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Recurrent Melioidosis in the Darwin Prospective Melioidosis Study: Improving Therapies Mean that Relapse Cases Are Now Rare

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The Darwin Prospective Melioidosis Study has documented 785 melioidosis cases over 23 years. Recurrent melioidosis occurred in 39/679 (5.7%) patients surviving initial infection; 29 patients suffered relapse of the original infection, and 10 presented with a new *Burkholderia pseudomallei* infection. With improved therapy, relapse has become rare in recent years.

Burkholderia pseudomallei, the causative agent of melioidosis, is endemic to the environment of Southeast Asia and northern Australia. Almost all *B. pseudomallei* infections are caused by environmental exposure to contaminated water or soils, with the commonest route being skin inoculation or, less frequently, inhalation or ingestion (1). Melioidosis can present as an acute, subacute, chronic, or recurrent disease and is fatal in between 10 and 50% of cases, depending on geographical region (2). Melioidosis treatment is protracted, and recurrent disease is a well-recognized concern, being documented for 13 to 23% of patients in Thailand (3–7) and in 6% of patients in Australia (3, 8, 9). Recurrent melioidosis can result either from relapse due to failure to clear an infection or from reinfection with a new *B. pseudomallei* strain.

Since 1 October 1989, the ongoing Darwin Prospective Melioidosis Study has documented all melioidosis cases in the tropical north of the Northern Territory of Australia, with the vast majority of cases managed at Royal Darwin Hospital in the capital city of Darwin (12.5° S). In the 23 years until 30 September 2012, there were 785 cases of culture-confirmed melioidosis, with 106 (13.5%) dying from their initial infection. Thirty-nine (5.7%) of the 679 survivors have subsequently presented with recurrent melioidosis (Table 1). We define recurrent melioidosis as culture-confirmed melioidosis occurring in a patient who re-presents following the due date for completion of their planned antibiotic therapy (10). Therapy consists of a minimum 2 weeks of intravenous antibiotics followed by a minimum 3 months of oral eradication therapy (2). Patients re-presenting during this period of therapy are considered to have recrudescent rather than recurrent melioidosis and have been excluded from our analysis. To classify recurrent melioidosis cases as either relapse or reinfection, both the initial and subsequent *B. pseudomallei* isolates were subjected to multilocus sequence typing (MLST) (11). Consecutive isolates were available for all but three cases (Table 1). This study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies School of Health Research (HREC 02/38).

Based on MLST and clinical factors, 29 (74%) of 39 recurrent melioidosis cases were attributed to relapse. Of these 29, 26 had identical sequence types (STs) for initial and recurrent isolates. Paired isolates were not available for 2 of the 29 cases (identification numbers [IDs] 121 and 238), but their time course and clinical history supported relapse (Table 1). An additional patient (ID 77), previously reported by Haase and coworkers, had discordant strains according to MLST but was thought to have relapsed from an initial infection with multiple strains (9). Of the 29 relapse cases, two patients relapsed twice, with one of these patients then presenting a third time with a fatal new infection with a different *B. pseudomallei* ST (i.e., reinfection after two relapses). Recurrent melioidosis in the remaining 10 patients (26%) was attributed to reinfection. Of these, 9 had discordant STs between initial and recurrent isolates. Paired isolates were not available for the remaining patient (ID 411), but the protracted time interval (6.6 years) between infections strongly supported reinfection.

The median time from first to second admission for relapse cases was 285 days (9.4 months), with an interquartile range of 416 days (~14 months) (range, 3.3 to 28 months). For reinfection cases, the median time between admissions was 1,643 days (~54 months), with an interquartile range of 1,158 days (~38 months) (range, 10.2 to 169.4 months). We constructed a Kaplan-Meier time-to-event curve analysis using the software program Stata version 12 (Stata Corporation, Texas) of relapse versus reinfection melioidosis patients, which demonstrated that reinfection cases show increased time between disease presentations compared with relapse patients (Fig. 1). Previous studies have reported median time spans for relapsed melioidosis of between 6 and 8 months, although it should be noted that studies conducted in Thailand have included some patients with very early relapse while still on therapy, which we would define as recrudescent melioidosis (3, 8). Despite the difference in case definitions, the increased time span for reinfection compared with relapse cases seen in our study is consistent with findings from Thailand (3), indicating that time is an important determinant of the nature of recurrent melioidosis.

Over the past decade, we have increasingly seen a reversal of attribution for recurrent melioidosis from predominantly relapse to predominantly reinfection. Of 375 melioidosis patients admitted prior to 30 September 2003, 24 (6.4%) have subsequently relapsed, in comparison to only 5 of 410 patients

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TABLE 1 Relapse and reinfection cases from the Darwin Prospective Melioidosis Study, 1 October 1989 through 30 September 2012[a]

Patient ID	Comment	Date of admission[c]	MSHR ID	No. of days between admissions	MLST
21	Relapse	Jan. 91	73	110	135
		May 91	99		135
24	Relapse	Feb. 91	80	203	480
		Sept. 91	118		480
52	Reinfection	Apr. 92	122	426	266
		June 93	223		452
77	Multiple infection—relapse	Jan. 94	240	244	453
		Sept. 94	335		792
81	Reinfection	Feb. 94	262	1,764	678
		Dec. 98	686		Not 678[b]
84	Relapse	Feb. 94	258	618	457
		Oct. 95	385		457
94	Relapse	Mar. 94	281	205	109
		Oct. 94	339		109
107	Relapse	Dec. 94	348	139	36
		May 95	377		36
109	Reinfection	Jan. 95	349	343	461
		Dec. 95	605		109
116	Relapse	Feb. 95	364	835	429
		June 97	1203		429
121	Missing paired isolate; classed as relapse due to time frame and disease presentation	May 95	668	109	NA
		Aug. 95	NA		NA
126	Relapse	Dec. 95	392	610	132
		Aug. 97	501		132
135	Relapse	Feb. 96	419	285	717
		Dec. 96	459		717
136	Reinfection	Mar. 96	420	5,152	36
		Apr. 10	3796		327
175	Relapse	Mar. 97	493	181	586
		Sept. 97	524		586
179	Relapse	Apr. 97	492	247	114
		Dec. 97	647		114
	2nd relapse	Feb. 00	934	763	114
	Reinfection	Apr. 05	2053	1,890	Not 114[b]
182	Relapse	Sept. 97	527	331	269
		Aug. 98	1202		269
208	Relapse	Mar. 98	559	657	109
	2nd relapse	Dec. 99	888		109
		Mar. 02	1390	805	109
211	Relapse	Mar. 98	628	852	327
		July 00	1047		327
215	Relapse	Mar. 98	663	210	36
		Oct. 98	918		36
216	Relapse	Mar. 98	634	309	128
		Jan. 99	795		128
218	Relapse	Apr. 98	641	668	729
		Feb. 00	932		729
238	Missing paired isolate; classed as relapse due to time frame and disease presentation	Dec. 98	867	237	103
		Aug. 99	NA		NA
259	Reinfection	Feb. 99	739	2175	576
		Jan. 05	1943		752
272	Relapse	Mar. 99	868	140	735
		Aug. 99	871		735
274	Relapse	Apr. 99	848	118	434
		Aug. 99	869		434
285	Relapse	Dec. 99	896	101	118
		Apr. 00	978		118
290	Reinfection	Jan. 00	912	1,521	337
		Mar. 04	1828		109
	2nd reinfection	Mar. 08	3029	1,470	333

(Continued on following page)

TABLE 1 (Continued)

Patient ID	Comment	Date of admission[c]	MSHR ID	No. of days between admissions	MLST
312	Relapse	May 00	1034	202	795
		Dec. 00	1068		795
316	Relapse	Sept. 00	1050	245	109
		May 01	1223		109
411	Missing paired isolate; classed as reinfection due to time frame	Mar. 04	1813	2,401	749
		Oct. 10	NA		NA
436	Reinfection	Feb. 05	1951	1,906	753
		Apr. 10	3919		902
448	Relapse	May 05	2077	339	109
		Apr. 06	2261		109
473	Relapse	Apr. 06	2255	413	798
		June 07	2595		798
480	Reinfection	May 06	2435	1,340	36
		Jan. 10	3694		279
482	Relapse	July 06	2406	458	109
		Oct. 07	2861		109
544	Relapse	Dec. 08	3271	686	279
		Oct. 10	4383		279
613	Relapse	Feb. 10	3705	492	811
		June 11	5162		811
703	Reinfection	Feb. 11	4694	311	466
		Dec. 11	5848		553

[a] Abbreviations: MSHR, Menzies School of Health Research; MLST, multilocus sequence typing; NA, not applicable. Relapse melioidosis cases are shaded for clarity.
[b] Sequence type differed from that of original strain by at least two MLST loci.
[c] Month and last two digits of year.

(1.2%) admitted from 1 October 2003 to 30 September 2012 (Fisher's exact test, $P = <0.001$). The observed decline of relapsed melioidosis is most likely due to improved use of efficacious antimicrobials, most notably a lengthened intravenous treatment phase for complex cases. Indeed, almost half of our melioidosis patients now receive at least 4 weeks of intravenous ceftazidime and/or meropenem for the primary treatment phase, with antibiotic choice and duration based on disease presentation and severity (2). For the last 3 years of our study (until 30 September 2012), we have treated 252 melioidosis patients, of which 29 cases (11.5%) were fatal. To date there has been only one episode of relapse in these 223 survivors. These data suggest that current antibiotic regimens are now truly eradicating *B. pseudomallei* infection in patients with melioidosis.

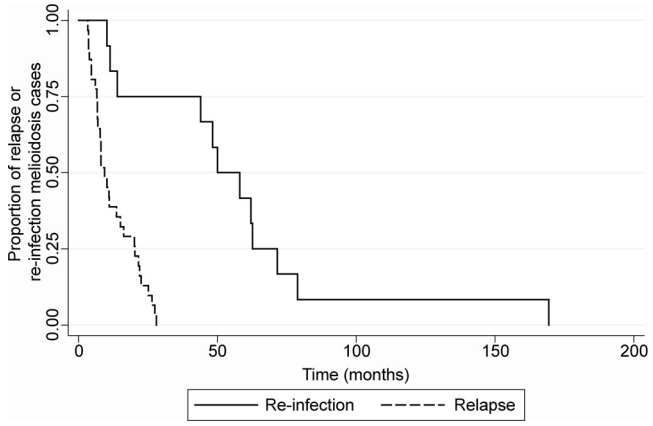

FIG 1 Kaplan-Meier time-to-event curve analysis of relapse versus reinfection melioidosis patients within the Darwin Prospective Melioidosis Study. Thirty-nine episodes of recurrent melioidosis occurred over the time frame of this study; 29 episodes were due to relapse of infection, and 10 were attributed to a new infection with *B. pseudomallei*. Patients presenting with reinfection are likely to have an increased time between disease presentations compared with that for relapse patients.

We recognize that a limitation of our study is the assumption that in all but one instance, individual infections are not caused by multiple *B. pseudomallei* strains. It is also possible that patients can be reinfected with a *B. pseudomallei* strain with an ST identical to that of their primary isolate, resulting in misattribution to relapse. However, the diversity of STs observed in the Northern Territory (12) and the increasing rarity of relapse cases seen in our study support the notion that reinfection with an identical ST would be an infrequent occurrence. More highly resolving molecular fingerprinting methods, such as whole-genome sequencing, would be required to differentiate such scenarios. Finally, we acknowledge that relapse may still occur in melioidosis patients diagnosed toward the end of our study, although it is now more than 16 months since the last case was admitted (June 2012).

Collectively, our data show that recurrent melioidosis in northern Australia is in decline and is now due predominantly to reinfection with a new strain of *B. pseudomallei* rather than to relapse with the original strain. The decreased rate of relapse cases within the Darwin Prospective Melioidosis Study over recent years can be attributed to improved antibiotic therapy and in particular prolongation of the intravenous phase.

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