

## Short Report: Molecular Characterization of Clinical *Burkholderia pseudomallei* Isolates from India

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**Abstract.** Multilocus sequence typing of seven isolates of *Burkholderia pseudomallei* from India showed considerable diversity, with six different sequence types. Possible dissemination of melioidosis by historical trading routes is supported by links to strains from Southeast Asia, China, and Africa and the presence of the *Burkholderia mallei* allele of the *bimA* gene.

*Burkholderia pseudomallei* is a soil saprophyte and the causative agent of melioidosis, an infectious disease that, while predominantly restricted to Southeast Asia and northern Australia, is increasingly being recognized in other tropical and subtropical regions.<sup>1,2</sup> In areas where the disease is endemic, the organism is commonly found in soil and surface water. Infections occur mostly by inoculation of the organism through puncture wounds in the skin and people with risk factors for melioidosis such as diabetes are especially susceptible to severe disease such as pneumonia and systemic sepsis.<sup>3</sup> Recent phylogenetic analysis of a global set of *B. pseudomallei* strains has suggested an Australian origin for *B. pseudomallei*, with subsequent spread to Southeast Asia and beyond.<sup>4</sup> Since 1953 melioidosis has been sporadically reported from India,<sup>5,6</sup> with one notable cluster initially attributed to plague.<sup>7</sup> Nevertheless, the population structure of Indian and other Asian strains of *B. pseudomallei* and their origins and relationships to strains from Southeast Asia and Australia remain undefined. The aim of this study was to analyze the molecular diversity of clinical *B. pseudomallei* strains from one location in southern India.

Seven clinical isolates of *B. pseudomallei* were collected from seven melioidosis patients admitted to Kasturba Hospital, Manipal, Karnataka State in southwest India between 2007 and 2009. Isolate 7 was obtained from an Austrian tourist with septicemic pneumonia, who was holidaying in India. Five patients had pulmonary involvement with septicemia and two had soft tissue infections (see Table 1). Three patients were diabetic and two had no identified risk factor for melioidosis. Ceftazidime was the most common antibiotic used. Two patients died and two were lost to follow-up.

Genomic DNA was obtained from culture isolates using phenol-chloroform extraction and further purified by QIAamp DNA minikit (Qiagen, Victoria, Australia). Real-time polymerase chain reaction was used to target TTS1 for species confirmation.<sup>8</sup> Molecular diversity was assessed by multilocus sequence typing (MLST)<sup>9</sup> and multilocus VNTR (variable number tandem repeat) analysis (MLVA-4).<sup>10</sup> A genetic marker linked to geographic origin (*Yersinia*-like fim-

briae [YLF]/*B. thailandensis*-like flagellum and chemotaxis [BTFC] locus) was amplified<sup>11</sup> and the *B. pseudomallei*- and *Burkholderia mallei*-type allele of the actin-based motility gene *bimA* was targeted as a potential virulence factor.<sup>12</sup>

Details of molecular results are shown in Table 1 and Figure 1. The MLST analysis resulted in six different sequence types (STs) from six *B. pseudomallei* isolates. All six were novel STs not previously listed on the global *B. pseudomallei* MLST database (<http://bpseudomallei.mlst.net/>). New ST numbers were assigned and comparisons were made with the global dataset. Two of the Indian STs were single locus variants (SLVs) (differing at one MLST locus) to each other and to isolates from Thailand, Kenya, and China. No MLST data were obtained from isolate 3 because of technical reasons, but isolates 2 and 3 shared the same MLVA-4 pattern suggesting an identical ST for these isolates.<sup>10</sup> Although such an identical MLV-4 type indicates a likely geographical or epidemiological link, no such link was found, with these two patients from locations around 50 km apart. All other isolates showed different MLVA-4 patterns. All seven *B. pseudomallei* isolates contained the YLF marker, which is predominant in Southeast Asia<sup>11</sup> and two of the isolates amplified the *B. mallei* allele of *bimA*, with the other five having the *B. pseudomallei* allele.

To date, only one other *B. pseudomallei* strain of Indian origin is listed on the MLST database. Although a small sample set, our MLST results indicate considerable diversity among *B. pseudomallei* collected over a small spatial and temporal range in southern India, with 6 of 7 having different STs. This suggests a non-recent introduction of *B. pseudomallei* into this area. The eBURST analysis clustered the Indian isolates with Asian *B. pseudomallei* strains and this was also supported by presence of the YLF geographical marker. The fact that some of the Indian *B. pseudomallei* isolates were SLVs to isolates from China and Kenya, supports the possibility of spread of *B. pseudomallei* along historical trading routes. *BimA* is involved in actin polymerization, which is crucial for intracellular motility and intercellular spread and is thus a potential virulence factor.<sup>12</sup> Both patients with the *B. mallei* allele of *bimA* presented with septicemic pneumonia. While the *B. mallei* allele occurs in some Australian *B. pseudomallei* strains, Sitthidet and others<sup>12</sup> found none of 64 strains from across Southeast Asia harboring this allele that is present in *B. mallei*, the causative bacterium of glanders. To our knowledge the two Indian *B. pseudomallei* strains noted here to have the

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TABLE 1  
Summary of patient information and molecular results of *Burkholderia pseudomallei* isolates

Isolate no.	Patient risk factors	Disease presentation (outcome)	ST	SLVs (country of origin of SLVs*)	YLF/ BTFC	<i>Bps/Bm</i> † allele of <i>bimA</i>
1	Diabetes, chronic renal disease	Septicemic pneumonia (lost to follow-up)	854	None	YLF	<i>Bm</i>
2	Chronic obstructive lung disease	Septicemic pneumonia (died)	855	None	YLF	<i>Bps</i>
3	Diabetes	Submandibular abscess (survived)	Na‡	Na	YLF	<i>Bps</i>
4	Diabetes	Septicemic pneumonia (survived)	856	None	YLF	<i>Bm</i>
5	Nil	Septicemic pneumonia (died)	857	None	YLF	<i>Bps</i>
6	Nil	Submandibular abscess (survived)	858	ST 859 (I, T, C, K)	YLF	<i>Bps</i>
7	Acute myeloid leukemia	Septicemic pneumonia (lost to follow-up)	859	ST 858 (I)	YLF	<i>Bps</i>

\* Indian sequence types (STs) are listed. C = China, I = India, K = Kenya, T = Thailand.

† *Bps* = *B. pseudomallei*; *Bm* = *B. mallei*.

‡ Na = not available. Isolate 2 and 3 had the same MLV-4 pattern, which suggests that they also had the same ST.<sup>10</sup>

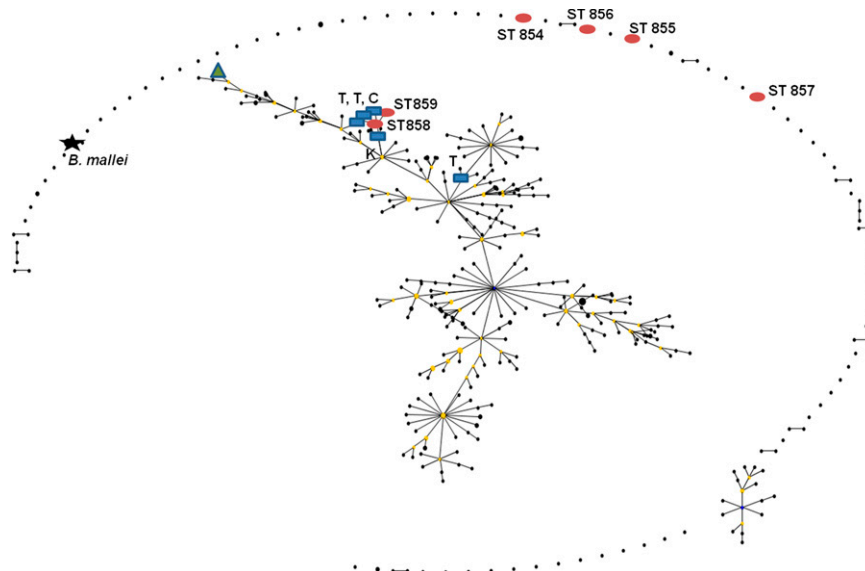


FIGURE 1. eBURST population snapshot of sequence types (STs) of *Burkholderia pseudomallei* isolates from India, Southeast Asia, China, and Africa. Red circles indicate *B. pseudomallei* isolates of this study with STs labeled, blue squares are SLVs to STs 858 and 859 with the letters denoting the country of origin (“T” Thailand, “C” China, and “K” Kenya). The green triangle is the only other isolate of Indian origin currently listed in the MLST database and was isolated in 1995. The black star indicates *Burkholderia mallei* isolates. This diagram contains 465 isolates with 409 STs.

*B. mallei*-type *bimA* allele are the first *B. pseudomallei* isolates of Asian origin reported to contain this allele. *Burkholderia mallei* is found in India, with sporadic reports of glanders in equines and it is recognized that *B. mallei* evolved from an ancestral *B. pseudomallei* strain.<sup>9</sup> Of interest, *B. mallei* is a double locus variant of Indian isolate 6 (ST 858).

In conclusion, this is the first report on the molecular characterization of *B. pseudomallei* isolates from southern India. These isolates were found to be diverse at MLST level and an Asian origin is likely. More work is needed to uncover the population structure of *B. pseudomallei* in India and potential links to historical trading routes.

Received March 22, 2011. Accepted for publication April 4, 2011.

Financial support: This work was supported by a project grant (no. 605820) from the Australian National Health and Medical Research Council, a project grant (UO1AI075568) from the National Institutes of Health, and a program grant (030662) from the Wellcome Trust.

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