Less Is More: *Burkholderia pseudomallei* and Chronic Melioidosis

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ABSTRACT The Gram-negative bacterium *Burkholderia pseudomallei* is the causative agent of melioidosis, a serious infectious disease of humans and animals. Once considered an esoteric tropical disease confined to Southeast Asia and northern Australia, research on *B. pseudomallei* has recently gained global prominence due to its classification as a potential bioterrorism agent by countries such as the United States and also by increasing numbers of case reports from regions where it is not endemic. An environmental bacterium typically found in soil and water, assessing the true global prevalence of melioidosis is challenged by the fact that clinical symptoms associated with *B. pseudomallei* infection are extremely varied and may be confused with diverse conditions such as lung cancer, tuberculosis, or *Staphyloccocus aureus* infection. These diagnostic challenges, coupled with lack of awareness among clinicians, have likely contributed to underdiagnosis and the high mortality rate of melioidosis, as initial treatment is often either inappropriate or delayed. Even after antibiotic treatment, relapses are frequent, and after resolution of acute symptoms, chronic melioidosis can also occur, and the symptoms can persist for months to years. In a recent article, Price et al. [mBio 4(4):e00388-13, 2013, doi:10.1128/mBio.00388-13] demonstrate how comparative genomic sequencing can reveal the repertoire of genetic changes incurred by *B. pseudomallei* during chronic human infection. Their results have significant clinical ramifications and highlight *B. pseudomallei*'s ability to survive in a wide range of potential niches within hosts, through the acquisition of genetic adaptations that optimize fitness and resource utilization.

GENOMIC FLEXIBILITY AND VARIATION ACROSS *BURKHOLDERIA PSEUDOMALLEI* STRAINS

Like many other bacterial pathogens, analysis of the *Burkholderia pseudomallei* genome has provided vital insights into its key virulence mechanisms. The "reference" *B. pseudomallei* genome, a clinical isolate from Thailand (K96243), was reported by the Sanger Centre almost 10 years ago (1). This landmark study revealed that the *B. pseudomallei* genome is exceptionally large, comprising two circular chromosomes and a genome size of 7.2 Mb (1), with >5,600 protein-coding genes and an impressive armamentarium of virulence genes, including multiple type III and type VI secretion systems, toxins, adhesins, and capsular polysaccharide clusters. Functional studies following this initial report have yielded many novel findings in *B. pseudomallei* biology, such as the identification of new toxins (2), and mechanisms of antibiotic resistance (3).

Recently, a whole-genome comparison of 11 *B. pseudomallei* strains from different geographic regions (Thailand, Singapore, Vietnam, and Australia) was reported (4). This study demonstrated the existence of a sizeable degree of variation in genome sizes among *B. pseudomallei* strains from diverse geographic locations, confirming a high level of intraspecies genetic diversity (Fig. 1). It has been proposed that genomic regions that are variably present across strains, formally referred to as the "accessory genome," may contain genes that specifically modify the ability of individual *B. pseudomallei* strains to cause different clinical symptoms or biological phenotypes (5). Besides accessory elements, microscale genetic variations in genes commonly present in multiple *B. pseudomallei* strains (the "core genome") were also observed. Interestingly, a subset of the latter genes exhibited signatures of positive selection, suggesting that their functions are adapting under evolutionary pressure. Examples of cellular processes highlighted through this approach included genes related to siderophore biosynthesis, iron metabolism, and cell adhesion. These results suggest that in its natural reservoir (soil), *B. pseudomallei* is likely to be constantly exposed to a variety of selective pressures that can modify its genome and improve its chances of survival.

GENETIC VARIATION DURING CHRONIC MELIOIDOSIS

Besides the comparison of geographically distinct isolates, another important area of *B. pseudomallei* research lies in investigating patterns of genetic variation between closely related *B. pseudomallei* strains during different phases of human infection, for example during chronic infection, as in the current study. Clinical evidence suggests that chronic melioidosis may represent a distinct clinical entity separate from acute melioidosis. Compared to acute *B. pseudomallei* infections, which are commonly associated with clinical risk factors such as diabetes, hazardous alcohol use, and renal disease (6), chronic *B. pseudomallei* infections are considered less severe but are more localized in site and difficult to eradicate (7, 8). Such observations are consistent with a model where *B. pseudomallei* strains causing chronic infection may have incurred additional genetic changes distinct from acute strains, allowing the bacteria to adapt within the infected host. Until recently, relatively few comprehensive genomic studies have focused on chronic melioidosis. The recent study by Price et al. (9) provides more-detailed insight of within-host evolution during a chronic infection period of >10 years. Consecutive isolates were collected and sequenced using next-generation technologies at three different time points from a single patient with a pulmonary infection. The first and third isolates (from sputum samples) were 139 months apart, with an intervening lung biopsy specimen iso-

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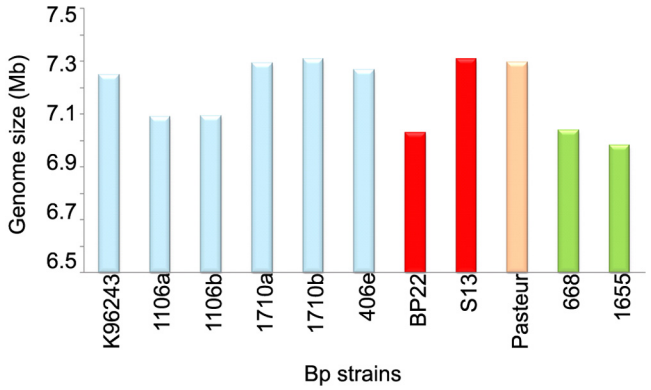

FIG 1 Genome size distributions of *B. pseudomallei* (Bp) strains across different geographic regions: Thailand (blue), Singapore (red), Vietnam (orange), and Australia (green).

late at 37 months from the initial infection. During this period, the patient was managed on intensive (ceftazidime and imipenem) and maintenance (doxycycline and amoxicillin-clavulanic acid [Augmentin]) antibacterial regimens. A genomic-scale comparative analysis across the three isolates revealed two findings—first, a signature of macrolevel genome reduction, and second, several microlevel genetic changes. Regarding the first finding (macroscale genome reduction), the investigators identified a pattern of reductive evolution within human hosts, involving loss of gene loci involved in specialized functions, such as facilitating bacterial response to various environmental conditions. Among the chronic isolates, four large genomic deletions were identified on *B. pseudomallei* chromosome II (Fig. 2) which has been shown to exhibit a chromosome-wide enrichment in genes related to accessory functions and niche-specific adaptations (9). This phenomenon of genome reduction has also been observed in other bacterial pathogens exhibiting a chronic disease phase. For example, large-scale genomic deletions are also characteristic of chronic *Pseudomonas aeruginosa* infection (10). Closer to home, the genome reductions observed in the *B. pseudomallei* chronic strains also show a striking parallel to *Burkholderia mallei*, a separate *Burkholderia* species originally derived from *B. pseudomallei* (11). Tellingly, both the *B. pseudomallei* chronic strains and *B. mallei* demonstrated genomic reduction across the same regions, such as the genes *BPSS1096* to *BPSS1112* and *BPSS1123* to *BPSS1203*, which contain gene clusters related to secondary metabolism. Because only 1 patient was studied, it remains to be seen if the specific genome reduction events observed in this series are stochastic or will be repeatedly observed in other cases of chronic melioidosis.

Besides macroscale genome reductions, the chronic melioidosis strains also exhibited several microscale genetic alterations, including insertions, deletions, and mutations. Genomic loci affected by indels were often directly or indirectly associated with pathogenesis, some examples being the *virG* gene of type VI secretion system I, the multidrug efflux lipoprotein AmrA, and various lipopolysaccharide (LPS) biosynthesis genes. Similarly, the chronic melioidosis strains exhibited a series of nonsynonymous single-nucleotide polymorphisms (SNPs) in genes related to adaptation such as primary sigma factor (*rpoD*), beta-lactam resistance (*penA*), and stress response (*rpoS*). Interestingly, while these findings collectively hint at a nonstochastic nature of *B. pseudomallei* evolution during chronic infection, it should be acknowledged that at present, the specific selective pressures in the host that might have induced such genomic changes remain unclear. One obvious selective pressure might come from attack by the host's immune system. Candidate modulators of host immunity in melioidosis might include the *TNF-α*, *IFN-γ*, and *TLR4* genes, as genetic knockouts of *TNF-α* and *IFN-γ* have been shown to increase *B. pseudomallei* susceptibility in mouse models (12), and genetic variation in Toll-like receptors (TLRs), in particular *TLR4* have been associated with host susceptibility to disease (13). However, the impact of these innate immunity pathways on the direct induction of genomic changes in *B. pseudomallei* remains to be experimentally demonstrated. Other plausible processes driving genomic changes in *B. pseudomallei* during infection might include differences in the host nutritional environment and the impact of antibiotic therapy.

The findings of Price et al. (9) demonstrate that *B. pseudomallei* can indeed acquire genetic alterations during human infection, and this general finding is also supported by independent studies examining *B. pseudomallei* evolution under other clinical scenar-

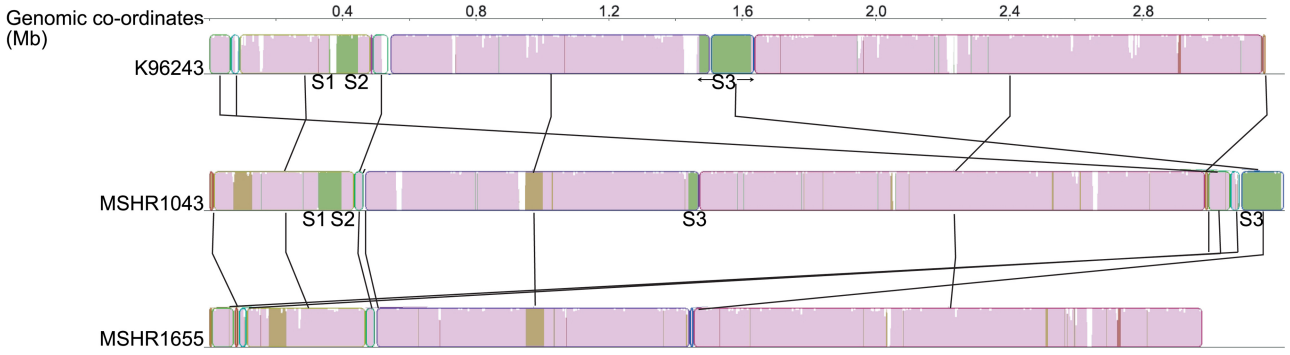

FIG 2 Macroscale genome reduction in a chronic melioidosis patient. The figure shows *B. pseudomallei* chromosome II from strains K96243 (reference), MSHR1043, and MSHR1655 (isolated 37 months later). The 3 *B. pseudomallei* isolates are depicted as a series of ordered locally collinear blocks (LCBs). The vertical lines connect homologous LCBs across the isolates. Conserved segments across all the three isolates are colored pink, while portions colored green are segments conserved across K96243 and MSHR1043. Segments conserved among MSHR1043 and MSHR1655 are colored brown. In strain MSHR1655, three genomic segments (marked S1, S2, and S3) found in strain MSHR1043 have undergone deletion. Sequence alignments and ordering was performed using Mauve 2.3.1 (18).

ios, including primary or relapsed melioidosis, or strains isolated from different body sites of the same patient. Comparisons of paired *B. pseudomallei* strains isolated during acute and relapsed disease have been reported (4, 14). In these studies, only a handful of genetic variations between acute and relapsed strains were identified. However, these genetic variations were biased toward protein-coding genes with more than 50% of the variations being nonsynonymous, suggesting the presence of active functional selection within the human host. For example, relapsed strains were associated with independent nonsynonymous mutations within the same *B. pseudomallei* gene *BPSS1483* (*tetR*, a transcriptional regulator), that was not present in the original infecting strain (14). Another study demonstrating *B. pseudomallei*'s ability to evolve within a human host was reported by Price et al. (15), who analyzed four patients diagnosed with acute disseminated melioidosis. A comparison of *B. pseudomallei* strains isolated from different tissue sites revealed considerable variation in the genome-wide distribution of variable tandem repeats, although it remains to be established if such variation represents neutral evolution or active selection. Taken collectively, these studies demonstrate *B. pseudomallei*'s remarkable ability to evolve genetically even within the same human host.

FUTURE PERSPECTIVES

Chronic melioidosis accounts for 8 to 12% of melioidosis cases in Thailand and Australia (6), with areas where melioidosis is not endemic (Europe and North America) contributing to the remainder (16, 17). There has been a paucity of published trials on antimicrobial treatment of chronic melioidosis, and it remains unclear whether treatment recommendations for this condition remain the same as for acute melioidosis (6). Studies such as those reported by Price et al. (9) are thus invaluable in providing evidence that chronic melioidosis is indeed different from its acute phase and highlighting potential avenues for chronic melioidosis treatment. More generally, the sequencing technologies applied here could also rapidly lead to powerful diagnostic tools for clinical use, including both early detection and effective treatment.

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