Multilocus Sequence Typing of Clinical Isolates of *Burkholderia pseudomallei*
Collected in Hainan, a Tropical Island of Southern China

Xu-Ming Wang,† Xiao Zheng,† Hua Wu,† Xiao-Jun Zhou,† Hui-Hui Kuang,‡ Hong-Li Guo,†
Kai Xu,† Tian-Jiao Li,† Ling-Li Liu,§ and Wei Li†,§,†

1Department of Clinical Laboratory, Hainan General Hospital, Haikou, People’s Republic of China; 2Hainan Department, The General Hospital of the Chinese People’s Liberation Army, Sanya, People’s Republic of China; 3State Key Laboratory for Infectious Disease Prevention and Control, Beijing, People’s Republic of China; 4National Institute for Communicable Disease Control and Prevention, Beijing, People’s Republic of China; 5Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, People’s Republic of China

Abstract. *Burkholderia pseudomallei* is a gram-negative bacterium that causes melioidosis. In this study, we examined the sequence types (STs) of 60 clinical isolates from patients with melioidosis in Hainan, a tropical island in southern China. The 60 clinical isolates were resolved into 30 STs. Among the STs, ST562 was also reported in Australia, and ST90 was also reported in the United States, probably from a traveler who had previously visited Asia. In addition, six novel STs were found in this study, including ST1395, ST1396, ST1397, ST1398, ST1399, and ST1443, whereas the remaining STs were mostly shared with other southeast Asian regions. Phylogenetic analysis of 60 isolates conducted using the eBURST v3 software segregated the 30 STs into three groups and 18 singletons. Our study shows genetic diversity of 60 clinical isolates of *B. pseudomallei* in Hainan Island.

INTRODUCTION

*Burkholderia pseudomallei* is a gram-negative environmental saprophyte that causes melioidosis. This organism is commonly isolated from soil and water in endemic areas such as southeast Asia and northern tropical Australia. Melioidosis usually occurs in the rainy season, particularly after a rainstorm or typhoon. In endemic areas, the greatest risk of developing melioidosis arises from frequent exposure to contaminated soil, water, or environmental aerosols. *Burkholderia pseudomallei* infection may be acquired through inhalation, subcutaneous inoculation, or direct exposure of wounded skin to contaminated material. Clinical presentations of melioidosis are highly variable, and can manifest as asymptomatic infection, localized skin abscesses, acute or chronic pneumonia, genitourinary, bone, and joint infections, or severe systemic sepsis with or without multiple abscesses in internal organs. Septic shock cases have a greater than 90% mortality rate. Because of the nonspecific disease presentation, lack of an available vaccine, and fear of deliberate release coupled with a high mortality rate, *B. pseudomallei* was upgraded to a Tier 1 select agent by the Centers for Disease Control and Prevention in October 2012 (http://www.selectagents.gov/).

Hainan Island is the largest tropical island in southern China. Situated in southeast Asia, it is close to endemic regions such as Thailand, Vietnam, Laos, and Hong Kong. Hainan has a tropical monsoon maritime climate characterized by high temperature and humidity. The rainy season of Hainan typically occurs between May and October, when frequent rainstorms and typhoons occur (http://www.weather.com.cn/hainan/qxjjj/03/341906.shtml). These factors combined have allowed Hainan to become a region of melioidosis endemicity.

Multilocus sequence typing (MLST) has been used to characterize the population structure and diversity of *B. pseudomallei*; however, there is little information regarding this organism available from China (http://pubmlst.org/bpseudomallei/). Therefore, this study was conducted to analyze the sequence type (ST) diversity of clinical strains of *B. pseudomallei* from Hainan in China.

MATERIALS AND METHODS

Ethics statement. In this study, we anonymized the melioidosis patient data. All clinical isolates were collected during routine melioidosis laboratory diagnosis; therefore, patients did not provide written informed consent.

Clinical isolates. A total of 60 clinical strains of *B. pseudomallei* were acquired from culture-confirmed melioidosis patients admitted to Hainan General Hospital, Haikou, and Hainan Department of the General Hospital of the Chinese People’s Liberation Army, Sanya. Information describing the 60 strains is shown in Table 1. Glycerol stocks of *B. pseudomallei* were stored at −80°C.

Bacterial diversity analysis. A total of 60 clinical isolates were typed by MLST as previously described. The number of alleles on seven housekeeping genes was determined by comparing the sequences to those at the *B. pseudomallei* MLST website (http://pubmlst.org/bpseudomallei/). The seven numbers were composed of the allelic profile of each strain, and each distinct allelic profile was assigned as a sequence type. New alleles and STs were submitted to the *B. pseudomallei* MLST database curator.

Phylogenetic analysis. The relatedness among 30 STs was displayed as a dendrogram by eBURST v3 (Ed Feil, University of Bath, Bath, United Kingdom) with six shared alleles defined as a group.

RESULTS AND DISCUSSION

Information describing the 60 clinical isolates. The 60 strains included in this study were isolated from 60 melioidosis patients...
patients in 15 different regions of Hainan excluding Basha, Qiongzhong, and Wuzhishan. All regions except Dingan, Tunchang, and Baoting were located along the coast (Figure 1). Because the population density of coastal areas was higher than that of inland areas, it remains unknown whether coastal areas are more conducive to \textit{B. pseudomallei} growth and persistence than inland regions. Environmental samples of both coastal and inland areas across Hainan are needed to better understand the true ecological distribution of \textit{B. pseudomallei} on this island. In Hainan, the presentations of melioidosis mainly included pneumonia, abscess formation, and septicaemia. Overall, 31 of 35 patients with septicemia died (Table 1).

### Table 1
Clinical information and STs of 60 clinical isolates of \textit{Burkholderia pseudomallei} in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample type</th>
<th>Location</th>
<th>Occurrence</th>
<th>Main clinical presentation</th>
<th>Outcome</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPPH 23</td>
<td>Blood</td>
<td>Haikou</td>
<td>November 2011</td>
<td>Septicemia</td>
<td>S</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 32</td>
<td>Bone marrow</td>
<td>Basha</td>
<td>July 2012</td>
<td>Septicemia, pneumonia</td>
<td>D</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 44</td>
<td>Blood, secretion</td>
<td>Ledong</td>
<td>April 2013</td>
<td>Septicemia, pneumonia, abscess</td>
<td>D</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 45</td>
<td>Blood, secretion</td>
<td>Ledong</td>
<td>May 2013</td>
<td>Abscess</td>
<td>S</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 65</td>
<td>Blood</td>
<td>Dongfeng</td>
<td>August 2014</td>
<td>Septicemia, pneumonia, abscess</td>
<td>D</td>
<td>50</td>
</tr>
<tr>
<td>HPPH 83</td>
<td>Sanies</td>
<td>Haikou</td>
<td>January 2004</td>
<td>Abscess</td>
<td>S</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 93</td>
<td>Sanies</td>
<td>Changjiang</td>
<td>November 2013</td>
<td>Septicemia, pneumonia, abscess</td>
<td>D</td>
<td>46</td>
</tr>
<tr>
<td>HPPH 50</td>
<td>Blood</td>
<td>Wanning</td>
<td>September 2013</td>
<td>Septicemia, pneumonia</td>
<td>S</td>
<td>50</td>
</tr>
<tr>
<td>HPPH 55</td>
<td>Blood</td>
<td>Haikou</td>
<td>February 2014</td>
<td>Septicemia, pneumonia</td>
<td>S</td>
<td>50</td>
</tr>
</tbody>
</table>

D = died; S = survived; STs = sequence types; U = unclear. In heavily shaded rows, STs was only found in Hainan. ST1395–1399 and ST1443 were the novel STs found in this study. ST562 was reported in both China and Australia; ST90 was also reported in the United States, probably from a traveler who had previously visited Asia.
The 60 clinical isolates belonged to 30 distinct STs, six of which had not been previously documented (Table 2). Among the six new STs, the **gmhD** locus of ST1443 was 99% similar to the allele-2 sequence, whereas the other new STs were generated by reassortment of existing alleles. These findings indicated that there was low genetic diversity within alleles, but alleles recombined frequently to form new STs. Among the 60 isolates, the loci of **ace**, **gltB**, **lepA**, **lipA**, and **ndh** had only three types of alleles, most of which were allele-1, but the loci of **gmhD** and **nark** had more than six alleles.

The remaining 24 STs were reported from other southeast Asian regions except ST562, ST90, and 10 STs that were only reported in China (Table 1). ST562 was shared with Australia and ST90 with the United States. It was previously reported that isolates of *B. pseudomallei* from Australia and Asia were distinct by MLST. Even isolates from the different regions of Australia were distinct. Because *B. pseudomallei* had not naturally been found in the environment in the United States, ST90 was probably from a traveler who had previously visited Asia. The ST562 may also have been caused by homoplasy in the MLST loci, or imported strains because Hainan is known for an international tourism. In view of the high level of genetic recombination, it may be a recombinant.

The ST distribution of 60 clinical isolates also differed (Figure 1). ST55 (eight strains), ST46 (seven strains), ST58 (six strains), and ST50 (five strains) were the most prevalent sequence types (Table 1). ST55 and ST46 were mainly found in southwest Hainan of Dongfang, Ledong, and Sanya, whereas ST58 was primarily distributed in southern Hainan and ST50 was primarily in northeastern Hainan. However,
more clinical isolates and environmental samples are needed to confirm these findings.

The relatedness of 30 STs. The 30 STs were divided into three groups and 18 singletons by eBURST. The majority of STs in the present study were singletons. With the exception of group 2, they did not form a radial expansion pattern (Figure 2). The 30 STs were compared with all of the STs present in the MLST database using eBURST (Figure 3). The results demonstrated a high level of diversity, with the 30 STs spread throughout almost all branches of the eBURST diagram.

In conclusion, the 60 clinical isolates from Hainan had a high level of diversity. Allele recombination may be an important factor leading to these diversities, and migration of humans may have also influenced the distribution of B. pseudomallei clones throughout Hainan.

Received April 11, 2016. Accepted for publication May 27, 2016.
Published online July 18, 2016.

Financial support: This work was funded by project no. Qiongwei-2013-Zizhu-059 from the Hainan Provincial Health Department and the National Natural Science Foundation of China (no. 81573208).

Authors’ addresses: Xu-Ming Wang, Hua Wu, Xiao-Jun Zhou, Hong-Li Guo, Kai Xu, Tian-Jiao Li, and Ling-Li Liu, Clinical Laboratory, Hainan General Hospital, Haikou, People’s Republic of China, E-mail: wxmhn1260@163.com, ssykkwahu@163.com, hainanbelicun@163.com, 18789609900@163.com, xukaisy@163.com, ltljhn@163.com, and liu_lingli@126.com. Xiao Zheng and Wei Li, Chinese Center for Disease Control and Prevention, Beijing, People’s Republic of China, E-mails: zhengxia@icdc.cn and ccliwpei@gmail.com. Hui-Hui Kuang, The General Hospital of the Chinese People’s Liberation Army, Sanya, People’s Republic of China, E-mail: huiziai1988@163.com.

REFERENCES