Opinion

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

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Among Gram-negative biodefence 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* (meliodosis) 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 and Peacock, 2006; Estes et al., 2007). At present, the recommended acute phase treatment for melioidosis includes β-lactam antibiotics such as ceftriaxone, amoxicillin-clavulanic acid or carbapenems (e.g. meropenem and imipenem; Peacock et al., 2008). Other efficacious therapeutic strategies 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 mechanisms documented for other bacteria (Walsh, 2003): (i) exclusion from the cell; (ii) enzymatic inactivation; (iii) target alterations or deletion; and (iv) 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% and 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 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 among Gram-negative biodefence 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 with their isogenic 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 and 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⁻¹) and trimethoprim (>32 μg ml⁻¹) (T. Mima and H. Schweizer, unpubl. obs.). In contrast, expression of the same efflux pump in *P. aeruginosa* only results in modest increases in resistance with MICs of 8 μg ml⁻¹ 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 isogenic *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 Bio-safety 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 isogenic *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*). *Burkholderia mallei* is extremely closely related to and widely considered a
clone of \textit{B. pseudomallei} but is generally more susceptible to antibiotics than \textit{B. pseudomallei} because most strains are lacking or not expressing some of the resistance mechanisms, for example the AmrAB–OprA efflux pump \cite{Nierman et al., 2004}. Once can therefore generalize that when a compound shows efficacy against \textit{B. pseudomallei} it is also efficacious against \textit{B. mallei}. In a sense, then, \textit{B. thailandensis} and \textit{B. pseudomallei} are suitable surrogates for \textit{B. mallei}.

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