CASE REPORT: RECOVERY FROM PERSISTENT SEPTICEMIC MELIOIDOSIS

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Abstract. Septicemic melioidosis is often fatal despite treatment with antibiotics such as ceftazidime to which Burkholderia pseudomallei, the causal pathogen, is sensitive in vitro. We report a near-fatal case of septicemic melioidosis with persistent B. pseudomallei bacteremia despite intravenous ceftazidime in which combination therapy with meropenem and ciprofloxacin, splenectomy and correction of metabolic acidosis allowed for hospital discharge. The choice of antibiotic agents was supported by intracellular minimum inhibitory concentration analysis using B. pseudomallei co-culture in Acanthamoeba trophozoites. The patient’s B. pseudomallei isolates were indistinguishable by pulsed-field gel electrophoresis from clinical and environmental isolates previously analyzed during investigation of a Western Australian melioidosis outbreak. A combination of antibiotics known to possess intracellular activity against B. pseudomallei, surgery and supportive critical care may provide a means of improving the probability of survival in persistent septicemic melioidosis.

INTRODUCTION

Melioidosis is a potentially fatal infection that is endemic in the tropical north of Australia.1 The severity of disease and risk of mortality is highest in persons with underlying chronic diseases such as diabetes mellitus and chronic renal failure, both of which are common in indigenous Australian communities.2,3 Acute septicemic melioidosis has a mortality of at least 34% under clinical trial conditions with ceftazidine, the current treatment of choice.4 However, the mortality of septicemic disease has been reported to be higher despite access to tertiary medical care and may exceed 90%.5

Burkholderia pseudomallei, the causative agent of melioidosis, is unusual in its ability to persist in macrophages and cause late onset septicemic infection after long disease-free intervals.6,7 Moreover, around 23% survivors of a first infection will subsequently develop a second, delayed onset infection despite apparently successful treatment.8 In this report, 44% of patients relapsed with septicaemia and 27% died. The principal therapeutic challenge in acute infection is to further reduce the immediate risk of fatal outcome. The risk of relapse in survivors presents a second major challenge. Therapeutic strategies have developed two distinct phases, targeted respectively at clinical resolution of acute infection and eradication of residual intracellular infection, to prevent relapse. In the above study relapses were more common following eight weeks or less of single agent therapy with amoxicillin/clavulanic acid than with a combination of chloramphenicol, doxycycline and co-trimoxazole for a longer period. Recent attempts to improve the outcome of initial therapy included a trial of the carbapenem antibiotic imipenem.9

We report a case of persistent septicemic melioidosis who recovered despite an apparently hopeless prognosis through a combination of intensive care, antibiotic therapy optimized by advanced laboratory techniques, and surgical intervention.

CASE REPORT

A 27 year old Aboriginal woman was admitted to hospital in the remote north of Western Australia with acute pneumonia. She had been unwell for three days prior to admission with a cough productive of yellow sputum with associated left lower pleuritic chest pain and shortness of breath, accompanied by fever, rigors, and night sweats. She had been residing in an area of known endemicity for melioidosis, had non-insulin dependent diabetes mellitus and was a heavy alcohol user.

Examination showed temperature of 39.2°C, blood pressure of 105/60 and a respiratory rate of 22/min. There was diminished air entry at the left lung base and moderate epigastric tenderness.

Investigations showed an elevated left hemidiaphragm on chest x-ray (Figure 1), normal electrolytes and renal function, total protein 85 g/l (60–80), albumin 25 g/l (35–50), alkaline phosphatase 493 u/L (35–135) and a normal ALT and AST. The C-reactive protein was 181 mg/l (<10). Complete blood count showed WBC 13.8 × 10⁹/l (4–11), hemoglobin 110 g/l (115–160), and an ESR of 120 mm/hr (<20).

She was given ceftriaxone and metronidazole initially with some improvement. Ceftazidime was substituted for ceftriaxone on day 3 when blood cultures grew Burkholderia pseudomallei. Despite the initial improvement her condition began to deteriorate and on day 9 she was transferred to this hospital by the Royal Flying Doctor Service.

On examination she was afebrile but appeared unwell and in distress. The chest was clear. The abdomen was distended, particularly in the right upper quadrant. A 2 × 1 cm tender lump was noted in the right calf. Meropenem 1 gram three times daily was substituted for ceftazidime and the metronidazole was discontinued. An abdominal CT scan (Figure 2) showed multiple collections in an enlarged spleen and small hepatic lesions.

Fever continued. At laparotomy the following day multiple splenic abscesses extending to the tail of the pancreas were found. The liver was enlarged and contained small abscesses in both right and left lobes. Splenectomy and distal pancurectomy were performed. Gross examination showed an enlarged spleen weighing 364 grams (Figure 3). Numerous abscess cavities were present in the spleen. In addition there were several areas of ischemic necrosis related to thrombosis in branches of the splenic artery (Figure 4). Histological examination confirmed the presence of multiple abscesses containing central purulent or fibrinous material, sur-
rounded by a rim of inflammatory cells including neutrophils and macrophages, many with an epithelioid appearance. Well-formed granulomas were not a feature although giant cells were identified scattered within the cellular exudate. Several arteries within the vicinity of the abscesses were occluded by thrombus with related areas of ischaemic necrosis. No organisms were identified on a tissue Gram stain. She was transferred to the Intensive Care Unit post-op-
eratively. Her clinical course was stormy, complicated by adult respiratory distress syndrome necessitating tracheostomy and by septic arthritis of her right knee and left ankle. *Burkholderia pseudomallei* was cultured from both sites. A further laparotomy was also required to remove abdominal packs and perform abdominal washout.

She was eventually discharged to the ward on day 22 still on meropenem in conjunction with fluconazole and ciprofloxacin. Bone scan confirmed osteomyelitis of the proximal right tibia and left talus.

She improved slowly and on day 26 her lines and tracheostomy were removed and she was maintained on oral ciprofloxacin, doxycycline, and trimethoprim/sulfamethoxazole. On day 32 she was discharged to her regional hospital for rehabilitation with a plan to treat with combination oral therapy for a total of six months.

**Susceptibility testing.** Minimum inhibitory concentrations of ceftazidime, imipenem, trimethoprim-sulfamethoxazole and ciprofloxacin were determined by E-strip (AB Biotest, Sweden) according to the manufacturer’s instructions (Table 1). Intracellular M.I.C.s were estimated using an amebic co-culture technique in which *Acanthamoeba astronyxis* trophozoites were used as a macrophage surrogate. In brief, $10^7$ colony forming units/mL *B. pseudomallei* cells in mid-log phase were added to $10^5$/mL amebic trophozoites and incubated at $20^\circ$C for 60 minutes to allow endocytosis of bacteria. Freshly reconstituted antibiotic was then added to a final concentration of 0.1, 1.0, 10.0, and 100 $\mu$g/mL. The suspension was mixed and incubated at $20^\circ$C for a further 60 minutes before centrifuging at $100 \times g$ for 5 minutes, discarding the supernatant and reconstituting the pelleted cells in 0.89% sodium chloride. Co-culture preparations were examined for intracellular bacteria by phase contrast microscopy at a magnification of 400 × immediately and after 24h incubation at $20^\circ$C (Figure 5). As a further control, *B. pseudomallei* type culture strain NCTC 10276 was tested in parallel with the patient’s strain at every stage.

**Molecular epidemiology.** Isolates from consecutive blood cultures taken over 10 days, joint aspirates and the patient’s spleen were compared with isolates from two septicemic patients in the original outbreak and two environmental isolates previously matched with the outbreak strain. A DNA macrorestriction fragment length polymorphism analysis was made using the protocol previously employed in the outbreak investigation. In short, bacterial DNA was extracted with the enzyme XbaI after treatment with proteinase K in a double extraction, each stage lasting 18 hr. Digests were then subjected to pulsed field gel electrophoresis. The finished gel was scanned and analysed using GelCompar (BioRad, CA) (Figure 6).

**DISCUSSION**

Ceftazidime has been regarded as the treatment of choice for septicemic melioidosis since publication of a prospective randomized clinical trial that showed a reduction in mortality to 34% from much higher figures with previously used regimens. However a mortality rate of 34% is still unacceptably high, particularly when around one-fourth of survivors are at risk of a septicemic recurrence. Alternative therapeutic approaches have been sought but, as yet, no regimen has been shown to produce a significant improvement in overall survival.

A prospective trial conducted in Thailand where melioidosis is most common indicated that imipenem, a carbapenem antibiotic, was at least as effective as ceftazidime dur-
Figure 4. Microscopic section of spleen; H&E × 200. The edge of an abscess cavity is seen (top right), surrounded by a zone of epithelioid histiocytes. Lymphocytes, histiocytes and macrophages infiltrate adjacent fibroblastic tissue (lower half).

Table 1

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>WAo97</th>
<th>NCTC 10276</th>
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</tr>
<tr>
<td>Imipenem</td>
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<td>4.0</td>
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<td>Co-trimoxazole</td>
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Intracellular MIC (μg/mL)

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<th>24 hr</th>
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</thead>
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<td>&gt;100</td>
</tr>
<tr>
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<tr>
<td>Co-trimoxazole</td>
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MIC = minimum inhibitory concentration.
WAo97 = patient’s culture strain of Burkholderia pseudomallei.
NCTC 10276 = B. pseudomallei-type culture strain.

Persistently septicemic melioidosis

Imipenem-treated patients were significantly less likely to fail therapy after 48 h, though this did not translate to improved overall survival. Imipenem was judged to be a satisfactory substitute for ceftazidime. Since relapsing infection may occur up to several decades after exposure, it may be some years before it is clear whether carbapenem therapy has made any difference to long-term outcomes in melioidosis. A more recent clinical trial comparing B. pseudomallei antibiotic-induced endotoxin release by ceftazidime with imipenem failed to show a difference in mortality between the two arms, though endotoxin release was higher in the ceftazidime treated group. The use of imipenem in the treatment of acute melioidosis was recently reported by another group.

Unfortunately, serious neurological adverse effects have been reported during imipenem use in critically ill patients. Meropenem has therefore replaced imipenem as the carbapenem of choice in a critical care setting. Although meropenem may have been used to treat patients with septicemic melioidosis, its use in human B. pseudomallei infection has not previously been reported. Faced with a persistently septicemic patient receiving optimal ceftazidime therapy, we were forced to look for an alternative regimen. Intravenous
Figure 5. Superimposed differential interference contrast and confocal laser scanning microscope images of *Burkholderia pseudomallei* cells after phagocytosis by *Acanthamoeba astronyxis* trophozoite (×2000). A large empty vacuole is present at the lower right of the amebic cell. Towards the bottom of the cell is a vacuole containing four bacteria, two of which appear bright white and in the same plane as adjacent amebic cytoplasm.

Figure 6. Pulsed-field gel electrophoresis of *Xba I* DNA macrorestriction digest of patient’s isolates alongside those from other patients in the WA melioidosis outbreak and environmental isolates of *Burkholderia pseudomallei*. The accompanying dendrogram shows the percent relatedness of isolates. From top to bottom, the lanes contain digest of isolates from: 1) the last fatal case in the outbreak cluster (WKo97, also known as WACC 56); 2) a late onset case epidemiologically linked to the original cluster; 3) the tap water isolate recovered during the outbreak; 4) the aerator isolate recovered during follow-up investigations into the environmental source of the outbreak, 5–9) isolates from two blood cultures more than a week apart, sputum, spleen and a surface exudate swab (knee); and 10) an isolate from a patient from the outbreak community who was diagnosed more than 8 months before the principal case cluster.
meropenem was commenced, and co-trimoxazole added on the basis of its effect in less severe *B. pseudomallei* infection. Ciprofloxacin was not used at this stage since recent publications have been critical of its use in both the acute and convalescent (or maintenance) settings. The problem of using beta-lactam antibiotics for treatment of intracellular bacterial infections has been reviewed elsewhere. There is little reason to believe that ceftazidime should be more active against intracellular *B. pseudomallei* than any other beta-lactam. However, it is surprising that a quinolone antibiotic with good intracellular penetration and intracellular anti-pseudomonal activity should be disappointing in clinical use for patients with melioidosis. Whatever the mechanism of functional resistance, conventional antibiotic susceptibility testing appears to be poor a predictor of intracellular antibacterial activity, as was seen in this case where breakpoint testing and e-test MIC did not explain treatment failure.

Our *in vitro* test of intracellular antibiotic activity using *Acanthamoeba* trophozoites as a macrophage surrogate was based on previous experience with *B. pseudomallei/Acanthamoeba* co-culture. The results confirmed an absence of intracellular ceftazidime activity at clinically sustainable concentrations but showed meropenem to have an inhibitory effect on intracellular *B. pseudomallei*. Co-trimoxazole had no useful intracellular effect when used alone. Ciprofloxacin had an intracellular effect at higher concentrations and was therefore substituted for co-trimoxazole. The effect of the combination of meropenem and ciprofloxacin was better than any other treatment of the *B. pseudomallei/Acanthamoeba* co-culture. As the patient’s splenectomy was performed shortly after the addition of intravenous ciprofloxacin to meropenem, we cannot be certain that the patient’s improvement was directly caused by the change of antimicrobial chemotherapy. Nevertheless, the patient progressed rapidly from a moribund condition with persistent *B. pseudomallei* bacteremia to eventual discharge from hospital with a sterile bloodstream. A favorable clinical outcome despite an apparently poor prognosis suggests that this antibiotic combination should be compared with ceftazidime in a prospective randomized clinical trial.

This case raises further questions regarding the biology of interactions between pathogen and host. While ceftazidime was shown to have an *in vitro* inhibitory effect on the *B. pseudomallei* clinical isolate and a type culture strain, the persistent bacteremia suggests a persistent sequestered focus of infection from which re-seeding of the bloodstream occurred. At a cellular level *B. pseudomallei*-infected macrophages could have played this role, and on a larger scale the splenic infection may have acted similarly. Since ceftazidime acts preferentially on replicating *B. pseudomallei* in an extracellular location, prolonged intravenous therapy of persistent bacteremia would be expected to give intracellular bacteria a survival advantage, increasing the risk of septicemic relapse at a later date. It therefore follows that optimal therapy can only be achieved by a combination of extracellular and intracellular bacterial eradication, as was attempted in this case.

The metabolic condition of the host at the time of bacteremia has a substantial bearing on the immediate prognosis. The majority of septicemic melioidosis occurs in patients with diabetes mellitus, chronic renal failure, or heavy alcohol use. All three of these known underlying risk factors may interfere with phagocyte function. Restoration of a normal plasma glucose might be expected to assist phagocyte function, and correction of the metabolic acidosis would be predicted to have reduced the mild acid stress that promotes expression of the putative virulence factor and invasin, acid phosphatase. If regulation of the general stress response in *B. pseudomallei* is similar to that in *Pseudomonas aeruginosa*, then correction of an acidosis may also have down-regulated the general bacterial stress response, consequently reducing expression of a variety of potential virulence factors.

The patient reported above is the daughter of two patients in a Western Australian outbreak of acute melioidosis. The details of that outbreak have been presented elsewhere. In brief, a cluster of five cases of culture-confirmed septicemic melioidosis occurred in a small community in remote northwestern Australia before the onset of the wet season at the end of 1997. Two more cases occurred in the same community during the following year, one of which was a re-emergence of an earlier infection unrelated to the case cluster. The other patient is believed to have delayed disease onset attributed to environmental exposure at the same time as the principal case cluster. Seroprevalence studies found no evidence of widespread asymptomatic seroconversion, but one individual seroconverted around the putative environmental exposure period. His residence was the site from which culture-positive tap water was obtained. The tap water isolate was indistinguishable from the clinical isolates by DNA macrorestriction analysis. Environmental investigations concluded just over one year after the principal case cluster implicated the community’s water supply system, whose treatment plant was found to be contaminated with an indistinguishable molecular type of *B. pseudomallei*. The furthest culture-positive upstream source of *B. pseudomallei* was an installation used to aerate drinking water and thus raise its acid pH prior to ground level storage and chlorination. A chlorination failure is known to have occurred during the putative exposure period.

In conclusion, we successfully managed a case of persistent bacteremic melioidosis by combining critical care, splenectomy, and an aggressive antimicrobial approach support-
ed by a novel method of intracellular susceptibility testing. The antimicrobial regimen we used needs further assessment in formal clinical trials. The public health implications of this single case, coming from a community supposedly recovering from an acute melioidosis outbreak, highlight the need for continuing surveillance.

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REFERENCES