Selective media and real-time PCR improves diagnosis of melioidosis in community-acquired pneumonia in a low-incidence setting in Kuala Lumpur, Malaysia

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Abstract

Introduction. *Burkholderia pseudomallei* (melioidosis) is an important cause of community-acquired pneumonia (CAP) in the tropics. Selective medium is recommended for laboratory diagnosis with non-sterile respiratory samples, while PCR is not routinely used due to variable reported performance. The effectiveness of these diagnostic modalities varies by site.

Aim. To compare selective media and real-time PCR (qPCR) with routine media in detecting *B. pseudomallei* in CAP respiratory samples in a low-incidence setting in Kuala Lumpur, Malaysia.

Methodology. Respiratory samples were routinely cultured on blood, chocolate and MacConkey agar (RESP-ROUTINE), and compared to culture on selective Ashdown medium (RESP-SELECTIVE) and qPCR. The gold standard was routine culture of *B. pseudomallei* from any site (ALL-ROUTINE).

Results. *B. pseudomallei* was detected in 8/204 (3.9%) samples. Overall sensitivity rates differed (*P*=0.03) for qPCR (100%), RESP-SELECTIVE (87.5%) and RESP-ROUTINE (50%). There was a trend towards lower median days to positive culture for RESP-SELECTIVE (1 day) compared to RESP-ROUTINE (2 days, *P*=0.08) and ALL-ROUTINE (2 days, *P*=0.06). Reagent costs for each additional detection were USD59 for RESP-SELECTIVE and USD354 for PCR.

Conclusions. In a low-incidence setting, selective culture of respiratory samples on Ashdown was more sensitive and allowed quicker identification than routine media, at reasonable cost. Blood cultures are critical, confirming four cases missed by routine respiratory culture. Selective medium is useful in early pneumonia (pre-sepsis) and resource-limited settings where blood cultures are infrequently done. Real-time PCR is costly, but highly sensitive and useful for high-risk patients with diabetes, cancer or immunosuppressants, or requiring ventilation or intensive care.

*Burkholderia pseudomallei* is an important soil saprophyte found in the tropics, particularly in Southeast Asia. It causes melioidosis in humans, which often manifests as pneumonia and sepsis with high mortality. Diagnosis by culture is the gold standard, but *B. pseudomallei* may be mistaken as a contaminant or missed in mixed cultures. The use of selective media is recommended for diagnostic culture, while PCR has variable reported performance and is not routinely used [1]. The clinical and cost-effectiveness of these diagnostic modalities in different settings depends on disease epidemiology, screening strategies and reagent costs. It is important to carry out local evaluations, particularly in resource-limited or low-incidence endemic areas where it may not be feasible to test all samples [2]. This prospective study aimed to compare selective media and real-time PCR (qPCR) with routine culture in detecting *B. pseudomallei* in respiratory samples from adults with community-acquired pneumonia (CAP) in a low-incidence setting.

Our centre is an urban teaching hospital in Kuala Lumpur, Malaysia, with five to ten melioidosis cases diagnosed annually. This study targeted CAP, the commonest presentation of...
Table 1. Performance characteristics of routine and selective culture media and PCR in 204 respiratory samples

<table>
<thead>
<tr>
<th>Diagnostic modality</th>
<th>No. of B. pseudomallei detected (prevalence, %)</th>
<th>Sensitivity/specificity (95% confidence intervals, %)*</th>
<th>Positive/negative predictive value (95% confidence intervals, %)</th>
<th>Sensitivity/specificity (95% credible intervals, %) using Bayesian latent class models</th>
<th>Median days to identificationb</th>
<th>Cost of reagents per sample (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESP-ROUTINE</td>
<td>4 (2.0%)</td>
<td>50 (15.5–84.6)/100 (100–100)</td>
<td>100 (100–100)/98.0 (96.1–99.9)</td>
<td>50 (19.8–80.2)/100 (100–100)</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>RESP-SELECTIVE</td>
<td>7 (3.4%)</td>
<td>87.5 (64.6–100)/100 (100–100)</td>
<td>100 (100–100)/95.9 (98.5–100)</td>
<td>85.8 (54.5–98.7)/100 (100–100)</td>
<td>1</td>
<td>0.87 (Ashdown)</td>
</tr>
<tr>
<td>PCR</td>
<td>8 (3.9%)</td>
<td>100 (100–100)/100 (100–100)</td>
<td>100 (100–100)/97.3 (73.3–100)</td>
<td>97.3 (73.5–100)/100 (100–100)</td>
<td>–</td>
<td>6.94</td>
</tr>
<tr>
<td>ALL-ROUTINE</td>
<td>8 (3.9%)</td>
<td>100 (100–100)/100 (100–100)</td>
<td>100 (100–100)/97.3 (73.5–100)</td>
<td>97.3 (73.5–100)/100 (100–100)</td>
<td>2</td>
<td>–</td>
</tr>
</tbody>
</table>

* a. Compared to the gold standard of ALL-ROUTINE (culture-confirmation of melioidosis from any site, including blood cultures)
  b. For positive cultures only; taken as time to presumptive identification of bacterial colonies by latex agglutination
medium alone. Earlier studies had shown that enrichment broth did not provide significant gains in isolation yield for sputum (1.2–10.2%), but gains were greater in other non-sterile samples [5, 10]. This is likely due to the high median bacterial load of 10^6 c.f.u. ml^-1 found in sputum [11].

qPCR sensitivity in sputum may be as low as 54–71% and even lower for specimens such as blood and throat swabs in some endemic settings [12, 13], which is why culture remains the gold standard. Our study showed high qPCR sensitivity in respiratory samples as seen in other centres [9, 14]. As qPCR takes only a few hours but at considerably higher cost, it can be reserved for patients at high risk of pulmonary melioidosis. Our multivariate analysis showed that independent predictors of pulmonary melioidosis among CAP patients were presence of diabetes (OR 17.6 [95% CI, 1.4–220.4], P = 0.03), cancer or immunosuppressants (OR 12.5 [1.3–121.7], P = 0.03), and need for assisted ventilation or intensive care (OR 11.2 [1.9–65.7], P = 0.01). Age, gender and renal disease were not independent predictors.

A limitation of our study is the small number of melioidosis cases. We had previously carried out a similar study of CAP samples in our centre using B. pseudomallei selective medium (BPSA) [8, 15], which has equivalent sensitivity to Ashdown medium [16]. For the present study, we switched to Ashdown medium to be in line with other regional laboratories [1]. If the results of the current and our past studies are combined to give a total of 13 melioidosis cases detected in 358 samples (3.6%), the overall combined sensitivity rate of RESP-SELECTIVE (91.7%) is significantly higher than RESP-Routine (50%; P = 0.03). The median days to positive culture for RESP-SELECTIVE (1 day) is significantly lower than RESP-Routine (2 days, z = -2.0, P = 0.046) and ALL-Routine (2 days, z = -2.3, P = 0.023). A further limitation is that storage of the samples at −80°C for later batched testing may have reduced subsequent detection of B. pseudomallei, although this would have the effect of underestimating sensitivity and overestimating time to positive culture for selective media and qPCR.

We conclude that in our setting with a low incidence of melioidosis, laboratory investigation of patients with CAP should include routine culture of respiratory samples on Ashdown medium. This selective agar is more sensitive and allows quicker identification of B. pseudomallei by a median 1 day than conventional culture, at reasonable cost for each additional detected case. Selective media would be especially useful in early pneumonia (localized infection or before sepsis) and in resource-limited settings where blood cultures are not routinely done. qPCR is costly, but highly sensitive and useful for high-risk patients with CAP, such as those with diabetes, cancer or immunosuppressants, or those requiring ventilation or intensive care.

Funding information
This study was partially supported by the Defense Threat Reduction Agency, USA under Broad Agency Announcement HDTRA1-6 (grant number HDTRA1-17-1-0027). The funder had no role in the study or publication.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Approval for this study was obtained from the Medical Ethics Committee of the University Malaya Medical Centre (no. 2016-1 2084).

References