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Comparison of Ashdown's Medium, *Burkholderia cepacia* Medium, and *Burkholderia pseudomallei* Selective Agar for Clinical Isolation of *Burkholderia pseudomallei*

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Ashdown's medium, *Burkholderia pseudomallei* selective agar (BPSA), and a commercial *Burkholderia cepacia* medium were compared for their abilities to grow *B. pseudomallei* from 155 clinical specimens that proved positive for this organism. The sensitivity of each was equivalent; the selectivity of BPSA was lower than that of Ashdown's or *B. cepacia* medium.

Burkholderia pseudomallei is a gram-negative soil saprophyte and the cause of melioidosis, a disease that is endemic in southeast Asia and northern Australia (4). Isolation of the organism from any sample is diagnostic for melioidosis and remains the "gold standard." Specimens from nonsterile sites may be the only specimen positive by culture, and the use of selective agar is necessary to reduce the overgrowth and masking of *B. pseudomallei* by commensal flora. Ashdown's agar is the most common selective medium in use in countries where the disease is endemic (1, 5). The presumptive identification of colonies of *B. pseudomallei* can be made from their characteristic purple color and dry and wrinkled appearance, which becomes evident after 24 to 48 h of incubation.

A second medium, *B. pseudomallei* selective agar (BPSA), which may enhance the growth of mucoid colonies compared with that on Ashdown's medium has recently been described (3), but this has not been extensively evaluated for the detection of *B. pseudomallei* in clinical samples. Neither medium is commercially available, and so they are not rapidly accessible to laboratories in areas of nonendemicity during the investigation of patients returning from abroad with suspected melioidosis or for use in the event of a suspected bioterrorist release of *B. pseudomallei*. *Burkholderia cepacia* medium, an agar most commonly used to identify *B. cepacia* complex from the sputum of individuals with cystic fibrosis, is widely available; but its ability to isolate *B. pseudomallei* in clinical samples has not been reported. The aim of this prospective study was to compare these three media in a clinical diagnostic setting in northeast Thailand and to determine their relative sensitivities and selectivities.

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Ashdown's agar was prepared as originally described (1), *B. cepacia* medium was prepared according to the manufacturer's recommendations (Mast Diagnostics, Merseyside, United Kingdom), and BPSA was prepared as described by Howard and Inglis (3). Patients were recruited prospectively between July and November 2003 by a study team at Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand. Patients with suspected melioidosis were actively sought during twice-daily ward rounds of the medical and intensive care wards. Blood and throat swab specimens for microbiological culture were taken from all patients; and urine, pus, and respiratory secretions were collected where available.

Specimens of urine, pus, respiratory secretions, and throat and wound swabs were plated directly onto the three media (BPSA, Ashdown's, and *B. cepacia* medium) in a random order (as defined by a random-number table) to minimize any systematic bias. Quantitative bacterial counts were performed by adding an equal volume of water to lyse pus cells and then serially diluting and spread plating 10 μl onto each of the three media. Samples from sterile sites (pus and aspirates) were also cultured using tryptic soy broth (TSB), and samples from nonsterile sites (respiratory secretions and throat and wound swabs) were cultured using selective enrichment broth (TSB containing 4% glycerol, 0.05% crystal violet, and colistin at a final concentration of 50 mg/liter). The broths were subcultured undiluted and after serial dilution onto the three media after 48 h incubation in air at 37°C. Blood cultures were performed using BacT/ALERT FA bottles (BioMérieux); these were incubated in air at 37°C and were subcultured undiluted onto the three media after 24 h, 48 h, and 7 days and on intervening days if the bottles became cloudy or the bottle indicator changed color. Blood cultures were examined by serial dilution when the bottle was cloudy or was considered positive for other reasons. All agar plates were incubated in air at 37°C and were examined after 24, 48, and 72 h. The growth

TABLE 1. Quantitative median values of *B. pseudomallei* colony counts

Medium	Quantitative median count (10^6 CFU/ml [IQR])[a]			Media compared	Statistical comparison *P* value		
	Day 1	Day 2	Day 3		Day 1	Day 2	Day 3
BPSA	227 (37–390)	235 (57–440)	280 (38–670)	BPSA vs Ashdown's medium	0.82	0.05	0.21
Ashdown's medium	205 (41–400)	231 (50–450)	289 (42–510)	*B. cepacia* medium vs Ashdown's medium	<0.0001	<0.0001	0.001
B. cepacia medium	280 (60–620)	281 (66–615)	300 (45–640)	BPSA vs *B. cepacia* medium	<0.0001	<0.0001	0.0003

[a] IQR, interquartile range. All statistical comparisons were performed by the Wilcoxon signed-rank test.

of *B. pseudomallei* and other organisms was recorded. The identification of *B. pseudomallei* was made on the basis of Gram stain, resistance to gentamicin and colistin, the inability to assimilate arabinose, and positivity by indirect immunofluorescence (7) and latex agglutination (6). A test with the API 20NE system was performed if any doubt remained; this has been reported to have a high degree of accuracy in our setting (2). Other microorganisms were identified by standard laboratory methodologies. Statistical analysis was performed by using the statistical program Intercooled STATA, version 8.0 (STATA, College Station, Tex.).

Cultures of 155 clinical specimens from 86 patients yielded *B. pseudomallei* on at least one selective medium (range, 1 to 4 positive samples per patient). Positive cultures were from the following specimen types: 56 blood samples (36%), 31 respiratory secretions (19%), 17 pus samples (11%), 25 throat swabs (16%), 12 wound swabs (8%), 13 urine specimens (8%), and 1 pleural fluid specimen (1%). Of these, 63 specimens (41%) were positive on primary culture (i.e., *B. pseudomallei* was grown on agar plated directly with the sample), while the remaining 92 specimens (59%) were positive only with samples from enrichment broth.

On qualitative comparison, the *B. pseudomallei* colonies grew on all three media, with two exceptions. In one case, a single colony of *B. pseudomallei* grew on BPSA but not on Ashdown's or *B. cepacia* medium; in another comparison, a single colony grew on Ashdown's medium but not BPSA or *B. cepacia* medium. This may reflect a stochastic phenomenon related to the low bacterial concentrations in the sample. A total of 115 samples examined by quantitative bacterial count were positive for *B. pseudomallei*. Colony counts on *B. cepacia* medium were significantly higher than those on either Ashdown's medium or BPSA on all 3 days (Table 1). There was no difference in colony counts between Ashdown's medium and BPSA.

After 18 h of incubation, there was obvious growth of *B. pseudomallei* on BPSA and *B. cepacia* medium, but only pinpoint growth was present on Ashdown's medium. At 24 h, colonies growing on *B. cepacia* medium were between 1 and 1.5 mm in diameter, on BPSA they were approximately 1 mm, and on Ashdown's medium they were approximately 0.5 mm. By 48 h, *B. pseudomallei* colonies were larger on BPSA and *B. cepacia* medium but were obvious on all three media. The colonial morphology of *B. pseudomallei* was often mucoid on BPSA, but these strains were also observed to grow on Ashdown's and *B. cepacia* media. A mixture culture of randomly selected clinical isolates of *B. pseudomallei* and *B. cepacia* was spread plated onto *B. cepacia* medium; it was not possible to distinguish reliably between these two species based on colony morphology after 2, 3, or 4 days of incubation in air at 37°C (data not shown).

In total, 524 Ashdown's and *B. cepacia* medium plates and 526 BPSA plates were inoculated with clinical samples. There was no difference in selectivity (based on ability to prevent the growth of other species), between Ashdown's medium and *B. cepacia* medium (Fisher's exact test, $P = 0.23$). BPSA was significantly less selective than either Ashdown's medium or *B. cepacia* medium (Fisher's exact test, $P < 0.0001$ in both cases) (Table 2). Organisms that were difficult to distinguish from *B. pseudomallei* (*Klebsiella* and *Pseudomonas* spp.) were identified to species level. The colony morphology of the majority of organisms that grew on one or more media was clearly different from that of *B. pseudomallei*, and these isolates were not further identified.

We propose that Ashdown's medium should remain the standard selective medium for clinical specimens in regions

TABLE 2. Other bacterial species and organisms growing on solid media

Organism	No. (%) of isolates recovered on the following medium (total no. of plates):		
	BPSA ($n = 526$)	Ashdown's medium ($n = 524$)	*B. cepacia* medium ($n = 524$)
Staphylococcus spp.	13 (2)	4 (1)	4 (1)
Enterococcus spp.	5 (1)	0 (0)	0 (0)
Klebsiella species	4 (1)	5 (1)	8 (2)
Pseudomonas aeruginosa	4 (1)	5 (1)	4 (1)
Other gram-negative bacilli, not further identified	7 (2)	0 (0)	0 (0)
Fungi	14 (3)	0 (0)	8 (2)
Mixed organisms[a]	20 (4)	7 (1)	13 (2)
Colonies, not further identified	96 (18)	52 (10)	51 (10)
Total	163 (31)	73 (14)	88 (17)

[a] Two or more species in addition to *B. pseudomallei.*

where melioidosis is endemic. *B. cepacia* medium represents a widely available, acceptable alternative in areas of nonendemicity for the isolation of *B. pseudomallei* from patients with suspected melioidosis. This medium also supports the growth of *B. mallei* and would be suitable for screening during a deliberate-release event involving *B. pseudomallei* and/or *B. mallei.*

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