Five-Year Surveillance for *Burkholderia pseudomallei* in Thailand from 2000 to 2004: Prevalence and Antimicrobial Susceptibility

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Objective: *To determine the prevalence and antibiotic susceptibility patterns of Burkholderia pseudomallei isolates in Thailand from 2000 to 2004.*
Material and Method: *the data on WHONET from 28 hospitals participated in the National Antimicrobial Resistance Surveillance Thailand (NARST) surveillance program, was reviewed and analyzed for the prevalence and antimicrobial susceptibility patterns.*
Results: *During the five-year surveillance, the prevalence of B. pseudomallei in clinical isolates was 69% in the Northeast, 14% in the North, 11.8 % in the Center, and 5% in the South. Compared to other regions, the prevalence rate in the Northeast had gradually increased from 2000 to 2004. Burirum Hospital had the highest prevalence rate in this area. The majority of isolates were obtained from blood (44.9%), pus (25.6%), respiratory tract (13.3%), and urinary tract (6.3%). The isolates from unusual sites including bone marrow, heart, and placenta were less commonly noted (< 1%). Based on in vitro susceptibility results, all isolates in each region expressed high susceptibility to ceftazidime (> 98.5%), amoxicillin/clavulanic acid (> 95%), cefoperazone/sulbactam (> 98%), imipenem (98.5%), and meropenem (98%), but express less susceptibility to trimethoprim-sulfamethoxazole (< 53%). However, the susceptibility of B. pseudomallei to trimethoprim/sulfamethoxazole determined by the disk diffusion method is unreliable; it must be performed by the minimal inhibitory concentration method.*
Conclusion: *With the exception of the Northeast, the prevalence rate of B. pseudomallei remains stable for all regions in Thailand. The isolates obtained from blood and pus represent more than two-thirds of all clinical isolates. Antimicrobial susceptibility patterns showed no evidence of increased resistance to antimicrobials most commonly prescribed for the treatment of melioidosis.*

Keywords: *Anti-infective agents, Burkholderia pseudomallei, Epidemiology, Microbial sensitivity tests, Population surveillance, Prevalence, Thailand*

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Burkholderia pseudomallei is an oxidase-positive, Gram-negative bacillus with a characteristic of smooth colonies on initial isolation, dry and wrinkle colonies on further incubation. Its ability to assimilate arabinose makes it different from the closely related but less pathogenic *B. thailandensis*[1,2]. It is a soil saprophyte found over Southeast Asia, Vietnam, Singapore, and northern Australia[3], and was identified in 4.4%, 6.1%, 20.4%, and 5.9% of soil samples collected from northern, central, northeastern, and southern

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regions of Thailand, respectively[4]. Acquisition of this organism from environmental source has been well recognized to cause a threatening disease, melioidosis, in humans.

The response to antimicrobial treatment of *B. pseudomallei* infections is often poor. The bacteria is known to be intrinsically resistant to a wide range of antimicrobial agents including β-lactam antibiotics, aminoglycosides, and macrolides[5,6], and treatment usually requires an initial parenteral therapy with ceftazidime, followed by a prolonged oral therapy with trimethoprim/sulfamethoxazole (TMP/SMX) and doxycycline[7,8]. In Thailand, severe infections caused by *B. pseudomallei* have been increasingly recognized each year, and acquired resistance to ceftazidime, TMP/SMX, and doxycycline has been reported[9,10]. A surveillance program, therefore, is essential for prevention, treatment, and control of this organism. Moreover, the distribution and trend of antimicrobial susceptibility would be a useful guide for appropriate antimicrobial therapy. Here, the authors report the prevalence and antimicrobial susceptibility patterns of *B. pseudomallei* from National Antimicrobial Resistance Surveillance, Thailand (NARST) surveillance program from 2000 to 2004.

Material and Method

Setting

In 1998, NARST of the Department of Medical Sciences of Thailand's Ministry of Public Health, was organized. The program was supported by the World Health Organization (WHO) to investigate the antimicrobial resistance situation of various microorganisms in Thailand. Strengthening as well as standardizing laboratory practices has been initiated. Several related activities included laboratory training on identification of pathogenic bacteria, antimicrobial susceptibility testing, and data collection using WHONET software. In the first phase, 33 hospitals had applied for and were accepted in the surveillance network. All data from each hospital including the types of specimen, bacterial isolation, and diameter of antimicrobial inhibition zone (in mm) were sent to NARST center (Nonthaburi, Thailand). Because only 28 hospitals had data available during the five-year surveillance period, the authors selected these hospitals (13 in the Center, 6 in the Northeast, 5 in the North, and 4 in the South) for data analysis.

Microbiology

Isolation and identification of *B. pseudomallei* were performed using the conventional culture and biochemical methods at the hospital laboratory, according to the recommendations of Gilardi and Koneman[11,12]. Antimicrobial susceptibility test to ceftazidime, amoxicillin/clavulanic acid, cefoperazone/sulbactam, imipenem, meropenem, and trimethoprim/sulfa-methoxazole (TMP/SMX) was determined by the disk diffusion method[13]. The interpretation for zone size was adapted from zone size interpretive standards for *Pseudomonas aeruginosa*, according to the Clinical Laboratory Standards Institute (CLSI) [formerly National Committee for Clinical Laboratory Standards (NCCLS)][14]. For quality assurance, proficiency testing (PT) with a panel of bacterial samples for both identification and antibiotic susceptibility has been provided to participating hospitals thrice a year from NARST center. There is 89% agreement in the PT for *B. pseudomallei* identification and 92% agreement in the antimicrobial susceptibility testing.

Statistical analysis

Prevalence of *B. pseudomallei* isolates and antimicrobial susceptibility from 2000 to 2004 were collected and grouped by WHONET data. Multiple isolates from different sites of a single patient were counted only once, and antimicrobial susceptibility determination of the first isolate was used in this study. Descriptive variables were presented in terms of number and percentage. The Chi-square for trend (Epi Info, 1993) was used to calculate the trend of isolation rates over time. Two tailed p-value of < 0.05 is considered statistically significant.

Results

Prevalence

A total of 4, 019 *B. pseudomallei* isolates from 28 hospitals were obtained from all clinical specimens. Each isolate represents a sample from a single patient. The highest number of isolates was obtained from the northeastern (69.1 %), the northern (14.1 %), the central (11.8 %), and the southern (5.0 %) region, respectively. The number of isolates with *B. pseudomallei* stratified by region during the five-year surveillance is shown in Table 1. Among the four regions, the northeastern region had significantly high infection rate per 100,000 population (Fig. 1). The infection rate in this area increased gradually from 2000 to 2004 ($p < 0.001$). Burirum and Sappasittiprasong Hospitals were more likely to have the two highest isolation rates in this area (Fig. 2). The data on *B. pseudomallei* isolations stratified by the region and year are summarized in Fig. 1, and the data on isolation for each hospital in

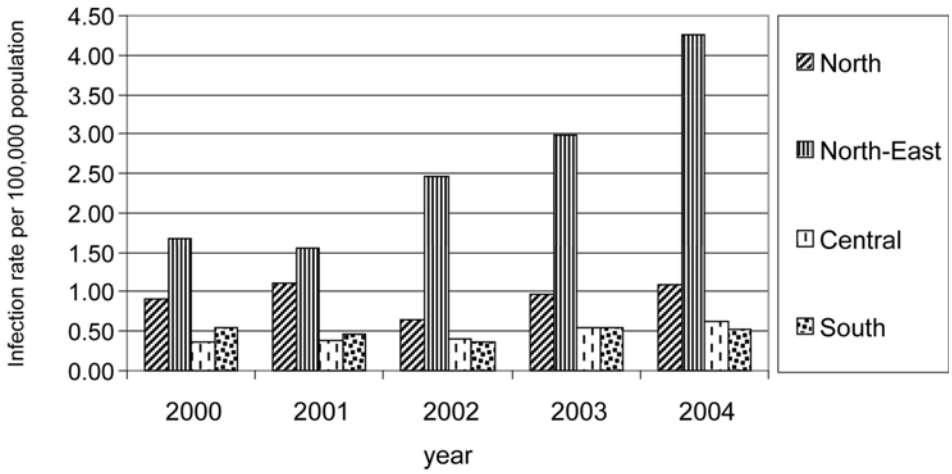

Fig. 1 *Burkholderia pseudomallei* isolation rate stratified by the region and year

the Northeast from 2000 to 2004 are summarized in Fig. 2.

Clinical epidemiology

The characteristics of patients with positive isolate for *B. pseudomallei*, stratified by region, hospital size, and site of infection, from 2000 to 2004 are shown in Table 1. The isolation from blood (45%), pus (25.5%), and respiratory tract (13%) represented the majority of isolates obtained in the present study. Most isolates were obtained in the Northeast (69%) and from hospitals with more than 500 beds (86%). Among 4,019 isolates, the uncommon isolation sites were identified in 21 isolates (0.5%) from the central nervous system, bone marrow, heart, placenta, and genital tract (Table 2). Among these isolates, central nervous system isolates (10 of 21, 48%) have been the most common isolation site.

Antimicrobial susceptibility

Determination of minimal inhibitory concentration (MIC) was not performed in all 28 hospitals. Therefore, the disk diffusion method was performed as a practical alternative in our surveillance system. An interpretation of the inhibition zone was applied according to the interpretative criteria for *Pseudomonas aeruginosa*. The antimicrobial susceptibility percentage results of *B. pseudomallei* in each year are shown in Table 3. Overall, the antimicrobial susceptibility results showed the high susceptibility to ceftazidime (> 98.5%), amoxicillin/clavulanic acid (> 95%), cefoperazone/sulbactam (> 98%), imipenem (98.5%), and meropenem

Table 1. Distribution, location, and common site of *Burkholderia pseudomallei* isolations in Thailand from 2000 to 2004

Characteristics	Number (%) (n = 4019)
Region	
North	566 (14.08)
Northeast	2,776 (69.07)
Center	475 (11.82)
South	202 (5.03)
Hospital size	
< 500 beds	566 (14.08)
≥ 500 beds	3,453 (85.92)
Site of isolation	
Blood	1,808 (44.99)
Pus	1,028 (25.58)
Respiratory tract	535 (13.31)
Urinary tract	252 (6.27)
Gland	54 (1.34)
Muskeletal system	54 (1.34)
Eye-ENT	19 (0.47)
Abdomien	13 (0.32)
Soft tissue	24 (0.60)
Other*	232 (5.78)

Note: ENT: ear, nose, throat; N: number of patients infected by *Burkholderia pseudomallei*

* Abscess, arm, armpit, aspirate, bile, bone, bone marrow, brain, bronchial wash, broncho-alveolar lavage, catheter, cerebrospinal fluid, dialysis fluid, drainage, gastric fluid, genital female, genital male, hand, heart, joint, liver, lung, placenta, pleural fluid, rectal, secretion, sputum, tracheal, wound, throat

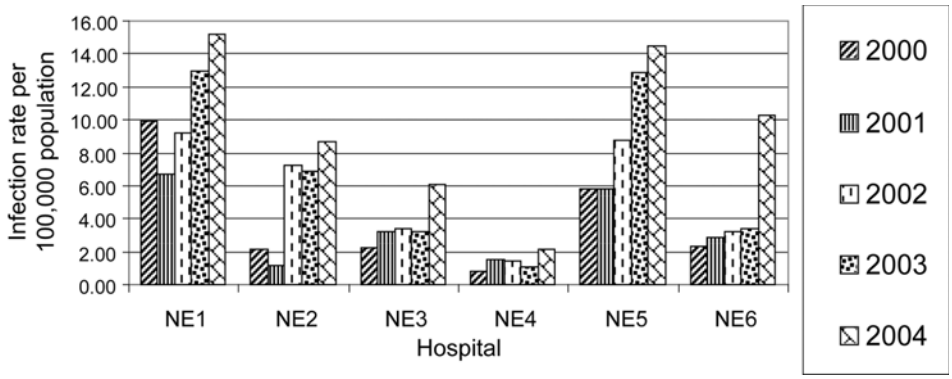

Fig. 2 *Burkholderia pseudomallei* isolation rate stratified by the hospitals in northeastern region

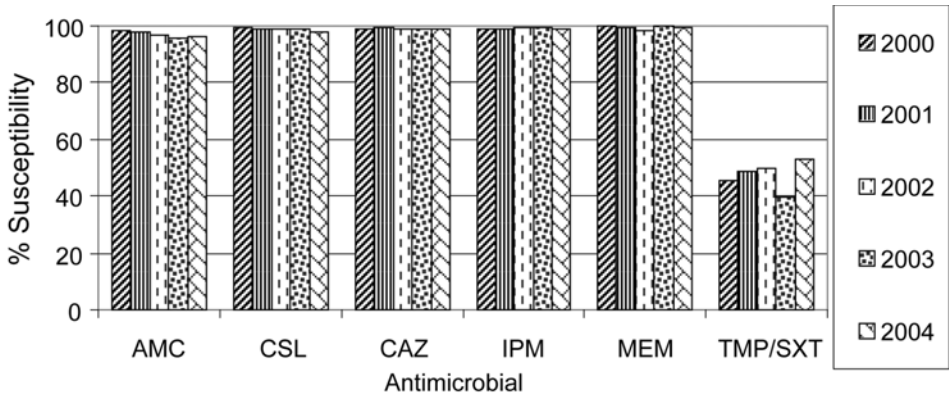

Fig. 3 Antimicrobial susceptibility of *Burkholderia pseudomallei* to antibiotics stratified by year. The inhibition zone interpretation was derived from the zone diameter interpretive standards for *Pseudomonas aeruginosa* complied by the Clinical Laboratory Standards Institute (CLSI)
AMC: amoxicillin/clavulanic acid, CSL: cefoperazone/sulbactam, CAZ: ceftazidime, IPM: imipenem, MEM: meropenem, TMP/SXT: trimethoprim/sulfamethoxazole

Table 2. Unusual isolation sites of *Burkholderia pseudomallei* isolates from 2000 to 2004

Unusual sites	Number (%) (n = 4,019)
Bone marrow	3 (0.075)
CNS	10 (0.249)
Heart	4 (0.100)
Placenta	1 (0.025)
Genital tract	3 (0.075)

Note: N: number of patient s' isolates that yielded *Burkholderia pseudomallei*, CNS: central nervous system, including cerebrospinal fluid and brain

(98%), but not to TMP/SMX (< 53%) in all regions in Thailand. Susceptibility of *B. pseudomallei* to each antibiotic during the entire study period remained stable (Fig. 3). In addition, there was no trend of a decrease in the inhibition zone of all isolates to six antimicrobial antibiotic agents (Table 4).

Discussion

The present study indicated that the prevalence, clinical epidemiology, and antimicrobial susceptibility of *B. pseudomallei* from 2000 to 2004 remained unchanged. Notably, there has been a trend in the increase in *B. pseudomallei* isolations in the Northeast. Although this may represent the true

Table 3. Antimicrobial susceptibility of *Burkholderia pseudomallei* isolates by region and year

Antimicrobial agent	Antimicrobial susceptibility (%)				
	2000	2001	2002	2003	2004
Four regions					
Amoxicillin/clavulanic acid	98.6	97.7	96.9	95.7	96.2
Cefoperazone/sulbactam	99.4	98.9	98.7	99.0	98.1
Ceftazidime	99.1	99.4	98.7	98.9	99.1
Imipenem	98.7	99.1	99.5	99.5	98.7
Meropenem	100.0	99.5	98.2	100.0	99.3
Trimethoprim/sulfamethoxazole	45.2	48.4	49.5	39.8	52.8
Northeastern region					
Amoxicillin/clavulanic acid	99.3	98.9	99.6	97.6	98.6
Cefoperazone/sulbactam	99.3	98.4	98.3	99.2	100.0
Ceftazidime	99.1	99.6	98.3	99.2	99.1
Imipenem	99.6	99.5	99.4	99.8	99.7
Meropenem	NA	50.0	100.0	100.0	100.0
Trimethoprim/sulfamethoxazole	46.9	50.8	52.7	41.8	58.1

Note: Antimicrobial susceptibility were calculated from number of susceptibility divided by total number of specimen that has been tested for each antibiotic; zone interpretation was derived from zone diameter interpretive standards for *Pseudomonas aeruginosa* according to the Clinical Laboratory Standards Institute
NA: not available

Table 4. 90-percent inhibition zone of *B. pseudomallei* isolates to antimicrobial by the year

Antibiotic	Inhibition zone (mm)				
	2000	2001	2002	2003	2004
AMC	15	24	24	24	22
CSL	23	27	26	27	26
CAZ	15	25	25	26	25
IPM	12	30	30	30	28
MEM	21	24	25	25	24
TMP/SMX	6	6	6	6	6

AMC: amoxicillin/clavulanic acid, CSL: cefoperazone/sulbactam, CAZ: ceftazidime, IPM: imipenem, MEM: meropenem, TMP/SXT: trimethoprim/sulfamethoxazole

prevalence of the disease in the Northeast, the authors are not able to exclude the false-positive result from other explanations, including an increased skill of technicians in the isolation of *B. pseudomallei* as well as an improvement in clinical recognition by clinicians. The fact that antimicrobial susceptibility showed no evidence of increased antimicrobial resistance patterns overtime supported the current guidelines for antimicrobial treatment of melioidosis in Thailand.

Recognized risk factors for *B. pseudomallei* infections include diabetes mellitus, thalassemia, renal disease (renal calculi or renal failure). Chronic lung disease, excessive alcohol consumption, and occupational exposure to surface water are other major factors associated with an increased risk for these severe infections[15,16]. The disease has many manifestations from indolent skin ulcers to visceral abscesses, pneumonia, and overwhelming septicemia[7,17-19]. Important clinical findings are geographically different in some extent. The high incidence of genitourinary infection with prostatic abscess in adult males prevailed in tropical Australia, whereas acute suppurative parotitis accounting for up to 40% in Thai children[20]. Although infections involving many other sites including the central nervous system, internal organs, and skin and soft tissues have been well described[21-23], cardiac involvement as well as bone and joint infection are uncommon[24]. The present study does confirm these prior studies and suggested that uncommon isolations remain uncommon during our surveillance period.

There are several limitations to the present study. The determination of MIC was not available in all 28 hospitals; the disk diffusion method was performed as a practical alternative in our surveillance

system. As there is no current standard on interpretation of the inhibition zone for *B. pseudomallei*, the authors have applied the interpretative criteria of *P. aeruginosa* in the present study. The lack of clinical information makes it impossible for us to distinguish clinical infection from colonization or to distinguish community-acquired infection from hospital-acquired infection. Nevertheless, the authors believe that our data represent a large number of isolates, representing the true prevalence, clinical epidemiology, and antimicrobial susceptibility of this microorganism in Thailand.

Given the findings from the present study, a continuous surveillance should be performed to evaluate the prevalence, clinical epidemiology, and antimicrobial susceptibility of this microorganism overtime. An improvement in the data collection including patients' clinical information and antimicrobial susceptibility testing using the MIC method should be implemented in the future surveillance. Whether the infections are hospital- or community- acquired should also be specified in the future surveillance data. Additional studies to identify factors associated with the increase in the prevalence of the disease in the Northeast as well as the reduced TMP/SMX susceptibility are needed.

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การเฝ้าระวังเชื้อ Burkholderia pseudomallei ในประเทศไทยในระยะ 5 ปี พ.ศ.2543-พ.ศ.2547: ความชุกและความไวต่อยาปฏิชีวนะ

วันทนา ปวีณกิตติพร, อนุชา อภิสารธนรักษ์, สุรางค์ เดชศิริเลิศ, สุวรรณา ตระกูลสมบูรณ์, อรทัย ทองมะลิ, ปฐม สวรรค์ปัญญาเลิศ, นลินี อัศวโภคี

วัตถุประสงค์*: เพื่อประเมินความชุกและความไวต่อยาปฏิชีวนะของเชื้อ Burkholderia pseudomallei ที่แยกได้ในประเทศไทยระหว่างพ.ศ. 2543-พ.ศ.2547*

วัสดุและวิธีการ*: ทบทวนและวิเคราะห์ข้อมูลที่เก็บรวบรวมด้วยโปรแกรม WHONET โดยโรงพยาบาลที่เข้าร่วมโครงการเฝ้าระวังของศูนย์เฝ้าระวังเชื้อดื้อยาต้านจุลชีพแห่งชาติของประเทศไทยจำนวน 28 แห่ง เพื่อหาความชุกและแบบแผนความไวต่อยาปฏิชีวนะ*

ผลการศึกษา*: ผลจากการเฝ้าระวังระยะ 5 ปี พบว่าเชื้อ Burkholderia pseudomallei ที่แยกได้จากผู้ป่วยในภาคตะวันออกเฉียงเหนือมีความชุกร้อยละ 69, ในภาคเหนือร้อยละ 14, ในภาคกลางร้อยละ 11.8 และในภาคใต้ร้อยละ 5 ภาคตะวันออกเฉียงเหนือมีอัตราความชุกของเชื้อเพิ่มขึ้นทุกปีตั้งแต่พ.ศ.2543-พ.ศ.2547เมื่อเปรียบเทียบกับภาคอื่น โดยโรงพยาบาลบุรีรัมย์มีความชุกของเชื้อสูงสุดในภูมิภาคนี้ ตัวอย่างผู้ป่วยที่มีการตรวจพบเชื้อส่วนใหญ่เป็นตัวอย่างเลือดร้อยละ 44.9, ตัวอย่างหนองร้อยละ 25.6, ตัวอย่างจากระบบทางเดินหายใจร้อยละ 13.3 และตัวอย่างระบบทางเดินปัสสาวะร้อยละ 6.3 สำหรับบริเวณที่มีการติดเชื้อไม่บ่อย (เช่น ไขกระดูก, หัวใจ, รก) มีการตรวจพบเชื้อนี้ต่ำ (น้อยกว่าร้อยละ 1) จากผลการทดสอบความไวของเชื้อทางห้องปฏิบัติการพบว่า เชื้อทุกสายพันธุ์ที่แยกได้ในแต่ละภูมิภาคมีความไวสูงต่อยา ceftazidime (มากกว่าร้อยละ 98.5), ยา amoxicillin/clavulanic acid (มากกว่าร้อยละ 95), ยา cefoperazone/sulbactam (มากกว่าร้อยละ 98), ยา imipenem (มากกว่าร้อยละ 98.5) และยา meropenem (ร้อยละ 98) แต่มีความไวต่ำต่อยา trimethoprim/sulfamethoxazole (น้อยกว่าร้อยละ 53) ซึ่งอาจมีสาเหตุจากผลการทดสอบความไวที่เชื่อถือได้น้อยและการแปลผลการดื้อยาเกินจริง*

สรุป*: ในทุกภูมิภาคของประเทศไทยยกเว้นภาคตะวันออกเฉียงเหนือมีอัตราความชุกของเชื้อ Burkholderia pseudomallei คงที่ ตัวอย่างเลือดและหนองเป็นตัวอย่างที่มีการตรวจพบเชื้อมากกว่า2ใน3ของตัวอย่างผู้ป่วยทั้งหมด และแบบแผนความไวของเชื้อบ่งชี้ว่าเชื้อไม่เพิ่มการดื้อต่อยาที่ใช้ในโรงพยาบาลรวมทั้งยาที่แนะนำให้ใช้ในการรักษาโรค mellioidosis*