The Journal of Microbiology (2012) Vol. 50, No. 3, pp. 434–443 DOI 10.1007/s12275-012-1555-1
Copyright ⓒ 2012, The Microbiological Society of Korea

Extracellular Stress and Lipopolysaccharide Modulate *Acinetobacter baumannii* Surface-Associated Motility[§]

Christin N. McQueary[1], Benjamin C. Kirkup[1,2], Yuanzheng Si[1], Miriam Barlow[3], Luis A. Actis[4], David W. Craft[1], and Daniel V. Zurawski[1*]

[1]*Division of Bacterial and Rickettsial Disease, Department of Wound Infections, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910, USA*
[2]*Uniformed Services University of the Health Sciences. F. Edward Hebert School of Medicine, Department of Medicine, Infectious Diseases Division*
[3]*School of Natural Sciences, University of California, Merced, CA 95343, USA*
[4]*Department of Microbiology, Miami University, Oxford, Ohio 45056, USA*

(Received November 7, 2011 / Accepted February 7, 2012)

***Acinetobacter baumannii* is a nosocomial bacterial pathogen, and infections attributed to this species are further complicated by a remarkable ability to acquire antimicrobial resistance genes and to survive in a desiccated state. While the antibiotic resistance and biofilm formation of *A. baumannii* is well-documented, less is known about the virulence attributes of this organism. Recent studies reported *A. baumannii* strains display a motility phenotype, which appears to be partially dependent upon Type IV pili, autoinducer molecules, and the response to blue light. In this study, we wanted to determine the prevalence of this trait in genetically diverse clinical isolates, and any additional required factors, and environmental cues that regulate motility. When strains are subjected to a wide array of stress conditions, *A. baumannii* motility is significantly reduced. In contrast, when extracellular iron is provided or salinity is reduced, motility is significantly enhanced. We further investigated whether the genes required for the production of lipopolysaccharide (*lpsB*) and K1 capsule (*epsA*/*ptk*) are required for motility as demonstrated in other Gram-negative bacteria. Transposon mutagenesis resulted in reduced motility by the insertion derivatives of each of these genes. The presence of the parental allele provided *in trans*, in the insertion mutant background, could only restore motility in the *lpsB* mutant. The production of core LPS directly contributes to the motility phenotype, while capsular polysaccharide may have an indirect effect. Further, the data suggest motility is regulated by extracellular conditions, indicating that *A. baumannii* is actively sensing the environment and responding accordingly.**

*For correspondence. E-mail: daniel.zurawski@amedd.army.mil; Tel.: +1-301-319-3110; Fax: +1-301-319-9801
[§]Supplemental material for this article may be found at http://www.springer.com/content/120956
The findings and opinions expressed herein belong to the authors and do not necessarily reflect the official views of the WRAIR, the U.S. Army, or the Department of Defense.

***Keywords*: motility, LPS, swarming, biofilm, nosocomial pathogen, extracellular stressors, bacteria, optical mapping

Introduction

Acinetobacter baumannii is a Gram-negative, coccobacillus; human opportunistic pathogen that causes severe infections and is often associated with traumatic war-related wounds (Dijkshoorn *et al.*, 2007; Scott *et al.*, 2007). These infections are a formidable threat because this pathogen survives several months on inanimate objects and is able to produce multidrug-resistant (MDR) and pan-drug resistant (PDR) derivatives (Cisneros and Rodriguez-Bano, 2002; Joly-Guillou, 2005; Montefour *et al.*, 2008). Immunocompromised patients worldwide, especially in intensive care units, are more susceptible to the nosocomial spread of *A. baumannii* and are significantly impacted by these infections (Dijkshoorn *et al.*, 2007; Peleg *et al.*, 2008). For example, there have been many outbreaks in civilian hospitals in the United States (Beavers *et al.*, 2009; La Forgia *et al.*, 2010; Perez *et al.*, 2010), Europe (Adams *et al.*, 2011; D'Arezzo *et al.*, 2011), South America (Dalla-Costa *et al.*, 2003; Prates *et al.*, 2011), Asia (Chang *et al.*, 2009; Choi *et al.*, 2010) and the Middle East (Marchaim *et al.*, 2007). In Asia, co-morbid conditions, such as diabetes mellitus, heavy smoking, and excess alcohol consumption also predispose individuals to *A. baumannii* infection (Falagas *et al.*, 2007; Alsultan *et al.*, 2009). Additionally, *A. baumannii* is a well-established war-related pathogen, recorded initially in the Korean War, with increasing prevalence in each of the subsequent conflicts (Lindberg *et al.*, 1955; Tong, 1972; Petersen *et al.*, 2007; Calhoun *et al.*, 2008). In the current conflicts in Iraq and Afghanistan, *A. baumannii* is one of the dominant Gram-negative bacteria to complicate wound healing in wounded military personnel (Gootz and Marra, 2008; Dallo and Weitao, 2010). The unfortunate outcomes of these infections include wound dehiscence, osteomyelitis, amputation, septicemia, and even mortality (Petersen *et al.*, 2007; Yun *et al.*, 2008; Guerrero *et al.*, 2010). Complicating the spread of this opportunistic pathogen is its exceptional ability to acquire foreign DNA, including antibiotic resistance genes (Peleg *et al.*, 2008). Perhaps the most foreboding examples of this are the recent identification of a strain of *A. baumannii* harboring the New Delhi β-metalloprotease (Karthikeyan *et al.*, 2010) and the emergence of colistin-resistant strains (Ko *et al.*, 2007; Park *et al.*, 2011).

The treatment of *A. baumannii* infections is further complicated by our ignorance of the molecular and cellular mechanisms of pathogenesis in the human host. While

there have been recent publications elucidating some virulence factors, such as phospholipase D (PLD) (Jacobs *et al.*, 2010), biofilm-associated protein (Bap) (Loehfelm *et al.*, 2008), outer membrane protein A (OmpA) (Choi *et al.*, 2005; Gaddy *et al.*, 2009), and penicillin-binding protein 7/8 (PBP-7/8) (Russo *et al.*, 2009), much still needs to be discovered about both the bacterial and host factors that are involved in forming biofilms, invading host cells, and ultimately establishing an infection. A recent observation that *A. baumannii* displays surface-associated motility provided some insight as to how this pathogen potentially scavenges nutrients in the human host (Mussi *et al.*, 2010).

Table 1. Strains used in this study

A. baumannii strain	Site of isolation	Source
846	Blood	MRSN
847	Blood	MRSN
848	Blood	MRSN
849	STS Bone	MRSN
853	Blood	MRSN
854	STS	MRSN
856	Tibia	MRSN
858	Femur	MRSN
859	Fibula	MRSN
860	Femur	MRSN
863	Warwound	MRSN
877	Warwound	MRSN
899	Warwound	MRSN
903	Tracheal aspiration	MRSN
906	Warwound	MRSN
907	Blood	MRSN
930	STS Bone	MRSN
939	STS Bone	MRSN
941	Warwound	MRSN
949	Sputum	MRSN
951	STS Bone	MRSN
953	Warwound	MRSN
954	Blood	MRSN
959	Bone	MRSN
960	STS	MRSN
961	Blood	MRSN
963	Blood	MRSN
1308	Blood	MRSN
1309	Blood	MRSN
1310	Blood	MRSN
ATCC 17978	CSF	ATCC
ATCC 19606	Urine	ATCC
AB0057	Blood	R. A. Bonomo
ATCC SDF	Human louse	L.A. Actis
ATCC AYE	Blood	L.A. Actis
A118	Blood	M. E. Tolmasky
ACICU	CSF	X. Huang
307-0294	Blood	A. A. Campagnari
307-0294::TN17		A. A. Campagnari
307-0294.30		A. A. Campagnari
307-0294.45		A. A. Campagnari
307-0294 ::TN17/pSS11		A. A. Campagnari
307-0294.30/pNLAC1::*ptk*		A. A. Campagnari
307-0294.45/pNLAC1::*epsA*		A. A. Campagnari

Interestingly, the term *Acinetobacter* derives from the Greek "akineto", meaning non-motile, yet gliding and twitching motility were originally described in environmental *Acinetobacter* spp.; however, *Acinetobacter* spp. are non-flagellated bacteria (Brisou, 1953; Halvorsen, 1963; Peleg *et al.*, 2008). Recent reports have shown that non-flagellated clinical isolates of *Acinetobacter* display surface motility; however, not all of the factors responsible for this movement have been identified (Mussi *et al.*, 2010; Clemmer *et al.*, 2011; Eijkelkamp *et al.*, 2011; McQueary and Actis, 2011). Multiple factors are utilized by other bacteria for movement including exopolysaccharide (EPS) production, extension and retraction of type IV pili (TFP), production of surfactants, and possibly lipopolysaccharide (LPS) shedding (Strom and Lory, 1993; Matsuyama *et al.*, 1995; Kearns and Losick, 2003; Caiazza *et al.*, 2005; Huang *et al.*, 2006; Chen *et al.*, 2007; Jamieson *et al.*, 2009; Yang *et al.*, 2010). These same factors also contribute to the pathogenicity of many bacteria. Polysaccharide production by *Vibrio cholerae* is necessary for motility and plays a role in its intestinal colonization (Ali *et al.*, 2000; Fong *et al.*, 2010). *Pseudomonas aeruginosa* uses the extension and retraction of TFP to mediate surface movement, which is required for its translocation of corneal epithelial layers (Alarcon *et al.*, 2009). *Serratia marcescens* produces a surfactant, serawettin, to enhance surface motility, and this motility also helps to protect it from antibiotics (Lindum *et al.*, 1998; Butler *et al.*, 2010). Lastly, the LPS O-antigen is required for swarming motility in *Salmonella enterica* serovar Typhimurium, as antibody inhibition of this component impeded motility and thus epithelial cell invasion (Toguchi *et al.*, 2000; Forbes *et al.*, 2008).

Here we report that clinical isolates of *A. baumannii* are capable of surface-associated motility. We further investigated extracellular influences and cellular components involved in this phenotype. While there are multiple, distinct patterns of motility associated with genetically related strains, the addition of several extracellular stressors reduced the "swarm-like" spread of *A. baumannii* across motility agar. In contrast, a reduction in salinity or the presence of extracellular iron enhanced the motility of all motile isolates and even induced the phenotype in isolates previously observed to be non-motile on motility agarose. Lastly, we investigated the motility of three transposon mutants which revealed that the presence of the LPS O-antigen and the K1 capsule also play a role in the motility of this pathogen. Therefore, it appears that the environment and multiple cellular factors significantly contribute to this newly identified *A. baumannii* phenotype.

Materials and Methods

Strains and growth conditions

Clinical isolates of *A. baumannii* were obtained from the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research (WRAIR). ATCC 17978 and ATCC 19606^T were purchased from the American Type Culture Collection

(ATCC). AB0057, ATCC SDF and ATCC AYE, A118, ACICU, and 307-0294 and its derivatives were generous gifts from Dr. Robert Bonomo, Dr. Luis Actis, Dr. Marcelo Tolmasky, Dr. Xiaozhe Huang, and Dr. Anthony Campagnari, respectively. All isolates used in this work (Table 1) were routinely cultured on Luria-Bertani (LB) broth or agar at 37°C.

OpGen mapping and phylogenetic tree assembly

A. baumannii strains were streaked to isolation from -80°C cryovials onto tryptic soy agar (TSA) containing 5% Sheep Blood Agar. Subsequently, single colonies were used to inoculate liquid TSA and grown overnight at 37°C. The genomic DNA from these strains was isolated and optically mapped as a commercial service (OpGen Inc., USA). To investigate the relatedness of the *A. baumannii* strains a cladogram was constructed using MapSolver (OpGen Inc.). The maps are aligned using a scale-sensitive algorithm that looks for regions of minimally 6 contiguous intervals between restriction sites such that the sites produced fragments of the same proportions. Pairwise comparisons of aligned maps produced a distance measure between the maps such that the distance was the total length of the alignable maps (both maps) divided by the sum of the total lengths of the maps. The resulting distance matrix was used to infer a cladogram (UPGMA). The cladogram was interpreted into a graphic format using iTol (Letunic and Bork, 2011).

Surface motility

Surface motility assays were completed in triplicate as described previously (Mussi *et al.*, 2010) with minor modifications. Briefly, motility plates were stabbed to the bottom with overnight LB agar cultures using an inoculation needle. The plates were incubated for 7 or 14 h at 37°C and the diameter of the cell halos due to motility on the surface of the agarose plates were measured. Motility was also measured after the addition of multiple extracellular stressors. The stressors were added at final concentrations of 100 μM 2,2'-dipyridyl (DIP), 1% ethanol, 300 mM NaCl, 50 μg/ml ampicillin, 40 μg/ml kanamycin, 4 μg/ml amikacin, 50 μM hydrogen peroxide, 0.8 mg/ml Congo red, or 50 ng/ml triclosan.

For the evaluation of non-stress conditions, motility was tested on motility plates with a final concentration of 100 μM $FeCl_3$ or without the addition of NaCl (0 mM final concentration) to the medium. Lastly, cells were stab inoculated into motility media (Rashid and Kornberg, 2000) solidified with 0.3% Noble agar (BD Difco™, USA), granulated agar (Difco) or agarose (GeneMate, BioExpress, USA) and Luria-Bertani or M9 minimal medium (Miller, 1972) containing 0.3% agarose.

Microtiter biofilm cultures

The biofilm assays were performed in triplicate as previously described (Heilmann *et al.*, 1996; Stepanovic *et al.*, 2000; Wakimoto *et al.*, 2004). Briefly, 3 ml of overnight bacterial cultures were grown in motility broth, which is the same medium as the motility plates but without the addition of agar or agarose, at 37°C shaking at 200 rpm. These cultures were diluted 1:50 in 100 μl of motility broth in a 96-well microtiter plate and incubated at 37°C at 20 rpm for 14 h. The absorbance of each well was determined on a Synergy 4 (BioTek, USA) multifunctional plate reader at 600 nm. The culture supernatants were aspirated and the plate gently washed twice with phosphate buffered saline (PBS) and air-dried for 10 min. The adherent cells were fixed with 100 μl of 95% methanol for 20 min. The plates were then washed 3 times with ddH_2O, 150 μl of 1% crystal violet were added to each well, followed by plate incubation at room temperature for 30 min. The plates were then washed again 3 times with ddH_2O, and the dye was solubilized in 200 μl 95% ethanol. The plates were sonicated using a Symphony ultra-sonic waterbath (VWR) for 10 min and the absorbance at 580 nm was determined.

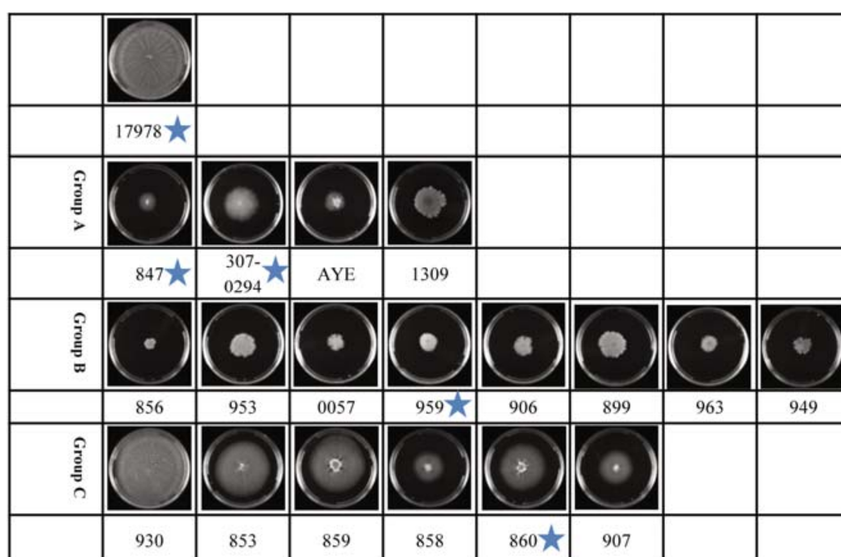

Fig. 1. Surface associated motility grouped according to pattern and phylogentic relatedness. A picture of the motility pattern of each isolate (categories A-C, ATCC 17978, and 307-0294) is placed next to each isolate. A star is placed next to the chosen isolate from each motility pattern group for subsequent figures.

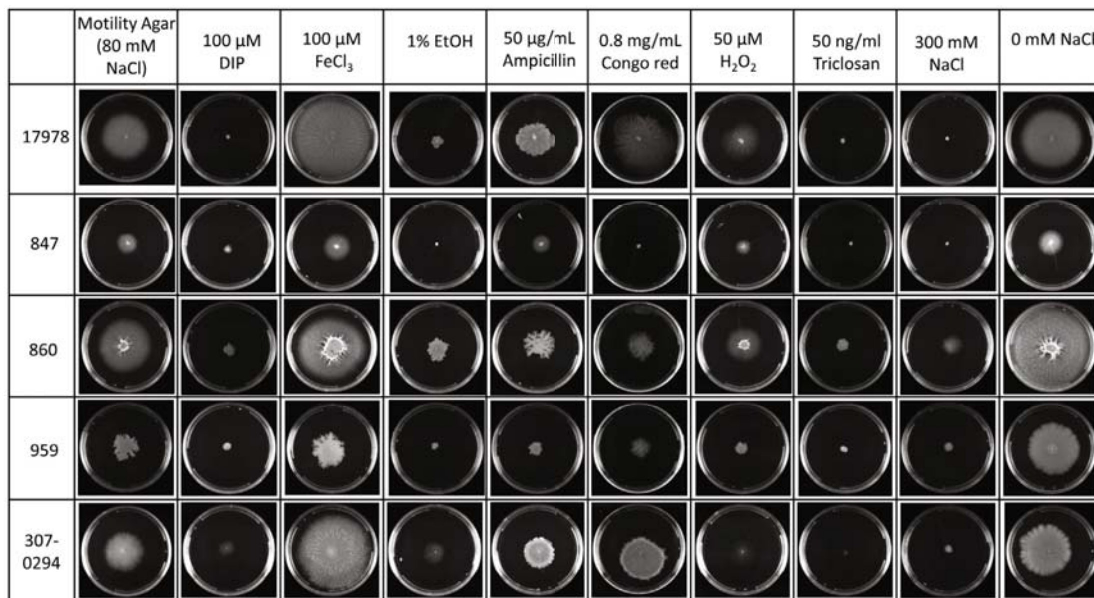

Fig. 2. Surface associated motility displayed by representative strains under various extracellular stress conditions. ATCC 17978, one representative isolate from each motility group, and 307-0294 were stab inoculated into motility agarose or motility agarose containing 100 µM DIP, 100 µM $FeCl_3$, 50 µg/ml ampicillin, 1% EtOH, 0.8 mg/ml Congo red, 50 ng/ml Triclosan, or 50 µM H_2O_2. Also, isolates were stab inoculated into swimming agarose with different salt concentrations (0 mM and 300 mM NaCl). The plates were incubated at 37°C for 7 or 14 h to visually display greatest differences.

Results

Cell motility phenotype is dependent on phylogenetic relatedness

A recent report suggested that motility was a potential virulence factor for *A. baumannii* (Mussi *et al.*, 2010); therefore, we wanted to assess this phenotype across a broad assortment of clinical strains. Thirty clinical isolates obtained from wounded soldiers and eight sequenced strains were stab inoculated into motility agarose, and subsequently these isolates were grouped based on the highly reproducible motility pattern that was displayed. The strains clustered into non-motile and 3 different motility patterns designated: A, B, and C. Pattern A is round with slightly jagged edges, and the cells are opaque and lighter in density. Pattern B has a dense grouping of cells that are rounded with pointed edges. Pattern C cells tend to move farther across the agarose surface, with dense cells at the site of inoculation that form striations as they radiate out from the center. ATCC 17978 was used as control due to its previously described motility (Mussi *et al.*, 2010), and 307-0294 was also used as a control for comparison to the insertion derivatives (Supplementary data Fig. S1). The motility exhibited by these isolates was surface-associated, and the different patterns were independent of inoculum concentration. However, the motility was dependent on the richness of the medium. ATCC 17978 displayed different patterns on swim agarose, LB agarose, and M9 agarose indicating motility patterns can be dependent on the enrichment of the medium (Supplementary data Fig. S2).

To investigate if the isolates displaying a similar pattern were related to one another, we assembled a cladogram based upon optical mapping of the *A. baumannii* genomes (Toguchi *et al.*, 2000). Interestingly, the relatedness of the clinical isolates and the motility pattern observed for each isolate tended to assemble near one another on a phylogenetic tree, although there are some exceptions (Supplementary data Fig. S1). This observation was especially clear for pattern "C" that has a flower-like pattern (853, 858, 859, 860, 907, and 930) and for the spoke-like pattern seen with isolates in pattern "B" (899, 906, 953, AB0057, 949, 963, and 959) (Supplementary data Fig. S1).

Motility is reduced by extracellular stressors

Since *A. baumannii* is exposed to different environments, including those of the human body as well as hospital surfaces, we assessed the impact of these extracellular conditions on motility. A number of different motility media were tried using different agar and agarose bases. After testing Noble agar, granulated agar, or agarose in the motility media, it was found that agarose allowed for greater motility by the isolates. One representative isolate from each motility pattern was selected and compared to that of a previously reported (Mussi *et al.*, 2010) motile isolate, ATCC 17978 and 307-0294 (Fig. 2, column 1). Figure 2, column 1 represents the untreated controls.

Because motility patterns were dependent upon the medium richness (Supplementary data Fig. S2) and the stress of blue light (Mussi *et al.*, 2010), we hypothesized that extracellular stress applied to the bacteria (nutrient, salinity, etc.) should alter the observed motility patterns. First, we prepared motility media with the addition of 100 µM DIP, which chelates the extracellular iron present in the medium and has been shown to alter other *A. baumannii* attributes

Table 2. Measurements of diameter (mm) of the cell halos of each strain under the given conditions. The data are Means±SD of experiments that were performed in triplicate.

A. baumannii strain	Motility agarose	DIP	$FeCl_3$	EtOH	Ampicillin	Congo Red	H_2O_2	Triclosan	300 mM Nacl	0 mM NaCl
17978	33.3 ± 5.8	3.7 ± 0.6	61.7 ± 5.8	11.0 ± 3.6	20.3 ± 3.2	14.3 ± 3.2	22.3 ± 4.5	4.7 ± 0.6	4.0 ± 1.0	54.3 ± 5.1
847	21.0 ± 1.0	4.7 ± 0.6	28.3 ± 2.5	3.3 ± 0.6	7.7± 3.1	4.7 ± 0.6	16.3 ± 4.5	3.7 ± 0.6	3.0 ± 1.0	29.0 ± 3.6
860	37.7 ± 6.4	12.7 ± 1.5	57.3 ± 4.0	16.3 ± 3.2	20.3 ± 3.1	21.7 ± 3.1	32.3 ± 7.2	14 ± 2.0	13.7 ± 2.1	59.0 ± 4.0
959	33.7 ± 5.5	7.7 ± 0.6	51.3 ± 3.5	8.0 ± 1.0	14.3 ± 1.5	25.0 ± 2.6	25.0 ± 2.0	7.3 ± 0.6	9.0 ± 1.0	55.7 ± 6.0
307-0294	33.7 ± 5.5	13.3 ± 2.1	53.3 ± 7.6	17.3 ± 7.5	21.0 ± 6.0	18.3 ± 2.5	22.3 ± 2.1	6.7 ± 1.5	11.7 ± 3.5	47 ± 4.4

such as the expression of iron regulated functions and biofilm formation (Tomaras *et al.*, 2003; Nwugo *et al.*, 2011). The addition of DIP reduced the diameter of the motility zones and the pattern displayed by each isolate regardless of the strain (Fig. 2, column 2). The reduction in motility was not due to a reduction in growth as demonstrated by growth curves of *A. baumannii* inoculated in motility broth plus or minus 100 μM DIP (data not shown). The observed pattern ranges from non-motile (959) to intermediate motility (ATCC 17978). Next, we tried the addition of antibiotics postulating that even if the strains possess an antibiotic resistance gene to the antibiotic tested, the bacteria would still be under stress. As thought, the addition of a sub-inhibitory concentration of ampicillin reduced the motility of all isolates, though to varying degrees (Fig. 2, column 5). We observed the same reduction in motility in the presence of other antibiotics, such as kanamycin and tetracycline (data not shown). The patterns were also altered from slight reduction in striations (847) to production of a pattern observed in another isolate (860) to the production of a completely different and previously unobserved pattern (ATCC 17978) (Fig. 2, column 5).

Recently, it has been shown that 1% ethanol stimulated the virulence of *A. baumannii* (Smith *et al.*, 2007); therefore, we tested whether this stress would also have a negative impact on motility. The addition of 1% ethanol greatly impeded the motility of all isolates with little (860) to no motility (847) observed (Fig. 2, column 4). Like the other stress conditions tested, the isolates had different motility patterns when compared to the control. We next tried the addition of Congo red to the motility medium because this dye has been shown to bind to the surface of other Gram-negative bacteria and inhibit the production of exopolysaccharide (Da Re and Ghigo, 2006), a cellular component shown to be required for motility in other bacteria (Huang *et al.*, 2006). The addition of 0.8 mg/ml Congo red produced a wide variety of changes in motility of all the isolates. The variations ranged from a new, previously unobserved pattern produced at the same diameter on motility agarose (ATCC 17978) to no motility (847) to a change into a pattern observed in other isolates (860) (Fig. 2, column 6). It is also well-known that increases in osmolarity are a stress condition often sensed by *A. baumannii* and other bacteria (Hood *et al.*, 2010); therefore, we sought to determine the effect of increased sodium chloride on motility. Motility agarose contains 80 mM NaCl but an increase to 300 mM drastically impeded the motility of all the *A. baumannii* isolates. Most strains were no longer motile (such as 847) and a few displayed very limited motility (959) (Fig. 2, column 9). An antimicrobial agent often used for disinfecting surfaces, triclosan, showed a significant inhibition of motility in *Proteus* species (Firehammer, 1987). Therefore, this agent was tested against the *A. baumannii* clinical isolates and proven to also significantly inhibit this trait. There was slight movement in 860 while the other isolates showed no movement away from the site of inoculation (Fig. 2, column 8). Lastly, oxidative stress may be generated by other disinfection reagents and is encountered in the host in the form of the innate immune response; therefore, the motility of *A. baumannii* in response to this stress was of interest (Forman and Torres, 2001). The motility of these isolates in response to hydrogen peroxide was reduced but not abolished, as all the isolates appeared to move approximately half the distance away from the inoculation site compared to the motility on motility agarose (Fig. 2, column 7). A quantitative presentation of these results can be found in Table 2.

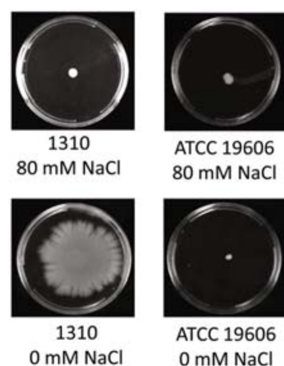

Fig. 3. Induction of motility in a non-motile isolate. *A. baumannii* 1310 is non-motile after inoculation on motility agarose but in the absence of sodium chloride (0 mM NaCl) displays motility. In contrast, the non-motile ATCC 19606^T does not display this attribute after incubation for 24 h at 37°C in the presence or absence of salt.

The removal of stress enhances motility

Due to the decreased motility in the presence of extracellular stress, it was important to determine if the reduction or removal of stress, an inverse condition, could induce or increase motility. First, we wanted to reduce the NaCl since a high NaCl environment had produced such a dramatic reduction in motility. Therefore, we prepared motility agarose without the addition of any NaCl as compared to the 80 mM NaCl found in the standard motility medium. The low

Table 3. Measurements of diameter (mm) of the cell halos of each strain under the given conditions. The data are Means±SD of experiments that were performed in triplicate.

A. baumannii strain	Motility agar (80 mM NaCl)	Motility agar (0 mM NaCl)
19606	4.7 ± 0.58	5.7 ± 0.58
1310	6 ± 1.0	50.3 ± 1.5

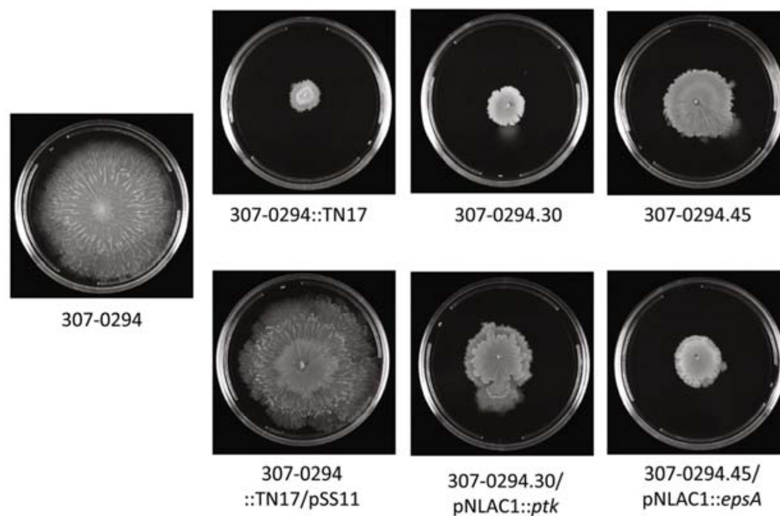

Fig. 4. Motility patterns displayed by LPS and capsular polysaccharide mutants. Three transposon derivatives of strain 307-0294, and the complemented strains, observed on motility agarose after 14 h of growth at 37°C. The reduction in motility displayed by the mutants indicates the use of several cellular components in *A. baumannii* surface-associated motility.

sodium chloride motility media enhanced the motility of almost all the isolates (Fig. 2, column 10). The patterns for most of the isolates remained the same, but the diameter of motility zones increased, with the exception of 959 which produced a new pattern on this medium. Interestingly, some isolates, such as 1310 that are non-motile on motility agarose, were found to be motile on 0 mM NaCl motility agarose (Fig. 3, column 1). However, this motility is not initiated in all non-motile isolates, such as ATCC 19606^T (Fig. 3 column 2), which remained non-motile on this medium.

Since the iron limitation also had a drastic effect on the motility of all isolates in our previous experiments and the requirements of iron for *A. baumannii* survival is well-established (Zimbler *et al.*, 2009), we thought that adding additional iron would also represent a reduction in environmental stress. As expected, the addition of extracellular iron increased motility over that observed on motility agarose (Fig. 2, column 3). A quantitative presentation of these results can also be found in Table 3.

Lipopolysaccharide and exopolysaccharide are involved in surface-associated motility

Other Gram-negative bacteria require the production of LPS and EPS for motility (Huang *et al.*, 2006) and the reduction of motility by *A. baumannii* after the addition of Congo red to motility plates prompted us to investigate the motility of mutants deficient in LPS or capsular polysaccharide production. Three transposon mutants of the *A. baumannii* 307-0294 strain, 307-0294::TN17, 307-0294.30, and 307-0294.45, contain interruptions in *lpsB*, *ptk*, and *epsA*, respectively (Luke *et al.*, 2010). The *lpsB* mutant has a deep lipopolysaccharide core mutation and the ptk and epsA mutants are deficient in the production of capsule, as shown in Russo et al., where Ptk is a tyrosine kinase that activates capsule production in other bacterial species and EpsA is involved in polysaccharide/capsule export (Cuthbertson *et al.*, 2009; Luke *et al.*, 2010; Russo *et al.*, 2010). When these strains were stab inoculated into motility agarose and compared to the parental strain, *A. baumannii* 307-0294 (Fig. 1, group A), each mutant exhibited a defect in motility (Fig. 4). All three mutant strains were capable of some motility away from the site of inoculation, but the diameter of movement was drastically diminished and the pattern contained fewer striations of cells when compared with the parental strain 307-0294. Insertion derivatives that were complemented *in trans* with the parental alleles of *lpsB*, *ptk*, and *epsA* were also tested on motility agarose. Interestingly, only the motility of the LPS mutant was restored to wild-type levels, while the motility of the two other mutants appeared unchanged (Fig. 4). A quantitative presentation of these results can be found in Table 4. These results suggest that LPS production has a direct role in the motility phenotype, while the interruption of the genes involved in K1 capsule production and export may have had a downstream effect on other genes or gene products required for motility. Additionally, the copy number of the complementing plasmids may have altered the balance between the amount of capsule produced and other gene products involved in the cell motility.

Biofilm formation under stress conditions

With the observation that motility was significantly reduced under certain stress conditions, we hypothesized that biofilm formation would inversely increase under these stress conditions. It has been shown in *Escherichia coli* (Lee et *al.*, 2007) and *P. aeruginosa* (Banin *et al.*, 2005; Patriquin *et al.*, 2008) that exposure to specific compounds, such as indole and iron, can stimulate biofilm production while decreasing

Table 4. Measurements of diameter (mm) of the cell halos of each strain under the given conditions. The data are Means±SD of experiments that were performed in triplicate.

307-0294	307-0294::TN17	307-0294.30	307-0294.45	307-0294 ::TN17/pSS11	307-0294.30/ pNLAC1::*ptk*	307-0294.45/ pNLAC1::*epsA*
74.0±6.1	20.0±5.6	22.0±9.5	39.0±6.6	73.0±6.2	40.7±8.1	26.3±5.0

motility. All the isolates were able to form biofilm under all the conditions and media tested (data not shown). However, there were great variations in the amount of attachment to polystyrene. There was no correlation between increases or decreases in biofilm in the presence of certain extracellular stressors, and there was no correlation between the amount of biofilm and a given isolate's surface-associated motility.

Discussion

We infer from our data that motile *A. baumannii* isolates utilize multiple factors to move across the surface of motility agarose. Other microorganisms also use many attributes to move along a surface, such as *Myxococcus xanthus*, which requires TFP, LPS O-antigen, and a fibril extracellular matrix of exopolysaccharide (EPS) in S-motility on soft and wet surfaces (Lancero *et al.*, 2004). *V. paradoxus* EPS and *S. enterica* serovar Typhimurium secrete an unidentified wetting agent that helps propel them over an agar surface (Chen *et al.*, 2007; Jamieson *et al.*, 2009). Therefore, it is possible that *A. baumannii* may also use these factors in conjunction to move across a soft, semi-solid surface. TFP has also been shown to be involved in the motility of *P. aeruginosa* and *Neisseria gonorrhoeae* (Burrows, 2005). Genes coding for TFP have been identified in multiple *A. baumannii* genomes (Smith *et al.*, 2007; Adams *et al.*, 2008), and their role in motility was recently explored in another publication and shown to potentially contribute to the motility phenotype (Clemmer *et al.*, 2011). However, the insertion mutant that was analyzed in that study was made in *pilT*, which is a potential regulator of pilus retraction, and not in the gene encoding the pilin subunit itself, *pilA*. The data presented in this manuscript determined that the absence of LPS or capsular polysaccharide (Luke *et al.*, 2010; Russo *et al.*, 2010), resulted in impaired motility, but did not completely abolish motility in the 307-0294 strain. Therefore, it appears that other factors are involved in the motility of this organism beyond just Type IV-mediated motility.

The addition of extracellular stressors also supports the hypothesis that there are multiple factors involved in the motility of *A. baumannii* since regardless of the stress, motility was impeded and/or altered. The stressors added to the motility media were examples of potential stresses the cells may encounter *in vivo* or *in vitro*. The addition of an iron chelator, DIP, creates a free-iron-poor environment which is often encountered in the host. DIP significantly reduced the motility of all motile isolates. This is not due to a decrease in growth rate, as growth curves in this medium did not show any defects (data not shown). The addition of this chelator may block the release of LPS and thus the surface moisture does not increase, owing to the cells' inability to glide over the surface. In *E. coli* and *S. enterica* serovar Typhimurium investigators reported that saturation of the iron chelator transferrin blocked the release of LPS that typically occurs in the presence of this protein (Ellison *et al.*, 1988). Iron chelators can cause a bacteriostatic effect, which is not the case in this medium as growth was not inhibited. However, it is possible that the presence of iron-bound DIP may have prevented the release of LPS from *A. baumannii,* and therefore, reduced motility. *A. baumannii* may encounter an iron-rich environment after a traumatic injury (Liu *et al.*, 2004) or an injured patient receives a blood transfusion from stored blood, which contains high levels of free iron (Hod *et al.*, 2010). This iron-rich environment may promote the virulence of this pathogen as the presence of this extracellular micronutrient significantly enhanced motility. *A. baumannii* would not need to scavenge for this nutrient from a fixed position, but instead could disseminate through the host via motility. Also, a saline environment is found in the host, present in both body fluids and on skin. Salt is also ubiquitous within the hospital environment in the form of drug formulations, wound dressings, and intravenous fluids (Hood *et al.*, 2010). High salt has shown to induce a stress response in *A. baumannii* and therefore would likely impede motility, as was observed (Hood *et al.*, 2010). As expected, the removal of this stress not only restored motility but significantly enhanced motility by the isolates.

A. baumannii may encounter reactive oxygen species in multiple environments, such as the release from macrophages or other innate immune cells at the site of tissue injury and vaporized and dry mist-generated hydrogen peroxide disinfection systems which have shown to be efficacious against *A. baumannii* in the hospital setting (Forman and Torres, 2001; Ray *et al.*, 2010; Piskin *et al.*, 2011). As expected, this stress drastically reduced the motility of the tested strains away from the site of inoculation. In the hospital environment there is elevated use of antimicrobials both as a prophylactic application for patients with extensive injuries and to treat current infections; moreover, the antimicrobial triclosan is abundantly present in hand soaps. These agents may not always abolish or mitigate infections, but the stress of their presence can alter bacterial physiology. As expected, the addition of antibiotics or triclosan to the motility agarose plates significantly reduced the motility of these strains. Lastly, *A. baumannii* can be exposed to low levels of ethanol from antibacterial hand gels that are not thoroughly rubbed in or inadequately sanitized hospital surfaces (Dixon, 2008). So while exposure of *A. baumannii* to the stress of 1.0% ethanol increases the virulence of the pathogen (Smith *et al.*, 2007), it appears to impede motility as well.

Although extracellular stress correlates with motility, this link is not exhibited with biofilm formation. Additionally, motility does not inversely correlate with biofilm formation as observed in other motile isolates (O'Toole and Kolter, 1998). The lack of correlation of biofilm formation with respect to an extracellular stress or motility is not surprising given the lack of correlation between biofilm and other cellular properties of *A. baumannii* that have been observed previously (McQueary and Actis, 2011).

In summary, our study provides a comprehensive analysis of motility displayed by a diverse set of *A. baumannii* clinical isolates. Further, we elucidated how this motility is affected by environmental signals and required gene products. We have highlighted the frequency with which this trait is observed in clinical isolates as well as how much variation exists between different strains of *A. baumannii* (Fig. 1). The next challenge will be to link the motility phenotype to virulence. If it can be determined that *A. baumannii* utilizes motility

in vivo to establish a more robust infection, then the work presented here, and in other studies (Mussi *et al*., 2010; Clemmer *et al*., 2011), may have identified potential targets that could be exploited for treatment.

Acknowledgements

We would like to thank Anthony Campagnari for the strains he provided and for his review and critique of this manuscript. We would also like to thank the MRSN, Robert Bonomo, Xiaozhe Huang, and Marcelo Tolmasky for providing *Acinetobacter baumannii* strains. We would like to gratefully acknowledge the funds from the Defense Medical Research and Development Program (DMRDP) that supported this work.

References

Adams, M.D., Goglin, K., Molyneaux, N., Hujer, K.M., Lavender, H., Jamison, J.J., MacDonald, I.J., Martin, K.M., Russo, T., Campagnari, A.A., and *et al.* 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J. Bacteriol.* **190**, 8053–8064.

Adams, D., Yee, L., Rimmer, J.A., Williams, R., Martin, H., and Ovington, C. 2011. Investigation and management of an *A. baumannii* outbreak in ICU. *Br. J. Nurs.* **20**, 140, 142, 144–147.

Alarcon, I., Evans, D.J., and Fleiszig, S.M. 2009. The role of twitching motility in *Pseudomonas aeruginosa* exit from and translocation of corneal epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **50**, 2237–2244.

Ali, A., Johnson, J.A., Franco, A.A., Metzger, D.J., Connell, T.D., Morris, J.G.Jr., and Sozhamannan, S. 2000. Mutations in the extracellular protein secretion pathway genes (eps) interfere with rugose polysaccharide production in and motility of *Vibrio cholerae*. *Infect. Immun.* **68**, 1967–1974.

Alsultan, A.A., Hamouda, A., Evans, B.A., and Amyes, S.G. 2009. *Acinetobacter baumannii*: emergence of four strains with novel bla (OXA-51-like) genes in patients with diabetes mellitus. *J. Chemother.* **21**, 290–295.

Banin, E., Vasil, M.L., and Greenberg, E.P. 2005. Iron and *Pseudomonas aeruginosa* biofilm formation. *Proc. Natl. Acad. Sci. USA* **102**, 11076–11081.

Beavers, S.F., Blossom, D.B., Wiemken, T.L., Kawaoka, K.Y., Wong, A., Goss, L., McCormick, M.I., Thoroughman, D., and Srinivasan, A. 2009. Comparison of risk factors for recovery of *Acinetobacter baumannii* during outbreaks at two Kentucky hospitals, 2006. *Public Health Rep.* **124**, 868–874.

Brisou, J. 1953. Essay on the system of the genus *Achromobacter*. *Ann. Inst. Pasteur (Paris)* **84**, 812–814.

Burrows, L.L. 2005. Weapons of mass retraction. *Mol. Microbiol.* **57**, 878–888.

Butler, M.T., Wang, Q., and Harshey, R.M. 2010. Cell density and mobility protect swarming bacteria against antibiotics. *Proc. Natl. Acad. Sci. USA* **107**, 3776–3781.

Caiazza, N.C., Shanks, R.M., and O'Toole, G.A. 2005. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. *J. Bacteriol.* **187**, 7351–7361.

Calhoun, J.H., Murray, C.K., and Manring, M.M. 2008. Multidrug-resistant organisms in military wounds from Iraq and Afghanistan. *Clin. Orthop. Relat. Res.* **466**, 1356–1362.

Chang, H.L., Tang, C.H., Hsu, Y.M., Wan, L., Chang, Y.F., Lin, C.T., Tseng, Y.R., Lin, Y.J., Sheu, J.J., Lin, C.W., and *et al.* 2009. Nosocomial outbreak of infection with multidrug-resistant *Acinetobacter baumannii* in a medical center in Taiwan. *Infect. Control Hosp. Epidemiol.* **30**, 34–38.

Chen, B.G., Turner, L., and Berg, H.C. 2007. The wetting agent required for swarming in *Salmonella enterica* serovar typhimurium is not a surfactant. *J. Bacteriol.* **189**, 8750–8753.

Choi, W.S., Kim, S.H., Jeon, E.G., Son, M.H., Yoon, Y.K., Kim, J.Y., Kim, M.J., Sohn, J.W., and Park, D.W. 2010. Nosocomial outbreak of carbapenem-resistant *Acinetobacter baumannii* in intensive care units and successful outbreak control program. *J. Korean Med. Sci.* **25**, 999–1004.

Choi, C.H., Lee, E.Y., Lee, Y.C., Park, T.I., Kim, H.J., Hyun, S.H., Kim, S.A., Lee, S.K., and Lee, J.C. 2005. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. *Cell Microbiol.* **7**, 1127–1138.

Cisneros, J.M. and Rodriguez-Bano, J. 2002. Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin. Microbiol. Infect.* **8**, 687–693.

Clemmer, K.M., Bonomo, R.A., and Rather, P.N. 2011. Genetic analysis of surface motility in *Acinetobacter baumannii*. *Microbiology* **157**, 2534–2544.

Cuthbertson, L., Mainprize, I.L., Naismith, J.H., and Whitfield, C. 2009. Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in Gram-negative bacteria. *Microbiol. Mol. Biol. Rev.* **73**, 155–177.

D'Arezzo, S., Principe, L., Capone, A., Petrosillo, N., Petrucca, A., and Visca, P. 2011. Changing carbapenemase gene pattern in an epidemic multidrug-resistant *Acinetobacter baumannii* lineage causing multiple outbreaks in central Italy. *J. Antimicrob. Chemother.* **66**, 54–61.

Da Re, S. and Ghigo, J.M. 2006. A CsgD-independent pathway for cellulose production and biofilm formation in *Escherichia coli*. *J. Bacteriol.* **188**, 3073–3087.

Dalla-Costa, L.M., Coelho, J.M., Souza, H.A., Castro, M.E., Stier, C.J., Bragagnolo, K.L., Rea-Neto, A., Penteado-Filho, S.R., Livermore, D.M., and Woodford, N. 2003. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. *J. Clin. Microbiol.* **41**, 3403–3406.

Dallo, S.F. and Weitao, T. 2010. Insights into *Acinetobacter* war-wound infections, biofilms, and control. *Adv. Skin Wound Care* **23**, 169–174.

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

Dixon, B. 2008. There's the rub: infection control that spreads infection. *Lancet Infect. Dis.* **8**, 91–91.

Eijkelkamp, B.A., Hassan, K.A., Paulsen, I.T., and Brown, M.H. 2011. Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions. *BMC Genomics* **12**, 126.

Ellison, R.T.3rd, Giehl, T.J., and LaForce, F.M. 1988. Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferrin. *Infect. Immun.* **56**, 2774–2781.

Falagas, M.E., Karveli, E.A., Kelesidis, I., and Kelesidis, T. 2007. Community-acquired *Acinetobacter* infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **26**, 857–868.

Firehammer, B.D. 1987. Inhibition of growth and swarming of *Proteus mirabilis* and *Proteus vulgaris* by triclosan. *J. Clin. Microbiol.* **25**, 1312–1313.

Fong, J.C., Syed, K.A., Klose, K.E., and Yildiz, F.H. 2010. Role of *Vibrio* polysaccharide (vps) genes in VPS production, biofilm formation and *Vibrio cholerae* pathogenesis. *Microbiology* **156**, 2757–2769.

Forbes, S.J., Eschmann, M., and Mantis, N.J. 2008. Inhibition of *Salmonella enterica* serovar typhimurium motility and entry into epithelial cells by a protective antilipopolysaccharide monoclonal

immunoglobulin A antibody. *Infect. Immun.* **76**, 4137–4144.

Forman, H.J. and Torres, M. 2001. Redox signaling in macrophages. *Mol. Aspects Med.* **22**, 189–216.

Gaddy, J.A., Tomaras, A.P., and Actis, L.A. 2009. The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. *Infect. Immun.* **77**, 3150–3160.

Gootz, T.D. and Marra, A. 2008. *Acinetobacter baumannii*: an emerging multidrug-resistant threat. *Expert Rev. Anti. Infect. Ther.* **6**, 309–325.

Guerrero, D.M., Perez, F., Conger, N.G., Solomkin, J.S., Adams, M.D., Rather, P.N., and Bonomo, R.A. 2010. *Acinetobacter baumannii*-associated skin and soft tissue infections: recognizing a broadening spectrum of disease. *Surg. Infect. (Larchmt)* **11**, 49–57.

Halvorsen, J.F. 1963. Gliding Motility in the Organisms *Bacterium anitratum* (B5w), *Moraxella lwoffi* and *Alkaligenes haemolysans*, as compared to *Moraxella nonliquefaciens*. *Acta. Pathol. Microbiol. Scand.* **59**, 200–204.

Heilmann, C., Gerke, C., Perdreau-Remington, F., and Gotz, F. 1996. Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. *Infect. Immun.* **64**, 277–282.

Hod, E.A., Zhang, N., Sokol, S.A., Wojczyk, B.S., Francis, R.O., Ansaldi, D., Francis, K.P., Della-Latta, P., Whittier, S., Sheth, S., and *et al.* 2010. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood* **115**, 4284–4292.

Hood, M.I., Jacobs, A.C., Sayood, K., Dunman, P.M., and Skaar, E.P. 2010. *Acinetobacter baumannii* increases tolerance to antibiotics in response to monovalent cations. *Antimicrob. Agents Chemother.* **54**, 1029–1041.

Huang, T.P., Somers, E.B., and Wong, A.C. 2006. Differential biofilm formation and motility associated with lipopolysaccharide/exopolysaccharide-coupled biosynthetic genes in *Stenotrophomonas maltophilia*. *J. Bacteriol.* **188**, 3116–3120.

Jacobs, A.C., Hood, I., Boyd, K.L., Olson, P.D., Morrison, J.M., Carson, S., Sayood, K., Iwen, P.C., Skaar, E.P., and Dunman, P.M. 2010. Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. *Infect. Immun.* **78**, 1952–1962.

Jamieson, W.D., Pehl, M.J., Gregory, G.A., and Orwin, P.M. 2009. Coordinated surface activities in *Variovorax paradoxus* EPS. *BMC Microbiol.* **9**, 124.

Joly-Guillou, M.L. 2005. Clinical impact and pathogenicity of *Acinetobacter*. *Clin. Microbiol. Infect.* **11**, 868-873.

Karthikeyan, K., Thirunarayan, M.A., and Krishnan, P. 2010. Coexistence of blaOXA-23 with blaNDM-1 and armA in clinical isolates of *Acinetobacter baumannii* from India. *J. Antimicrob. Chemother.* **65**, 2253–2254.

Kearns, D.B. and Losick, R. 2003. Swarming motility in undomesticated *Bacillus subtilis*. *Mol. Microbiol.* **49**, 581–590.

Ko, K.S., Suh, J.Y., Kwon, K.T., Jung, S.I., Park, K.H., Kang, C.I., Chung, D.R., Peck, K.R., and Song, J.H. 2007. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J. Antimicrob. Chemother.* **60**, 1163–1167.

La Forgia, C., Franke, J., Hacek, D.M., Thomson, R.B.Jr., Robicsek, A., and Peterson, L.R. 2010. Management of a multidrug-resistant *Acinetobacter baumannii* outbreak in an intensive care unit using novel environmental disinfection: a 38-month report. *Am. J. Infect. Control* **38**, 259–263.

Lancero, H., Caberoy, N.B., Castaneda, S., Li, Y., Lu, A., Dutton, D., Duan, X.Y., Kaplan, H.B., Shi, W., and Garza, A.G. 2004. Characterization of a *Myxococcus xanthus* mutant that is defective for adventurous motility and social motility. *Microbiology* **150**, 4085–4093.

Lee, J., Bansal, T., Jayaraman, A., Bentley, W.E., and Wood, T.K. 2007. Enterohemorrhagic *Escherichia coli* biofilms are inhibited by 7-hydroxyindole and stimulated by isatin. *Appl. Environ. Microbiol.* **73**, 4100–4109.

Letunic, I. and Bork, P. 2011. Interactive tree of life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res.* **39**, W475–478.

Lindberg, R.B., Wetzler, T.F., Newton, A., Howard, J.M., Davis, J.H., and Strawitz, J. 1955. The bacterial flora of the blood stream in the Korean battle casualty. *Ann. Surg.* **141**, 366–368.

Lindum, P.W., Anthoni, U., Christophersen, C., Eberl, L., Molin, S., and Givskov, M. 1998. N-Acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* **180**, 6384–6388.

Liu, J.B., Tang, T.S., and Xiao, D.S. 2004. Changes of free iron contents and its correlation with lipid peroxidation after experimental spinal cord injury. *Chin. J. Traumatol.* **7**, 229–232.

Loehfelm, T.W., Luke, N.R., and Campagnari, A.A. 2008. Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. *J. Bacteriol.* **190**, 1036–1044.

Luke, N.R., Sauberan, S.L., Russo, T.A., Beanan, J.M., Olson, R., Loehfelm, T.W., Cox, A.D., St Michael, F., Vinogradov, E.V., and Campagnari, A.A. 2010. Identification and characterization of a glycosyltransferase involved in *Acinetobacter baumannii* lipopolysaccharide core biosynthesis. *Infect. Immun.* **78**, 2017–2023.

Marchaim, D., Navon-Venezia, S., Leavitt, A., Chmelnitsky, I., Schwaber, M.J., and Carmeli, Y. 2007. Molecular and epidemiologic study of polyclonal outbreaks of multidrug-resistant *Acinetobacter baumannii* infection in an Israeli hospital. *Infect. Control Hosp. Epidemiol.* **28**, 945–950.

Matsuyama, T., Bhasin, A., and Harshey, R.M. 1995. Mutational analysis of flagellum-independent surface spreading of *Serratia marcescens* 274 on a low-agar medium. *J. Bacteriol.* **177**, 987–991.

McQueary, C.N. and Actis, L.A. 2011. *Acinetobacter baumannii* biofilms: Variations among strains and correlations with other cell properties. *J. Microbiol.* **49**, 243–250.

Miller, J. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold spring Harbor, New York, N.Y., USA.

Montefour, K., Frieden, J., Hurst, S., Helmich, C., Headley, D., Martin, M., and Boyle, D.A. 2008. *Acinetobacter baumannii:* an emerging multidrug-resistant pathogen in critical care. *Crit. Care Nurse* **28**, 15–25; quiz 26.

Mussi, M.A., Gaddy, J.A., Cabruja, M., Arivett, B.A., Viale, A.M., Rasia, R., and Actis, L.A. 2010. The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. *J. Bacteriol.* **192**, 6336–6345.

Nwugo, C.C., Gaddy, J.A., Zimbler, D.L., and Actis, L.A. 2011. Deciphering the iron response in *Acinetobacter baumannii*: A proteomics approach. *J. Proteomics* **74**, 44–58.

O'Toole, G.A. and Kolter, R. 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* **30**, 295–304.

Park, Y.K., Choi, J.Y., Shin, D., and Ko, K.S. 2011. Correlation between overexpression and amino acid substitution of the PmrAB locus and colistin resistance in *Acinetobacter baumannii*. *Int. J. Antimicrob. Agents* **37**, 525–530.

Patriquin, G.M., Banin, E., Gilmour, C., Tuchman, R., Greenberg, E.P., and Poole, K. 2008. Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. *J. Bacteriol.* **190**, 662–671.

Peleg, A.Y., Seifert, H., and Paterson, D.L. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* **21**, 538–582.

Perez, F., Endimiani, A., Ray, A.J., Decker, B.K., Wallace, C.J., Hujer, K.M., Ecker, D.J., Adams, M.D., Toltzis, P., Dul, M.J., and *et al.* 2010. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *J. Antimicrob. Che-*

mother. **65**, 1807–1818.

Petersen, K., Riddle, M.S., Danko, J.R., Blazes, D.L., Hayden, R., Tasker, S.A., and Dunne, J.R. 2007. Trauma-related infections in battlefield casualties from Iraq. *Ann. Surg.* **245**, 803–811.

Piskin, N., Celebi, G., Kulah, C., Mengeloglu, Z., and Yumusak, M. 2011. Activity of a dry mist-generated hydrogen peroxide disinfection system against methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii. Am. J. Infect. Control.* **37**, 525–530.

Prates, C.G., Martins, A.F., Superti, S.V., Lopes, F.S., Ramos, F., Cantarelli, V.V., and Zavascki, A.P. 2011. Risk factors for 30-day mortality in patients with carbapenem-resistant *Acinetobacter baumannii* during an outbreak in an intensive care unit. *Epidemiol. Infect.* **139**, 411–418.

Rashid, M.H. and Kornberg, A. 2000. Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. USA* **97**, 4885–4890.

Ray, A., Perez, F., Beltramini, A.M., Jakubowycz, M., Dimick, P., Jacobs, M.R., Roman, K., Bonomo, R.A., and Salata, R.A. 2010. Use of vaporized hydrogen peroxide decontamination during an outbreak of multidrug-resistant *Acinetobacter baumannii* infection at a long-term acute care hospital. *Infect. Control Hosp. Epidemiol.* **31**, 1236–1241.

Russo, T.A., Luke, N.R., Beanan, J.M., Olson, R., Sauberan, S.L., Macdonald, U., Schultz, L.W., Umland, T.C., and Campagnari, A.A. 2010. The K1 capsular polysaccharide of *Acinetobacter baumannii* 307-0294 is a major virulence factor. *Infect. Immun.* **78**, 3993–4000.

Russo, T.A., MacDonald, U., Beanan, J.M., Olson, R., MacDonald, I.J., Sauberan, S.L., Luke, N.R., Schultz, L.W., and Umland, T.C. 2009. Penicillin-binding protein 7/8 contributes to the survival of *Acinetobacter baumannii in vitro* and *in vivo. J. Infect. Dis.* **199**, 513–521.

Scott, P., Deye, G., Srinivasan, A., Murray, C., Moran, K., Hulten, E., Fishbain, J., Craft, D., Riddell, S., Lindler, L., and *et al.* 2007. An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. *Clin. Infect. Dis.* **44**, 1577–1584.

Smith, M.G., Gianoulis, T.A., Pukatzki, S., Mekalanos, J.J., Ornston, L.N., Gerstein, M., and Snyder, M. 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev.* **21**, 601–614.

Stepanovic, S., Vukovic, D., Dakic, I., Savic, B., and Svabic-Vlahovic, M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J. Microbiol. Methods* **40**, 175–179.

Strom, M.S. and Lory, S. 1993. Structure-function and biogenesis of the type IV pili. *Annu. Rev. Microbiol.* **47**, 565–596.

Toguchi, A., Siano, M., Burkart, M., and Harshey, R.M. 2000. Genetics of swarming motility in *Salmonella enterica* serovar typhimurium: critical role for lipopolysaccharide. *J. Bacteriol.* **182**, 6308–6321.

Tomaras, A.P., Dorsey, C.W., Edelmann, R.E., and Actis, L.A. 2003. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. *Microbiology* **149**, 3473–3484.

Tong, M.J. 1972. Septic complications of war wounds. *JAMA* **219**, 1044–1047.

Wakimoto, N., Nishi, J., Sheikh, J., Nataro, J.P., Sarantuya, J., Iwashita, M., Manago, K., Tokuda, K., Yoshinaga, M., and Kawano, Y. 2004. Quantitative biofilm assay using a microtiter plate to screen for enteroaggregative *Escherichia coli. Am. J. Trop. Med. Hyg.* **71**, 687–690.

Yang, Z., Lux, R., Hu, W., Hu, C., and Shi, W. 2010. PilA localization affects extracellular polysaccharide production and fruiting body formation in *Myxococcus xanthus. Mol. Microbiol.* **76**, 1500–1513.

Yun, H.C., Branstetter, J.G., and Murray, C.K. 2008. Osteomyelitis in military personnel wounded in Iraq and Afghanistan. *J. Trauma* **64**, S163–168; discussion S168.

Zimbler, D.L., Penwell, W.F., Gaddy, J.A., Menke, S.M., Tomaras, A.P., Connerly, P.L., and Actis, L.A. 2009. Iron acquisition functions expressed by the human pathogen *Acinetobacter baumannii. BioMetals* **22**, 23–32.