Pneumonia and Septicemia Caused by *Burkholderia thailandensis* in the United States

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*Burkholderia thailandensis* is closely related to *Burkholderia pseudomallei*, the causative agent of melioidosis. It is generally considered avirulent and previously has been reported to occur only in Southeast Asia. We report the first case of pneumonia and septicemia caused by *B. thailandensis* in the United States.

CASE REPORT

A healthy 2-year-old male was a restrained passenger in a single-car motor vehicle accident in northeast Texas in 2003. The overturned car came to rest in a drainage ditch beside the road, and the child was submerged for approximately 2 min. He was pulseless and apneic upon extraction from the vehicle. He was resuscitated, transported, and admitted to an intensive care unit, where he developed aspiration pneumonitis. He was treated with cefuroxime, and 3 days later, he was febrile for the first week of hospitalization, with a predominant pulmonary infiltrates present on chest radiographs at the time of discharge eventually resolved, and the patient is clinically well.

The blood isolate from this patient was submitted to the Centers for Disease Control and Prevention (CDC) for confirmatory identification. Standard biochemical tests were presumptive for *B. pseudomallei* (12); however, the isolate (CDC3015869) assimilated arabinose by use of minimal salt solution with 10% L-arabinose and therefore was identified as *Burkholderia thailandensis* (13).

We performed molecular characterization of this isolate using real-time PCR, 16S rRNA gene sequencing, multilocus sequence typing (MLST), and DNA-DNA hybridization. We tested the isolate by using a recently developed real-time PCR assay which amplifies a region of *orf2* of the *B. pseudomallei* type III secretion system gene cluster (10). This assay is specific for *B. pseudomallei* and does not amplify DNA from other *Burkholderia* spp., including *B. thailandensis*. DNA from CDC3015869 was not amplified using the type III secretion system PCR.

16S rRNA gene sequencing was performed as previously described (6) and yielded a sequence of approximately 1.5 kbp for CDC3015869. BESTFIT analysis (Wisconsin package, v. 10.3; Accelrys, San Diego, CA) indicated 99.4% identity with the *B. thailandensis* type strain (GenBank accession no. BSU91838), with a 2-bp insertion and 9-bp differences. An analysis comparing the 16S rRNA gene sequence of CDC3015869 with those of related *Burkholderia* strains in the CDC collection indicated that it was identical to strain CDC2721121, which was isolated from a pleural wound from a 76-year-old Louisiana man in 1997. Additionally, there was only a 1-bp difference (99.9% identity) between the 16S rRNA gene sequences of CDC3015869/CDC2721121 and that of isolate recovered in 1982 from the intestines of a foal in France. Analysis comparing all three 16S rRNA gene sequences indicated they clustered together and most closely with *B. thailandensis* (Fig. 1).

Although sequencing of the 16S rRNA gene can reveal differences among *Burkholderia* species, MLST is based upon...
differences in the sequences of seven housekeeping genes
has been shown to be not only useful for subtyping strains of
\textit{B. pseudomallei} but also capable of resolving genetic differences
among close relatives of \textit{B. pseudomallei} (6, 7, 8). CDC3015869
and CDC2721121 were identical by MLST and were desig-
nated sequence type (ST) 101 (7, 8). Strain 82172 was previ-
ously designated ST 73, and although it did not share any
alleles with the six STs of \textit{B. thailandensis} previously identified,
it clustered most closely with \textit{B. thailandensis} based on analysis
of the concatenated sequences of the MLST loci (8). ST 101
shares two alleles, \textit{gltB} and \textit{gmhD}, with ST 73, and both STs
cluster with other \textit{B. thailandensis} isolates (Fig. 2). The allele
designations and sequences are available on the MLST website
(http://bpseudomallei.mlst.net).

DNA-DNA hybridization was performed as previously de-
scribed (2). The relative binding ratios (RBR) of CDC2721121
were 100% to both CDC3015869 and 82172 and 96% to the
type strain of \textit{B. thailandensis} but only 76% to the type strain of
\textit{B. pseudomallei} under the optimum reassociation conditions
(65°C) (Table 1). Divergence for strains CDC3015869 and
82172 was <1, and the RBR remained high (91% to 95%)
under stringent reassociation (80°C) conditions. The diver-
gence for \textit{B. pseudomallei} was 4.5, and RBR dropped to 59%
under the stringent reassociation conditions.

On the basis of our phenotypic and molecular data, inclu-
ding DNA-DNA hybridization, CDC3015869 is conclusively
identified as \textit{B. thailandensis}. Based on MLST, all three strains
described above were closely related to \textit{B. thailandensis} isolates
from Southeast Asia, although they form a distinct subcluster.

\textit{Burkholderia pseudomallei} is the causative agent of melioido-
sis, a human and animal disease endemic in Southeast Asia and
northern Australia. It has been reported to occur in tropical
latitudes between 20°N and 20°S, including Central and South
America (4). Melioidosis can present with a variety of clinical
manifestations, including acute pneumonia with septicemia.
Septicemic disease can have high mortality even after aggres-
sive antimicrobial therapy is initiated (4). Infection can result
from cutaneous inoculation, inhalation, or ingestion; circum-
cstances involving heavy inoculations, such as near drowning,
may shorten onset of infection (4).

\textit{B. pseudomallei} is a gram-negative rod and soil saprophyte.
Environmental investigations to collect and characterize soil-
borne \textit{B. pseudomallei} in Thailand resulted in isolation of a
closely related yet distinct \textit{B. pseudomallei}-like organism, now
recognized as \textit{B. thailandensis} (3). The most reliable biochem-
ical test for differentiation of these two species is assimilation
of arabinose by \textit{B. thailandensis} (11). Numerous \textit{Burkholderia}-
specific PCR assays can also differentiate the two organisms
(4). Genetically, both 16S rRNA gene sequencing and DNA-
DNA hybridization studies have confirmed the designation of
\textit{B. thailandensis} as a unique species (3, 14). \textit{B. thailandensis} is

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{16S_rRNA_gene_sequence_analysis.png}
\caption{16S rRNA gene sequence analysis. The neighbor-joining phylogenetic tree of \textit{Burkholderia thailandensis} strains was based on compar-
isons with 16S rRNA gene sequences of related bacteria. \textit{Pseudomonas aeruginosa} DSM 50071 (GenBank accession no. X06604) was used as an
outgroup for this analysis. Sequences were aligned and trimmed and gaps removed using the Wisconsin package, v. 10.3. Analysis was then done
with MEGA 3.1 (Kimura 2-parameter, 1,000-step bootstrap) (http://www.megasoftware.net/). The bar indicates 2% sequence dissimilarity.}
\end{figure}
much less virulent in animal models than *B. pseudomallei*, with a mean 50% lethal dose of $10^9$ CFU/mouse, versus 182 CFU/mouse for *B. pseudomallei* (11). With *B. thailandensis* generally considered avirulent, disease due to *B. thailandensis* is extremely rare. No *B. thailandensis* isolates were identified among 1,200 patients with melioidosis (11). There has been at least one reported case of melioidosis where *B. thailandensis* was cultured from the purulent material of an amputated knee sustained after a motorcycle accident in Thailand (5, 9).

Our data demonstrate that infection with *B. thailandensis* can occur in humans and corroborate two other similar reports from Thailand (5, 9). It is likely that the Texas case of pneumonia and septicemia resulted from aspiration of drainage ditch water at the accident site. Similar routes of infection, including vehicular accidents (9) and near drowning (1, 4), are described for melioidosis. We were unable to isolate *B. thailandensis* from soil or water samples collected 1 year after the accident at the Texas site; therefore, we could not establish evidence of environmental persistence in the United States. No information documenting exposure risks, such as military service or reported motor vehicle accident, is available for the LA case, so the source of infection remains unknown.

On the basis of our phenotypic and molecular data, including DNA-DNA hybridization, the isolates described here are definitively identified as *B. thailandensis*. Further studies are needed to identify the virulence factors associated with these and other pathogenic *B. thailandensis* isolates and to determine their molecular and evolutionary relationships to other *B. thailandensis* and *B. pseudomallei* strains.

Melioidosis was diagnosed when this case was presumed to
be caused by *B. pseudomallei*. Emergency response preparedness procedures emphasize the importance of definitively identifying the causative organism of disease. In this case, arabinose assimilation proved to be a simple, straightforward, and useful method for differentiating *B. pseudomallei* and *B. thailandensis*. When *B. pseudomallei* is presumptively identified, the use of tests such as arabinose assimilation in clinical laboratories, as well as *B. pseudomallei*-specific PCR assays, 16S rRNA gene sequencing, and MLST in reference laboratories, can differentiate *B. pseudomallei* from close relatives such as *B. thailandensis*. Our findings emphasize the importance of obtaining definitive identification of the causative organism in order to monitor for emergence of novel pathogens and to rule out possible infection by a bioterror agent.

**Nucleotide sequence accession numbers.** The 16S rRNA gene sequences of CDC3015869, CDC2721121, and 82172 were deposited in GenBank with accession no. DQ388535, DQ388536, and DQ388537, respectively.

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**REFERENCES**


**TABLE 1. DNA-DNA hybridization of Burkholderia spp. and Burkholderia-like strains**

<table>
<thead>
<tr>
<th>Source of unlabeled DNA</th>
<th>Result with labeled DNA from strain CDC2721121 (LA)</th>
<th>RBR&lt;sup&gt;a&lt;/sup&gt; (%) (65°C)</th>
<th>D&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RBR&lt;sup&gt;a&lt;/sup&gt; (%) (80°C)</th>
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</thead>
<tbody>
<tr>
<td>CDC2721121 (LA)</td>
<td>100</td>
<td>0.0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>CDC3015869 (TX)</td>
<td>100</td>
<td>1.0</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>82172 (France)</td>
<td>100</td>
<td>0.5</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td><em>Burkholderia thailandensis</em> ATCC 700388</td>
<td>96</td>
<td>1.0</td>
<td>91</td>
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</tr>
<tr>
<td><em>Burkholderia pseudomallei</em> ATCC 23343</td>
<td>76</td>
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<td>59</td>
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<tr>
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<td>5.5</td>
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<tr>
<td><em>Burkholderia cepacia</em> ATCC 25416</td>
<td>35</td>
<td>10.5</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The RBR is the amount of double-stranded DNA formed between labeled and unlabeled DNAs from different strains divided by the amount of double-stranded DNA formed between labeled and unlabeled DNA from the same strain.

<sup>b</sup> Divergence (D) within related sequences was calculated on the assumption that each 1°C decrease in the thermal stability of a DNA duplex is caused by 1% of unpaired bases within that duplex. Divergence was calculated to the nearest 0.5%.