, more recent studies have shown a lower-than expected incidence of toxic complications from this drug class. These improvements are felt to be due to the use of lower doses, different drug formulations, and careful ICU monitoring (135). Additional studies have shown that polymyxins can be administered via the nebulized form to treat respiratory infections (131;135;136). The main side effect of inhaled colistin is bronchoconstriction. Unfortunately as the use of polymyxins has increased, the concern for emergence of *A. baumannii* strains resistant to this class of antibiotics has grown. Recent data from an outbreak of MDR *A. baumannii* in South Korea has demonstrated high rates of in vitro resistance to colistin (137). Fortunately, polymyxin resistance by *A. baumannii* in the United States has remained low with less than 2% of isolates showing resistance to polymyxin B (13).

Potential future treatment options for MDR *A. baumannii* may center upon the development and usage of synthetic peptides that can disrupt bacterial cell membranes. In many cases these peptides are isolated from natural sources including phagocytes and amphibian skin (138). It can prove to be extremely difficult to manufacture these compounds in large quantities and in some cases they are prone to rapid metabolism and can prove to be immunogenic (138). Unfortunately, the pace of discovery of new antibiotics and their delivery to the clinical market has shown a significant decline in the past 25 years (See Figure 9). Furthermore, the number of new antimicrobials targeted towards Gram-negative pathogens has been outpaced by those agents directed towards Gram-positive pathogens.

Figure 10. IDSA campaign logo for the antibiotic development initiative.

According to the most recent report by the Antimicrobial Availability Task Force (AATF) of the Infectious Diseases Society of America, there are no compounds in the pipeline at an advanced state of development for the treatment of multidrug-resistant *A. baumannii*. **This led the panel to conclude that *A. baumannii* is a prime example of "a mismatch between unmet medical needs and the current antimicrobial research and development pipeline" (12;139).** This month, the IDSA released its bold plan (see Figure 10) calling for the development of 10 new antibiotic agents by the year 2020 (named "The 10 x '20 Initiative") (140).

Citing the increased levels of antibacterial resistance as a severe threat to the practice of healthcare, the IDSA has called for an innovative and multidisciplinary approach involving government and commercial stakeholders to foster the development of new antibiotic agents that can help effectively control MDR pathogens, including *A. baumannii*. The position statement describes how the antibiotic discoveries in the 1930s and 1940s were a 'transformative moment in human history' and that the pace of discovery led physicians that witnessed that era to describe the achievements as an "awesome acquisition of power" for patients and their providers (141).

IX. Conclusions

The emergence of *A. baumannii* from an organism of little clinical relevance to its establishment as a pathogen which poses a significant challenge to clinicians on a worldwide scope has occurred with little fanfare. Only in the past five years have clinicians, researchers, and the press begun to appreciate the significant problems posed by this previously-ignored pathogen. Recent work has begun to clarify the attributable morbidity and mortality associated with an *A. baumannii* infection. However a great deal of research and exploration must be completed to better understand the pathogenic mechanisms

employed by *A. baumannii* to cause significant human disease. While the types of clinical infections caused by *A. baumannii* do not differ greatly from those seen with other Gram-negative pathogens, the extreme drug resistance patterns demonstrated by this bacterium often leave clinicians with few therapeutic choices. Recognizing the global threat of resistant pathogens like *A. baumannii,* The World Health Organization has identified drug resistance as one of the three greatest threats to human health (140).

Clinicians treating a patient with an *A. baumannii* infection must assume that other nearby patients are colonized by the bacterium and at risk for developing a clinically-significant infection. As a result, stringent and at times, innovative, infection control procedures must be adopted to help prevent the spread of *A. baumannii*. Despite these measures, the organism has found ways to spread in epidemic fashion throughout hospitals, across cities, and from country to country. If the past 20 years serve as an indication of the potential for this pathogen, then *A. baumannii* needs to be considered a pathogen of the future. The IDSA has recognized the threat posed by this organism and a host of others that plague the medical establishment and has called for accelerated development of new antimicrobials to combat these pathogens.

Unfortunately, *A. baumannii* has consistently demonstrated that when challenged with antibiotics it can acquire and exploit resistance mechanisms at a faster rate and with more success than most of the other hospital-encountered pathogens. Unless a better understanding of the virulence mechanisms utilized by this pathogen to both colonize the hospital environment and cause significant human disease is attained, *A. baumannii* will continue to plague our healthcare system with increasing severity.

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