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RESEARCH NOTE

Detection of *Burkholderia pseudomallei* in Sputum using Selective Enrichment Broth and Ashdown's Medium at Kampong Cham Provincial Hospital, Cambodia [version 1; peer review: 2 approved]

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V1 First published: 11 Dec 2014, 3:302
<https://doi.org/10.12688/f1000research.5935.1>

Latest published: 15 May 2015, 3:302
<https://doi.org/10.12688/f1000research.5935.2>

Abstract

Melioidosis infection, caused by *Burkholderia pseudomallei*, is increasingly reported in Cambodia. We hypothesized that implementation of an enhanced sputum testing protocol in a provincial hospital diagnostic microbiology laboratory would increase detection of *B. pseudomallei*. We tested 241 sputum specimens that were deemed acceptable for culture, comparing culture in selective enrichment broth followed by sub-culture on Ashdown's medium to standard culture methods. Two specimens (0.8%) were positive for *B. pseudomallei* using the enhanced protocol whereas one specimen (0.4%) was positive using standard methods. These findings demonstrate that *B. pseudomallei* is rarely detected in sputum at this hospital. The low frequency of *B. pseudomallei* in sputum specimens precludes drawing any conclusions about the relative benefits of an enhanced sputum testing protocol at this site. Promoting clinician awareness of the infection and encouraging utilization of diagnostic microbiology services are likely to be important factors in facilitating identification of melioidosis.

Keywords

Burkholderia pseudomallei , melioidosis , pneumonia , diagnostic microbiology , lung , sputum , Cambodia

Open Peer Review

Approval Status

1

2

version 2

(revision)

15 May 2015

version 1

11 Dec 2014

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University of Oxford, Oxford, UK

2. **Jan Jacobs**, Institute of Tropical Medicine,
Antwerp, Belgium

Any reports and responses or comments on the article can be found at the end of the article.

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Competing interests: No competing interests were disclosed.

Grant information: This study was funded by support from the University of Washington to TEW.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Nhem S, Letchford J, Meas C *et al.* **Detection of *Burkholderia pseudomallei* in Sputum using Selective Enrichment Broth and Ashdown's Medium at Kampong Cham Provincial Hospital, Cambodia [version 1; peer review: 2 approved]** F1000Research 2014, 3:302 <https://doi.org/10.12688/f1000research.5935.1>

First published: 11 Dec 2014, 3:302 <https://doi.org/10.12688/f1000research.5935.1>

Introduction

Melioidosis, infection with the Gram negative bacterium *Burkholderia pseudomallei*, has increasingly been described in Cambodia in recent years^{1–6}. Infection is caused by inoculation, inhalation, or ingestion of *B. pseudomallei*, an environmental saprophyte⁷. In one single center Cambodian study, melioidosis (defined as growth of *B. pseudomallei* in any clinical specimen) was lethal in more than half of cases, many of whom had received inappropriate initial antibiotic therapy². The clinical presentation of melioidosis is notoriously variable, but pneumonia and sepsis occur commonly⁷. Sub-acute and chronic forms of melioidosis may be confused with tuberculosis and it is important to distinguish these diseases⁸. *B. pseudomallei* is resistant to penicillin, ampicillin, first- and second-generation cephalosporins and aminoglycosides⁷. Use of quinolones or third-generation cephalosporins to treat melioidosis is associated with poorer outcomes^{9–11}. Thus, many of the first line antimicrobial therapies administered for pneumonia and sepsis do not adequately treat melioidosis. The treatment course for melioidosis is prolonged, requiring initial intensive therapy for at least 10–14 days with intravenous ceftazidime, imipenem, or meropenem, followed by 3–6 months of oral eradication therapy with trimethoprim-sulfamethoxazole⁷. Moreover, ceftazidime and carbapenems are relatively expensive and may not be universally accessible in Cambodia. Therefore, rapid identification of the bacterium is essential to guide clinical management. In pulmonary melioidosis, culture of sputum may provide critical diagnostic information. In this report, we present our experience implementing an enhanced sputum testing protocol in a Cambodian provincial hospital diagnostic microbiology laboratory to increase detection of *B. pseudomallei*.

Kampong Cham Provincial Hospital is a 260 bed referral hospital approximately 120 kilometers by road northeast of Phnom Penh. The diagnostic microbiology laboratory at the hospital began offering culture and antibiotic susceptibility testing in 2009. Several cases of *B. pseudomallei* have been isolated at the laboratory since then. Given resource limitations, standard media used to culture sputum specimens in this laboratory are blood, chocolate, and MacConkey agars. In northeast Thailand, where melioidosis is highly endemic⁷, the use of selective enrichment broth and Ashdown's medium has enhanced the detection of *B. pseudomallei* from sputum^{12,13}. We hypothesized that the use of selective enrichment broth and Ashdown's medium at Kampong Cham Provincial Hospital would similarly increase the identification of cases of pulmonary melioidosis.

Methods

To compare the use of selective enrichment broth and Ashdown's medium to standard sputum culture at Kampong Cham Provincial Hospital, we performed specific testing for *B. pseudomallei* on all sputum specimens submitted to the laboratory and accepted for bacterial culture between March 25 and September 30, 2013, following a previously described protocol^{12,14}. We chose these dates to include much of the rainy season, when melioidosis is diagnosed more frequently². Specimens accepted for culture had fewer than 10 epithelial cells per low power field or moderate to high numbers of polymorphonuclear cells on Gram stain. Specimens were inoculated into selective enrichment broth containing Tryptic Soy Broth 10g,

glycerol 40ml, crystal violet 0.1% 5ml, and colistin 50mg per liter of distilled water for two days at 37°C in aerobic conditions. Specimens were then sub-cultured onto Ashdown's medium (Tryptic Soy Broth 10g, agar 15g, glycerol 40ml, crystal violet 0.1% 5ml, neutral red 1% 5ml, and gentamicin 4mg per liter of distilled water) and incubated for four days at 37°C in aerobic conditions. Colonies that grew were tested using the oxidase test; oxidase-positive colonies were tested using a highly sensitive and specific monoclonal antibody-based latex agglutination test for *B. pseudomallei*^{15,16}. In parallel, as per routine laboratory practice, all sputum specimens were also cultured onto sheep blood, chocolate, and MacConkey agars.

Results

Two hundred and forty one sputum specimens deemed acceptable for culture were received by the laboratory during the study period and tested using the enhanced protocol. *B. pseudomallei* was isolated from one specimen (0.4%) using standard media and from two specimens (0.8%) using the enhanced protocol. The single specimen positive using standard media was also positive using the enhanced protocol; the enhanced technique therefore detected one additional isolate of *B. pseudomallei* compared to the standard protocol. In this case, *Klebsiella pneumoniae* grew on standard media, raising the possibility that overgrowth of *K. pneumoniae* precluded detection of *B. pseudomallei* in the absence of selective enrichment broth. Among the 241 sputum specimens collected during the study period, *B. pseudomallei* accounted for two (1.6%) of the 122 that were culture positive. Other bacteria isolated from the sputum samples during the study period were *K. pneumoniae* (54), *Pseudomonas* species (37), *Enterobacter* species (7), *Acinetobacter* species (5), *Staphylococcus aureus* (4), *Stenotrophomonas maltophilia* (3), *Streptococcus pneumoniae* (3), *Haemophilus influenzae* (3), *Escherichia coli* (3), *K. ozaenae* (3), unknown Enterobacteriaceae (2), *Vibrio alginolyticus* (1), and a non-fermenting Gram negative bacillus (1). On standard media, three other specimens were positive for *B. pseudomallei* during the study period: a pleural fluid specimen from one patient with a *B. pseudomallei*-positive sputum culture, and pus from two other individuals.

Dataset 1. Detection of *B. pseudomallei* in Sputum using Selective Enrichment Broth and Ashdown's Medium Compared to Standard Culture

<http://dx.doi.org/10.5256/f1000research.5935.d40423>

Specimens (Column A) were inoculated into selective enrichment broth before sub-culture onto Ashdown's medium (Column B). Colonies that grew were tested using the oxidase test (Column C); oxidase-positive colonies were tested using a monoclonal antibody-based latex agglutination test for *B. pseudomallei* (Column D). The final results of this testing method are given in Column E. All sputum specimens were also cultured onto sheep blood, chocolate, and MacConkey agars. The results of standard culture are given in Column F. NRF: normal respiratory flora.

Conclusions/Discussion

These data confirm that respiratory melioidosis is detected in patients presenting to Kampong Cham Provincial Hospital although the overall number of sputum specimens positive for *B. pseudomallei*

was low. Our rate of detection of *B. pseudomallei* in sputum samples submitted to the laboratory is similar to a previously published study evaluating the etiology of clinical respiratory infection at this hospital³. This study of patients with acute lower respiratory tract infection identified melioidosis in four of 422 (0.9%) patients from the hospital. The diagnosis was determined by culture of *B. pseudomallei* from sputum or blood at an off-site laboratory.

In light of the small number of specimens positive for *B. pseudomallei* we could not ascertain an advantage to the use of the enhanced sputum testing protocol. However, the ramifications of identification of *B. pseudomallei* are substantial. Although ceftazidime and imipenem may not be available in Cambodian hospital pharmacies and cost prevents the outside purchase by most patients, mortality from melioidosis is greatly diminished when appropriate antibiotic therapy is instituted¹⁷. Others have reported a benefit of a similar diagnostic testing strategy. For example, the use of selective enrichment broth and *B. pseudomallei* selective agar to test 154 respiratory samples in a referral center in Kuala Lumpur identified three cases of *B. pseudomallei* that were not detected using routine media¹⁸. Thus, the relative benefit of the enhanced sputum testing protocol may be greater in circumstances where *B. pseudomallei* is isolated more commonly.

A limitation to our investigation is the relatively small total number of sputum specimens submitted to the laboratory during the six month period. As the hospital microbiology laboratory has only been operational since 2009, this may reflect the established clinical practice of not relying on microbiology data to make treatment decisions. We further observed that the majority of sputum specimens came from patients presenting to the hospital's tuberculosis screening area and ward. This suggests that in many cases clinicians may have been considering the diagnosis of tuberculosis. Melioidosis and tuberculosis may be difficult to distinguish clinically⁸ and both infections should be considered in patients with chronic symptoms of respiratory infection in Cambodia. However, most respiratory melioidosis is associated with symptoms for less than two months and the mean incubation period in acute melioidosis is nine days^{19,20}. We speculate that sputum from melioidosis patients presenting elsewhere in the hospital with acute clinical syndromes of pneumonia and sepsis may not have been submitted for culture. This may have reduced our rate of detection of melioidosis in this study. Furthermore, this highlights the importance of informing clinicians of the utility of diagnostic microbiology testing, preferably before initiation of treatment.

Several other techniques should also be considered in the diagnosis of pulmonary melioidosis. Many cases of pulmonary melioidosis have concurrent bacteremia¹⁹ and *B. pseudomallei* bacteremia is reported in Cambodia. In a recent study, *B. pseudomallei* accounted for 12.6% of clinically significant positive blood cultures collected from adults between July 2007 and December 2010 at the Sihanouk Hospital Centre of HOPE, Phnom Penh²¹. From January to December 2013, *B. pseudomallei* was isolated in 18 of 2,230 (0.8%) blood cultures submitted to microbiology laboratories at five Cambodian

government hospitals, with the majority of *B. pseudomallei* cultures (15/434, 3.5%) occurring at Takeo Provincial Hospital (unpublished data). Throat swabs cultured in selective enrichment broth, while insensitive, are easily obtained and highly specific for the diagnosis of melioidosis^{22,23}. Thus, routinely culturing blood and throat swabs in patients with pneumonia and sepsis may enhance the diagnosis of respiratory melioidosis in Cambodia.

In conclusion, these data confirm that *B. pseudomallei* is a respiratory pathogen in Kampong Cham province but show that it is rarely detected in sputum samples submitted to the diagnostic microbiology laboratory at Kampong Cham Provincial Hospital. As a result, our study cannot determine the utility of an enhanced sputum testing protocol at this site. However, the benefit of the enhanced testing protocol may be more apparent elsewhere in Cambodia where melioidosis is more common. In addition, given the severity of melioidosis and importance of identifying the infection to guide treatment, several other factors should be considered in order to increase detection of the disease. In particular, clinician awareness of the diverse presentations of melioidosis and routine utilization of diagnostic microbiology services for patients presenting with pneumonia and sepsis should be promoted.

Data availability

F1000Research: Dataset 1. Detection of *B. pseudomallei* in Sputum using Selective Enrichment Broth and Ashdown's Medium Compared to Standard Culture, [10.5256/f1000research.5935.d40423](https://doi.org/10.5256/f1000research.5935.d40423)²⁴

Author contributions

TEW conceived the study. SN, JCMc, EJB, and TEW designed the protocol with the support of CM. SN, JL, and ST carried out the study. SN, JL, and TEW analyzed the data. All authors participated in the generation or revision of the manuscript, and approved the final version.

Competing interests

No completing interests were disclosed.

Grant information

This study was funded by support from the University of Washington to TEW.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank Ms. Vanaporn Wuthiekanun for providing the sputum testing protocol and advice. We also thank Dr. Narisara Chantratita for providing latex agglutination tests and Dr. Michael Prouty for support. We are grateful to the Kampong Cham Provincial Hospital diagnostic microbiology laboratory staff for their dedication to the project.

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Current Peer Review Status:

Version 1

Reviewer Report 06 February 2015

<https://doi.org/10.5256/f1000research.6343.r7217>

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Jan Jacobs

Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

This is a well-designed and clearly written paper about describing the incremental yield of selective culture for *Burkholderia pseudomallei* (BPM) in sputum samples routinely submitted for culture in a Provincial hospital in Cambodia, where melioidosis is endemic.

I have the following comments:

1. Most readers will not be familiar with melioidosis. It may be useful to state more explicitly in the Introduction that culture for the time being is the only reliable way for diagnosis of melioidosis and that recovery of BPM from any sample and in any quantity always means infection: this is especially important as for most other bacteria grown from non-sterile samples (as respiratory secretions), colonization and oral flora contamination must be excluded, which may be done by, among other criteria, based on density of growth. Second, based on this observation, selective media have been recommended as part of routine sputum culture in endemic areas.
2. The main weakness of this study is that there are no data about indications of sampling and selection of patients and from the Discussion can be learned that probably no well-defined indications have been used. So it is not clear whether all patients were suspected of lower respiratory tract infection and acutely ill, or if they selected, for instance, based upon initial treatment success and ability to pay for the culture etc.. The authors should emphasize this non-selection somewhat more, and, for instance, display data or at least an idea about the proportion of patients cultured in relation to the total (estimated) numbers of eligible patients with acute respiratory tract infections. Likewise, some data about the patients' profile (age (median/range or IQR) and gender would be of interest, as well as the distribution between hospitalized/outpatients - the spectrum of organisms cultured as mentioned in the Results suggests a high proportion of hospitalized patients.
3. In line with the above, I think the authors should reconsider amending their conclusion: they are right by stating that selective enrichment culture media would be more rewarding in settings with high(er) incidence of melioidosis, but there is for the time being no sound

evidence about the to assume that the incidence of melioidosis in their place will be lower than, for instance, in Thailand. Moreover, and as the authors state in the Discussion above, sputum and other microbiological cultures are probably not yet exploited to the best extent by the clinicians and probably also cost-prohibitive for many patients, reason why identification and selection of at-risk patients and pre-test probability may have been more important than overall incidence of melioidosis (as the authors state in the Discussion). In other words, if the enhanced testing protocol would have applied on for instance, middle-aged patients with diabetes presenting with overt signs of pneumonia/sepsis, incremental yield and cost-effectiveness may be much higher. So I suggest to consider rephrasing "the benefit ...may be more apparent ... in patients selected upon risk factors and clinical presentation..." Likewise, the Abstract (final sentence) may be rephrased to focus more on selected indications for sputum sampling.

4. The authors may add some information about the referral population of Kampong Cham hospital: rural, agriculture...

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response () 07 May 2015

T Eoin West, University of Washington, Seattle, USA

Thank you for this constructive review.

1. We agree and have modified the introduction to clarify these points as suggested.
2. This is indeed a shortcoming of this study; unfortunately we do not have any data on the patients who provided the sputum samples tested for these patients. Sputum samples are submitted to the laboratory from both clinic and hospitalized patients. Our future studies aim to investigate patient characteristics and indications for sampling.
3. We agree with these very helpful comments, and have modified the abstract and discussion as suggested.
4. We have now added this information as suggested.

Competing Interests: None

Reviewer Report 20 January 2015

<https://doi.org/10.5256/f1000research.6343.r7216>

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David A. B. Dance

Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Churchill Hospital, University of Oxford, Oxford, UK

This paper describes an evaluation of the use of a selective enrichment method to detect *Burkholderia pseudomallei* in sputum samples in a provincial hospital in Cambodia. The additional yield compared with conventional cultures was relatively low (only one of 241 samples tested).

The paper is well written and presents the results clearly. It would have been improved by the inclusion of an assessment of the additional costs of using selective culture. Clearly the benefits in this hospital are relatively small (although potentially life-saving for the one additional patient with melioidosis who was detected), but cannot be extrapolated to other areas because the distribution of melioidosis may be remarkably focal (for example in Thailand the incidence is very low in the central region but high in the northeast). It is also unclear why a selective enrichment technique was used as opposed to direct culture onto solid media, since enrichment culture was not shown significantly to increase the *B. pseudomallei* isolation rate from sputum compared with plating directly onto Ashdown's medium in a previous study (Wuthiekanun *et al.*, 1990). It might also have been possible to have been more selective about the samples that were cultured and to have analysed patient characteristics in greater detail, although the majority of samples were thought to have been from patients suspected of having TB, a group in whom it has previously been suggested that testing for melioidosis (using a similar enrichment technique) might be warranted (Suntornsut *et al.*, 2013).

Although this is a relatively small scale study with relatively unremarkable results, it warrants being in the public domain. Laboratories in melioidosis-endemic areas should still be encouraged to consider looking for *B. pseudomallei* in appropriate samples and should conduct their own assessment of the costs and benefits of doing so based on local epidemiology.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response () 07 May 2015

T Eoin West, University of Washington, Seattle, USA

Thank you for the helpful comments.

We opted not to perform cost-benefit analyses in light of our small numbers of positive samples and wide confidence intervals around point estimates. We agree that these calculations are important to perform in the future in larger studies.

We chose to use selective broth based on Wuthiekanun V *et al*, Am J Trop Med Hyg 77(5), 2007, 812-13. This study reported that "a total of 94 of 120 (78%) respiratory secretions were positive for *B. pseudomallei*, of which 33 (35%) were positive from enrichment broth alone."

Unfortunately we do not have any data on patient characteristics. Our future studies aim to investigate patient characteristics and indications for sampling.

Competing Interests: None

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