Clinical Microbiology and Infection 24 (2018) 84.e1–84.e4

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

A simple laboratory algorithm for diagnosis of melioidosis in resource-constrained areas: a study from north-central Vietnam

T.T. Trinh [1, *], T.S. Hoang [1], D.A. Tran [2], V.T. Trinh [3], A. Göhler [4], T.T. Nguyen [5], S.N. Hoang [6], R. Krumkamp [7, 8], L.T.N. Nguyen [9], J. May [7, 8], P.M. Doan [10], C.D. Do [10], T.A. Que [2], I. Steinmetz [4, 11]

[1)] *Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Viet Nam*
[2)] *General Hospital of Nghe An Province, Viet Nam*
[3)] *General Hospital of Ha Tinh Province, Viet Nam*
[4)] *Friedrich Loeffler Institute for Medical Microbiology, Greifswald, Germany*
[5)] *General Hospital of Quang Binh Province, Viet Nam*
[6)] *General Hospital of Quang Tri Province, Viet Nam*
[7)] *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
[8)] *German Centre for Infection Research (DZIF), Hamburg-Borstel-Lübeck, Germany*
[9)] *Hue Central Hospital, Hue, Viet Nam*
[10)] *Bach Mai Hospital, Hanoi, Viet Nam*
[11)] *Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria*

ARTICLE INFO

Article history:
Received 12 April 2017
Received in revised form
26 June 2017
Accepted 26 July 2017
Available online 3 August 2017

Editor: G. Greub

Keywords:
Burkholderia pseudomallei
Diagnosis
Epidemiology
Melioidosis
North-central Vietnam

ABSTRACT

Objectives: Melioidosis may be endemic in many tropical developing countries, but diagnosis of the disease is currently unreliable in resource-limited areas. We aimed to validate a simple and cheap laboratory algorithm for the identification of *Burkholderia pseudomallei* from clinical specimens in parts of Vietnam where the disease has not previously been reported.
Methods: In June 2015, we conducted training courses at five general hospitals in north-central provinces in order to raise awareness of the disease and to introduce a simple and cheap laboratory identification algorithm for *B. pseudomallei* including the three-antibiotic disc test.
Results: Until the end of the year (7 months later), 94 suspected *B. pseudomallei* strains resistant to gentamicin and colistin but sensitive to amoxicillin/clavulanic acid were detected in clinical specimens from 70 patients. All strains were further confirmed as *B. pseudomallei* by using a specific TTSS1 real-time PCR assay and *recA* sequencing analysis. Among positive blood cultures, positive rates with *B. pseudomallei* ranged from 3.4% (5/147) to 10.2% (32/312) in the various clinics. A total of 82.8% (58/70) patients were bacteraemic, with a mortality of 50% (18/36) among patients with known outcome. No death occurred in nonbacteraemic patients.
Conclusions: Our results demonstrate that the introduction of a simple and easy-to-perform laboratory algorithm for the identification of *B. pseudomallei* from clinical samples, together with clinical awareness raising, can lead to the diagnosis of a significant number of melioidosis cases in resource-limited clinical laboratories which previously did not identify the pathogen. **T.T. Trinh, Clin Microbiol Infect 2018;24:84.e1–84.e4**

Introduction

Melioidosis is a life-threatening infectious disease of the tropics and subtropics caused by the soil bacterium *Burkholderia pseudomallei*. The overall case fatality rate for the disease can reach 40% even if drugs of choice are provided; the fatality rate can be up to 80% if diagnosis is not performed accurately and subsequent

* Corresponding author. T. T. Trinh, Department of Vietnam Type Culture Collection, Institute of Microbiology and Biotechnology, Vietnam National University, 144 Xuan Thuy, Cau Giay, Hanoi, Viet Nam.
E-mail address: tttrung@vnu.edu.vn (T.T. Trinh).

http://dx.doi.org/10.1016/j.cmi.2017.07.029
1198-743X/

antibiotic treatment is not appropriate [1]. Because of diverse clinical presentations, diagnosis of melioidosis has to rely on the identification of *B. pseudomallei* in the laboratory, with a positive culture of *B. pseudomallei* from clinical specimens as the reference standard [2]. *B. pseudomallei* is a Gram-negative, oxidase-positive bacillus resistant to gentamicin (although susceptible isolates occur [3]) and colistin, but susceptible to amoxicillin/clavulanic acid. The currently recommended diagnosis includes biochemical tests such the API 20NE test and emphasizes the need for specific antibody-based agglutination or lateral flow assays for *B. pseudomallei* confirmation [1]. The in-house preparation and additional introduction of currently recommended selective media such as Ashdown agar and costly biochemical identification systems in the daily routine may comprise a major barrier for the detection of *B. pseudomallei* in clinical specimens in many potential endemic areas. Moreover, antibody-based tests are not widely available.

Recently it has been predicted that melioidosis can cause disease in 165 000 people, with 89 000 deaths worldwide [4]. In northeast Thailand, the disease is known to be a major cause of community-acquired septicaemia and is classified as the third most common cause of death from infectious diseases [5,6]. Although north-central Vietnam is located in the same geographical belt of the tropics as northeast Thailand and is adjacent to Laos, where melioidosis cases have been increasingly detected [7], the disease has not yet been reported from this part of Vietnam. Limited resources, lack of laboratory expertise and lack of clinical awareness are likely the main factors responsible for possible underreporting. To overcome these problems, we introduced a simple laboratory algorithm for the identification of *B. pseudomallei* accompanied by teaching clinical presentations of melioidosis in five clinical laboratories and hospitals in north-central Vietnam.

Methods

From 2 to 19 June 2015, lectures and hands-on training workshops on melioidosis were conducted at four provincial general hospitals of Nghe An, Ha Tinh, Quang Binh and Quang Tri, as well as one central hospital in Hue city. These hospitals serve an area of 40 327 km^2 with a population of 6.6 million (Supplementary Table S1). The lectures were orientated towards possible clinical presentations of the disease in order to raise awareness amongst local clinicians. Because studies from other endemic areas showed an increased number of melioidosis cases after heavy rainfall [8,9], the training was started before the rainy season. In order to achieve maximum adherence to the protocol in those resource-limited settings, we focused on the introduction to laboratory staff of a simple and comprehensible laboratory algorithm for the presumptive identification of *B. pseudomallei* from clinical samples.

All samples were taken as part of routine clinical care and decisions. Blood cultures were routinely subcultured on Columbia blood agar. Pus, sputum, urine and body fluids were directly cultured on MacConkey agar, chocolate agar and chromogenic media. After 48 hours' incubation at 37°C, single bacterial colonies with a metallic-sheen appearance were subjected to cytochrome oxidase testing and Gram staining. Oxidase-positive, Gram-negative bacilli were then tested for susceptibility against antibiotic discs of gentamicin (10 μg), colistin (10 μg) and amoxicillin/clavulanic acid (20/10 μg) according to Clinical and Laboratory Standards Institute recommendations. Interpretation was based on zone diameter interpretive standards for *Pseudomonas aeruginosa* and *Enterobacteriaceae* [10,11]. Suspected *B. pseudomallei* strains were defined as oxidase-positive, Gram-negative bacilli, resistant to gentamicin and colistin but sensitive to amoxicillin/clavulanic acid (zone diameter, 21–26 mm). For final identification, strains were transferred to the Institute of Microbiology and Biotechnology in Hanoi, where *B. pseudomallei*–specific TTSS1 real-time PCR assay [12] and *rec*A sequence analysis using BUR1 and BUR2 primers [13] were performed.

The study was approved by the research ethics committee of Bach Mai Hospital (1004/QD-BM 2014), a large referral hospital in Hanoi and a subordinate unit of the Ministry of Health. The study aimed to increase the quality of melioidosis diagnoses during routine clinical care and decisions through training measures. Informed consent of patients was not required. The participating hospitals signed agreements to take part in the study.

Results

By using this laboratory algorithm from June to December 2015 (7 months), 94 suspected *B. pseudomallei* strains were detected from the clinical specimens of 70 patients. All strains were confirmed by TTSS1 real-time PCR and showed a *rec*A gene sequence identical to that of *B. pseudomalle*i K96243. Seventy-six *B. pseudomallei* strains (80.9%) were isolated from blood, six (6.4%) from pus, five (5.3%) from sputum, two (2.1%) from urine and five (5.3%) from other body fluids ($n = 1$ cerebrospinal fluid, $n = 1$ pleural fluid, $n = 2$ bronchial washes, $n = 1$ synovial fluid). Of 1097 positive blood cultures obtained during the study, 76 samples (6.9%) were positive for *B. pseudomallei*. Positivity rates ranged from 3.4% (5/147) to 10.2% (32/312) among communities (Supplementary Table S1). From the 70 melioidosis patients, most cases ($n = 48$; 68.6%) were admitted during September, October and November, with a peak ($n = 23$; 32.9%) in October (Supplementary Fig. S1). Twenty-three patients (32.9%) were diagnosed at the provincial general hospital of Nghe An, 27 patients (38.6%) at Ha Tinh, six patients (8.6%) at Quang Binh, five patients (7.1%) at Quang Tri and nine patients (12.9%) at the central hospital of Hue city. Assuming that the patients' residential address is most likely the place where infection was acquired, mapping the patient's home addresses revealed 32 cases (45.7%) in Ha Tinh province (Fig. 1).

Fifty-eight patients (82.9%) had blood culture samples positive for *B. pseudomallei* (Table 1). Of those, 29 patients (50.0%) had documented intravenous treatment with at least one of the currently recommended antibiotics for severe melioidosis, namely ceftazidime, meropenem or imipenem [14]. In the remaining 29 (50%), treatment was either not adequate or treatment data were not available. Outcome of 19.0% (11/58) patients was not recorded. A total of 19.0% (11/58) of patients presenting with severe sepsis was transferred to other hospitals, with unknown outcome. Of all blood culture–positive patients with known outcome, 50.0% (18/36) of the patients died, and six deaths occurred within 48 hours after admission. Fifty percent (18/36) of the patients with known outcome recovered. Among patients with known outcome, 29% (5/17) died when receiving adequate treatment. A total of 37.5% (3/8) patients died with inadequate treatment, and 90% (10/11) of patients with unavailable treatment data died.

Of the 12 nonbacteraemic patients, 11 patients (91.7%) received ceftazidime, meropenem or amoxicillin/clavulanic acid. All patients recovered (Table 1).

Discussion

Although the first case of melioidosis was described more than a century ago, the disease is likely to be underreported in many parts of the world. Most clinical microbiology laboratories in potential endemic areas are resource limited. We therefore simplified the diagnostic procedure by teaching typical morphology characteristics of *B. pseudomallei* on different routine agar media and by introducing a simple bacterial identification including the three-

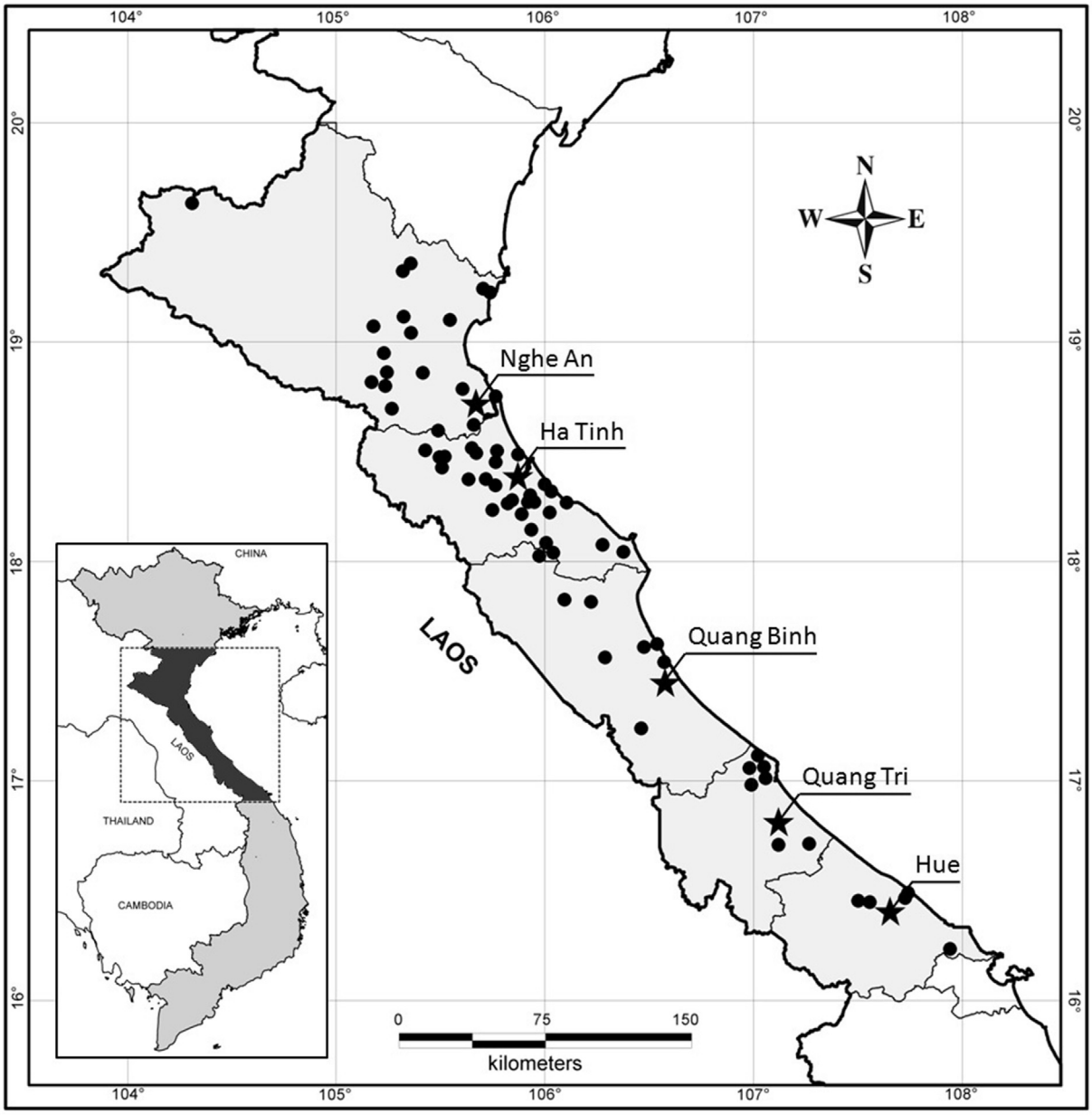

Fig. 1. Geographical distribution map based on home addresses of melioidosis patients diagnosed in north-central Vietnam, June to December 2015. Dots indicate homes of patients. Asterisks indicate locations of five general hospitals, with their names indicated. Map constructed by MapInfo 7.8 (MapInfo, Troy, NY, USA).

antibiotic disc test. This approach proved to be highly specific in this context, because all 94 oxidase-positive, Gram-negative bacilli with gentamicin and colistin resistance but amoxicillin/clavulanic sensitivity were confirmed as *B. pseudomallei* using molecular methods. Following this simple laboratory algorithm, three general hospitals of Ha Tinh, Quang Binh and Quang Tri in which *B. pseudomallei* had never before been identified were detected 38 (54.3%) of the 70 melioidosis cases within 7 months. The remaining 32 cases (45.7%) detected in the other two hospitals of Nghe An and Hue represent a considerable increase compared to the previous laboratory reports of fewer than five cases per year in each hospital. Most affected patients were admitted during the time of seasonal rainfall, with a peak in October.

Clinical presentations, risk factors and the high mortality of melioidosis cases (18/36, 50.0%, of cases with known outcome) in this study are consistent with other endemic areas, where septicaemia with pneumonia are the most common clinical presentation. The same is true for diabetes [5], which was frequently observed in melioidosis patients (19/70). In our study group, 10% of patients with melioidosis were children, including cases of suppurative parotitis, as reported from other endemic areas of Southeast Asia [15].

It has been predicted that *B. pseudomallei* is likely to be present in the environment throughout Vietnam, and melioidosis is probably highly endemic [4]. To date most cases of melioidosis in Vietnam have been reported from the north and south of the country rather than the central region [9,16]. To our knowledge, this is the first report of a series of melioidosis cases detected in north-central Vietnam. Limitations of our study include the fact that the high proportion of patients with positive blood cultures is probably the result of the nonuse of selective media. Such media would have possibly increased the detection of *B. pseudomallei* from nonsterile

Table 1
Demographic data, clinical presentations, risk factors and outcomes in 70 melioidosis patients diagnosed in north-central Vietnam from June to December 2015

Characteristic	Total ($n = 70$)	Septicaemia ($n = 58$)	No septicaemia ($n = 12$)
Demographic information			
Age (years)			
Mean ± SD	43.6 ± 19.5	46.0 ± 18.2	33.8 ± 25.0
Range	1–90	1–90	3–79
Children (aged ≤15 years)	7 (10.0%)	4	3
Male sex	47 (67.1%)	37	10
Rice occupation	43 (61.4%)	36	7
Organ involvement[a]			
Lungs	26 (55.3%)	22	4
Skin and soft tissue	7 (14.9%)	4	3
Genitourinary tract	3 (6.4%)	1	2
Bone and joint	2 (4.3%)	2	0
Lymph nodes	2 (4.3%)	0	2
Suppurative parotitis	2 (4.3%)	1	1
Central nervous system	1 (2.1%)	1	0
Middle ear	1 (2.1%)	1	0
Liver	1 (2.1%)	1	0
Kidney	1 (2.1%)	1	0
Prostatitis	1 (2.1%)	0	1
Known risk factors			
Diabetes	19 (54.3%)	18	1
Chronic lung disease	4 (11.4%)	3	1
Chronic renal disease	3 (8.6%)	3	0
Chronic liver disease	2 (5.7%)	1	1
Immunosuppressive drug use	4 (11.4%)	2	2
Alcoholism	2 (5.7%)	1	1
Malignancy	1 (2.9%)	1	0
Outcomes			
Recovery overall	30/70 (42.9%)	18/58 (31.0%)	12/12 (100%)
Recovery in cases with known outcome	30/48 (62.5%)	18/36 (50.0%)	—
Death overall	18/70 (25.7%)	18/58 (31.0%)	0
Death in cases with known outcome	18/48 (37.5%)	18/36 (50.0%)	—
Unknown	22/70 (31.4%)	22/58 (37.9%)	0

[a] Four patients had multiple organ involvement: one had prostatitis and renal abscess, and three others had pneumonia with skin abscess.

samples such as sputum, throat and wound swabs, resulting in the detection of more localized infections. Furthermore, follow-up studies of the melioidosis patients were not carried out after their hospital discharge or transfer, making it hard to determine the true mortality rate and the effectiveness of antibiotic treatment.

The introduction of selective media and antibody-based tests for rapid culture confirmation would probably further increase the detection rate and speed up the final diagnosis in our study region. However, a comparison of the performance of various diagnostic algorithms was not our aim in this study. The three-antibiotic disc test seems to be a valuable tool to start detecting melioidosis cases in resource-limited settings where the epidemiologic situation is unclear. Better diagnostic methods and improved awareness are likely to substantially reduce mortality. The introduction and evaluation of more sophisticated diagnostic algorithms can be based on the results obtained with the three-antibiotic disc test. The implementation of this simple and cheap method to other potentially endemic and resource-limited areas might be helpful as a first step towards understanding the global distribution of melioidosis.

Acknowledgement

We thank N. N. Minh for his help in constructing the map of patient's homes.

Transparency declaration

Supported in part by the Vietnamese Ministry of Science and Technology and the German Federal Ministry of Education and Research through the project RENOMAB (Research Network on Melioidosis and *Burkholderia pseudomallei;* reference 01DP13007). All authors report no conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.07.029.

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