ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2011, p. 5388–5391 Vol. 55, No. 11
0066-4804/11/$12.00 doi:10.1128/AAC.05517-11

Survey of Antimicrobial Resistance in Clinical *Burkholderia pseudomallei* Isolates over Two Decades in Northeast Thailand▽†

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Received 11 August 2011/Accepted 20 August 2011

A 21-year survey conducted in northeast Thailand of antimicrobial resistance to parenteral antimicrobial drugs used to treat melioidosis identified 24/4,021 (0.6%) patients with one or more isolates resistant to ceftazidime ($n = 8$), amoxicillin-clavulanic acid ($n = 4$), or both drugs ($n = 12$). Two cases were identified at admission, and the remainder were detected a median of 15 days after starting antimicrobial therapy. Resistance to carbapenem drugs was not detected. These findings support the current prescribing recommendations for melioidosis.

Burkholderia pseudomallei is an environmental Gram-negative bacillus and the cause of melioidosis, which is prevalent across much of southeast Asia and northern Australia. Common features include bacteremia, pneumonia, hepatosplenic abscesses, septic arthritis, and skin or soft tissue infections, and mortality is 14 to 43% (6, 12). *B. pseudomallei* is innately resistant to a large number of antimicrobial agents, including all macrolides, all narrow-spectrum cephalosporins, most penicillins, all polymyxins, and the aminoglycosides (13, 14, 16). Although a proportion of *B. pseudomallei* isolates are susceptible to fluoroquinolones by *in vitro* testing (7), clinical evidence indicates that fluoroquinolones are not effective (1, 3, 18). Antimicrobial therapy for melioidosis is divided into an acute phase and an eradication phase (11). The current recommendation for the acute phase is parenteral antimicrobial agents for ≥10 days with ceftazidime (CAZ) or a carbapenem (imipenem [IPM] or meropenem [MEM]), the same agents that are recommended for empirical therapy of suspected cases. Amoxicillin-clavulanic acid (AMC) may be used as an alternative, although it is associated with a higher rate of treatment failure (19). The aim of this study was to perform a 21-year survey of *B. pseudomallei* resistance rates to CAZ, AMC, and IPM or MEM and confirm the appropriateness of the current empirical parenteral regimen.

We identified consecutive patients with culture-confirmed melioidosis presenting to Sappasithiprasong Hospital in Ubon Ratchathani in northeast Thailand between 1 January 1987 and 31 December 2007 as part of ongoing surveillance for clinical and diagnostic trials (2, 4, 17, 19, 22). Samples taken at admission included blood, throat swab, respiratory secretions, pus, urine, and swabs from surface lesions (as appropriate). Laboratory culture and bacterial identification were performed as described previously (21). Repeat blood culture was performed at the end of the first and second week after diagnosis in those patients who remained febrile or developed apparently new foci of infection on therapy. Sites positive for *B. pseudomallei* at admission were sampled weekly until negative. For the purposes of the analysis, clinical specimens obtained prior to and within 3 days of starting parenteral treatment were grouped as "admission" samples.

Susceptibility testing was performed during the study period using the Kirby Bauer disk diffusion method. Interpretative standards for zone sizes are not available for *B. pseudomallei.* We used the Clinical and Laboratory Standards Institute threshold zone sizes for members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* (5): IPM or MEM (≥16 mm, 15 to 14 mm, and ≤13 mm for susceptible, intermediate resistant, and resistant, respectively), CAZ (≥18, 17 to 15, and ≤14 mm, respectively) and AMC (≤18, 17 to 14, and ≤13 mm, respectively). Testing for carbapenems commenced in 1994 and was performed using an imipenem disk between 1994 and 2005 and a meropenem disk thereafter (reflecting local prescribing practice). All isolates with a reduced zone size to any drug that was consistent with intermediate or full resistance were further evaluated by Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Etest was used due to its simplicity and reliability (9, 20, 23). Etest with MEM

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▽ Published ahead of print on 29 August 2011.

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TABLE 1. Details of 24 cases infected with *B. pseudomallei* that were resistant to one or more recommended parenteral antimicrobial drugs for initial therapy

Case	Yr	Strain ID[a]	Specimen type	No. of days after admission that specimen was collected	MIC (mg/liter)[b]			Genotyping result[c]	
					CAZ	AMC	MEM	PFGE banding pattern	MLST
1	1987	316a	Blood	6	2	2/1	0.75		
		316c	Blood	24	**48**	2/1	1	Same as 316a	
2	1988	365a[d]	Urine	2	**12**	**24/12**	1		
		365c	Blood	3	2	2/1	1	Same as 365a	
3	1988	402a	Blood	0	2	2/1	0.5		
		402g	Blood	9	**48**	1/0.5	0.75	Same as 402a	
		402h	Throat swab	9	**24**	1.5/0.75	0.75	Same as 402a	
4	1988	405a[d]	Blood	11	**16**	**32/16**	2	Not performed	
5	1989	490b	Sputum	2	2	2/1	0.5		
		490f	Sputum	24	**512**	2/1	0.75	Same as 490b	
6	1989	533a	Sputum	4	6	4/2	1		
		533eii	Sputum	28	**512**	3/1.5	0.75	Same as 533a	
7	1989	577a	Throat swab	1	1.5	6/3	0.75		
		577b	Blood	8	6	**512/256**	1.5	Same as 577a	
		577ci	Sputum	20	**512**	1.5/0.75	1	Same as 577a	
		577cii	Sputum	20	8	**512/256**	0.75	Same as 577a	
		577d	Sputum	32	6	**64/32**	0.75	Same as 577a	
8	1991	858ai	Blood	3	1.5	2/1	0.75		
		858g	Blood	20	**64**	2/1	1.5	Same as 858ai	
		858d	Synovial fluid	27	**48**	2/1	0.75	Same as 858ai	
		858fi	Blood	27	**64**	2/1	1	Same as 858ai	
9	1992	942a	Blood	0	2	2/1	0.75		
		942dii	Surface swab	6	**64**	**24/12**	2	Same as 942a	
10	1992	956a	Blood	1	3	2/1	0.5		
		956c	Blood	15	**48**	1.5/0.75	0.5	Same as 956a	
11	1992	975a	Sputum	0	1.5	2/1	0.5		All three isolates ST 873
		975ci	Sputum	13	**12**	**16/8**	2	2 bands different from 975a	
		975d	Sputum	17	**32**	2/1	0.5	Same as 975a	
12	1992	979a	Throat swab	0	3	1.5/0.75	0.75		
		979bi	Sputum	7	8	**12/6**	1	Same as 979a	
		979bii	Tracheal suction	31	**512**	4/2	3	Same as 979a	
13	1992	984a	Sputum	0	1	2/1	0.5		
		984d	Sputum	6	8	8/4	1.5	Same as 984a	
		984di	Sputum	6	8	**24/12**	1.5	Same as 984a	
14	1992	995a	Parotid pus	0	2	1.5/0.75	0.38		
		995e	Parotid pus	21	**16**	**24/12**	1	14 bands different from 995a	Different ST from 995a[e]
15	1992	1005a	Sputum	0	2	4/2	0.75		
		1005d	Sputum	12	**12**	**24/12**	1.5	Same as 1005a	
16	1998	2085a	Blood	1	2	2/1	1		
		2085g	Blood	29	6	**12/6**	1.5	Same as 2085a	
17	1999	2374a	Sputum	1	6	3/1.5	1		
		2374b	Sputum	3	1.5	**12/6**	0.5	6 bands different from 2374a	Different ST from 2374a[e]
18	1999	2381a	Throat swab	9	1	1.5/0.75	0.38		
		2381c	Sputum	18	8	**12/6**	3	6 bands different from 2381a	Different ST from 2381a[e]

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TABLE 1—*Continued*

Case	Yr	Strain ID[a]	Specimen type	No. of days after admission that specimen was collected	MIC (mg/liter)[b]			Genotyping result[c]	
					CAZ	AMC	MEM	PFGE banding pattern	MLST
		2381d	Swab	22	1	**12/6**	0.5	6 and 9 bands different from 2381a and 2381c, respectively	Different ST from 2381a and 2381c[e]
19	2001	2690a	Sputum	18	**512**	1.5/0.75	1	Not performed	
20	2003	3013a	Sputum	1	3	3/1.5	0.5		Both isolates ST 874
		3013c	Sputum	19	**32**	**16/8**	2	1 band different from 3013a	
21	2006	3964b	Blood	1	2	2/1	0.75		
		3964c	Tracheal suction	19	**48**	1.5/0.75	1	Same as 3964b	
		3964d	Blood	19	**64**	2/1	0.75	Same as 3964b	
22	2006	4095a	Sputum	1	2	3/1.5	1		
		4095c	Pleural fluid	23	**48**	**48/24**	2	Same as 4095a	
23	2007	4226a	Sputum	1	8	6/3	1		
		4226b	Throat swab	1	**16**	**12/6**	2	Same as 4226a	
		4226c	Sputum	11	**16**	**12/6**	1.5	Same as 4226a	
24	2007	4609a	Sputum	1	1.5	1.5/0.75	0.75		
		4609e	Tracheal suction	22	**24**	**24/12**	2	Same as 4609a	

[a] Strain ID, strain identification.
[b] The interpretive criteria for MICs for the different antimicrobial drugs were as follows: for ceftazidime (CAZ), ≤8, 16, and ≥32 mg/liter for susceptible, intermediate resistant, and resistant, respectively; for amoxicillin-clavulanic acid (AMC), ≤8/4, 16/8, and ≥32/16 mg/liter (amoxicillin value given before the slash and clavulanic acid value given after the slash), respectively; and meropenem (MEM), ≤4, 8, and ≥16 mg/liter, respectively. Nonsusceptible MICs are indicated in boldface type.
[c] Abbreviations: PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; ST, sequence type.
[d] Resistant on admission.
[e] Likely to represent simultaneous infection with more than one strain of *B. pseudomallei*.

was used for carbapenems. The following cutoffs for MICs were used to define for susceptible, intermediate resistant, and resistant: ≤4, 8, and ≥ 16 mg/liter for MEM, respectively; ≤8, 16, and ≥ 32 mg/liter for CAZ, respectively; and ≤8/4, 16/8, and ≥32/16 mg/liter for AMC, respectively.

Bacterial genotyping was performed using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) as described previously (8). *B. pseudomallei* isolates with resistance to one or more antimicrobials underwent PFGE, together with at least one susceptible isolate from the same patient. Typing was not performed when paired isolates were not available. PFGE banding patterns were compared for susceptible and resistant isolates from the same patient. Isolates from the same patient with identical banding patterns were regarded as genotypically indistinguishable, but isolates with one or more bands different were further characterized using a two-stage MLST protocol. The two most variable MLST loci (*gmhD* and *narK*) were sequenced and compared. If one or both loci differed in the pair of isolates under consideration, this was interpreted as indicating different (undefined) sequence types (ST). In the event that the two isolates had matching alleles at these two loci, the five remaining loci were sequenced to determine the full 7-gene profile, and the sequence type was determined using the *B. pseudomallei* MLST website (http://bpseudomallei.mlst.net).

Isolates from the same patient with an identical PFGE banding pattern or ST were regarded as originating from the same clone. If the isolates were cultured during two distinct episodes, this was considered to represent relapse. Patients with two or more genotypes identified during a single episode were defined as having mixed infection, and those with different genotypes isolated in two distinct episodes were defined as having reinfection.

B. pseudomallei was isolated from 4,030 patients presenting for the first time with melioidosis. Isolates from nine patients failed to grow on Mueller-Hilton agar (used for susceptibility testing) and were excluded from further analysis. Twenty-four patients (24/4,021 [0.6%]) had one or more cultures positive for *B. pseudomallei* with a reduced zone size on disk diffusion testing to CAZ ($n = 8$), AMC ($n = 4$), or both CAZ and AMC ($n = 12$). This was confirmed by Etest (Table 1). No carbapenem resistance was documented. In-hospital mortality was 54% (13 of 24 cases). Primary resistance to CAZ and/or AMC (resistance identified at admission on first episode) was very rare and occurred in only two patients (2/4,021 [0.05%]; resistance to CAZ in both cases). Neither patient (cases 2 and 4) had a documented history of antimicrobial consumption prior to specimen collection. A further patient (case 19) had a first isolate cultured by us on day 18 of hospital admission that was resistant to CAZ; this patient had received ceftazidime for 5 days prior to referral to Sappasithiprasong Hospital, but we did not have the original isolate to determine whether this was primary resistance. In the remaining 21 cases, resistance emerged during antimicrobial therapy (Table 1), 20 (95%) of whom were on the drug to which resistance developed. The median duration of treatment with ceftazidime and/or AMC in

these 21 cases prior to detection of resistance was 15 days (interquartile range [IQR], 7 to 21 days; range, 3 to 33 days).

Genotyping was used to compare the initial susceptible and emergent resistant isolate pairs. An identical PFGE banding pattern for the initial susceptible strain and the emergent resistant strain was observed in 16 of 21 patients. Of the remaining 5 patients with isolates that differed by one or more bands, 2 patients had the initial susceptible strain and the emergent resistant strain sharing the same ST (cases 11 and 20), but 3 patients did not (cases 14, 17, and 18). These results suggest that in 18 of 21 cases (86%), the resistant subpopulation arose from their own founder inoculum, but that in 3 patients, infection was polyclonal (caused by more than one strain of *B. pseudomallei*).

A total of 196 patients presented with recurrent infection during the study period, of which 5 (2.6%) had isolates cultured that were resistant to AMC ($n = 4$) or both CAZ and AMC ($n = 1$). None of these patients had a resistant isolate cultured during their primary episode. The four isolates with resistance to AMC were identified at the time of readmission, and all four patients had been treated previously with this drug. The single isolate with resistance to CAZ and AMC was detected after treatment with IPM for 14 days and following isolation at presentation of an isolate that was susceptible to both drugs. Genotyping demonstrated that 3 patients had relapse and 2 had reinfection.

Our findings indicate that *B. pseudomallei* associated with human infection that has primary resistance to CAZ or AMC is rare, which supports the continued use of these drugs as empirical or first- and second-line therapy for both primary and recurrent melioidosis infection in Thailand. The rate of primary resistance to ceftazidime that we observed (0.05% [2/4,225]) is comparable to that observed in Malaysia (0.5% [1/182]) and Australia (0.6% [1/170]) (10, 15). The rate of primary resistance to CAZ or AMC in patients presenting with recurrent infection is higher (2.0% [4/196]) but is still uncommon, and we propose that empirical CAZ remains an acceptable choice pending culture results. We recommend that patients who do not respond to CAZ therapy have repeat cultures taken and treatment switched to a carbapenem drug.

We gratefully acknowledge the support provided by staff at the Mahidol-Oxford Tropical Medicine Research Unit and Sapprasithiprasong Hospital.

This study was supported by The Wellcome Trust. G.C.K.W.K. is supported by a Wellcome Trust Clinical Research Training Fellowship. S.J.P. is supported by the NIHR Cambridge Biomedical Research Centre.

REFERENCES

1. **Chaowagul, W., Y. Suputtamongkul, M. D. Smith, and N. J. White.** 1997. Oral fluoroquinolones for maintenance treatment of melioidosis. Trans. R. Soc. Trop. Med. Hyg. **91:**599–601.
2. **Cheng, A. C., et al.** 2007. A randomized controlled trial of granulocyte colony-stimulating factor for the treatment of severe sepsis due to melioidosis in Thailand. Clin. Infect. Dis. **45:**308–314.
3. **Chetchotisakd, P., W. Chaowagul, P. Mootsikapun, D. Budhsarawong, and B. Thinkamrop.** 2001. Maintenance therapy of melioidosis with ciprofloxacin plus azithromycin compared with cotrimoxazole plus doxycycline. Am. J. Trop. Med. Hyg. **64:**24–27.
4. **Chierakul, W., et al.** 2005. Two randomized controlled trials of ceftazidime alone versus ceftazidime in combination with trimethoprim-sulfamethoxazole for the treatment of severe melioidosis. Clin. Infect. Dis. **41:**1105–1113.
5. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. M100-S20, vol. 30(1), p. 40-53. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Currie, B. J., L. Ward, and A. C. Cheng.** 2010. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Negl. Trop. Dis. **4:**e900.
7. **Dance, D. A., V. Wuthiekanun, W. Chaowagul, and N. J. White.** 1989. The antimicrobial susceptibility of *Pseudomonas pseudomallei*. Emergence of resistance in vitro and during treatment. J. Antimicrob. Chemother. **24:**295–309.
8. **Godoy, D., et al.** 2003. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J. Clin. Microbiol. **41:**2068–2079.
9. **Inglis, T. J., F. Rodrigues, P. Rigby, R. Norton, and B. J. Currie.** 2004. Comparison of the susceptibilities of *Burkholderia pseudomallei* to meropenem and ceftazidime by conventional and intracellular methods. Antimicrob. Agents Chemother. **48:**2999–3005.
10. **Jenney, A. W., G. Lum, D. A. Fisher, and B. J. Currie.** 2001. Antibiotic susceptibility of *Burkholderia pseudomallei* from tropical northern Australia and implications for therapy of melioidosis. Int. J. Antimicrob. Agents **17:**109–113.
11. **Limmathurotsakul, D., and S. J. Peacock.** 2011. Melioidosis: a clinical overview. Br. Med. Bull. **99:**125–139.
12. **Limmathurotsakul, D., et al.** 2010. Increasing incidence of human melioidosis in Northeast Thailand. Am. J. Trop. Med. Hyg. **82:**1113–1117.
13. **Livermore, D. M., P. Y. Chau, A. I. Wong, and Y. K. Leung.** 1987. β-Lactamase of *Pseudomonas pseudomallei* and its contribution to antibiotic resistance. J. Antimicrob. Chemother. **20:**313–321.
14. **Moore, R. A., D. DeShazer, S. Reckseidler, A. Weissman, and D. E. Woods.** 1999. Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. Antimicrob. Agents Chemother. **43:**465–470.
15. **Sam, I. C., K. H. See, and S. D. Puthucheary.** 2009. Variations in ceftazidime and amoxicillin-clavulanate susceptibilities within a clonal infection of *Burkholderia pseudomallei*. J. Clin. Microbiol. **47:**1556–1558.
16. **Schweizer, H. P.** 2003. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. Genet. Mol. Res. **2:**48–62.
17. **Simpson, A. J., et al.** 1999. Comparison of imipenem and ceftazidime as therapy for severe melioidosis. Clin. Infect. Dis. **29:**381–387.
18. **Steward, J., et al.** 2005. Comparison of gatifloxacin, moxifloxacin and ciprofloxacin for treatment of experimental Burkholderia pseudomallei infection. J. Antimicrob. Chemother. **55:**523–527.
19. **Suputtamongkol, Y., et al.** 1994. Ceftazidime vs. amoxicillin/clavulanate in the treatment of severe melioidosis. Clin. Infect. Dis. **19:**846–853.
20. **Thamlikitkul, V., and S. Trakulsomboon.** 2009. In vitro activity of doripenem against *Burkholderia pseudomallei*. Antimicrob. Agents Chemother. **53:**3115–3117.
21. **Walsh, A. L., and V. Wuthiekanun.** 1996. The laboratory diagnosis of melioidosis. Br. J. Biomed. Sci. **53:**249–253.
22. **White, N. J., et al.** 1989. Halving of mortality of severe melioidosis by ceftazidime. Lancet **ii:**697–701.
23. **Wuthiekanun, V., et al.** 2005. Trimethoprim/sulfamethoxazole resistance in clinical isolates of *Burkholderia pseudomallei*. J. Antimicrob. Chemother. **55:**1029–1031.