Greer, Rachel C; Wangrangsimakul, Tri; Amornchai, Premjit; Wuthiekanun, Vanaporn; Laongnual-panich, Achara; Dance, David AB; Limmathurotsakul, Direk; (2018) Misidentification of Burkholderia pseudomallei as Acinetobacter species in northern Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene, 113 (1). pp. 48-51. ISSN 0035-9203 DOI: https://doi.org/10.1093/trstmh/try108

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/4649568/

DOI: https://doi.org/10.1093/trstmh/try108

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Misidentification of *Burkholderia pseudomallei* as *Acinetobacter* species in northern Thailand

Rachel C. Greer\(^a\), Tri Wangrangsimakul\(^b\), Premjit Amornchais, Vanaporn Wuthiekanun\(^a\), Achara Laongnualpanich\(^c\), David A. B. Dance\(^b\), Direk Limmathurotsakul\(^a\)

\(^a\)Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; \(^b\)Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK; \(^c\)Chiang Rai Prachanukroh Hospital, Chiang Rai, Thailand; \(^d\)Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; \(^e\)Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane Capital, Lao People’s Democratic Republic; \(^f\)Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

*Corresponding author: Tel: +66 2 203 6333; E-mail: tri@tropmedres.ac

Received 25 June 2018; revised 8 August 2018; editorial decision 5 September 2018; accepted 10 September 2018

Background: *Burkholderia pseudomallei* is the causative agent of melioidosis, a disease endemic throughout the tropics.

Methods: A study of reported *Acinetobacter* spp. bacteraemia was performed at Chiang Rai provincial hospital from 2014 to 2015. Isolates were collected and tested for confirmation.

Results: A total of 419 putative *Acinetobacter* spp. isolates from 412 patients were re-identified and 5/419 (1.2%) were identified as *B. pseudomallei*. Four of the five patients with melioidosis died. An estimated 88/419 (21%) isolates were correctly identified as *Acinetobacter* spp.

Conclusions: Misidentification of *Acinetobacter* spp. as *B. pseudomallei* or other bacteria is not uncommon and programmes to address these shortfalls are urgently required.

Keywords: *Acinetobacter*, *Burkholderia pseudomallei*, laboratory quality assurance, melioidosis, misidentification

Introduction

*Burkholderia pseudomallei*, an environmental Gram-negative bacterium, is the causative agent of melioidosis.\(^1\) There are an estimated 165 000 human cases with 89 000 deaths annually worldwide.\(^2\) It is thought to be endemic in northern Thailand; however, only sporadic reports have emerged to date.\(^2\) Transmission occurs through percutaneous inoculation, inhalation or aspiration.\(^1\) Risk factors include diabetes mellitus, alcoholism, chronic lung disease and chronic kidney disease.\(^3\)

Clinical misdiagnosis of melioidosis regularly occurs due to its protean manifestations, ranging from mild fever to fatal septic shock.\(^1,3\) Diagnosis is typically made by culture of clinical specimens and identification of *B. pseudomallei* using conventional microbiological techniques including Gram stain, oxidase test, biochemical tests and antibiotic susceptibility tests.\(^4,5\) Additionally, latex agglutination, identification kits (e.g. API20NE), matrix-assisted light desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and molecular tests can provide further confirmation.\(^5\) However, laboratory misidentification can occur due to lack of awareness, inadequate quality assurance or limited resources. Treatment involves an intensive phase with intravenous (IV) ceftazidime or meropenem for 10–14 d followed by an eradication phase of 3–6 months with oral co-trimoxazole or co-amoxiclav.\(^1\) Here we report five culture-confirmed cases whose *B. pseudomallei* had been misidentified as *Acinetobacter* spp.

Materials and methods

A study of reported *Acinetobacter* spp. bacteraemia was conducted at Chiang Rai provincial hospital, in northern Thailand, from December 2014 to December 2015. Clinical blood culture isolates originally identified as *Acinetobacter* spp. by the local hospital laboratory using conventional microbiological techniques were prospectively collected. These isolates were subcultured and
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Risk factors</th>
<th>Temperature (°C)</th>
<th>HR (⁄min)</th>
<th>BP (mmHg)</th>
<th>RR (⁄min)</th>
<th>SOFA score</th>
<th>Presenting symptoms</th>
<th>Examination findings</th>
<th>Radiological findings</th>
<th>Working diagnoses</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>M</td>
<td>Smoker, chronic obstructive pulmonary disease, lung abscess</td>
<td>37.5</td>
<td>122</td>
<td>100/60</td>
<td>40</td>
<td>8</td>
<td>Fever, cough, dyspnoea, abdominal pain</td>
<td>Reduced breath sounds on the left; right upper quadrant abdominal pain</td>
<td>CXR: left lung abscess; US: hepatic parenchymal disease</td>
<td>Chronic lung abscess with acute pneumonia, septic shock, multi-organ failure</td>
<td>Died within 24 h of admission</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>M</td>
<td>Smoker, diabetes mellitus (new diagnosis)</td>
<td>35.0</td>
<td>66</td>
<td>69/45</td>
<td>20</td>
<td>9</td>
<td>Fever, abdominal pain, vomiting</td>
<td>Marked right upper quadrant tenderness with hepatomegaly</td>
<td>CXR: right upper zone infiltration</td>
<td>Septic shock, acute respiratory distress syndrome, multi-organ failure</td>
<td>Died on day 6 of admission</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>M</td>
<td>Smoker, alcoholism, cirrhosis</td>
<td>37.8</td>
<td>114</td>
<td>125/73</td>
<td>18</td>
<td>2</td>
<td>Fever</td>
<td>Hepatomegaly</td>
<td>Not done</td>
<td>Ongoing melioidosis, urinary tract infection</td>
<td>Recovered</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>M</td>
<td>Diabetes mellitus</td>
<td>36.6</td>
<td>125</td>
<td>130/68</td>
<td>Intubated and ventilated</td>
<td>14</td>
<td>Fever, cough, dyspnoea</td>
<td>Right lung crepitation</td>
<td>CXR: bilateral lung infiltrates</td>
<td>Septic shock, diabetic ketoacidosis, pneumonia, multi-organ failure</td>
<td>Died within 48 h of admission</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>M</td>
<td>Chronic kidney disease</td>
<td>38.5</td>
<td>107</td>
<td>109/73</td>
<td>Intubated and ventilated</td>
<td>13</td>
<td>Fever, cough, dyspnoea</td>
<td>Widespread wheezing and crepitation</td>
<td>CXR: right lung infiltration</td>
<td>Septic shock, pneumonia, multi-organ failure</td>
<td>Died on day 6 of admission</td>
</tr>
</tbody>
</table>

BP: blood pressure; CXR: chest X-ray; HR: heart rate; M: male; RR: respiration rate; SOFA: sequential organ failure assessment; US: ultrasound.
Results and discussion

A total of 419 blood culture isolates reported as *Acinetobacter* spp. were obtained from 412 patients, of which 88/419 isolates (21%) were found to be oxidase positive (*Acinetobacter* spp. should be oxidase negative). Five of these (5.7%) were identified as *B. pseudomallei* based on the typical colonial appearance on Ashdown agar, positive latex agglutination test and VITEK 2 and MALDI-TOF profiles. The remaining oxidase-positive isolates comprised a variety of environmental bacteria, including *Achromobacter denitrificans* (22/88 [25.0%]), *Ralstonia mannitolilytica* (16/88 [18.2%]) and *Ochrobactrum anthropi* (9/88 [10.2%]). Of the 331/419 oxidase-negative isolates (79%), a sample of 60 isolates was tested using MALDI-TOF, confirming the identification as *Acinetobacter* spp. in 16/60 (26.7%) and *Stenotrophomonas maltophilia* in 43/60 (71.7%), with 1 isolate unidentified. If the sample set was representative, an estimated 88/419 (21.0%) isolates were initially correctly identified as *Acinetobacter* spp.

Clinical summaries of the five misidentified melioidosis cases are presented in Table 1 and details of the blood culture results for each patient in Table 2. Admission blood cultures were reported within 22–44 h of collection as *Acinetobacter* spp., while antibiotic susceptibility results were reported 16–24 h later. All had underlying disease associated with an increased risk of melioidosis. Four cases had severe disease, were elderly, developed multi-organ failure and died (patients 1, 2, 4 and 5). The initial empirical treatment in these patients included IV ceftriaxone or IV piperacillin/tazobactam with additional antibiotics in some cases (e.g. amikacin, roxithromycin, azithromycin, doxycycline and vancomycin). Only two of the four severe cases received melioidosis-appropriate antibiotics—IV meropenem in both cases—with delays of 1 and 4 d from admission (patients 2 and 5, respectively). The other two severe cases died within 48 h of admission (patients 1 and 4). The patient who survived was younger, had mild disease, had previously been diagnosed with genitourinary tract melioidosis and was already on appropriate treatment (IV ceftazidime), which was continued despite the misleading admission blood culture result (patient 4).

In this report we confirm the presence of melioidosis in northern Thailand and demonstrate that laboratory misidentification of *Acinetobacter* spp. as *B. pseudomallei* or other bacteria remains commonplace. The predicted mortality from melioidosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Collection time (day 0=admission)</th>
<th>Report time (hours after collection)</th>
<th>Results</th>
<th>Antibiotic susceptibility (S = Sensitive, I = Intermediate sensitivity, R = Resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Day 0</td>
<td>37 h</td>
<td><em>Acinetobacter lwofii</em></td>
<td>S—ceftazidime, imipenem, cefoperazone/sulbactam R—amikacin, gentamicin, co-trimoxazole</td>
</tr>
<tr>
<td>2</td>
<td>Day 0</td>
<td>28 h</td>
<td>Mixed growth: <em>A. lwofii</em>, <em>Staphylococcus epidermidis</em></td>
<td>S—ceftazidime, imipenem, piperacillin-tazobactam, cefoperazone/sulbactam, ciprofloxacin, ertapenem, meropenem, cefotaxime, ceftriaxone, co-trimoxazole R—amikacin, gentamicin</td>
</tr>
<tr>
<td>3</td>
<td>Day 0</td>
<td>99 h</td>
<td><em>Burkholderia pseudomallei</em></td>
<td>NB—susceptibility results for <em>Acinetobacter lwofii</em> only S—ceftazidime, imipenem, cefoperazone/sulbactam R—amikacin, gentamicin, co-trimoxazole</td>
</tr>
<tr>
<td>4</td>
<td>Day 0</td>
<td>40 h</td>
<td><em>Acinetobacter baumannii</em></td>
<td>S—ceftazidime, doripenem, imipenem, piperacillin-tazobactam, cefoperazone/sulbactam, ertapenem, meropenem, cefotaxime R—amikacin, gentamicin, co-trimoxazole</td>
</tr>
<tr>
<td>5</td>
<td>Day 0</td>
<td>22 h</td>
<td><em>A. baumannii</em></td>
<td>S—ceftazidime, doripenem, imipenem, piperacillin-tazobactam, cefoperazone/sulbactam, ertapenem, meropenem, cefotaxime R—amikacin, gentamicin, co-trimoxazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 h</td>
<td><em>A. lwofii</em></td>
<td>I—cefotaxime R—amikacin, gentamicin, co-trimoxazole</td>
</tr>
</tbody>
</table>

NB: antibiotic susceptibility results were generally reported 16–24 h after the blood culture flagged positive.
Conclusions

Making the correct diagnosis is vital to patient outcome and fundamental to any effective surveillance and antimicrobial stewardship programmes. The diagnosis and management of melioidosis remain suboptimal in many endemic regions. The misidentification of \( B. \) pseudomallei and other bacteria demonstrated in this report highlights the need to prioritize and strengthen laboratory capacity and quality. Following discussions with local stakeholders, plans are under way to provide additional training, improve quality assurance and introduce consistent use of new diagnostics (e.g., latex agglutination test, API20NE and VITEK) within the microbiology laboratory. Efforts to increase the clinical awareness of melioidosis, including the importance of effective and timely treatment in at-risk patients, are also required.

Authors’ contributions: DL conceived the study. RCG, AL and DL formulated the study protocol. RCG and TW coordinated and collected the data. PA and VW coordinated and performed the laboratory tests. RCG, TW, VW, AL, DABD and DL analysed and interpreted the results. RCG and TW drafted the manuscript. DABD and DL critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. RCG and TW are guarantors of the paper.

Acknowledgements: The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint first authors. We thank Dr Thanittha Chatsuwan and Areearat Theiprakhang for laboratory support and Nidanuch Tasak and Piangnet Jaiboon for their help with data collection. We are grateful to the clinical and laboratory staff of Chiang Rai Prachanukroh Hospital, Chiang Rai, Thailand, for their interest and participation in the study.

Funding: This work was supported by the Wellcome Trust, UK (grants 106698/2/14/Z (to DABD) and 101103/2/13/Z (to DL)).

Competing interests: None declared.

Ethical approval: Ethical approval was obtained from the Chiang Rai Prachanukroh Hospital (approval letter references CR0032.102/ RESEARCH/29012 and 22283), the Faculty of Tropical Medicine, Mahidol University (TMEC 14-040 and 16-041) and the Oxford Tropical Research Ethics Committee (OXTREC 546-14 and 5106-16). Individual informed consent was not required. This study was carried out in compliance with the Declaration of Helsinki.

References