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Australian and Thai Isolates of *Burkholderia pseudomallei* Are Distinct by Multilocus Sequence Typing: Revision of a Case of Mistaken Identity▽

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A recent study using multilocus sequence typing (MLST) of *Burkholderia pseudomallei* isolates found a sequence type (ST60) to be common to both Thailand and Australia, contradicting earlier studies showing complete distinction between isolates from these regions. The ST60 isolates reportedly from Australia had been obtained for MLST from United Kingdom and U.S. collections. We have located and characterized the original Australian isolates; they were collected in 1983, and they are neither ST60 nor *B. pseudomallei* isolates. The *B. pseudomallei* MLST database has been corrected, and there is no ST common to isolates verified as obtained from Australia or from Thailand.

B. pseudomallei, the environmental bacterium that causes melioidosis, is widely dispersed throughout Southeast Asia and northern Australia (8). The true extent of its global distribution remains very unclear (1), with a recent case cluster of melioidosis in Brazil (6) confirming the need to consider melioidosis beyond the well-known regions of endemicity.

Multilocus sequence typing (MLST) has been used to clarify the genetic relationships of *B. pseudomallei* isolates from different locations (3). MLST initially showed that isolates of *B. pseudomallei* from Thailand and those from northern Australia were distinct (2, 7). However, a recent study comparing a historical *B. pseudomallei* collection from Southeast Asia to that of the MLST database (http://bpseudomallei.mlst.net/) concluded that Australian isolates may not be distinct from those of Thailand (4). This conclusion was based on the finding that MLST ST60 was common to environmental isolates from both countries. The authors stated that this finding was profoundly significant to melioidosis epidemiology and research, since the prior finding that Australian *B. pseudomallei* isolates were distinct from other isolates sourced elsewhere was no longer the case.

We have now tracked down the Australian *B. pseudomallei* ST60 isolates and show that the results are a case of mistaken identity. Furthermore, we confirm that to date, all known Australian *B. pseudomallei* STs are indeed distinct from all known Thai STs. The incorrect attribution of a strain to a specific geographic location or clinical finding can have major ramifications, and the scenario we describe illustrates how critical it is to ensure the accuracy of strain data deposited in publicly accessible data sets such as the MLST databases.

The five "Australian" ST60 isolates used in the recent study (4) were all listed in the MLST database as being from environmental sources. Three had been obtained for MLST in the laboratory of B.G.S. from the large *B. pseudomallei* collection at the Central Public Health Laboratory, United Kingdom, and two had been obtained by the Centers for Disease Control (CDC), United States. We established, through the curator of the MLST database (D.G.), that the two CDC isolates were actually the same as two strains from the United Kingdom, having been part of a collection sent to the CDC from the United Kingdom. Indeed, it is now recognized that there was some duplication in the *B. pseudomallei* MLST database as a result of the same strains being deposited separately in the United Kingdom and in the U.S. collections with different identifiers (data not shown).

Having established that there were only three "Australian" ST60 strains, we then ascertained their histories. These three strains had been cultured by A.D.T. in 1983 from soil from a single property in southeast Queensland, Australia, as part of an investigation into an outbreak of melioidosis in pigs. These isolates, D228, D260, and D304, were sent to D.A.D. in the United Kingdom in 1992. Testing by both A.D.T. and D.A.D., including testing by an API 20NE kit (bioMerieux, Paris, France), the lack of agglutination with *B. pseudomallei* antisera and the lack of virulence in guinea pigs had confirmed that these three were not *B. pseudomallei* isolates, although they had not been identified to a species level.

We have now been able to retrieve and reculture two of these 1983 strains from A.D.T.'s long-term collection; stored D228 was nonviable. Both D260 and D304 are clearly not *B. pseudomallei* strains. They are oxidase-positive gram-negative

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bacilli that cannot be identified to a species level with both an API 20NE and an API 20E (bioMerieux, Paris, France) kit. They are agglutination negative with *B. pseudomallei* antisera and are negative with the *B. pseudomallei*-specific type III secretion system PCR (5). Furthermore, MLST confirmed they were not *B. pseudomallei* strains and were completely different than the ST60 strain. Only four of the seven MLST loci were able to be sequenced using the normal MLST primers, and the allelic sequences obtained (which were identical for both D260 and D304) were very divergent from any allele in the MLST database.

It is now evident that there was a strain identity mixup when the "Australian" ST60 strains were incorporated into the United Kingdom collection and were included in the original MLST study (3). What were thought to be the "Australian" ST60 strains were presumably ST60 strains from another source. While the real identities of the strains typed as ST60 remain speculative, it is likely that they were isolates from Thailand, since Thai isolates make up the vast majority of the isolates in the United Kingdom collection. ST60 is one of the more common STs found in Thailand (7).

Two other STs have been attributed to both Australia and Southeast Asia in the past. There were two "Australian" ST23 isolates listed in the database that we have found to also be United Kingdom and CDC duplicates of a single isolate from the same collection sent by A.D.T. to D.A.D. in 1992. The original isolate, X1003, was isolated by A.D.T. in 1978 from a goat in Townsville, Queensland. We have retrieved and recultured this isolate and found it belongs to a novel ST of *B. pseudomallei*, ST517, not ST23, confirming that another isolate substitution error is likely to have occurred. Another "Australian" isolate, typed as ST84, was cultured from a patient with an uncertain travel history, and as discussed by McCombie et al., this isolate was likely from an infection acquired in Southeast Asia (4).

The misidentified isolates have been removed from the MLST website database. At present, there are 178 STs from Australia and 224 STs from Thailand represented in the database, and there is complete separation of Australian and Thai STs among those isolates whose origins are verifiable from original sources. *B. pseudomallei* strain collections in laboratories in regions of nonendemicity often contain a mixture of isolates from travelers to regions of endemicity who present with melioidosis, isolates obtained from other collections, and isolates from laboratories in regions of endemicity. Because of the implications of errors such as those described here, we call for a system of provenance to document the identity, the associated clinical information, and the chain of custody of isolates in global databases.

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REFERENCES

1. **Cheng, A. C., and B. J. Currie.** 2005. Melioidosis: epidemiology, pathophysiology, and management. Clin. Microbiol. Rev. **18:**383–416.
2. **Cheng, A. C., D. Godoy, M. Mayo, D. Gal, B. G. Spratt, and B. J. Currie.** 2004. Isolates of *Burkholderia pseudomallei* from northern Australia are distinct by multilocus sequence typing, but strain types do not correlate with clinical presentation. J. Clin. Microbiol. **42:**5477–5483.
3. **Godoy, D., G. Randle, A. J. Simpson, D. M. Aanensen, T. L. Pitt, R. Kinoshita, and B. G. Spratt.** 2003. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J. Clin. Microbiol. **41:**2068–2079.
4. **McCombie, R. L., R. A. Finkelstein, and D. E. Woods.** 2006. Multilocus sequence typing of historical *Burkholderia pseudomallei* isolates collected in Southeast Asia from 1964 to 1967 provides insight into the epidemiology of melioidosis. J. Clin. Microbiol. **44:**2951–2962.
5. **Novak, R. T., M. B. Glass, J. E. Gee, D. Gal, M. J. Mayo, B. J. Currie, and P. P. Wilkins.** 2006. Development and evaluation of a real-time PCR assay targeting the type III secretion system of *Burkholderia pseudomallei*. J. Clin. Microbiol. **44:**85–90.
6. **Rolim, D. B.** 2005. Melioidosis, northeastern Brazil. Emerg. Infect. Dis. **11:** 1458–1460.
7. **Vesaratchavest, M., S. Tumapa, N. P. Day, V. Wuthiekanun, W. Chierakul, M. T. Holden, N. J. White, B. J. Currie, B. G. Spratt, E. J. Feil, and S. J. Peacock.** 2006. Nonrandom distribution of *Burkholderia pseudomallei* clones in relation to geographical location and virulence. J. Clin. Microbiol. **44:**2553–2557.
8. **White, N. J.** 2003. Melioidosis. Lancet **361:**1715–1722.