Research Article

Identification of factors needed by a clinical isolate of *Acinetobacter baumannii* to resist antibacterial compounds

Celena M. Gwin[1], Natalia Prakash, and Nathan W. Rigel

Department of Biology, Hofstra University, Hempstead, NY 11549

Abstract. In recent years, *Acinetobacter baumannii* has emerged as a major threat to human health. Yet studies to uncover the molecular mechanisms used by *A. baumannii* to mediate human infections have only recently begun. For example, the methods used by *A. baumannii* to resist antibacterial treatment are not fully understood. Using a candidate approach, we sought to identify proteins found in the *A. baumannii* cell envelope that are important for resistance to antimicrobial compounds. We screened selected mutants in genes needed for assembly of the bacterial cell envelope, biofilm formation, or capsule production for sensitivity to different antibiotics and detergents. Compared to wild-type, these mutants displayed an increased sensitivity to bacitracin yet not to vancomycin, both antibiotics that target the cell wall. Only the *ompA* and *lnt* mutants had significantly increased sensitivity to the DNA gyrase inhibitor novobiocin. Similarly, there was also a spectrum of sensitivity to bile salts, with *ompA* and *lnt* mutants being most sensitive while *pgaA*, *pgaB*, and *capA* mutants showed a modest growth defect. While we do not fully understand the specificity of these sensitivity profiles, our findings suggest a potential use for these antimicrobial compounds in genetic selections for mutants that disrupt cell envelope biogenesis. This approach could potentially inform future efforts to combat multi-drug resistant *Acinetobacter* infections.

Introduction

Components of the bacterial cell envelope are the first line of defense microbes have to combat antimicrobial compounds (Silhavy et al., 2010). For example, the outer membrane (OM) of Gram-negative bacteria is a particularly formidable barrier. Composed of lipopolysaccharide (LPS) in the outer leaflet and phospholipids in the inner leaflet, passage of large molecules across this asymmetric bilayer is highly restricted (May and Silhavy, 2017; Okuda et al., 2016). Importantly, this permeability barrier is an important defense against entry of anti-bacterial compounds like antibiotics and detergents (May and Silhavy, 2017). In addition to the OM, many Gram-negative bacteria also produce extracellular polysaccharide capsules that provide another layer of protection (Woodward and Naismith, 2016). These capsules are often among the most important virulence factors produced by bacterial pathogens like *Neisseria meningitidis* and *Klebsiella pneumoniae* (Paczosa and Mecsas, 2016; Tzeng et al., 2016). These capsules provide protection against phagocytosis by human immune cells, allowing

Correspondence to: nathan.w.rigel@hofstra.edu
[1]**Current address:** Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536

Bios 90(3) 149–157, 2019

propagation of infection. Formation of biofilms is another strategy deployed by microbes to enable colonization, with a notable example being how *Pseudomonas aeruginosa* infects lungs of cystic fibrosis patients (Rybtke et al., 2015). Studies have shown that the emerging human pathogen *Acinetobacter baumannii* is capable of deploying all of these countermeasures to successfully infect humans (Iwashkiw et al., 2012; Kwon et al., 2017; Smani et al., 2014; Wang-Lin et al., 2017). Coupled with an affinity for acquiring drug resistance determinants horizontally, *A. baumannii* represents an urgent threat to human health (Andersson et al., 2017; Maciel et al., 2017; Shin and Park, 2017).

Faced with the dangers posed by *A. baumannii*, there has recently been an increase in studies devoted to understanding *A. baumannii* pathogenesis. An important tool to aid these efforts is the availability of an ordered transposon mutant library in a clinical isolate of *A. baumannii* (Gallagher et al., 2015). Ordered mutant libraries have the potential to rapidly facilitate genetic analysis of non-model organisms like *A. baumannii*. Comprehensive genetic analyses are critically important to gain insight into the molecular mechanisms used by *A. baumannii* to cause human infections and to resist antibiotic treatment. An important requirement for genetic analysis in bacteria is the ability to design selections and screen for particular mutants. Measuring the relative sensitivity to chemicals like antibiotics and detergents is a convenient way to hunt for mutants in the laboratory. Such chemical genetic approaches have been important for understanding basic bacterial physiology and have been applied to drug discovery efforts (Anders et al., 2014; French et al., 2016; Jones et al., 2017; Silver, 2011). We reasoned that disrupting factors important for cell envelope biogenesis are likely to display robust phenotypes when exposed to antibiotics and detergents and thus facilitate subsequent analyses.

To initiate our studies, we scanned the *A. baumannii* mutant library for known and predicted virulence factors involved in biogenesis of the bacterial cell envelope. We identified factors implicated in forming biofilms (*pgaA and pgaB*; Itoh et al., 2005; Wang et al., 2004), producing capsules (*capA*; George et al., 2015; Olivares-Illana et al., 2008), and acylating membrane lipoproteins (*lnt*; Buddelmeijer and Young, 2010; Robichon et al., 2005). We also selected *ompA*, a known *A. baumannii* virulence factor important for motility and antibiotic resistance (Kwon et al., 2017; Smani et al., 2014). With these mutants in hand, we subjected them to treatment with different anti-bacterial compounds. Compared to wild-type, both the *ompA* and *lnt* mutants displayed aberrant colony morphologies and sensitivity to three different compounds. In contrast, the remaining mutants had more modest phenotypes, showing significant sensitivity to only two compounds. Taken together, our findings show that formation of the bacterial cell envelope is critical for *A. baumannii* to survive treatment with antibacterial compounds. In addition, our results will enable future genetic screens to dissect the molecular mechanisms by which *A. baumannii* causes disease.

Table 1. Strains used in this study (source: Gallagher et al., 2015)

Strain	Genotype
AB5075	wild-type
pgaA	*pgaA*109::T26
pgaB	*pgaB*172::T26
capA	*capA*172::T26
ompA	*ompA*157::T26
lnt	*lnt*153::T26

The T26 cassette confers resistance to tetracycline.

Materials and Methods

Bacterial strains and growth conditions

All *A. baumannii* strains used in this study are listed in Table 1, and were obtained from the University of Washington *A. baumannii* Mutant Collection (Gallagher et al., 2015). All strains were grown in LB broth or on LB agar plates (Lennox; Fisher Scientific) at 30°C prior to antibiotic/detergent susceptibility testing as described below.

Kirby-Bauer antibiotic sensitivity assays

Bacterial strains were tested for antibiotic sensitivity using disc diffusion assays. Overnight cultures of each strain were used to inoculate the entire surface of Mueller Hinton (BD) agar plates using sterile swabs. After allowing the plates time to dry, sterile paper discs impregnated with various antibiotics (BD BBL) were placed on the agar surface. Plates were incubated overnight at 30°C. The next day, zones of growth inhibition were measured. All antibiotics were tested in triplicate. The antibiotics used (and their dosages per disc) were bacitracin (10 µg), erythromycin (15 µg), novobiocin (30 µg), and vancomycin (30 µg). Mean values from triplicate trials were then analyzed by Student's *t*-test for statistical significance.

Detergent sensitivity assays

To test for detergent sensitivity, overnight cultures of each bacterial strain were grown in LB at 30°C. The next day, a loopful of each strain was streaked for single colonies on MacConkey agar plates (BD, contains 1.5 g/L bile salts). For comparison, each strain was also streaked onto an LB agar plate. All plates were incubated overnight at 30°C and growth was assessed the next day.

Results

Acinetobacter baumannii AB5075 encodes genes with predicted roles in cell envelope biogenesis

The advent of high density mutagenesis and deep sequencing technologies have delivered an abundance of information about the microbial world. These data sets have allowed prediction of gene function of non-model organisms using data from well-studied organisms like *Escherichia coli*. Using the *E. coli* genes as the query, the *A. baumannii* strain AB5075 genome was searched for genes involved in building and maintaining components of a typical Gram-negative bacterial cell envelope. As expected, there were homologs to most of the key cell envelope biogenesis machinery. Among these were homologs of genes required for synthesizing and assembling LPS, outer membrane proteins (OMPs), lipoproteins, capsules, and biofilms. One exception was the absence of an obvious *bamC* homolog. This is not surprising as *bamC* is among the least well-conserved members of the OMP assembly machine known as the Bam complex (Ricci and Silhavy, 2012). As many of these factors are essential for growth, constructing loss-of-function mutations in such genes would be impossible. Thus, we focused our studies on the non-essential genes where mutants were already available. Several genes were chosen for subsequent study including *pgaA* and *pgaB* (biofilm adhesins), *capA* (capsule biosynthesis), *ompA* (major porin), and *lnt* (lipoprotein maturation).

Mutations that disrupt the bacterial cell envelope display a spectrum of sensitivity to antimicrobial compounds

In order to develop a chemical genetic framework to study *A. baumannii* mutants, each mutant was challenged with different detergents and antibiotics. First, a baseline for normal growth was established under standard laboratory conditions. When grown on LB agar plates, the *pgaA*, *pgaB*, and *capA* mutants demonstrated robust growth, similar to what we observed with wild-type *A. baumannii* (Fig. 1). These mutant strains all grow as densely wild-type in the heavy streaks and yield isolated colonies of similar sizes. However, both the *ompA* and *lnt* mutants exhibited growth defects as exhibited by altered colony morphology. Colonies of the *ompA* mutant appeared extremely mucoid. This type of colony morphology is often associated with induction of a cellular stress response (Sikdar et al., 2013). The single colonies of the *lnt* mutant are much smaller than wild-type, indicating that this mutant has a growth defect. In Gram-negative bacteria like *E. coli*, *lnt* is essential for growth. However, a recent report demonstrated that in some Gram-negative bacteria, mutants that lack *lnt* are viable (LoVullo et al., 2015). Given that several bacterial lipoproteins are essential for growth,

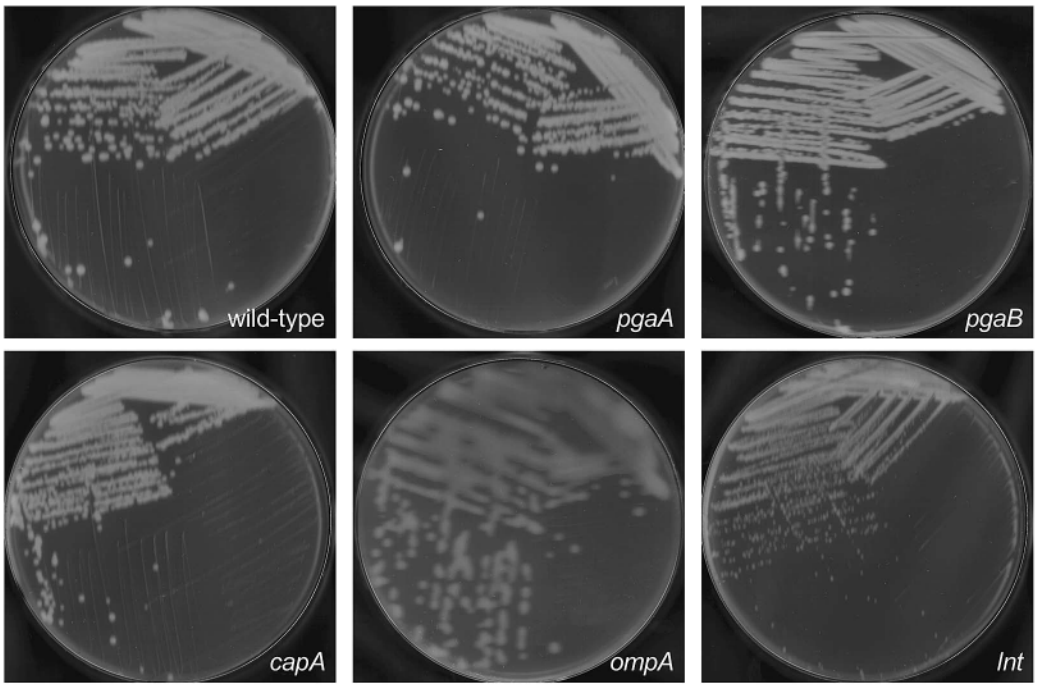

Figure 1. Growth of wild-type and mutant *A. baumannii* strains on LB agar plates. Broth cultures of the indicated strains were grown overnight in LB at 30°C. The next day, a loopful of each culture was streaked for single colonies onto LB agar plates. Plates were incubated overnight at 30°C prior to imaging.

it is not surprising that *lnt* is also important for robust growth.

With a baseline established, growth of each strain was observed on MacConkey agar plates. MacConkey agar is traditionally used to select for and differentiate among Gram-negative enteric bacteria. MacConkey agar contains bile salts, which act as a detergent and can solubilize membranes. Normally, Gram-negative bacteria with an intact cell envelope grow quite well on MacConkey agar. However, mutants with defective cell envelopes often display severe growth defects associated with increased membrane permeability (Ruiz et al., 2006). As shown in Figure 2, wild-type *A. baumannii* was able to grow well on MacConkey agar. However, the *pgaA*, *pgaB*, *capA* mutants did not grow as well, only showing the most robust growth in the heaviest part of the streak plate. In particular, growth of the *pgaA* mutant is thin in the heavy streak and actually yields single colonies. Robust growth was not observed for either the *ompA* or *lnt* mutants. In fact, both mutants showed poor growth even in the heaviest streak. The observed difference in growth on MacConkey is consistent with each mutation compromising structural integrity of the cell envelope.

Next, antibiotic disc sensitivity assays were performed using the Kirby-Bauer technique. *A. baumannii* AB5075 is a multi-drug resistant clinical isolate that encodes resistance elements for beta-lactams and aminoglycosides, among other antibiotics (Gallagher et al., 2015). To separate defects in the cell envelope from these resistance elements, we tested our collection of mutants for sensitivity to bacitracin, vancomycin, erythromycin, and novobiocin. Bacitracin and vancomycin are particularly useful for probing cell envelope integrity, as they are both large antibiotics that are generally excluded by the outer membrane when it is fully intact. Compared to wild-type *A. baumannii*, all mutants tested were significantly more sensitive to bacitracin (Fig. 3A). In particular, the *ompA* and *lnt* mutants exhibited the greatest level of

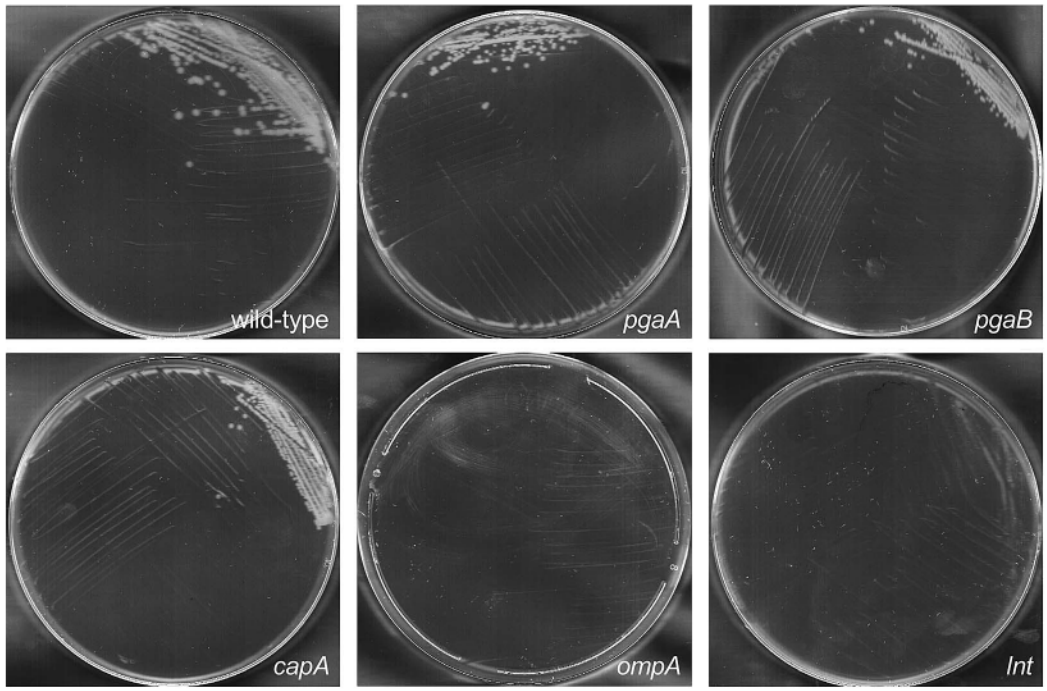

Figure 2. Growth of wild-type and mutant *A. baumannii* strains on MacConkey agar plates. Broth cultures of the indicated strains were grown overnight in LB at 30°C. The next day, a loopful of each culture was streaked for single colonies onto MacConkey agar plates. Plates were incubated overnight at 30°C prior to imaging.

sensitivity. Interestingly, a different trend was observed with vancomycin. All strains tested were equally sensitive to vancomycin (Fig. 3B). Since both bacitracin and vancomycin inhibit cell wall synthesis, albeit in different ways, each strain was challenged with two additional classes of antibiotics. When challenged with erythromycin, which binds to the 50S ribosomal subunit and inhibits translation, all strains exhibited the same degree of sensitivity (Fig. 3C). However, both the *ompA* and *lnt* mutants showed significant sensitivity when challenged with novobiocin, which inhibits DNA replication upon binding to DNA gyrase (Fig. 3D). In contrast, the *pgaA*, *pgaB*, and *capA* mutants were no more sensitive to novobiocin than wild-type *A. baumannii*.

Discussion

To effectively kill a bacterial cell, antibacterial agents must penetrate a variety of defensive measures. In Gram-negative bacteria, the cell envelope is among the most powerful barriers preventing successful antimicrobial chemotherapy (May and Silhavy, 2017; Okuda et al., 2016; Silhavy et al., 2010). In order to develop new antibiotics, a thorough understanding of bacterial cell physiology is essential. Using a genetic approach, we sought to identify factors important for *A. baumannii* to resist exposure to antibacterial compounds. Over the course of our studies, we observed a spectrum of phenotypes associated with various cell envelope mutants. Some mutants like *ompA* and *lnt* had growth defects even in the absence of significant chemical challenge. Not surprisingly, these two mutants were also the most susceptible to the broadest variety of antimicrobial compounds. Both mutants were strongly inhibited by the bile salts found in MacConkey agar, failing to grow even in the heaviest part of a streak plate. When challenged with antibiotics, both mutants also showed the greatest sensitivity. Bacitracin and novobiocin were both

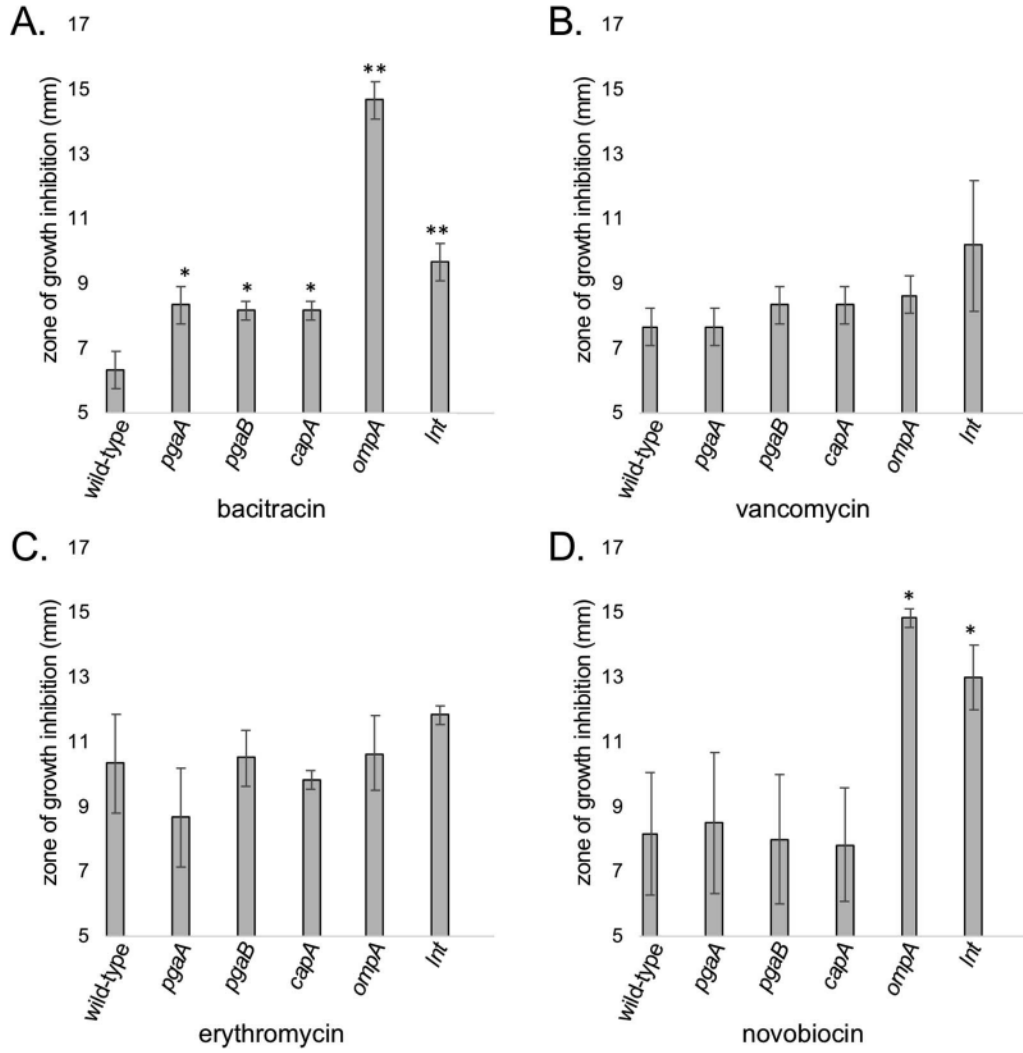

Figure 3. Antibiotic sensitivity profiles of *A. baumannii* mutants. The indicated strains were seeded onto Mueller Hinton agar plates and then discs with bacitracin (A), vancomycin (B), erythromycin (C), or novobiocin (D) were placed on the agar surface. After overnight incubation at 30°C, zones of growth inhibition around each disc were measured. The data represent the mean of triplicate trials from a representative experiment, and the error bars represent the standard deviation. Significance was calculated using Student's *t*-test; * $p < 0.05$, ** $p < 0.01$.

effective at inhibiting growth of both the *ompA* and *lnt* mutants.

Of all the genes we studied, *ompA* has been the most well characterized in *A. baumannii*. OmpA has been shown to be the major non-specific porin found in the *A. baumannii* outer membrane (Sugawara and Nikaido, 2012). Like all OMPs, OmpA forms a beta-barrel with a central pore, though the small pore size (~2 nm) makes OmpA relatively impermeable to toxic compounds like antibiotics and detergents. Interestingly, mutants lacking *ompA* show increased membrane permeability as observed here and in other reports (Kwon et al., 2017; Sugawara and Nikaido, 2012). While increased permeability to antibiotics has been noted before, there has been no clear description of the molecular underpinnings behind this phenotype. Given the broad sensitivity of *ompA* mutants to a wide variety of antibiotics and

detergents, it is possible that the absence of this major OMP somehow destabilizes the outer membrane of *A. baumannii*. Structural studies of the *A. baumannii* outer membrane are underway and will likely shed light on this possibility (Koning et al., 2013).

To our knowledge, this is the first report of the phenotypes associated with an *lnt* mutant in any species or strain of *Acinetobacter*. Given the essential nature of *lnt* in most Gram-negative bacteria, and the fitness costs associated with losing *lnt* in those organisms where it is possible, we were not surprised to observe the growth defects associated with the *lnt* mutant. Like all typical Gram-negative bacteria, *A. baumannii* encodes numerous lipoproteins that are predicted to be essential for growth. It is already established that the third and final acylation step accomplished by Lnt is required for efficient lipoprotein trafficking via the Lol system (Narita and Tokuda, 2017). Without this acyl tail, sorting of di-acylated lipoproteins is dramatically reduced (Robichon et al., 2005). Thus, all functions performed by lipoproteins, including OMP and LPS assembly, will be compromised in turn. Such generalized disruption of the cell envelope explains the broad sensitivity phenotypes exhibited by the *lnt* mutant in our studies. We are currently using the *lnt* mutant as a tool to investigate how lipoproteins are sorted in *Acinetobacter* species. Interestingly, bacteria that can tolerate loss of *lnt* also contain an alteration in their lipoprotein sorting (Lol) genes in which the *lolC* and *lolE* genes are fused into a single gene called *lolF* (Chahales and Thanassi, 2015; LoVullo et al., 2015). A similar fusion event has occurred in *A. baumannii*, which likely explains the ability to disrupt *lnt*. More experiments are needed to understand how these changes influence how lipoproteins are sorted in *A. baumannii*, and will likely inform studies of lipoprotein trafficking in Gram-negative bacteria more broadly.

A more complicated story emerges when looking at the *pgaA*, *pgaB*, and *capA* mutants. In contrast to *ompA* and *lnt*, these other mutants displayed more modest sensitivity phenotypes. On the continuum between wild-type and *ompA*/*lnt*, the *pgaA*, *pgaB*, and *capA* mutants fell somewhere in between. For example, on MacConkey plates these three mutants were able to grow, though not quite as robustly as wild-type. When challenged with antibiotics, these three mutants grew just as well as wild-type when exposed to erythromycin, novobiocin, and vancomycin. However, bacitracin was effective at inhibiting growth of *pgaA*, *pgaB*, and *capA*. This suggests a level of specificity unique to bacitracin that we do not fully understand. Both bacitracin and vancomycin are large cyclic peptides that inhibit cell wall synthesis. Their mechanism of action is slightly different however. Bacitracin interferes with bactoprenol, the carrier molecule that transports peptidoglycan subunits to the periplasm for incorporation into the growing peptidoglycan cell wall (Stone and Strominger, 1971). Vancomycin binds to the peptides that extend from N-acetylmuramic acid residues, thus blocking crosslinking of adjacent peptidoglycan chains (Watanakunakorn, 1984). This difference in mechanism of action does not provide an obvious explanation for the selective sensitivities we observed. Since cell wall formation occurs in the periplasm, presumably both antibiotics must reach the same subcellular compartment in order to function. While we do not have any evidence suggesting vancomycin is selectively excluded by the *A. baumannii* cell envelope, this remains a formal possibility. Taken together, our findings identified four genes with important roles in conferring resistance to antibacterial compounds: *pgaA*, *pgaB*, *capA*, and *lnt*. By challenging these mutants with a variety of antibacterial compounds, we have identified molecules that will be useful in ongoing selections and screens for mutants, which will be used to illuminate how the *A. baumannii* cell envelope is assembled. Furthermore, this provides the opportunity to investigate differential effects of antibiotics working in similar pathways. Pursuing these studies will yield a clearer understanding of how bacteria like *A. baumannii* survive attack by antibacterial compounds.

Acknowledgments: This work was supported by start-up funds from Hofstra University and

by funds from the National Science Foundation under Grant No. 1615822 to NWR. CMG was supported in part by a fellowship from TriBeta.

References

Anders, L., Guenther, M.G., Qi, J., Fan, Z.P., Marineau, J.J., Rahl, P.B., Lovén, J., Sigova, A.A., Smith, W.B., Lee, T.I., et al. (2014). Genome-wide localization of small molecules. Nat. Biotechnol. 32, 92–96.

Andersson, J.A., Sha, J., Kirtley, M.L., Reyes, E., Fitts, E.C., Dann, S.M., and Chopra, A.K. (2017). Combating multi-drug resistant pathogens with host-directed non-antibiotic therapeutics. Antimicrob. Agents Chemother. e01943–1917.

Buddelmeijer, N., and Young, R. (2010). The essential *Escherichia coli* apolipoprotein N-acyltransferase (Lnt) exists as an extracytoplasmic thioester acyl-enzyme intermediate. Biochemistry (Mosc.) 49, 341–346.

Chahales, P., and Thanassi, D.G. (2015). A more flexible lipoprotein sorting pathway. J. Bacteriol. 197, 1702–1704.

French, S., Mangat, C., Bharat, A., Côté, J.-P., Mori, H., and Brown, E.D. (2016). A robust platform for chemical genomics in bacterial systems. Mol. Biol. Cell 27, 1015–1025.

Gallagher, L.A., Ramage, E., Weiss, E.J., Radey, M., Hayden, H.S., Held, K.G., Huse, H.K., Zurawski, D.V., Brittnacher, M.J., and Manoil, C. (2015). Resources for genetic and genomic analysis of emerging pathogen *Acinetobacter baumannii*. J. Bacteriol. 197, 2027–2035.

George, S.E., Nguyen, T., Geiger, T., Weidenmaier, C., Lee, J.C., Liese, J., and Wolz, C. (2015). Phenotypic heterogeneity and temporal expression of the capsular polysaccharide in *Staphylococcus aureus*. Mol. Microbiol. 98, 1073–1088.

Itoh, Y., Wang, X., Hinnebusch, B.J., Preston, J.F., and Romeo, T. (2005). Depolymerization of beta-1,6-N-acetyl-D-glucosamine disrupts the integrity of diverse bacterial biofilms. J. Bacteriol. 187, 382–387.

Iwashkiw, J.A., Seper, A., Weber, B.S., Scott, N.E., Vinogradov, E., Stratilo, C., Reiz, B., Cordwell, S.J., Whittal, R., Schild, S., et al. (2012). Identification of a general O-linked protein glycosylation system in *Acinetobacter baumannii* and its role in virulence and biofilm formation. PLoS Pathog. 8, e1002758.

Jones, M.B., Nierman, W.C., Shan, Y., Frank, B.C., Spoering, A., Ling, L., Peoples, A., Zullo, A., Lewis, K., and Nelson, K.E. (2017). Reducing the bottleneck in discovery of novel antibiotics. Microb. Ecol. 73, 658–667.

Koning, R.I., de Breij, A., Oostergetel, G.T., Nibbering, P.H., Koster, A.J., and Dijkshoorn, L. (2013). Cryo-electron tomography analysis of membrane vesicles from *Acinetobacter baumannii* ATCC19606 T. Res. Microbiol. 164, 397–405.

Kwon, H.I., Kim, S., Oh, M.H., Na, S.H., Kim, Y.J., Jeon, Y.H., and Lee, J.C. (2017). Outer membrane protein A contributes to antimicrobial resistance of *Acinetobacter baumannii* through the OmpA-like domain. J. Antimicrob. Chemother. 72, 3012–3015.

LoVullo, E.D., Wright, L.F., Isabella, V., Huntley, J.F., and Pavelka, M.S. (2015). Revisiting the Gram-negative lipoprotein paradigm. J. Bacteriol. 197, 1705–1715.

Maciel, W.G., da Silva, K.E., Croda, J., Cayô, R., Ramos, A.C., de Sales, R.O., de Souza, G.H. de A., Bortolotto Bampi, J.V., Limiere, L.C., Casagrande, J.C., et al. (2017). Clonal spread of carbapenem-resistant *Acinetobacter baumannii* in neonatal intensive care unit. J. Hosp. Infect. 98(3), 300–304.

May, K.L., and Silhavy, T.J. (2017). Making a membrane on the other side of the wall. Biochim. Biophys. Acta 1862, 1386–1393.

Narita, S.-I., and Tokuda, H. (2017). Bacterial lipoproteins; biogenesis, sorting and quality control. Biochim. Biophys. Acta 1862, 1414–1423.

Okuda, S., Sherman, D.J., Silhavy, T.J., Ruiz, N., and Kahne, D. (2016). Lipopolysaccharide transport and assembly at the outer membrane: the PEZ model. Nat. Rev. Microbiol. 14, 337–345.

Olivares-Illana, V., Meyer, P., Bechet, E., Gueguen-Chaignon, V., Soulat, D., Lazereg-Riquier, S., Mijakovic, I., Deutscher, J., Cozzone, A.J., Laprévote, O., et al. (2008). Structural basis for the regulation mechanism of the tyrosine kinase CapB from *Staphylococcus aureus*. PLoS Biol. 6, e143.

Paczosa, M.K., and Mecsas, J. (2016). Klebsiella pneumoniae: Going on the offense with a strong defense. Microbiol. Mol. Biol. Rev. 80, 629–661.

Ricci, D.P., and Silhavy, T.J. (2012). The Bam machine: a molecular cooper. Biochim. Biophys. Acta 1818, 1067–1084.

Robichon, C., Vidal-Ingigliardi, D., and Pugsley, A.P. (2005). Depletion of apolipoprotein N-acyltransferase causes mislocalization of outer membrane lipoproteins in *Escherichia coli*. J. Biol. Chem. 280, 974–983.

Ruiz, N., Wu, T., Kahne, D., and Silhavy, T.J. (2006). Probing the barrier function of the outer membrane with chemical conditionality. ACS Chem. Biol. 1, 385–395.

Rybtke, M., Hultqvist, L.D., Givskov, M., and Tolker-Nielsen, T. (2015). *Pseudomonas aeruginosa* biofilm infections: community structure, antimicrobial tolerance and immune response. J. Mol. Biol. 427, 3628–3645.

Shin, B., and Park, W. (2017). Antibiotic resistance of pathogenic *Acinetobacter* species and emerging combination therapy. J. Microbiol. Seoul Korea 55, 837–849.

Sikdar, R., Simmons, A.R., and Doerrler, W.T. (2013). Multiple envelope stress response pathways are activated in an *Escherichia coli* strain with mutations in two members of the DedA membrane protein family. J. Bacteriol. 195, 12–24.

Silhavy, T.J., Kahne, D., and Walker, S. (2010). The bacterial cell envelope. Cold Spring Harb. Perspect. Biol. *2*, a000414.

Silver, L.L. (2011). Challenges of antibacterial discovery. Clin. Microbiol. Rev. 24, 71–109.

Smani, Y., Fàbrega, A., Roca, I., Sánchez-Encinales, V., Vila, J., and Pachón, J. (2014). Role of OmpA in the multidrug resistance phenotype of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 58, 1806–1808.

Stone, K.J., and Strominger, J.L. (1971). Mechanism of action of bacitracin: complexation with metal ion and C55-isoprenyl pyrophosphate. Proc. Natl. Acad. Sci. USA 68, 3223–3227.

Sugawara, E., and Nikaido, H. (2012). OmpA is the

principal nonspecific slow porin of *Acinetobacter baumannii*. J. Bacteriol. 194, 4089–4096.

Tzeng, Y.-L., Thomas, J., and Stephens, D.S. (2016). Regulation of capsule in *Neisseria meningitidis*. Crit. Rev. Microbiol. 42, 759–772.

Wang, X., Preston, J.F., and Romeo, T. (2004). The *pgaABCD* locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. J. Bacteriol. 186, 2724–2734.

Wang-Lin, S.X., Olson, R., Beanan, J.M., MacDonald, U., Balthasar, J.P., and Russo, T.A. (2017). The capsular polysaccharide of *Acinetobacter baumannii* is an obstacle for therapeutic passive immunization strategies. Infect. Immun. 85(12), e00591–17.

Watanakunakorn, C. (1984). Mode of action and in-vitro activity of vancomycin. J. Antimicrob. Chemother. 14 Suppl D, 7–18.

Woodward, L., and Naismith, J.H. (2016). Bacterial polysaccharide synthesis and export. Curr. Opin. Struct. Biol. 40, 81–88.

Received 3 December 2017; revised 11 December 2018; accepted 22 January 2019.