Laboratory safety: handling *B. pseudomallei* isolates without a biosafety cabinet

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Background

Burkholderia pseudomallei is a soil-dwelling bacterium endemic to parts of Australia, and the causative agent of melioidosis (1). Laboratory-acquired infections have occurred and as such an international consensus guidelines suggests handling of the organism in a Biosafety Level (BSL) 3 facility within a class II biological safety cabinet (BSC). However, there is regional divergence in practice. The authors sought to assess the risk to laboratory staff while handling *B. pseudomallei* isolates outside of a class II BSC.

This research was approved by the Royal Brisbane & Women's Ethics Committee LNR/2020/QRBW/61126.

Methods

Seroprevalence

Staff members who had previously handled *B. pseudomallei* isolates completed a questionnaire designed to assess an estimated total exposure over the preceding 10-year period. Additionally, an indirect haemagglutination assay (IHA) for detection of total antibody and an enzyme immunoassay (EIA) detecting immunoglobulin G (IgG) was performed (2).

Bio-aerosol detection

For detection of *B. pseudomallei* aerosols a *Burkholderia thailandensis* type strain was used as a substitute for safety purposes. This substitution was considered likely to be bioequivalent due to previously reported size and volume data between species (3). Air sampling was performed using the MicroBio MB1 (Cantium Scientific, Kent United Kingdom) single-stage sieve impactor at a flow rate of 100L/min, Figure 1. Aerosol generating experiments included plate opening and tilting, oxidase reaction, catalase reaction, 0.5 McFarland suspension creation, susceptibility lawn plating, and mass spectrometer (MS) target spot creation. Each experiment occurred within a class II BSC. Tryptic soy agar (TSA) collection plates were subsequently incubated aerobically at 35°C for 5 days.

Table 1. Environmental bio-aerosols detected per experiment in CFU/m^3

Experiment	Growth (Hours)	CFU/m^3				
Replicate		1	2	3	4	5
Plate opening	24	7	20	20	7	34
	48	34	20	20	7	14
	72	20	7	14	-	-
McFarland creation	24	12	9	5	7	6
	48	6	13	0	12	0
	72	27	41	20	-	-
Catalase	24	0	20	0	20	7
	48	7	0	14	14	27
	72	34	20	41	-	-
Oxidase	24	7	0	0	0	0
	48	20	20	14	0	27
Susceptibility lawn	24	0	0	20	20	14
	48	0	0	7	7	14
MS spot	24	20	48	7	27	14
	48	41	7	27	20	20
Control	-	41	10	26	20	26

Results

Seroprevalence

In total, 30 participants were enrolled in this study. The estimated total handling events from all participants over a 10-year period was 1,419. Total handling events outside a BSC were 1,267 (89%). All participant serological results were negative, representing a 0% seroprevalence amongst laboratory staff (95% confidence interval: 0 – 12%).

Bio-aerosol detection

In total 78 bio-aerosol detection experiments were performed. Table 1 demonstrates the type of handling, organism growth in hours, and total environmental organism detected. Approximately 12,000L of environmental air was sampled during the course of the 78 handling experiments. While numerous environmental organisms were detected, no TSA experiment plates demonstrated growth of *B. thailandensis*. Therefore, the rate of *B. thailandensis* detection was <1 CFU/m^3 per experiment. Across all experiments the total detectable *B. thailandensis* approximated to < 9 x 10-5 CFU/m^3.

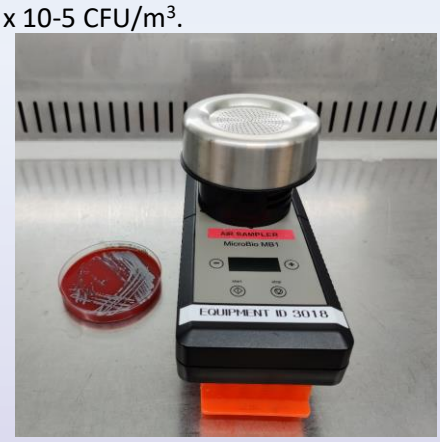

Figure 1. Air sampler above an open *B. thailandensis* culture plate

Discussion

The average ventilation rate of a scientist is approximately 11.8–12.3L/min (4). Assuming each laboratory handling procedure results in 1 – 5 CFU *B. pseudomallei*/m^3, a scientist working with one isolate would potentially inhale 1.25 – 6.25 x 10^{-2} CFU/min. However, our results suggest a significantly lower number of aerosols generated per procedure and therefore an inhalation exposure of < 1.6 x 10^{-4} CFU/min. To our knowledge there are no reported cases of laboratory-acquired melioidosis in Queensland. Additionally, the Townsville laboratory has directly identified over 400 clinical isolates in the preceding 30 years without incident.

Conclusion

This study suggests a low risk of laboratory-acquired melioidosis when handling *B. pseudomallei* on an open bench. However, individual laboratories will need to undertake a risk assessment and take region-specific guidelines into consideration.

References:

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