Iranian Journal of Microbiology
Volume 14 Number 3 (June 2022) 430-433

CASE REPORT

Case of misdiagnosed melioidosis from Hue city, Vietnam

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Received: February 2022**, Accepted:** May 2022

ABSTRACT

Melioidosis is an emerging infection, a potentially fatal tropical disease caused by *Burkholderia pseudomallei* in humans and animals, endemic in Southeast Asia and northern Australia. Diagnosis remains problematic due to its similarity to many other infections. The lack of clinical awareness and correct microbiological diagnosis contributes to the misidentification of melioidosis. We present a melioidosis case, which was misdiagnosed with pneumonia and septicemia due to *Aeromonas salmonicida*, leading to ineffective prolonged-course antibiotic treatment for the patient.

Keywords: Diagnostic errors; Melioidosis; *Burkholderia pseudomallei*; *Aeromonas salmonicida*; 16S ribosomal RNA

INTRODUCTION

Melioidosis, also known as Whitmore's disease, is an infectious disease caused by the aerobic, Gram-negative bacillus *B. pseudomallei*. The organism is widely disseminated in water and muddy soils of tropical and subtropical areas, especially in Southeast Asia (including Viet Nam) and northern Australia. It is spread to humans and animals through inhalation of contaminated debris or direct contact with the contaminated source. Melioidosis has various clinical manifestations like skin and respiratory tract infections, multisystem abscess formation, and even bacteremia with septic shock (1). Melioidosis diagnosis remains a challenge because of its similarity to other infections, especially tuberculosis, and the limitation of conventional bacterial identification methods (2). It is estimated that melioidosis can kill almost 89,000 people per year worldwide (3). Cases have been reported from various parts of Viet Nam. However, misdiagnosis and mistreatment have usually occurred, which may cause melioidosis-related morbidity and mortality to remain quite high in Viet Nam (4). Here, we report a case of melioidosis which was misdiagnosed for a long, causing ineffective treatment and long-term physical consequences.

CASE REPORT

A 48-year-old mason was admitted to the Hue University of Medicine and Pharmacy hospital, Hue city, Viet Nam, presenting fever, cough and breathlessness for three days. He had a history of type 2 diabetes mellitus with poor glycemic control.

On arrival at the hospital, his vital signs were as follows; body temperature 39.7°C, respiratory rate 28 breaths/min, heart rate 128 bpm, and blood pressure 100/60 mm Hg. He coughed with mucopurulent sputum. Chest examination revealed decreased breath sounds on the left lung base and no bilateral crackles; other physical examination findings were unremark-

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able. Laboratory findings showed several hematological and biochemical parameters were deranged, such as total leukocyte count 15.9 G/L (Neutrophil predominance), platelet 78 G/L, C-reactive protein (CRP) (324.6 mg/l) and hemoglobin A1c (HbA1c) 9.09%. On his left chest X-ray and ultrasound, there is a small pleural effusion and a consolidated lung base (Fig. 1).

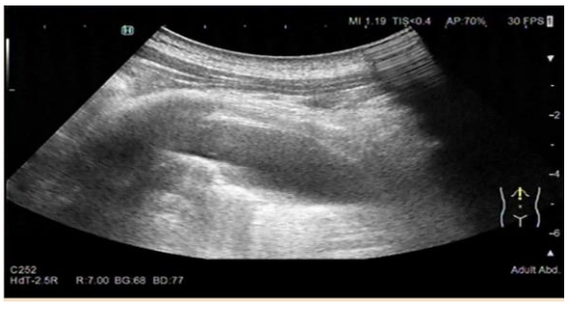

Fig. 1. Ultrasound image left thorax showing a small pleural effusion.

The Ziehl-Neelsen stain from sputum for 3 days gave a negative result for acid-fast bacilli. Sputum, blood and pleural fluid were collected and sent for cultures. The microbiologic smear and culture of the cloudy pleural fluid showed no visible organisms and a negative culture. However, a few Gram-negative bacilli and abundant white blood cells were seen in Gram stains from the sputum. After 4 days of incubation, turbidity was observed in the blood culture bottle. The blood-broth mixture was then subcultured on blood agar and Drigalski agar media and incubated overnight. Both sputum and blood cultures showed smooth white non-hemolytic colonies with entire margin on blood agar plate and non-fermenting colonies on Drigalski agar (Fig. 2). Gram staining showed Gram-negative bacilli with no specific characteristics (Fig. 3). The organism was catalase-positive and oxidase-positive. The isolate was further processed using API 20NE strip (bioMérieux, France) and identified as *Aeromonas salmonicida*. Kirby-Bauer disc diffusion method was used for antimicrobial susceptibility testing.

He was treated with cefoperazone-sulbactam, azithromycin and gentamicin upon arrival. Antibiotics were changed to piperacillin-tazobactam, fosfomycin, meropenem and ciprofloxacin according to susceptibility test results. Although he received intensive care unit (ICU) support and antibiotics treatment, the clinical response was improved but remained unstable. He occasionally had a high fever (40°C) or tachypnea. He was still coughing with thick mucopurulent sputum.

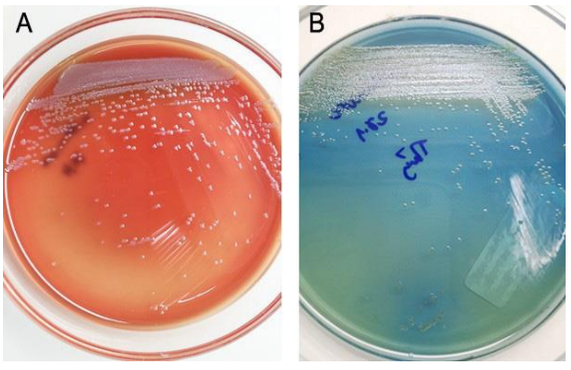

Fig. 2. Colony isolated from blood-broth on blood agar (A) and Drigalski agar (B) media after 24 h.

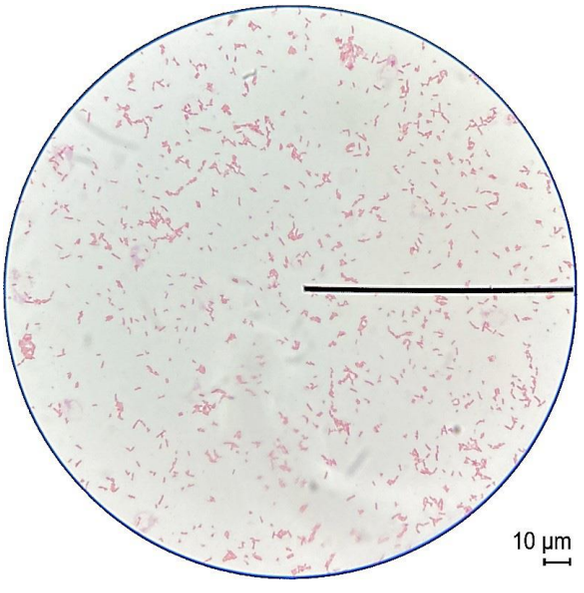

Fig. 3. Gram negative bacilli on 100× objective lens, with immersion oil (isolated from blood broth)

This isolate was subjected into the project of evaluation of 16S rRNA gene sequencing for species. The 16S rRNA gene sequencing showed that our isolation was matched with *B. pseudomallei* strain ATCC 23343 16S ribosomal RNA, partial sequence (NCBI reference sequence: NR_043553.1; NCBI: txid28450; database: 16S ribosomal RNA, identical site: 99.8%, pairwise identified: 99.8%). Furthermore, in the meantime, the second sputum sample was sent for culture and also gave the same colony characteristics as the previous report. Therefore, we tested the bacterial colony with an immunochromatographic assay: Active Melioidosis Detect™ (InBios, USA) which

showed a positive result (Fig. 4). Antimicrobial susceptibility testing for *B. pseudomallei* was performed by Kirby-Bauer disc diffusion method as CLSI guidelines. The isolate was found to be sensitive to amoxicillin/clavulanic acid, ceftazidime, meropenem, sulfamethoxazole/trimethoprim and piperacillin/tazobactam. Eventually, the patient was transferred to the Hue Central hospital with specialized facilities for further treatment.

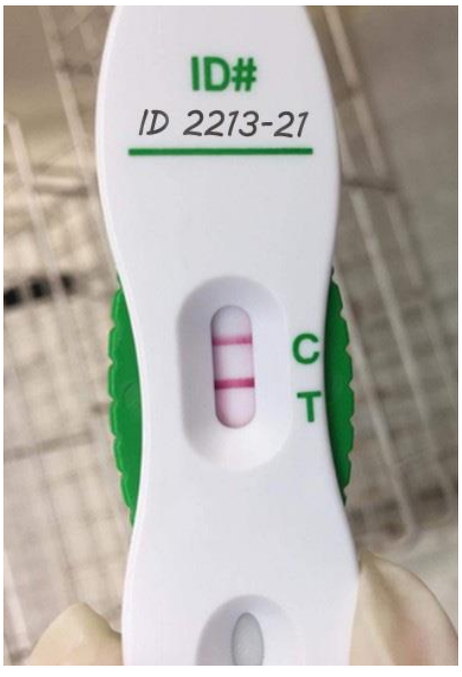

Fig. 4. Active Melioidosis Detect™ (InBios, USA) test positive result.

DISCUSSION

Melioidosis is a challenging disease due to difficulties in diagnosis and prolonged treatment (5, 6). Acute melioidosis is associated with high mortality, especially with the delayed right diagnosis and effective antibiotic treatment, highlighting the need for early confirmation of diagnosis (7-9). However, the clinical recognition of melioidosis is usually difficult for physicians because of its similarity to other infections, particularly tuberculosis (6). Identification of *B. pseudomallei* also poses challenges for the clinical microbiology laboratory. Although culture currently remains the gold standard method for the melioidosis diagnosis (2), even with positive cultures, *B. pseudomallei* can be misidentified due to a shortage of properly validated diagnostic test reagents and limitations of several commonly used diagnostic methods (2, 10). *B. pseudomallei* is included in the commercially available identification systems (API 20NE, VITEK® 2GN CARD REF. 21341) database, with variably reported accuracies and reported to be misidentified as other organisms (2, 11, 12).

In our case, it is clinically difficult to distinguish pneumonia due to melioidosis from tuberculosis or more common forms of pneumonia. Of challenge to the laboratory, the organism exhibited similar colony characteristics as other non-*Pseudomonas aeruginosa* non-fermenters. The colonies did not display the typical characteristic – the wrinkled appearance of *B. pseudomallei* on both blood and Drigalski agar plates upon incubation beyond 24-48 hours. The organism appeared as small Gram-negative bacilli with unspecific characteristics for *B. pseudomallei* (non "safety pin" like appearance) on Gram staining. Since the isolate was non-fermenting Gram-negative rods with the positive oxidase test, the API 20NE system was appropriately chosen for the bacterial identification according to the manufacturer's instructions (12). However, the isolate was identified as *Aeromonas salmonicida* instead of *B. pseudomallei.*

There are two possible influences on the misdiagnosis of melioidosis in this case. Firstly, the API 20NE system - a common tool used in first-line diagnostic laboratories was reported to misidentify *B. pseudomallei* isolates as other species when used precisely according to the manufacturer's instructions (11, 13, 14). Secondly, the unsuspected clinical and organism characteristics induced the missing of the combination between the API 20NE system and several effective, simple screening such as susceptibility testing (usually resistant to aminoglycosides and colistin or polymyxin but susceptible to amoxicillin/clavulanic acid) or the immunochromatographic assay in the absence of automated microbiological systems. Incorrect identification of *B. pseudomallei* as *Aeromonas salmonicida* led to ineffective prolonged-course antibiotic treatment in this patient. Besides, 16S rRNA gene sequencing with high accuracy is considered to detect broad pathogens including *B. pseudomallei*, particularly in non-melioidosis-endemic areas if available (2, 16).

CONCLUSION

Clinicians and laboratory staff should be highly

aware of the possibility of melioidosis in endemic areas due to its various clinical manifestations. In addition, this case highlights the need for rapid and correct diagnostic tests as well as the combination of different diagnostic tests for the timely confirmation of melioidosis in clinical microbiology laboratories.

ACKNOWLEDGEMENTS

The 16S rRNA gene sequencing in this study was supported by project No. DHH 2020-04-122 of Hue University, Hue, Viet Nam.

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