Published in final edited form as:
Microb Biotechnol. 2012 September ; 5(5): 581–583. doi:10.1111/j.1751-7915.2012.00334.x.

When it comes to drug discovery not all Gram-negative bacterial biodefense pathogens are created equal: *Burkholderia pseudomallei* is different

Herbert P. Schweizer
Department of Microbiology, Immunology and Pathology, and Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research, Colorado State University, Fort Collins, CO, USA

Amongst Gram-negative biodefense pathogens, *Yersinia pestis* (plague), *Francisella tularensis* (tularemia) and, to a lesser extent, *Brucella* species (brucellosis) are most widely studied. In contrast, *Burkholderia mallei* (glanders) and *B. pseudomallei* (melioidosis) have garnered less attention. While the underlying reasons are multifaceted, for example perceived importance of an organism being listed as a Category A versus B pathogen, *B. pseudomallei* poses formidable and unique challenges pertaining to development of therapeutic countermeasures. It is fair to say that, in general, *Y. pestis*, *F. tularensis* and *Brucella* species are susceptible to most classes of antibiotics and that the main challenge with these organisms is rapid and accurate diagnosis to enable initiation of proper therapeutic interventions. In contrast, therapeutic countermeasures for *B. pseudomallei* are limited because of intrinsic resistance (Wuthiekanun & Peacock, 2006, Estes *et al.*, 2010). At present, the recommended acute phase treatment for melioidosis includes β-lactam antibiotics such as ceftazidime, amoxicillin-clavulanic acid or carbapenems (e.g., meropenem and imipenem)(Peacock *et al.*, 2008). Other efficacious therapeutics such as trimethoprim-sulfamethoxazole are reserved for eradication phase treatment or potential prophylaxis (Peacock et al., 2008). To complicate matters, *Burkholderia* species are intrinsically resistant to polymyxins and therefore there is no drug of last resort such as colistin that is being used to treat infections by panresistant so-called superbugs.

Fundamentally, *B. pseudomallei* is not unique from other bacteria and intrinsic resistance is achieved using multiple, documented mechanisms (Walsh, 2003): 1. Exclusion from the cell; 2) Enzymatic inactivation; 3) Target alterations or deletion; and 4) Active efflux from the cell. A fifth mechanism, namely metabolic bypass of the effected enzyme by complementation with an insensitive equivalent has not yet been reported in *B. pseudomallei*. Resistance mechanisms can act in synergy to achieve significant levels of resistance. For example, drug efflux is most effective in bacteria with reduced outer membrane permeability (Nikaido, 2001), for example *Acinetobacter baumanii*, *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia.* The outer membrane permeability in these bacteria is between 1–11% of that observed in *Escherichia coli* (Hancock, 1998). Reduced outer membrane permeability is primarily due to the exclusionary properties of porins (Pages *et al.*, 2008) and lipopolysaccharide (LPS)(Raetz *et al.*, 2007). LPS contributes to high-level polymyxin resistance in species such as *Burkholderia* (Novem *et al.*, 2009) or mutant strains of *P. aeruginosa* and *S. enterica* serovar Typhimurium where the lipid A portion is modified, e.g. by modification with 4-amino-4-deoxyarabinose (Raetz et al., 2007). In summary, the cell envelope of Gram-negative

*Correspondence: Dr. Herbert P. Schweizer, Department of Microbiology, Immunology and Pathology, Colorado State University, IDRC at Foothills Campus, 0922 Campus Delivery, Fort Collins, CO, 80523, USA, Herbert.Schweizer@colostate.edu.

bacteria, especially the outer membrane, is a major barrier for antibiotics and its contributions to antimicrobial susceptibility are complex (Fig. 1).

Why is *B. pseudomallei* unique amongst Gram-negative biodefense pathogens with respect to drug discovery efforts? Although outer membrane permeability has not yet been directly assessed in *B. pseudomallei*, the intrinsic resistance of this bacterium to many antibiotics can most likely be directly attributed to synergy between exclusion and active efflux from the cell. This notion is supported by the finding that antibiotic susceptibilities of efflux pump expressing strains compared to their isogenetic pump mutant counterparts are vastly different and could not simply be explained by expression of efflux pumps alone. For example, aminoglycoside and macrolide susceptibilities of wild-type and AmrAB-OprA efflux pump mutant strains differ up to 100-fold and 16-fold, respectively (Moore *et al.*, 1999, Trunck *et al.*, 2009). Similarly, the clindamycin susceptibility of *B. pseudomallei* is greatly (>16-fold) affected by the expression status of the BpeAB-OprB efflux pump (Mima & Schweizer, 2010). Although outer membrane barrier properties may look alike, our experiences indicate that even bacteria like *B. pseudomallei* and *P. aeruginosa* with similar outer membrane permeabilities behave quite differently in terms of antibiotic susceptibility profiles. Expression of the BpeEF-OprC efflux pump in *B. pseudomallei* results in high level resistance (as judged by minimal inhibitory concentrations [MIC]) to chloramphenicol (512 μg/mL) and trimethoprim (>32 μg/mL)(Mima and Schweizer, unpublished observations). In contrast, expression of the same efflux pump in *P. aeruginosa* only results in modest increases in resistance with MICs of 8 μg/mL for both chloramphenicol and trimethoprim (Kumar *et al.*, 2006). This rather dramatic difference is not due to lack of transcription or translation, but likely because the outer membrane properties of *B. pseudomallei* and *P. aeruginosa* are quite different despite similar relative outer membrane permeabilities of *Pseudomonas* and *Burkholderia* species.

Our experiences have shown us that commonly used Gram-negatives bacteria such as *E. coli* and *P. aeruginosa, including* TolC or pump mutants to assess roles of efflux, are often inappropriate surrogates for drug anti-*B. pseudomallei* discovery efforts. To this end, we have generated isogenetic *B. pseudomallei* efflux pump proficient (expressing) and deficient mutants in either the virulent (and therefore select agent) strain 1026b (DeShazer *et al.*, 1997) or its derivative Bp82 (Propst *et al.*, 2010) which is excluded from select agent listings and can be handled in a $BSL2^{+}$ laboratory with local Institutional Biosafety Committee jurisdiction. We have employed these strains to test novel compounds for anti-*B. pseudomallei* activity. The ketolide cethromycin showed efficacy against clinical and environmental strains but expression of the AmrAB-OprA efflux pump resulted in high-level resistance (Mima *et al.*, 2011b). In contrast, the activity of the new monosulfactam BAL30072 was not significantly affected by efflux (Mima *et al.*, 2011a).

In our hands, the less pathogenic but closely related BSL2 agent *B. thailandensis* (Brett *et al.*, 1998, Yu *et al.*, 2006) is an appropriate surrogate for *B. pseudomallei*. It for example possesses the equivalent cadre of efflux pumps and we have generated the corresponding panel of isogenetic *B. thailandensis* efflux pump proficient (expressing) and deficient mutants.

Conclusions

Whole cell screening is an important step in the drug discovery process. Our findings with *B. pseudomallei* indicate that it is imperative to choose proper strains for whole cell screening. Even seemingly closely related species or species with similar outer membrane permeabilities may possess quite disparate cell envelope properties. One must especially be careful about choice of surrogate strains and recognize that Gram-negatives are not all

created equal. For example, in the context of drug discovery efforts *E. coli* strains may be perfectly good surrogates for *Y. pestis* and *F. tularensis*, but in most instances they are likely inappropriate surrogates for *B. pseudomallei*. By choosing inappropriate surrogates, properties of antibiotics may be misjudged (e.g., propensity for efflux) or antibiotics with activity against the targeted bacterium may be entirely missed. Modern genetic technologies facilitate construction of suitable screening strains which may include proper surrogates (e.g. *B. thailandensis* for *B. pseudomallei*). *B. mallei* is extremely closely related to and widely considered a clone of *B. pseudomallei* but is generally more susceptible to antibiotics than *B. pseudomallei* because most strains are lacking or not expressing some of the resistance mechanisms, for example the AmrAB-OprA efflux pump (Nierman *et al.*, 2004). Once can therefore generalize that when a compound shows efficacy against *B. pseudomallei* it is also efficacious against *B. mallei*. In a sense, then, *B. thailandensis* and *B. pseudomallei* are suitable surrogates for *B. mallei*.

Acknowledgments

I am grateful to the students and postdocs at Colorado State University that have contributed to various aspects of this work. Funding was provided by NIH NIAID grant AI065357.

References

Brett PJ, DeShazer D, Woods DE. *Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species. Int J Syst Bacteriol. 1998; 48:317–320. [PubMed: 9542103]

DeShazer D, Brett P, Carlyon R, Woods D. Mutagenesis of *Burkholderia pseudomallei* with Tn*5*-OT182: isolation of motility mutants and molecular characterization of the flagellin structural gene. J Bacteriol. 1997; 179:2116–2125. [PubMed: 9079894]

Estes DM, Dow SW, Schweizer HP, Torres AG. Present and future therapeutic strategies for melioidosis and glanders. Expert Rev Anti Infect Ther. 2010; 8:325–338. [PubMed: 20192686]

Hancock REW. Resistance mechanisms in *Pseudomonas aeruginosa* and other non-fermentative bacteria. Clin Infect Dis. 1998; 27(Suppl 1):S93–S99. [PubMed: 9710677]

Kumar A, Chua KL, Schweizer HP. Method for regulated expression of single-copy efflux pump genes in a surrogate *Pseudomonas aeruginosa* strain: identification of the BpeEF-OprC chloramphenicol and trimethoprim efflux pump of *Burkholderia pseudomallei* 1026b. Antimicrob Agents Chemother. 2006; 50:3460–3463. [PubMed: 17005832]

Mima T, Kvitko BH, Rholl DA, Page MGP, Desarbre E, Schweizer HP. In vitro activity of BAL30072 against *Burkholderia pseudomallei*. Int J Antimicrob Agents. 2011a; 38:157–159. [PubMed: 21596528]

Mima T, Schweizer HP. The BpeAB-OprB efflux pump of *Burkholderia pseudomallei* 1026b does not play a role in quorum sensing, virulence factor production, or extrusion of aminoglycosides but is a broad-spectrum drug efflux system. Antimicrob Agents Chemother. 2010; 54:3113–3120. [PubMed: 20498323]

Mima T, Schweizer HP, Xu ZQ. In vitro activity of cethromycin against Burkholderia pseudomallei and investigation of mechanism of resistance. J Antimicrob Chemother. 2011b; 66:73–78. [PubMed: 20965890]

Moore RA, DeShazer D, Reckseidler S, Weissman A, Woods DE. Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. Antimicrob Agents Chemother. 1999; 43:465–470. [PubMed: 10049252]

Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, Ulrich RL, Ronning CM, Brinkac LM, Daugherty SC, Davidsen TD, Deboy RT, Dimitrov G, Dodson RJ, Durkin AS, Gwinn ML, Haft DH, Khouri H, Kolonay JF, Madupu R, Mohammoud Y, Nelson WC, Radune D, Romero CM, Sarria S, Selengut J, Shamblin C, Sullivan SA, White O, Yu Y, Zafar N, Zhou L, Fraser CM. Structural flexibility in the *Burkholderia mallei* genome. Proc Natl Acad Sci USA. 2004; 101:14246–14251. [PubMed: 15377793]

Nikaido H. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. Semin Cell Dev Biol. 2001; 12:215–223. [PubMed: 11428914]

Novem V, Shui G, Wang D, Bendt AK, Sim SH, Liu Y, Thong TW, Sivalingam SP, Ooi EE, Wenk MR, Tan G. Structural and biological diversity of lipopolysaccharides from *Burkholderia pseudomallei* and *Burkholderia thailandensis*. Clin Vaccine Immunol. 2009; 16:1420–1428. [PubMed: 19692625]

Pages JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat Rev Microbiol. 2008; 6:893–903. [PubMed: 18997824]

Peacock SJ, Schweizer HP, Dance DAB, Smith TL, Gee JE, Wuthiekanun V, DeShazer D, Steinmetz I, Tan P, Currie BJ. Consensus guidelines on the management of accidental laboratory exposure to *Burkholderia pseudomallei* and. Burkholderia mallei Em Infect Dis. 2008; 14:e2.

Propst KL, Mima T, Choi KH, Dow SW, Schweizer HP. A *Burkholderia pseudomallei* D*purM* mutant is avirulent in immune competent and immune deficient animals: candidate strain for exclusion from Select Agent lists. Infect Immun. 2010; 78:3136–3143. [PubMed: 20404077]

Raetz CR, Reynolds CM, Trent MS, Bishop RE. Lipid A modification systems in gram-negative bacteria. Annu Rev Biochem. 2007; 76:295–329. [PubMed: 17362200]

Trunck LA, Propst KL, Wuthiekanun V, Tuanyok A, Beckstrom-Sternberg SM, Beckstrom-Sternberg JS, Peacock SJ, Keim P, Dow SW, Schweizer HP. Molecular basis of rare aminoglycoside susceptibility and pathogenesis of *Burkholderia pseudomallei* clinical isolates from Thailand. PLoS Negl Trop Dis. 2009; 3:e0000519.

Walsh, C. Antibiotic resistance. American Society for Microbiology Press; Washington, D.C: 2003.

Wuthiekanun V, Peacock SJ. Management of melioidosis. Expert Rev Anti Infect Ther. 2006; 4:445–455. [PubMed: 16771621]

Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, Nierman WC, Tan P. Genomic patterns of pathogen evolution revealed by comparison of *Burkholderia pseudomallei,* the causative agent of melioidosis, to avirulent *Burkholderia thailandensis*. BMC Microbiol. 2006; 6:46. [PubMed: 16725056]

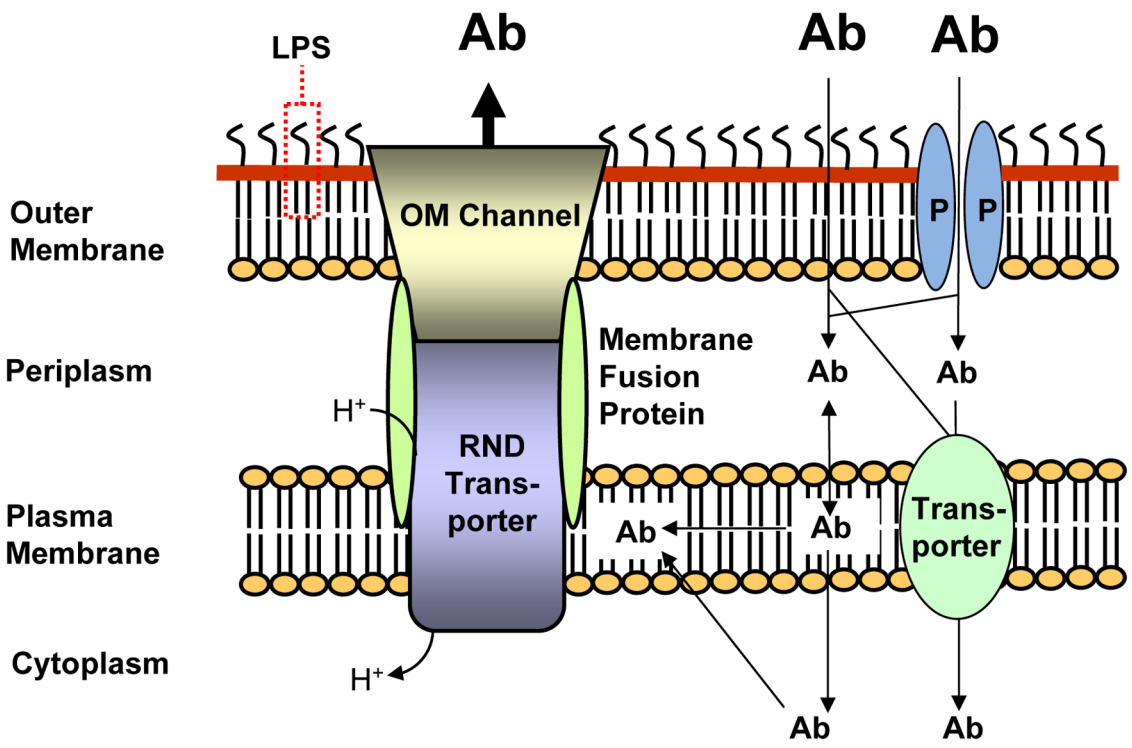

Fig. 1. The cell envelope of Gram-negative bacteria is a major barrier for antibiotics

The cell envelope of Gram-negative bacteria consists of the plasma membrane, the periplasm and the outer membrane. The outer membrane is the major barrier for antibiotics (Ab). Some antibiotics penetrate this membrane either through porins (P) or by passive diffusion through the outer membrane phospholipid (inner leaflet)-lipid A (outer leaflet) bilayer. The lipopolysaccharide (LPS) forms another barrier for many antibiotics but polycationic compounds such as gentamicin and colistin are being transported through the outer membrane via interaction with LPS in a process called self-promoted uptake. Antibiotic molecules then enter the cell from the periplasm either via partition into and passive diffusion through the plasma membrane or are actively transported via transporters into the cytoplasm. Efflux pumps of the resistance nodulation cell division (RND) superfamily are major players in antibiotic resistance of Gram-negative bacteria. These tripartite systems span the entire cell envelope and are composed of an RND transporter, a membrane fusion protein and an outer membrane (OM) channel. It is generally accepted that RND transporters acquire substrates from the plasma membrane. Efflux via RND pumps is driven by the proton gradient. The setup illustrated in this figure explains why synergy between exclusionary outer membrane and/or cell envelope properties is a powerful mechanism leading to high-level antibiotic resistance in non-enteric Gram-negative bacteria. Although antibiotics may be present outside the bacterial cell in high concentration (illustrated by large bold letters), passive influx through the various compartments of the cell coupled to active efflux via a cell envelope-spanning channel results in low intracellular of antibiotics (illustrated by smaller letters).