# Evaluation of antibiotic susceptibility of *Burkholderia pseudomallei* isolated from melioidosis patients in northern Vietnam

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***Abstract:***

**Melioidosis is a potentially fatal infectious disease caused by the Gram-negative bacterium *Burkholderia pseudomallei*. This disease is prevalent in northern Australia and Southeast Asia, including Vietnam. Therapeutic options are limited to a selected group of six antibiotics: amoxicillin/clavulanic acid, ceftazidime, doxycycline, imipenem, meropenem, and trimethoprim/sulfamethoxazole. Although recent efforts have been made to detect cases of melioidosis, the antibiotic resistance of *B. pseudomallei* in Vietnam has been poorly investigated. In this study, the susceptibility of 53 *B. pseudomallei* strains isolated from melioidosis patients in northern Vietnam was determined using the broth microdilution assay and the E-test method. All strains (100%) were susceptible to doxycycline (minimum inhibitory concentration - MIC range: 0.5-4 µg/ml), imipenem (MIC range: 0.25-1 µg/ml), meropenem (MIC range: 0.5-4 µg/ml), and trimethoprim/sulfamethoxazole (MIC range: 0.032/0.608-0.75/14.25 µg/ml). One strain (1.9%) exhibited intermediate resistance to ceftazidime, and two strains (3.8%) demonstrated intermediate resistance to amoxicillin/clavulanic acid. Our findings indicate that the majority of clinical *B. pseudomallei* strains isolated from melioidosis patients in northern Vietnam remain susceptible to the six antibiotics currently recommended for the treatment of this disease.**

***Keywords:*** **antimicrobial susceptibility, broth microdilution, *Burkholderia pseudomallei*, E-test, melioidosis, minimum inhibitory concentration, northern Vietnam.**

***Classification numbers:*** **3.2, 3.4, 3.6**

## 1. Introduction

*Burkholderia pseudomallei* is a saprophytic soil, Gram-negative bacillus and the causative agent of melioidosis, a potentially fatal infection [1]. Melioidosis manifests with diverse clinical symptoms, including pneumonia, chronic infections, localised abscesses, and sepsis [1, 2]. Disease mortality ranges from 14 to 40% and can reach 80% in the absence of effective antibiotic treatment [3]. To date, no commercially available vaccine exists for melioidosis.

*B. pseudomallei* exhibits intrinsic resistance to various antibiotic classes, including β-lactams (penicillin, ampicillin, cephalosporins), aminoglycosides (gentamicin, tobramycin, streptomycin), quinolones, polymyxins (polymyxin B and colistin), and macrolides [1, 4]. Therapeutic options for melioidosis are limited to six recommended antibiotics: amoxicillin/clavulanic acid, ceftazidime, doxycycline, imipenem, meropenem, and trimethoprim/sulfamethoxazole. Treatment typically involves two phases: the intensive phase and the eradication phase [5]. During the intensive phase, patients receive intravenous administration of antibiotics, with ceftazidime being the first-line therapy. In some cases, parenteral ceftazidime is supplemented with oral or parenteral trimethoprim/sulfamethoxazole. If the patient's condition deteriorates under ceftazidime therapy, meropenem or imipenem may be used as alternatives. The eradication phase involves at least three months of oral trimethoprim/sulfamethoxazole, or in certain cases, doxycycline or amoxicillin/clavulanic acid, to prevent recurrence [6, 7].

The first case of melioidosis in Vietnam was documented in 1925. Since then, Vietnam has been recognised as an

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endemic area for the disease, with an estimated 10,430 cases of melioidosis and 4,703 related deaths annually [8]. Recent efforts have been made to improve the diagnosis of melioidosis in clinical settings, leading to the identification of a significant number of cases [9, 10]. However, the antibiotic resistance of *B. pseudomallei* in Vietnam remains poorly studied, with limited research conducted in northern regions. A report by P.H. Nhung, et al. (2019) [11] showed 312 *B. pseudomallei* strains isolated from patients admitted to Bach Mai Hospital were susceptible to ceftazidime, amoxicillin/clavulanic acid, and imipenem, although two strains (0.6%) showed intermediate resistance to doxycycline, and 34 strains (10.9%) were resistant to trimethoprim/sulfamethoxazole [11]. Another study on 75 clinical strains at the National Hospital for Tropical Diseases reported susceptibility rates of 82.2% for trimethoprim/sulfamethoxazole, 93.3% for ceftazidime, 88.4% for amoxicillin/clavulanic acid, 93.2% for imipenem, 96.3% for meropenem, and 100% for doxycycline [12]. Notably, a recent report [13] found low sensitivity rates of 54.5% for trimethoprim/sulfamethoxazole, 74% for ceftazidime, and 65.7% for amoxicillin/clavulanic acid in *B. pseudomallei* strains isolated from 39 paediatric patients admitted to Vietnam National Children's Hospital.

The high resistance rates and discrepancies in the aforementioned studies in Vietnam warrant attention. Early studies in Thailand overestimated resistance rates [14], which was later re-evaluated to be much lower [15]. Additionally, low resistance rates with ceftazidime, imipenem, amoxicillin/clavulanic acid, and doxycycline were reported in Taiwan (China) [16], Singapore [17], Thailand [18], and Australia [19]. We hypothesise that the high resistance rates reported for Vietnamese *B. pseudomallei* strains may be attributable to technical errors. To investigate this, we conducted antimicrobial susceptibility testing (AST) on 53 clinical *B. pseudomallei* strains using six recommended antibiotics. Minimum inhibitory concentrations (MICs) were determined for amoxicillin/clavulanic acid, ceftazidime, doxycycline, imipenem, and meropenem via the broth microdilution assay, and for trimethoprim/sulfamethoxazole using the E-test method.

## 2. Materials and methods

### *2.1. Bacterial strains*

Between 2015 and 2017, a total of 53 clinical *B. pseudomallei* strains were isolated from melioidosis patients admitted to 12 provincial hospitals, including Bac Giang (n=18), Dien Bien (n=1), Hai Duong (n=4), Hai Phong (n=5), Hoa Binh (n=1), Hung Yen (n=1), Ninh Binh (n=1), Phu Tho (n=2), Quang Ninh (n=6), Tuyen Quang (n=2), Vinh Phuc (n=11), and Yen Bai (n=1) (Fig. 1). The *B. pseudomallei* strains were identified based on colony morphology, gram staining, oxidase tests, and a specific TTSS1 real-time polymerase chain reaction (PCR) assay [20]. Two reference strains, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, were used for quality control during antimicrobial susceptibility testing (AST).

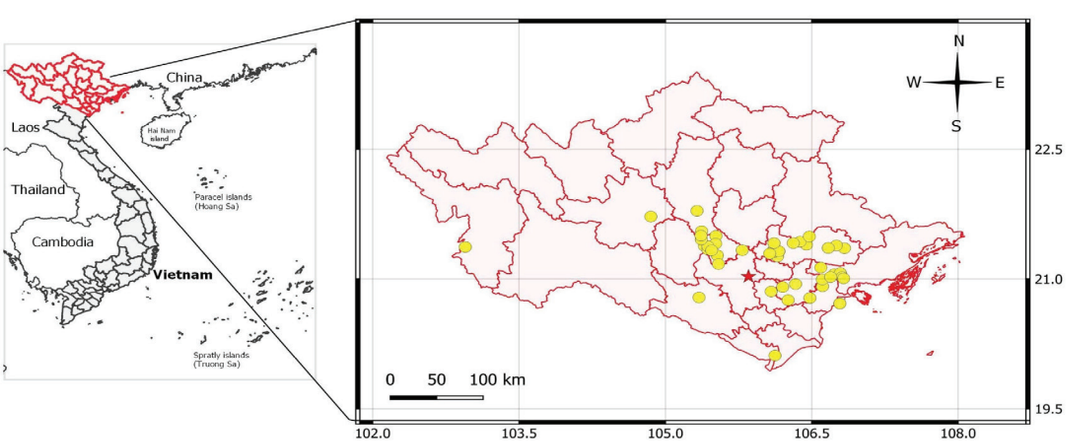


**Fig. 1. Geographic distribution of melioidosis patients (n=53).** Yellow circles represent the patient's home addresses. Asterisk is Hanoi capital. The map was constructed by QGIS version 3.22.1.

### *2.2. Antimicrobial susceptibility testing*

Antibiotic powders, including amoxicillin, potassium clavulanate, ceftazidime, doxycycline, imipenem, and meropenem, were purchased from Sigma-Aldrich (USA). The broth microdilution assay was conducted using 96-well plates (Corning, USA). E-test strips for trimethoprim/sulfamethoxazole (ratio 1:19) were obtained from bioMérieux (France). MICs were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M45 guidelines for *B. pseudomallei*, with the following criteria: amoxicillin-clavulanate ≤8/4 (S), 16/8 (I), ≥32/16 (R); ceftazidime ≤8 (S), 16 (I), ≥32 (R); doxycycline ≤4 (S), 8 (I), ≥16 (R); imipenem ≤4 (S), 8 (I), ≥16 (R); meropenem (based on the breakpoints of imipenem) ≤4 (S), 8 (I), ≥16 (R); and trimethoprim-sulfamethoxazole ≤2/38 (S), ≥4/76 (R) [21]. Each test procedure was repeated at least twice for intermediate and resistant results.

*Bacterial preparation:* From frozen vials, bacterial cultures were streaked onto Mueller-Hinton agar (MHA) plates and incubated at 37°C for 18-24 hours. Bacterial colonies were dissolved in 0.85% saline and adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to $2\times10^8$ CFU/ml.

*Broth microdilution assay:* Broth microdilution assay was conducted following the guidelines of CLSI M45 [21]. A working stock of the antibiotics was prepared and diluted in cation-adjusted Mueller-Hinton broth (cMHB). Positive and negative controls were added to the last positions of the plates. A total of 100 µl of antibiotic stock was added to the first column of a sterile 96-well plate, with the remaining wells filled with 50 µl cMHB. Using a multi-channel pipette, the antibiotic solution was serially twofold diluted to generate 10 concentrations ranging from 0.06 to 32 µg/ml. The solutions were mixed by pipetting up and down at least 10 times. After mixing, 50 µl from the last column was discarded to ensure a uniform volume of 50 µl in all wells. A 0.5 McFarland cell suspension in saline was diluted into 4.9 ml cMHB, and 50 µl of this inoculum was added to each well, followed by incubation at 37°C for 16-20 hours. MIC values were recorded as the lowest antibiotic concentration at which no visible bacterial growth was observed.

*E-test:* The E-test (bioMérieux, France) for trimethoprim/sulfamethoxazole was performed according to the manufacturer's instructions. Bacterial suspensions were evenly spread onto MHA plates using a cotton swab and a plate rotator. An E-test strip was placed in the centre of the inoculated plate. Within 15 minutes, the plate was inverted and incubated at 35±2°C.

The results were read after 16-20 hours of incubation, with MICs recorded at the point of 80% inhibition. Interpretation followed CLSI M45 breakpoints [21].

## 3. Results and discussion

### *3.1. Results*

All 53 clinical *B. pseudomallei* strains (100%) were susceptible to doxycycline (MIC range: 0.5-4 µg/ml), imipenem (MIC range: 0.25-1 µg/ml), and meropenem (MIC range: 0.5-4 µg/ml). E-test results for trimethoprim/sulfamethoxazole confirmed susceptibility in all 53 strains (100%), with MIC values ranging from 0.032/0.608 to 0.75/14.25 µg/ml. Notably, one strain (1.9%) exhibited intermediate resistance to ceftazidime, and two strains (3.8%) showed intermediate resistance to amoxicillin/clavulanic acid. The MIC values for these strains were 16 µg/ml for ceftazidime and 16/8 µg/ml for amoxicillin/clavulanic acid. Despite these observations, the majority of *B. pseudomallei* strains tested remain susceptible to all six antibiotics examined in this study (Tables 1, 2).

**Table 1. Antimicrobial susceptibility testing of clinical *B. pseudomallei* strains isolated in northern Vietnam (n=53).**

| Antimicrobial agents | Susceptible (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Amoxicillin/clavulanic acid | 96.2 | 3.8 | 0 |
| Ceftazidime | 98.1 | 1.9 | 0 |
| Doxycycline | 100 | 0 | 0 |
| Imipenem | 100 | 0 | 0 |
| Meropenem | 100 | 0 | 0 |
| Trimethoprim/sulfamethoxazole | 100 | 0 | 0 |

**Table 2. Minimum inhibitory concentration distributions of clinical *B. pseudomallei* strains isolated in northern Vietnam (n=53).**

| Antibiotics | Minimum inhibitory concentration (µg/ml) | | | | | | | | | | | | | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *0.06* | *0.125* | | *0.25* | *0.5* | *1* | | *2* | *4* | *8* | | *16* | *32* | | |
| Amoxicillin/clavulanic acid (2:1)[a] | | | | | | | | | 4 | 47 | | 2 | | 8 | 8 |
| Ceftazidime | | | | | | 8 | | 44 | | | | 1 | | 2 | 2 |
| Doxycycline | | | | | 11 | 33 | | 5 | 4 | | | | | 1 | 2 |
| Imipenem | | | | 21 | 29 | 3 | | | | | | | | 0.5 | 0.5 |
| Meropenem | | | | | 5 | 43 | | 2 | 3 | | | | | 1 | 1 |
| | MIC (µg/ml) | | | | | | | | | | | | | $MIC_{50}$ | $MIC_{90}$ |
| | .016 | .023 | .032 | .047 | .064 | .094 | .125 | .19 | .25 | .38 | .50 | .75 | 1.0 | | |
| Trimethoprim/ sulfamethoxazole (1:19)[b] | | | 2 | 2 | 12 | 9 | 17 | 5 | 3 | 1 | 1 | 1 | | 0.125 | 0.25 |

[a]: MIC values present the concentration of amoxicillin; [b]: MIC values present the concentration of trimethoprim.

### *3.2. Discussion*

The treatment of melioidosis requires prolonged, high-dose antibiotic therapy to ensure complete eradication of the infection. Therapeutic options are limited to a specific group of six antibiotics, with ceftazidime being the primary treatment, and trimethoprim/sulfamethoxazole, doxycycline, or amoxicillin/clavulanic acid serving as secondary options [6]. Consequently, the emergence of antibiotic resistance in *B. pseudomallei* poses a significant challenge in managing melioidosis. In this study, we assessed the antibiotic susceptibility of *B. pseudomallei* strains isolated from melioidosis patients in northern Vietnam. Among the 53 clinical strains tested, 100% were susceptible to imipenem, meropenem, and trimethoprim/sulfamethoxazole. One strain (1.9%) exhibited intermediate resistance to ceftazidime, and two strains (3.8%) demonstrated intermediate resistance to amoxicillin/clavulanic acid.

Trimethoprim/sulfamethoxazole is a critical antibiotic for the eradication phase of melioidosis treatment [7]. Using the E-test, we found all clinical *B. pseudomallei* strains to be susceptible to this antibiotic. The low resistance rate observed in our study aligns with findings from Taiwan (China) (0%) [16], Laos (0.8%), Cambodia (0%) [22], and Thailand (0.33%) [15], suggesting that true resistance to trimethoprim/sulfamethoxazole in *B. pseudomallei* is exceptionally rare across Asia. These findings are consistent with a recent study in southern Vietnam, which reported that all 33 *B. pseudomallei* strains isolated from paediatric patients at Children's Hospital II in Ho Chi Minh city were susceptible to trimethoprim/sulfamethoxazole [10].

However, our findings differ from earlier studies in northern Vietnam, which reported relatively high resistance rates to trimethoprim/sulfamethoxazole: 10.9% [11], 16.4% [12], and 18.2% [13]. We acknowledge potential technical errors in previous susceptibility testing for *B. pseudomallei* in Vietnam, particularly regarding quality control, method selection, and result interpretation, which may have contributed to inaccurate reporting. Although we attempted to re-test isolates previously reported as resistant, we were unable to obtain them.

Ensuring the validity of experimental results necessitates robust quality control, especially when evaluating antibiotic resistance. Unfortunately, not all Vietnamese studies have reported their quality control methods. Furthermore, the choice of testing and interpretation method is crucial. In developing countries where melioidosis is endemic, AST often relies on the disc diffusion method rather than broth microdilution, E-test, or automated systems due to resource limitations and the impracticality of routinely determining MICs for a broad range of antibiotics [23]. While the disc diffusion method generally provides reliable results for most antibiotics, it can overestimate resistance to trimethoprim/sulfamethoxazole [22]. Notably, the study by T.Y. Le, et al. (2023) [13] did not detail the specific testing method employed. K.T. Nguyen, et al. (2022) [12]'s

study utilised both disc diffusion and E-test methods for determining resistance to trimethoprim/sulfamethoxazole in *B. pseudomallei*. Importantly, strains should not be classified as 'resistant' based solely on disc diffusion results; alternative methods such as the E-test or broth microdilution are recommended for more accurate assessment [14].

Laboratories commonly refer to the methodologies and breakpoints provided by the two major global systems: the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST). CLSI recommends the broth microdilution method for testing *B. pseudomallei* and interprets disc diffusion results based on criteria for closely related strains [21], EUCAST, on the other hand, has recently published guidelines for interpreting disc diffusion testing specifically for *B. pseudomallei* [24]. While differences in susceptibility profiles between these two methods are recognised [25], we chose to adhere to CLSI guidelines to ensure consistency with previous studies conducted in Vietnam [11-13].

The high susceptibility rate to ceftazidime (98.1%) in this study is comparable to earlier studies from Singapore (>99%) [17], Malaysia (>95%) [26], and Thailand (>99%) [18, 27]. Even with amoxicillin/clavulanic acid, we observed a high susceptible rate in 96.2% of clinical strains, aligning with reported rates of over 97% in Singapore [17], 93% in Taiwan (China) [16], and more than 99% in Thailand [27]. Furthermore, all tested strains in this study were susceptible to imipenem, meropenem, and doxycycline. These findings are consistent with reports from other countries [16, 19, 26], confirming that imipenem, meropenem, and doxycycline remain highly effective options for treating melioidosis.

## 4. Conclusions

In conclusion, our findings confirmed that *B. pseudomallei* isolated from melioidosis patients admitted to provincial hospitals in northern Vietnam remain susceptible to the six currently recommended antibiotics for treatment of melioidosis. These results provide robust support for the effectiveness of the existing treatment guidelines, allowing clinicians to proceed with confidence in their therapeutic approach.

## CRediT author statement

Nguyen Phuong Anh: Data curation, Investigation, Writing; Vu Thi Ngoc Anh: Validation, Reviewing, Editing; Tran Thi Le Quyen: Data curation, Supervision; Bui Nguyen Hai Linh: Visualisation, Software; Trinh Thanh Trung: Conceptualisation, Methodology, Supervision.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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