Open Forum Infectious Diseases

MAJOR ARTICLE

Epidemiological and Clinical Characteristics of Melioidosis Caused by Gentamicin-Susceptible *Burkholderia pseudomallei* in Sarawak, Malaysia

Tonnii L. L. Sia,[1,a] Anand Mohan,[2,3,a] Mong-How Ooi,[3,4] Su-Lin Chien,[5] Lee-See Tan,[5] Charles Goh,[5] Daniel C. L. Pang,[6] Bart J. Currie,[7] Jin-Shyan Wong,[8,9] and Yuwana Podin[3]

[1]Infectious Disease Unit, Department of Medicine, Sarawak General Hospital, Kuching, Sarawak, Malaysia, [2]Department of Paediatrics, Bintulu Hospital, Bintulu, Sarawak, Malaysia, [3]Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Sarawak, Malaysia, [4]Department of Paediatrics, Sarawak General Hospital, Kuching, Sarawak, Malaysia, [5]Department of Pathology, Bintulu Hospital, Bintulu, Sarawak, Malaysia, [6]Bintulu Medical Centre, Bintulu, Sarawak, Malaysia, [7]Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, Northern Territory, Australia, [8]Borneo Medical Centre, Kuching, Sarawak, Malaysia, and [9]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Sarawak, Malaysia.

Background. *Burkholderia pseudomallei*, the causative agent of melioidosis, is intrinsically resistant to a broad range of antibiotics, including aminoglycosides. In Sarawak, Malaysia, a high proportion of melioidosis cases are caused by gentamicin-susceptible isolates. There are limited epidemiological and clinical data on these infections.

Methods. We conducted a retrospective study of culture-confirmed melioidosis among adults admitted to Bintulu Hospital in Sarawak, Malaysia, from January 2011 until December 2016.

Results. One hundred forty-eight adults with culture-confirmed melioidosis were identified. Of 129 (87%) tested, 84 (65%) had gentamicin-susceptible *B pseudomallei*. The average annual incidence of melioidosis was 12.3 per 100 000 population, with marked variation between districts ranging from 5.8 to 29.3 per 100 000 population. Rural districts had higher incidences of melioidosis and overwhelmingly larger proportions of gentamicin-susceptible *B pseudomallei* infection. Significantly more patients with gentamicin-susceptible infection had no identified risk factors, with diabetes less frequently present in this group. Ninety-eight percent had acute presentations. Pneumonia, reported in 71%, was the most common presentation. Splenic abscesses were found in 54% of those imaged. Bacteremia was present in 88%; septic shock occurred in 47%. Forty-five (35%) patients died. No differences in clinical, laboratory, or outcome characteristics were noted between gentamicin-susceptible and gentamicin-resistant infections.

Conclusions. Gentamicin-susceptible *B pseudomallei* infections are common in Sarawak and dominate in the high-incidence rural interior regions. Clinical manifestations and outcomes are the same as for gentamicin-resistant *B pseudomallei* infections. Further studies are required to determine if all gentamicin-susceptible *B pseudomallei* infections in Sarawak are clonal and to ascertain their environmental drivers and niches.

Keywords. melioidosis; *Burkholderia pseudomallei*; gentamicin-susceptible; clinical characteristics; Sarawak.

Melioidosis is an important cause of infectious disease mortality in the Western Pacific region and in Southeast Asia (SEA) [1, 2]. Although historically known to be endemic to SEA and northern Australia, cases are now increasingly being reported worldwide [3, 4]. Melioidosis is caused by the environmental saprophyte *Burkholderia pseudomallei* (*Bp*), a gram-negative bacillus that is intrinsically resistant to a broad range of antibiotics, including aminoglycosides [5].

In 2014, we reported the presence of rare gentamicin-susceptible *Bp* among a large proportion of clinical isolates from central Sarawak, Malaysian Borneo [6]. Interestingly, all gentamicin-susceptible isolates examined were genetically typed as either multilocus sequence type (ST) 881 or its single-locus variant ST997. The mechanism of the susceptibility to gentamicin was ascertained to be a novel mutation within the gene *amrB*, which encodes an essential component of the AmrAB-OprA multidrug efflux pump, with the mutation resulting in prevention of the efflux of gentamicin and macrolides that occurs in wild-type *Bp*.

Limited data are available on the disease burden and the epidemiological and clinical characteristics of melioidosis among adults in Sarawak. Importantly, the epidemiological and clinical characteristics of infection caused by gentamicin-susceptible

Received 24 May 2021; editorial decision 31 August 2021; accepted 2 September 2021.

[a]T. L. L. S. and A. M. contributed equally to this work.

Correspondence: Y. Podin, Institute of Health and Community Medicine, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia (pyuwana@unimas.my).

Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab460

isolates, and whether differences exist compared to infection with gentamicin-resistant *Bp*, remain unknown. To answer these questions, we conducted a retrospective study of adults with melioidosis admitted to Bintulu Hospital in Sarawak, Malaysia.

METHODS

Study Site and Population

Bintulu Hospital is a 302-bed hospital that provides medical, surgical and intensive care services to adults and children living in Bintulu Division (consists of Bintulu and Tatau districts, and the subdistrict of Sebauh) and Belaga district (consists of Belaga and Sungai Asap subdistricts) of Kapit Division in Sarawak, Malaysian Borneo. This 32 000-km^2 area located within the central region of Sarawak has a total population of approximately 256 000. Although a large proportion of this population resides in Bintulu town (the largest town in the region), rural communities living in traditional longhouses and workers (mainly migrant) living in plantations are present in all districts. This rural population relies mainly on untreated water (eg, from rivers or gravity-fed water supplies from streams) for daily consumption.

We retrospectively identified all culture-confirmed melioidosis cases among adults aged ≥15 years admitted from January 2011 to December 2016 through a manual search of the microbiology laboratory logbooks and electronic database. Medical records of identified cases were retrieved and bacterial, epidemiological, and clinical details were collected using standardized case report forms. In addition, admission chest radiographs of patients with melioidosis pneumonia were retrieved and reviewed.

Case Definitions

Acute melioidosis was diagnosed if symptoms were present for <2 months, while chronic melioidosis was diagnosed if symptoms were present for ≥2 months before admission. Diabetes mellitus was defined by the presence of fasting plasma glucose ≥7.0 mmol/L and/or random plasma glucose ≥11.1 mmol/L, or glycated hemoglobin ≥6.5% [7]. Chronic kidney disease and acute kidney injury were defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) definitions [8, 9]. In this study, melioidosis risk factors assessed included diabetes mellitus, chronic kidney disease, congestive cardiac failure, bronchiectasis, chronic obstructive pulmonary disease, malignancy, immunosuppressive therapy, and acquired or congenital immunodeficiency diseases. Septic shock was defined as the presence of hypotension not responsive to fluid replacement with end organ dysfunction. A melioidosis-active antibiotic was defined as either ceftazidime or a carbapenem.

Microbiological Methods

As part of routine clinical practice in Bintulu Hospital, blood samples collected from patients were subjected to the BACTEC blood culture system according to the manufacturer's instructions (Becton Dickinson). Positive growth was subcultured onto blood agar, chocolate agar, and MacConkey agar. Samples from other sources (pus, sputum, endotracheal secretions, pleural fluid) were cultured directly on blood agar, chocolate agar, and MacConkey agar. *Bp* was identified with either API 20NE (bioMérieux) or BBL Crystal Identification Systems (Becton Dickinson). Antibiotic susceptibility of *Bp* isolates was determined by either Etest (bioMérieux) or Kirby-Bauer disk diffusion test (Becton Dickinson), adapting interpretative criteria from *Burkholderia cepacia* and *Pseudomonas aeruginosa* according to Clinical and Laboratory Standard Institute (CLSI) guidelines.

Molecular Characterization of Bacterial Isolates

The presence of nucleotide sequences encoding *Burkholderia* intracellular motility factor A (BimABm or BimABp) and the *Burkholderia thailandensis*–like flagellum and chemotaxis and the *Yersinia*-like fimbrial (YLF) gene clusters were determined using previously published methods [10]. A previously published allele-specific polymerase chain reaction (AS-PCR) assay was used to interrogate *Bp* isolates for the presence of amrB mutants that confer susceptibility to aminoglycosides and macrolides [6].

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 23. The Mann-Whitney *U* test was used for numerical variables, and either the χ^2 test or Fisher exact test was used for categorical variables. Correlations between number of culture-confirmed melioidosis cases and average monthly rainfall was examined using Pearson correlation coefficient test. Population data were obtained from the Malaysian Census Data 2010. Meteorological data were obtained from the Malaysian Meteorological Department.

Ethics Statement

The study was approved by the Malaysian Medical Research Ethics Committee (NMRR-16-1029-31390). All data analyzed were anonymized.

Patient Consent Statement

The study involved a retrospective review of medical records and no intervention in patient care. The study was approved by the Malaysian Medical Research Ethics Committee and not deemed to require consent.

RESULTS

Bacterial Characteristics

During the 6-year study period, 148 adults with culture-confirmed melioidosis were identified. Of these, 129 (87%) had *Bp* isolates tested for gentamicin susceptibility; all analyses were

limited to these patients. *Bp* was isolated from a single specimen in 112 cases, 2 specimen types in 16 cases, and 3 specimen types in 1 case. Blood was the commonest source of a positive isolate (n = 113). Others included respiratory secretions (n = 20), pus (n = 10), lung aspirate (postmortem) (n = 2), and peritoneal fluid and urine (n = 1 each).

Of the 129 patients, 84 (65%) had infection with gentamicin-susceptible isolates. Antibiotic susceptibility of *Bp* isolates was determined by disk diffusion in all patients and by Etest in 76; susceptibility findings corresponded in both tests in all cases. The 76 cases tested by Etest included 49 (64%) gentamicin-susceptible isolates (minimum inhibitory concentration [MIC] = 0.5–3.0 μg/mL) and 27 (36%) gentamicin-resistant isolates (MIC = 24–256 μg/mL).

Seventy-eight *Bp* isolates, including 49 (63%) gentamicin-susceptible and 29 (37%) gentamicin-resistant isolates, had molecular characterization performed. No differences were found between gentamicin-susceptible and gentamicin-resistant isolates, with all isolates possessing the $bimA_{Bp}$ variant and the YLF gene cluster. AS-PCR assay on the isolates confirmed that all 49 gentamicin-susceptible isolates had a mutation at the *amrB* gene, which conferred the susceptibility.

Population Incidences and Epidemiological Characteristics

The average annual incidence of melioidosis was 12.3 per 100 000 adult population, with the highest incidence recorded in 2016 (19.4 per 100 000 adult population). Marked variation in average annual incidence rates was observed in the different districts, ranging from 5.8 to 29.3 per 100 000 adult population, with the highest incidence rates recorded in Belaga, Tatau, and Sungai Asap (Figure 1). The proportion of gentamicin-susceptible *Bp* infection was significantly higher in Belaga (23/26 [88%]), Tatau (25/28 [89%]), and Sungai Asap (11/11 [100%]) than in Bintulu district (10/39 [26%]) ($P < .001$).

No correlation was found between the monthly incidence and average rainfall ($r = -0.041$, $P = .73$) (Figure 2), and no

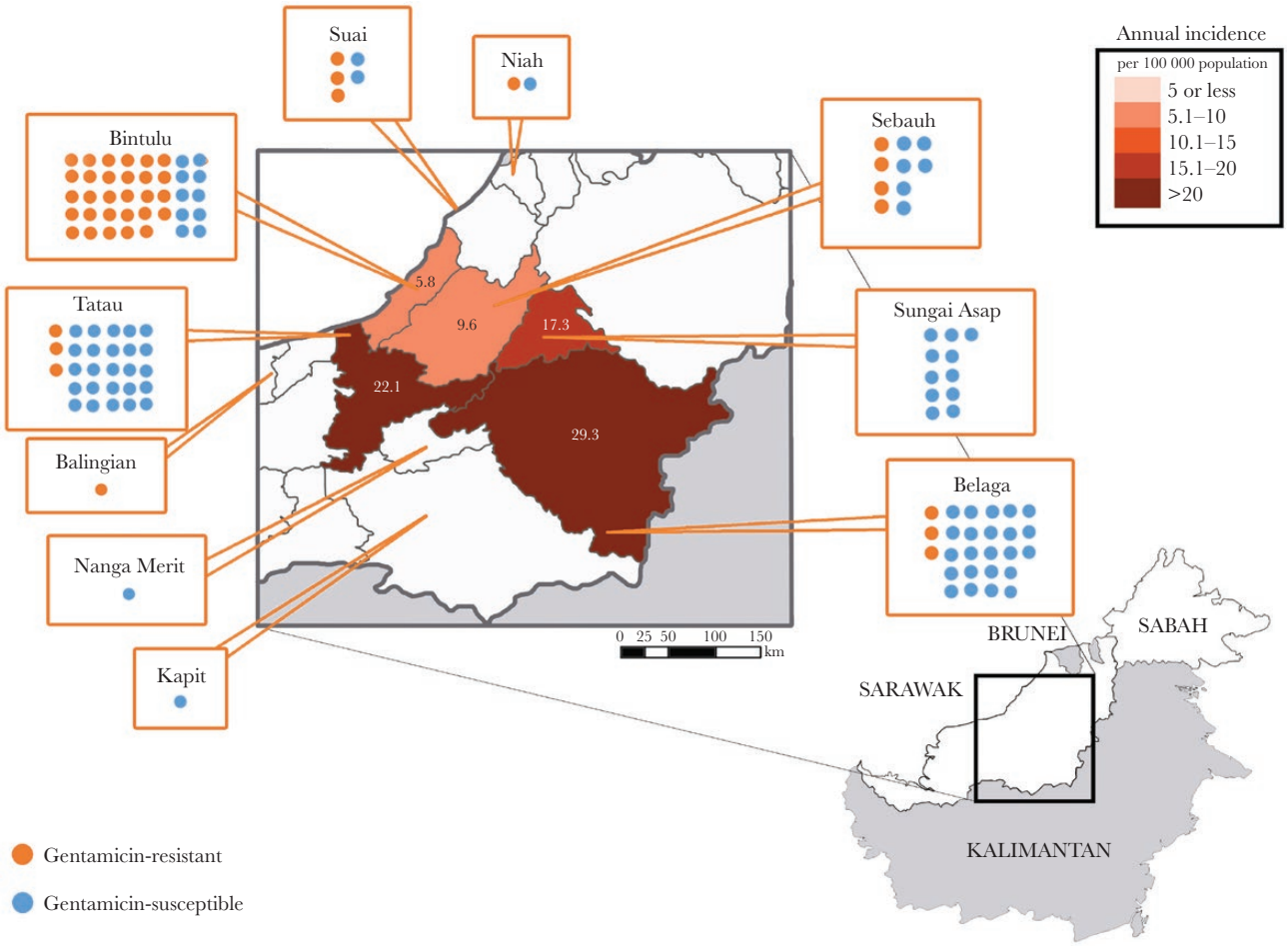

Figure 1. Number of cases by strain susceptibility and district, and the average annual incidence of adult melioidosis in districts in central Sarawak, Malaysia. The map of central Sarawak depicts the number of gentamicin-susceptible and gentamicin-resistant infections in each district or subdistrict. The average annual incidence of adult melioidosis in districts and subdistricts in the region is also shown. Incidences in districts with small numbers of cases (≤5 cases) were not calculated as most melioidosis cases in those districts are admitted to other hospitals and not to Bintulu Hospital. Five patients in the study had no data on address/location recorded.

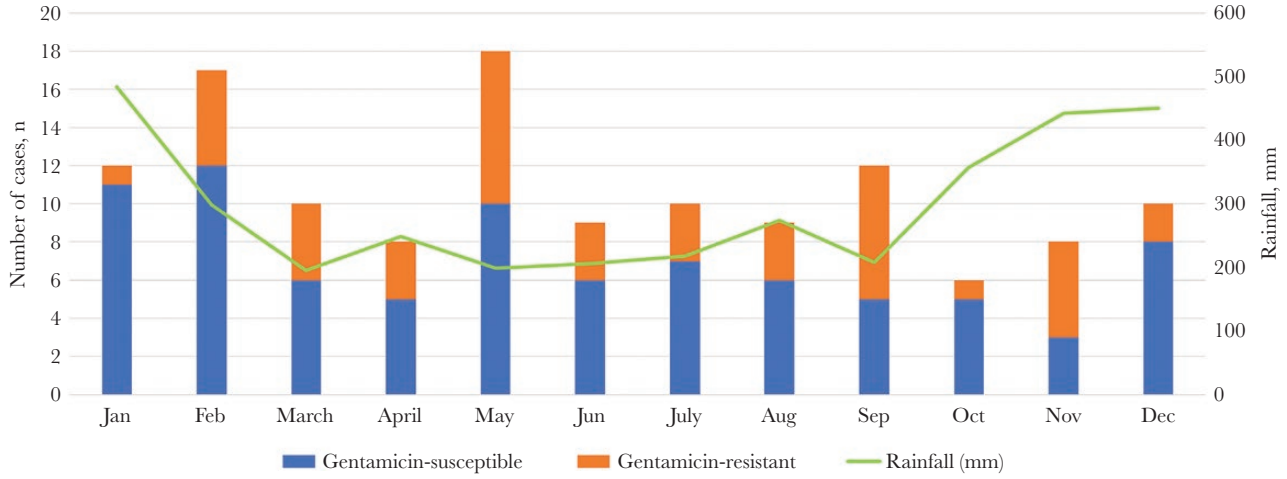

Figure 2. Distribution of 129 adult melioidosis cases and average rainfall by month. The bar chart shows the distribution of the 129 adult melioidosis cases according to the month of admission. Average monthly rainfall over the 6-year period (January 2011–December 2016) in Bintulu, Sarawak, is shown in the line graph. Cases are categorized into gentamicin-susceptible and gentamicin-resistant infections.

changes in the gentamicin susceptibility pattern were recorded throughout the study period.

Patient Demographics, Risk Factors, Clinical Manifestations, and Investigations

One hundred fourteen patients (88%) were aged ≤60 years, including 51 (40%) who were ≤40 years (Figure 3). No significant differences in age or sex were noted between gentamicin-susceptible and gentamicin-resistant infections (Table 1). Seventy-eight patients (60%) had at least 1 risk factor for melioidosis, and significantly more patients with gentamicin-susceptible *Bp* infection had no identified risk factors compared with patients with gentamicin-resistant infection (40/84 [48%] vs 11/45 [24%]; odds ratio {OR}, 2.8 [95% confidence interval {CI}, 1.3–5.9]; P = .01). Diabetes mellitus was the commonest risk factor identified, present in 63 (49%) patients, including 25 (40%) who were newly diagnosed during the admission for melioidosis. Diabetes was present less frequently in those with gentamicin-susceptible infections compared to those with gentamicin-resistant infections (35/84 [42%] vs 28/45 [62%]; OR, 0.4 [95% CI, .3–.9]; P = .03).

Among all presentations, 127 (98%) were acute while only 2 (2%) were chronic. The median duration of symptoms prior to admission was significantly longer in patients with gentamicin-susceptible infection (Table 1). Pneumonia was the most common presentation (71%). Undifferentiated

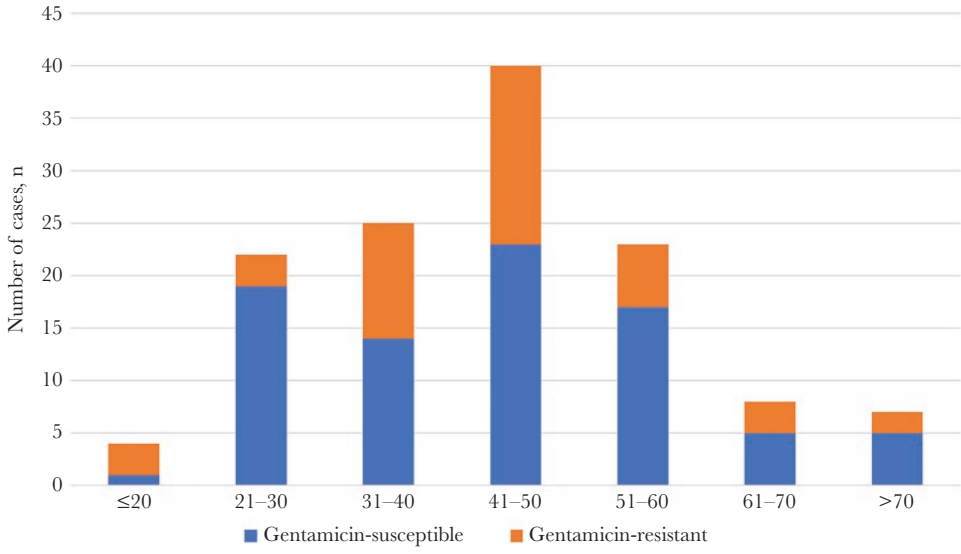

Figure 3. Distribution of the 129 adult melioidosis cases by age and gentamicin susceptibility of isolates. Bar chart shows the distribution of cases according to age. Cases are categorized into gentamicin-susceptible and gentamicin-resistant infections.

Table 1. Demographic, Presenting Clinical Features on Admission, Subsequent Findings, and Outcome of Adults With Melioidosis Based on *Burkholderia pseudomallei* Gentamicin Susceptibility

Characteristic	All Patients	Gentamicin-Susceptible *B pseudomallei* Infection	Gentamicin-Resistant *B pseudomallei* Infection	*P* Value
No. of patients	129	84	45	
Demography				
Age, y, median (IQR)	44.0 (34.0–52.0)	44.0 (32.0–52.8)	45.0 (37.0–51.0)	.94
Male sex	95 (74)	61 (73)	34 (76)	.71
Malaysian citizen	93 (72)	59 (70)	34 (76)	.51
Preexisting conditions/risk factors[a]				
Diabetes mellitus	63 (49)	35 (42)	28 (62)	.03
Chronic kidney disease	11 (9)	8 (10)	3 (7)	.58
Lymphoma	3 (2)	1 (1)	2 (4)	.24
Congestive cardiac failure	2 (2)	1 (1)	1 (2)	.65
Bronchiectasis	2 (2)	0 (0)	2 (4)	.05
COPD	1 (1)	0 (0)	1 (2)	.17
No identified risk factor[b]	51 (40)	40 (48)	11 (24)	.01
Duration of illness before admission, d, median (IQR)	7.0 (3.0–13.5)	7.0 (4.0–14.0)	4.0 (2.0–7.0)	.02
Presenting manifestation				
Pneumonia	91 (71)	61 (73)	30 (67)	.48
Undifferentiated fever	17 (13)	12 (14)	5 (11)	.61
Soft tissue abscess	12 (9)	9 (11)	3 (7)	.45
Genitourinary	4 (3)	1 (1)	3 (7)	.08
Osteomyelitis	2 (2)	1 (1)	1 (2)	.65
Dacrocystitis	2 (2)	0 (0)	2 (4)	.05
Neurological	1 (1)	0 (0)	1 (2)	.17
Investigations				
Hemoglobin, g/dL, median (IQR)	12.2 (10.6–13.6)	12.2 (10.7–13.5)	12.2 (10.4–13.8)	.73
WBC count, $\times 10^9$/L, median (IQR)	10.4 (7.2–15.1)	10.5 (7.4–14.8)	10.3 (6.8–15.6)	.86
Platelet count, $\times 10^9$/L, median (IQR)	188 (107–268)	190 (117–269)	175 (93–263)	.94
Urea, mmol/L, median (IQR)	7.0 (4.5–14.0)	6.9 (4.5–14.2)	7.7 (3.9–13.7)	.42
Creatinine, µmol/L, median (IQR)	103 (66–192)	100 (66–184)	107 (67–206)	.85
Abdominal imaging[c]				
Spleen abscesses only	28 (33)	22 (39)	6 (21)	.08
Liver abscesses only	5 (6)	1 (2)	4 (14)	.02
Both spleen and liver abscesses	18 (21)	12 (21)	6 (21)	.93
No liver/spleen abscesses	34 (40)	21 (38)	13 (45)	.51
Subsequent findings and outcome				
Bacteremia	113 (88)	74 (88)	39 (87)	.81
Septic shock	61 (47)	36 (43)	25 (56)	.16
Acute kidney injury	57 (44)	35 (42)	22 (49)	.43
Died	45 (35)	27 (32)	18 (40)	.43

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: *B pseudomallei*, *Burkholderia pseudomallei*; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; WBC, white blood cell count.

[a]Some patients had >1 preexisting condition/risk factor.

[b]Data do not include hazardous alcohol use as too few cases had this information recorded.

[c]Eighty-five patients had abdominal ultrasonography (gentamicin-susceptible *B pseudomallei* infection, n = 56; gentamicin-resistant *B pseudomallei* infection, n = 29).

fever, soft tissue abscesses, and pneumonia accounted for 93% of all melioidosis presentations. Neurological presentations were rare (n = 1). No significant differences were noted in the clinical presentations, laboratory parameters, and radiological findings (abdominal ultrasound and chest radiographs) of patients with gentamicin-susceptible and gentamicin-resistant infection (Table 1). Of 85 patients who had abdominal imaging, 51 (60%) had abdominal visceral abscesses; splenic abscesses were present in 54% (46/85) and liver abscesses in 27% (23/85).

Chest radiographs of all 91 melioidosis pneumonia patients were analyzed. All presented with acute melioidosis, including 62%, 87%, and 92% who had symptoms for ≤1, ≤2, and ≤3 weeks, respectively. Fifty-two (57%) had multilobar disease, all involving both lungs. Involvement of a single lobe occurred in 38 (42%): 22 (58%) had discrete patchy infiltrates, whereas 16 (42%) had dense lobar consolidation. The upper lobe was affected in 12 of 38 (32%). Pleural effusions were present in 12 of 91 cases. One patient had a normal chest radiograph.

Clinical Course and Outcome

Forty-five (35%) patients died. Death occurred within a median of 3 (interquartile range, 2–13) days following admission and was unrelated to preadmission illness duration. Mortality was similar in patients from the various districts. Those aged >70 years had a higher mortality (5/7 [71%] vs 40/122 [33%]; $P = .04$). Mortality was not related to presence of risk factors or to gentamicin susceptibility of isolates: 15 of the 45 (33%) fatal cases had no identified risk factor, and mortality was 32% (27/84) in the gentamicin-susceptible cohort and 40% (18/45) in the gentamicin-resistant cohort ($P = .30$). Furthermore, mortality in patients without risk factors was similar with both gentamicin-susceptibility patterns (11/40 [28%] vs 4/11 [36%]; $P = .45$). Overall, no significant differences were noted in the epidemiological or clinical characteristics of fatal gentamicin-susceptible and gentamicin-resistant cases.

Bacteremia was present in 113 (88%) patients overall and in 98% of the 45 fatal cases ($P = .01$). Septic shock was strongly associated with mortality, recorded in 89% (40/45) of fatal cases but in only 25% (21/84) of survivors ($P < .001$). Among 61 septic shock patients, the case fatality rate was 66%. Acute kidney injury was significantly associated with mortality, recorded in 34 (76%) fatal cases but in only 23 (27%) survivors ($P < .001$). Patients with pneumonia had a higher mortality than patients with other presentations (38/91 [42%] vs 7/38 [18%]; OR, 3.2 [95% CI, 1.3–8.0]; $P = .01$).

Among fatal cases, 84% received ventilatory/intensive care support. Of the 7 patients not receiving intensive care, all were aged >70 years or had severe debilitating comorbidities, and escalation of care was withheld. Only 58% of the fatal cases received a melioidosis-active antibiotic at admission. However, overall, patients who received melioidosis-active antibiotics upon admission had similar mortality to patients who did not receive melioidosis-active antibiotics (26/70 [37%] vs 19/59 [32%]; $P = .55$). Eight (18%) fatal cases never received a melioidosis-active antibiotic; all succumbed within 3 days of admission and before culture results were known. Only 1 patient overall (with gentamicin-susceptible *Bp* infection) received gentamicin, in combination with melioidosis-active antibiotics.

Among survivors, complete follow-up data were available in only 58% (49/84) of cases. No relapses were recorded in these patients during the 6-month follow-up period.

DISCUSSION

This study from Sarawak, Malaysian Borneo, compares melioidosis infections caused by rare gentamicin-susceptible *Bp* with infections caused by the more typical gentamicin-resistant isolates. There were no significant differences in clinical manifestations, severity, or outcomes between infections from gentamicin-susceptible and gentamicin-resistant *Bp*. What was found were markedly higher incidences of melioidosis and a vastly greater proportion of gentamicin-susceptible *Bp* in the rural interior regions (Belaga, Tatau, and Sungai Asap districts) in comparison to the coastal Bintulu region, which includes Bintulu town.

In our previous study, we showed that all gentamicin-susceptible *Bp* isolates from this region and others in Sarawak belonged to a single multilocus sequence type, ST881, or its single-locus variant ST997, whereas gentamicin-resistant *Bp* isolates included a diversity of sequence types [6]. Whether this still holds true for the isolates in this subsequent study is unknown as the isolates have not been genotyped. Nevertheless, all gentamicin-susceptible isolates that were analyzed were confirmed to possess a single-nucleotide polymorphism mutation in the *amrB* gene through a previously published AS-PCR assay, inferring that these isolates may in fact belong to ST881 or ST997. In a recent melioidosis study in Kapit, another rural district located in central Sarawak (Figure 1), 92% of infections were due to gentamicin-susceptible isolates, consistent with the findings in the rural areas in our study [11]. In other melioidosis-endemic regions in Australia, Asia, and SEA, including peninsular Malaysia, studies of clinical *Bp* isolates have demonstrated usually high levels of strain diversity [12–16]. It is hypothesized that the predominance of gentamicin-susceptible *Bp* seen in rural districts in Sarawak has resulted from expansion of a clonal *Bp* population, with a single efflux pump mutation preventing the efflux of gentamicin that normally occurs in wild-type *Bp*. However, whole genome sequencing of current and future strains from across the region will be required to test this hypothesis as it is noted that several other genetic variations have been described to cause gentamicin susceptibility [17]. Furthermore, to date, the gentamicin-susceptible ST881 found in Sarawak has not been reported from other locations, with the exception of a single case in a Chinese traveler returning from working in the Malaysian jungle [18].

While it is recognized that distinct genetic populations of *Bp* may be spatially clustered and restricted to specific geographical regions [14, 19–21], the reasons for selection and propagation of ST881 to be the predominant genotype in Sarawak remain unknown. Any specific environmental niche for ST881 has yet to be found and indeed environmental sampling in Sarawak to date has been unable to recover ST881, even when using culture media avoiding gentamicin (Y. Podin, unpublished data). The clinical data from this study show no evidence of attenuated virulence with gentamicin-susceptible *Bp*, consistent with a prior study of another gentamicin-susceptible *Bp* that retained full virulence in an acute mouse melioidosis model [17]. It is conceivable that gentamicin-susceptible strains are more infective compared to other *Bp* strains in the Sarawak environment, but a biological reason for this is not apparent. While nonrandom distributions of *Bp* strains in clinical samples compared to those in environmental samples in previous studies have alluded to this possibility of increased strain

infectivity [22, 23], studies from Australia have not found such differential infectivity/virulence between environmental and clinical *Bp* [24].

The restriction of ST881 to Sarawak is in sharp contrast to the other wild-type STs (gentamicin-resistant *Bp*) described from Sarawak, which have been found to be widely dispersed through SEA [6]. This is similar to recent studies from Laos and Myanmar [25, 26], which suggest a dynamic process of more recent possibly anthropogenically driven regional dispersal of *Bp,* in addition to the ancient intercontinental dispersals originally from Australia, where *Bp* is thought to have arisen [12]. The spread of *Bp* in the Americas is considered a more recent phenomenon, with recent evidence suggesting that *Bp* may now be endemic in the United States [4].

That incidence rates are higher in the rural inland areas of Sarawak in comparison to the coastal Bintulu region, and the substantially higher proportion of gentamicin-susceptible *Bp* in the rural areas is likely to reflect a combination of environmental factors and human host behaviors facilitating infection. The rural regions consist of subsistence indigenous populations, plantation workers with ongoing deforestation for crops such as oil palm, and some large-scale construction projects such as the Bakun dam with concomitant environmental changes and population resettlements. While the link between increased melioidosis and environmental perturbation has been long recognized [27], any specific drivers of emergence and propagation of ST881 remain to be elucidated. Higher overall rates of melioidosis in rural populations are also well recognized and reflect increased exposure to the environment in occupational and daily living activities and likely also ingestion of untreated water containing *Bp* [28].

In northern Australia and northeast Thailand, 81% and 75% of melioidosis cases, respectively, are reported to occur during the wet season [29, 30]. This contrasts with the lack of seasonal variation in the incidence of melioidosis in Sarawak seen in this study. This may reflect the contrast between the wet/dry tropics of northern Australia and northeast Thailand, where prolonged dry periods occur over some months, and the rainfall pattern in Sarawak (Figure 2).

In Sarawak, 40% of all patients with melioidosis had no identified risk factors and the rate was 48% in those infected with gentamicin-susceptible *Bp.* Nevertheless, overall, 49% of patients had diabetes as a risk factor, and in 40% of these the diabetes was diagnosed only after admission with melioidosis. The rate of diabetes was consistent with other studies [31] but was significantly lower in those infected with gentamicin-susceptible *Bp* at 42%. In the recent melioidosis study in Kapit, the absence of risk factors was reported in nearly 50% of cases [11], a figure substantially higher than described in most other studies [31]. The lower rate of diabetes and the higher proportion without identified risk factors in those infected with gentamicin-susceptible *Bp* may in part reflect the rural population that accounted for the vast majority of these cases, with a higher exposure burden to environmental *Bp* in the rural areas. Interestingly, these findings correlate well with our previous pediatric melioidosis study, where high incidences of severe and fatal disease were found in children with no risk factors, caused overwhelmingly (97%) by gentamicin-susceptible isolates [32]. One limitation of the present study, however, was that the prevalence of hazardous alcohol use was not determined as most patients did not have data recorded.

Nearly all (98%) patients with melioidosis in Sarawak had acute presentations, and 71% had pneumonia as the presenting diagnosis. Bacteremia and septic shock were found in 88% and 47% of patients, respectively. In other endemic regions, up to 15% of cases have chronic presentations, and pneumonia, recognized as the most common manifestation, is found in 51%–61% of cases [33]. The higher prevalence of acute presentations, pneumonia, bacteremia, and septic shock in Sarawak than in Australia and Thailand may well reflect that those other, less severe cases of melioidosis are going undiagnosed. While undifferentiated fever with positive blood culture and soft tissue infections with culture of *Bp* from aspirates or pus also occurred, genitourinary, neurological, and cutaneous melioidosis were rare or absent. This spectrum of clinical manifestations is similar to that seen elsewhere in SEA, which is well documented to be distinct from that observed in Australia [24].

Sixty percent (51/85) of those who had abdominal imaging were found to have splenic and/or liver abscesses; splenic abscesses (54% of those imaged) were twice as common as liver abscesses. Thus, the spleen is the second most affected organ (after the lungs). Interestingly, the spleen was also found to be one of the commonest affected organs in children with melioidosis in this region [34]. In comparison, splenic abscesses are detected in only 25% and 5% of melioidosis patients in Thailand and Australia, respectively [29, 35]. The high rate of splenic involvement in Sarawak likely reflects the high prevalence of bacteremia, as does multilobar involvement, the most common manifestation of melioidosis pneumonia in Sarawak, noted in 57% of cases. This was similar to findings from Cambodia but contrasted with findings from northern Australia, where multilobar involvement was present in only 30% of cases [36, 37].

Worldwide, mortality from melioidosis has been reported to be between 9% and 70% [33], and the mortality in this study was 35%. The presence of host risk factors and older age have been reported to be the most important determinants of mortality from melioidosis, and in fact, death from melioidosis in those without risk factors is believed to be extremely uncommon if rapid diagnosis, appropriate antibiotics, and state-of-the-art management of sepsis are available [24, 29]. The relatively high mortality in those without risk factors in Sarawak may be due to several reasons. First, delays in diagnosis and initiation of appropriate antibiotics still occur despite the recognition of

central Sarawak as a melioidosis hotspot. Second, although intensive care is available, facilities and expertise are limited compared to those of better-resourced regions. Finally, pneumonia, bacteremia, and septic shock are all associated with increased mortality, and the very high prevalence of these manifestations in those diagnosed with melioidosis in Sarawak underpins the mortality of 35%.

This study has several limitations. First, selective culture media, such as threonine-basal salt solution plus colistin, was not routinely used [38]. This may have resulted in a reduced yield of *Bp* from nonsterile site specimens and led to fewer diagnoses of less severe/localized melioidosis infections. Second, for antibiotic susceptibility, an adaptive interpretation of the CLSI guidelines was used, as CLSI does not provide specific criteria for interpreting disk diffusion tests for *Bp*. Recently the European Committee on Antimicrobial Susceptibility Testing has defined specific disk diffusion zone diameter distributions and epidemiological cutoff values for *Bp* [39, 40]. Nevertheless, the specific interpretation of gentamicin susceptibility for *Bp* isolates from our study remains valid, given the clear separation of susceptible and resistant isolates on both Etest and disk diffusion. Finally, the study is unable to inform on the usefulness or harmfulness of gentamicin for treatment of gentamicin-susceptible *Bp*, as only 1 patient in the study received the antibiotic.

CONCLUSIONS

Two-thirds of melioidosis cases in central Sarawak were caused by gentamicin-susceptible *Bp*, which dominates in the high-incidence rural interior regions. Clinical manifestations and outcomes were the same as for infections with gentamicin-resistant *Bp*. Extremely high rates of bacteremia, pneumonia, splenic abscesses, and septic shock were observed, with overall mortality of 35%. Further studies are required to determine if all gentamicin-susceptible *Bp* infections in Sarawak are clonal and to ascertain their environmental drivers and niches.

Notes,

Acknowledgments. We thank the Director-General of Health, Malaysian Ministry of Health, for his approval and support of this work. We acknowledge the contribution of doctors, nurses, and laboratory technologists of Bintulu Hospital involved in the care of these patients. We thank the directors of Hospital Bintulu and Dr Hock-Hin Chua for the administrative assistance throughout the study. We are grateful to Dr Mohamed Adam Bin Hj Bujang for statistical input, Mary Jiew from the Department of Meteorology (Malaysia) for the rainfall data, and Sing-Jiat Ngu for the help with the figures.

Financial support. The laboratory investigation of this work was partly funded by the Special Funding for Research Institutes Scheme (I02/SpFRI/1426/16/3) awarded to Yuwana Podin by Universiti Malaysia Sarawak.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Hantrakun V, Kongyu S, Klaytong P, et al. Clinical epidemiology of 7126 melioidosis patients in Thailand and the implications for a National Notifiable Diseases Surveillance System. Open Forum Infect Dis **2019**; 6:ofz498.
2. Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol **2016**; 1:15008.
3. Alvarez-Hernandez G, Cruz-Loustaunau D, Ibarra JA, et al. Description of two fatal cases of melioidosis in Mexican children with acute pneumonia: case report. BMC Infect Dis **2021**; 21:204.
4. Cossaboom CM, Marinova-Petkova A, Strysko J, et al. Melioidosis in a resident of Texas with no recent travel history, United States. Emerg Infect Dis **2020**; 26:1295–9.
5. McEniry DW, Gillespie SH, Felmingham D. Susceptibility of *Pseudomonas pseudomallei* to new beta-lactam and aminoglycoside antibiotics. J Antimicrob Chemother **1988**; 21:171–5.
6. Podin Y, Sarovich DS, Price EP, et al. *Burkholderia pseudomallei* isolates from Sarawak, Malaysian Borneo, are predominantly susceptible to aminoglycosides and macrolides. Antimicrob Agents Chemother **2014**; 58:162–6.
7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care **2010**; 33(Suppl 1):S62–9.
8. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract **2012**; 120:c179–84.
9. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med **2013**; 158:825–30.
10. Sarovich DS, Price EP, Webb JR, et al. Variable virulence factors in *Burkholderia pseudomallei* (melioidosis) associated with human disease. PLoS One **2014**; 9:e91682.
11. Toh V, Tee SP, Lee SH. Clinical characteristics and predictors of mortality in patients with melioidosis: the Kapit experience. Trop Med Int Health **2021**; 26:664–71.
12. Chewapreecha C, Holden MT, Vehkala M, et al. Global and regional dissemination and evolution of *Burkholderia pseudomallei*. Nat Microbiol **2017**; 2:16263.
13. Corea EM, Merritt AJ, Ler YH, et al. Sri Lankan National Melioidosis Surveillance Program uncovers a nationwide distribution of invasive melioidosis. Am J Trop Med Hyg **2016**; 94:292–8.
14. McRobb E, Kaestli M, Price EP, et al. Distribution of *Burkholderia pseudomallei* in northern Australia, a land of diversity. Appl Environ Microbiol **2014**; 80:3463–8.
15. Zhu X, Chen H, Li S, et al. Molecular characteristics of *Burkholderia pseudomallei* collected from humans in Hainan, China. Front Microbiol **2020**; 11:778.
16. Zueter AR, Rahman ZA, Abumarzouq M, Harun A. Multilocus sequence types of clinical *Burkholderia pseudomallei* isolates from peninsular Malaysia and their associations with disease outcomes. BMC Infect Dis **2018**; 18:5.
17. Trunck LA, Propst KL, Wuthiekanun V, et al. Molecular basis of rare aminoglycoside susceptibility and pathogenesis of *Burkholderia pseudomallei* clinical isolates from Thailand. PLoS Negl Trop Dis **2009**; 3:e519.
18. Zong Z, Wang X, Deng Y, Zhou T. Misidentification of *Burkholderia pseudomallei* as *Burkholderia cepacia* by the VITEK 2 system. J Med Microbiol **2012**; 61:1483–4.
19. Baker A, Pearson T, Price EP, et al. Molecular phylogeny of *Burkholderia pseudomallei* from a remote region of Papua New Guinea. PLoS One **2011**; 6:e18343.
20. Rachlin A, Mayo M, Webb JR, et al. Whole-genome sequencing of *Burkholderia pseudomallei* from an urban melioidosis hot spot reveals a fine-scale population structure and localised spatial clustering in the environment. Sci Rep **2020**; 10:5443.
21. Sathkumara HD, Merritt AJ, Corea EM, et al. Clinical, bacteriologic, and geographic stratification of melioidosis emerges from the Sri Lankan National Surveillance Program. Am J Trop Med Hyg **2018**; 98:607–15.
22. Chewapreecha C, Mather AE, Harris SR, et al. Genetic variation associated with infection and the environment in the accidental pathogen *Burkholderia pseudomallei*. Commun Biol **2019**; 2:428.
23. Vesaratchavest M, Tumapa S, Day NP, et al. Nonrandom distribution of *Burkholderia pseudomallei* clones in relation to geographical location and virulence. J Clin Microbiol **2006**; 44:2553–7.
24. Currie BJ. Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. Semin Respir Crit Care Med **2015**; 36:111–25.
25. Rachlin A, Luangraj M, Kaestli M, et al. Using land runoff to survey the distribution and genetic diversity of *Burkholderia pseudomallei* in Vientiane, Laos. Appl Environ Microbiol **2020**; 87. doi:10.1128/AEM.02112-20.
26. Webb JR, Win MM, Zin KN, et al. Myanmar *Burkholderia pseudomallei* strains are genetically diverse and originate from Asia with phylogenetic evidence of re-introductions from neighbouring countries. Sci Rep **2020**; 10:16260.

27. Kaestli M, Mayo M, Harrington G, et al. Landscape changes influence the occurrence of the melioidosis bacterium *Burkholderia pseudomallei* in soil in northern Australia. PLoS Negl Trop Dis **2009**; 3:e364.
28. Limmathurotsakul D, Kanoksil M, Wuthiekanun V, et al. Activities of daily living associated with acquisition of melioidosis in northeast Thailand: a matched case-control study. PLoS Negl Trop Dis **2013**; 7:e2072.
29. Currie BJ, Ward L, Cheng AC. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Negl Trop Dis **2010**; 4:e900.
30. Suputtamongkol Y, Hall AJ, Dance DA, et al. The epidemiology of melioidosis in Ubon Ratchatani, northeast Thailand. Int J Epidemiol **1994**; 23:1082–90.
31. Wiersinga WJ, Virk HS, Torres AG, et al. Melioidosis. Nat Rev Dis Primers **2018**; 4:17107.
32. Mohan A, Podin Y, Tai N, et al. Pediatric melioidosis in Sarawak, Malaysia: epidemiological, clinical and microbiological characteristics. PLoS Negl Trop Dis **2017**; 11:e0005650.
33. Gassiep I, Armstrong M, Norton R. Human melioidosis. Clin Microbiol Rev **2020**; 33:e00006-19.
34. Mohan A, Manan K, Tan LS, et al. Detection of spleen abscesses facilitates diagnosis of melioidosis in Malaysian children. Int J Infect Dis **2020**; 98:59–66.
35. Maude RR, Vatcharapreechasakul T, Ariyaprasert P, et al. Prospective observational study of the frequency and features of intra-abdominal abscesses in patients with melioidosis in northeast Thailand. Trans R Soc Trop Med Hyg **2012**; 106:629–31.
36. Meumann EM, Cheng AC, Ward L, Currie BJ. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. Clin Infect Dis **2012**; 54:362–9.
37. Rammaert B, Beauté J, Borand L, et al. Pulmonary melioidosis in Cambodia: a prospective study. BMC Infect Dis **2011**; 11:126.
38. Wuthiekanun V, Smith MD, Dance DA, White NJ. Isolation of *Pseudomonas pseudomallei* from soil in north-eastern Thailand. Trans R Soc Trop Med Hyg **1995**; 89:41–3.
39. Dance DAB, Wuthiekanun V, Baird RW, et al. Interpreting *Burkholderia pseudomallei* disc diffusion susceptibility test results by the EUCAST method. Clin Microbiol Infect **2021**; 27:827–9.
40. Karatuna O, Dance DAB, Matuschek E, et al. *Burkholderia pseudomallei* multi-centre study to establish EUCAST MIC and zone diameter distributions and epidemiological cut-off values [manuscript published online ahead of print 9 July 2020]. Clin Microbiol Infect **2020**. doi:10.1016/j.cmi.2020.07.001.