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Burkholderia pseudomallei Isolates from Sarawak, Malaysian Borneo, Are Predominantly Susceptible to Aminoglycosides and Macrolides

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Melioidosis is a potentially fatal disease caused by the saprophytic bacterium *Burkholderia pseudomallei*. Resistance to gentamicin is generally a hallmark of *B. pseudomallei*, and gentamicin is a selective agent in media used for diagnosis of melioidosis. In this study, we determined the prevalence and mechanism of gentamicin susceptibility found in *B. pseudomallei* isolates from Sarawak, Malaysian Borneo. We performed multilocus sequence typing and antibiotic susceptibility testing on 44 *B. pseudomallei* clinical isolates from melioidosis patients in Sarawak district hospitals. Whole-genome sequencing was used to identify the mechanism of gentamicin susceptibility. A novel allelic-specific PCR was designed to differentiate gentamicin-sensitive isolates from wild-type *B. pseudomallei*. A reversion assay was performed to confirm the involvement of this mechanism in gentamicin susceptibility. A substantial proportion (86%) of *B. pseudomallei* clinical isolates in Sarawak, Malaysian Borneo, were found to be susceptible to the aminoglycoside gentamicin, a rare occurrence in other regions where *B. pseudomallei* is endemic. Gentamicin sensitivity was restricted to genetically related strains belonging to sequence type 881 or its single-locus variant, sequence type 997. Whole-genome sequencing identified a novel nonsynonymous mutation within *amrB*, encoding an essential component of the AmrAB-OprA multidrug efflux pump. We confirmed the role of this mutation in conferring aminoglycoside and macrolide sensitivity by reversion of this mutation to the wild-type sequence. Our study demonstrates that alternative *B. pseudomallei* selective media without gentamicin are needed for accurate melioidosis laboratory diagnosis in Sarawak. This finding may also have implications for environmental sampling of other locations to test for *B. pseudomallei* endemicity.

Melioidosis is a potentially fatal disease endemic in Southeast Asia, northern Australia, and other tropical regions. Melioidosis is caused by the saprophytic bacterium *Burkholderia pseudomallei*, commonly found in the environment in regions of endemicity, with infection generally occurring from contact with contaminated water or soils (1). Clinical presentations of melioidosis are highly variable and can manifest as asymptomatic infections, localized skin abscess formation, acute or chronic pneumonia, genitourinary, bone, and joint infections, or severe systemic sepsis, with or without foci of multiple abscesses in internal organs, with a mortality of >90% in septic shock cases (2, 3). Due in part to the high virulence of this organism and increased concerns for transmission by aerosolization, *B. pseudomallei* was upgraded to a tier 1 select agent by the U.S. Centers for Disease Control and Prevention in 2012 (http://www.selectagents.gov/).

B. pseudomallei is intrinsically resistant to a wide range of antibiotics, including many β-lactams, aminoglycosides, and macrolides (4–6). This array of drug resistance is conferred through a variety of mechanisms, including inactivating enzymes, cell exclusion, and broad-range efflux pumps. Although almost all *B. pseudomallei* strains are resistant to the aforementioned antibiotics, there have been reports of rare (~0.1%) aminoglycoside and macrolide susceptibility in isolates from Thailand (7, 8) and in a chronic-carriage patient from Australia (9). Aminoglycoside and macrolide resistance in *B. pseudomallei* is thought to be conferred solely by the multidrug efflux pump AmrAB-OprA (10, 11).

In the present study, we identified aminoglycoside and macrolide sensitivity in *B. pseudomallei* isolates of clinical origin from a wide geographical region within Sarawak, Malaysian Borneo. Antibiotic susceptibility was confined to closely related isolates based on multilocus sequence typing (MLST). Using whole-genome sequencing (WGS), we identified a nonsynonymous mutation within the multidrug efflux pump, AmrAB-OprA. Reversion of this mutation restored aminoglycoside and macrolide resistance.

MATERIALS AND METHODS

Ethics. Bacterial strain collection and research were approved by the Medical Research Ethics Committee and registered with the National Medical Research Registrar and the Clinical Research Centre, Ministry of Health of Malaysia. All clinical isolates were from routine melioidosis laboratory diagnosis, and hence no written consent was obtained from patients.

Bacterial strains. The *B. pseudomallei* strains used in this study are listed in Table 1. With the exception of MSHR7596, MSHR7597,

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