Melioidosis bioreconnaissance on three continents.

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Abstract

The bacterial infection known as melioidosis occurs sporadically throughout its endemic zone and only rarely manifests in case clusters or outbreaks. When these occur, the unusual concentration of cases presents an opportunity to advance understanding of the human, bacterial and environmental factors that result in disease. We consider how a series of expeditionary investigations have shaped our laboratory response to global melioidosis events over a period of two decades, and have enabled development of a bioreconnaissance concept for emerging infectious diseases.

Introduction: local expeditions

We were ill-prepared for a public health laboratory field investigation when the West Kimberley melioidosis outbreak occurred immediately before the Christmas holiday in 1997. It was clear something unusual was afoot as soon as a second blood culture from the same remote WA community grew *Burkholderia pseudomallei*, it was unclear what specific measures would help beyond informing the regional public health physician. At that point there was no clear investigational precedent for management of a culture-confirmed melioidosis case cluster. The three early fatalities prompted a fact-finding visit before the onset of the seasonal rains. The subsequent series of investigations has been documented in detail elsewhere [1-3]. In short, the initial field investigation concentrated on detection of an environmental source of *B. pseudomallei* that could explain the melioidosis case-cluster [2]. Subsequent public health laboratory studies and a follow up visit during decommissioning of specific water treatment installations identified a potential upstream environmental source of *B. pseudomallei* [3]. Two sporadic culture-positive cases occurred in individual from the area who were present in the affected community during the initial outbreak, one of which occurred over a year later [4]. No further cases were diagnosed from the community after those two sporadic cases in the following two decades of state-wide surveillance. On conclusion of the initial outbreak response, we conducted a three year study of community water sources in tropical northern Australian, but after comprehensive testing in a reference laboratory setting found no evidence for widespread *B. pseudomallei* contamination of community water sources in Northern Australia [5]. Field work during this study focused on sample collection only, all analytical work being performed after transport to the reference laboratory, which in some cases occurred after significant delays.
On conclusion of those environmental surveillance activities we ran a targeted study of major environmental disturbance on a mine site in the East Kimberley, where we were able to detect *B. pseudomallei* in restored land close to mine tailings [6]. Sero-surveillance results from that study found evidence for *B. pseudomallei* exposure while on leave, but not during periods of work on the site. Although the mine site study was the first to use deployable PCR assays on site during field work, including a micro-fluidic capillary electrophoresis analyser, a conventional PCR thermal cycler, a high speed centrifuge and dry block heater to a remote location. Transport of this bulky equipment was logistically complex (Figure 1). At that time, clinical microbiology lacked portable instruments for real time PCR assays, necessitating use of compact electrophoresis for analysis of amplified products of conventional PCR, following nucleic acid extraction from soil samples. Nevertheless, this work resulted in detection of *B. pseudomallei* - positive specimens by PCR while still on site allowing the positive sites to be revisited for intensive sampling during the same trip. The PCR - positive results were subsequently confirmed by culture in the public health laboratory, and linked to the West Kimberley outbreak strain of *B. pseudomallei* originally isolated eight years previously.

**Overseas expeditions**

A small cluster of melioidosis cases occurred in a single farming family in rural, Northeastern Brazil [7]. Initially thought to be a variant of plague, an astute pathologist considered melioidosis and found the microbiological support to identify *B. pseudomallei*. After an initial investigation had quickly ruled out a more sinister explanation, we were called to advise on the wider aspects of environmental investigation and risk management. Initially, this involved establishing a melioidosis investigation team under the auspices of the Ceará State Health Secretariat, with support from an infectious diseases hospital and the Federal University of Ceará. The first field trip preceded the development of WA field investigation capability and was therefore not PCR assay-enabled. Our logistic solution was shipment of in-house PCR assay reagents to NE Brazil. This proved to be logistically challenging due to a combination of cold chain sustainment and import restrictions on positive control material. Our first attempt to transfer a *B. pseudomallei* confirmatory assay as a reagents and method package was unsuccessful. Subsequent attempts were increasingly successful, leading to the Ceará group establishing self-sufficient laboratory confirmation of culture-positive melioidosis [8,9], standard laboratory methods [10], public health guidelines[11], and recognition of the emergence of sporadic melioidosis in the Americas [12].

**Recognition of undisclosed endemic *B. pseudomallei* infection in Sri Lanka**

Building on the Brazil partnership we developed a molecular methods training workshop during that series of visits that have since been used to train clinical and research laboratory staff in Malaysia and Sri Lanka [13]. These regional international collaborations have now been under way for over a decade, and in the case of Sri Lanka became the foundation of a national melioidosis surveillance
programme with profound implications for early diagnosis, treatment and prevention of melioidosis [14,15]. Most notably, nationwide melioidosis surveillance has exposed a relationship between clinico-pathological disease phenotype and \textit{B. pseudomallei} genotype (MLST). Through successive refinement in a series of domestic tests and deployments to Sri Lanka, the portable molecular diagnostic laboratory (now termed Lab-In-A-Box or LIAB) replaced bulky conventional PCR equipment with a single real-time thermal cycler and magnetic particle extraction (to eliminate the need for high speed centrifugation). This more compact equipment fitted in a simpler but still bulky shipment (Figure 2). Maintenance of cold-chain continued to be a problem with PCR reagents that had limited lifespan during transit into location and subsequent field use. Most recently, very compact real-time PCR systems with solid state light sources have reduced the LIAB system to a single consumer grade suitcase with internal foam padding, or even carry-on baggage (Figures 3 and 4).

**Closer to home, in suburban WA**

Our field work in Sri Lanka commenced in early 2008, and revealed a broad spectrum of clinical disease including cutaneous and soft tissue melioidosis. Cutaneous disease had been unusual in WA throughout this period. In 2012 a single case of cutaneous melioidosis occurred in a patient from southwestern WA with no recent history of travel to northern Australia or overseas. Detailed environmental investigation of the patient’s home and neighbourhood generated no insight into a potential \textit{B. pseudomallei} source. When two further culture positive cases of cutaneous melioidosis occurred in the same district almost two years later, we investigated a possible common source and identified a contaminated bottle of wound irrigation fluid which contained \textit{B. pseudomallei} of the same genotype as all cases in the cluster [16]. Removal of the contaminated bottle coincided with a cessation of case detections. There have been no further cases in the following four years and no cases of late onset septicaemia or relapsing soft tissue melioidosis. The long interval between the index case and the main case cluster has still not been explained, but did prepare the state public health laboratory for subsequent events because the MLST genotype was already on record when the second case occurred. Also notable was the use of previously validated MALDI-TOF mass spectroscopy [17] to rapidly identify suspect isolates from wound swabs which was possible before they gained their typical wrinkled colony appearance. Though no PCR assay capability was deployed on this occasion due to the proximity of the affected neighbourhood, the rapid laboratory response combined with willingness to task staff to support the Disease Control and lead the environmental investigation contributed to swift interruption of the outbreak. The option to deploy PCR assay capability in order to refine targeting of sample collection was available to us, had the outbreak continued unchecked.

**Conclusion**
Melioidosis usually occurs as a sporadic infection of remote and rural populations. Outbreaks are rare and provide an opportunity to learn more about its biology and particularly its ecology. Our involvement in a series of melioidosis case clusters spanning two decades illustrates the additional insights from field investigations. Case clusters provided us repeated opportunities to learn lessons, some of which have had enduring benefits for the affected communities, through strengthening disease control measures. The development of compact real-time PCR thermal cyclers has enabled the necessary components of a basic molecular diagnostic laboratory to pack into a single suitcase. Maintaining cold chain for reagents continues to present challenges but advances in stabilising PCR mastermix by lyophilisation presents an opportunity for a commercially-produced PCR assay for B. pseudomallei that could be run on site in any location to confirm culture, directly detect in clinical specimens, or to screen soil and water samples for detectable nucleic acid. There is still much to learn by closing in on the source of a melioidosis case cluster, using new molecular investigational methods, and rapidly escalating support for a targeted response. Continual fine tuning of this cooperative emerging disease response has improved melioidosis diagnosis, treatment and prevention. Nature may be full of surprises, but as experience has demonstrated, fortune favours the bioreconnaissance-enabled laboratory.

References


Figure 1. The evolution of a deployable molecular diagnostic laboratory for melioidosis bioreconnaissance. Early deployment using conventional PCR and microfluidic capillary electrophoresis encountered problems with shipment as checked baggage and high cost for separate courier delivery.
Figure 2. The evolution of a deployable molecular diagnostic laboratory for melioidosis bioreconnaissance. Downsizing by introduction of real-time PCR and magnetic particle NA extraction simplified transport by reducing the number of items and the total weight.
Figure 3. The evolution of a deployable molecular diagnostic laboratory for melioidosis bioreconnaissance. Use of compact real-time PCR instruments to reduce the deployable equipment to a single domestic grade suitcase.