Melioidosis is a potentially fatal infectious disease caused by an organism of the soil, Burkholderia pseudomallei (formerly Pseudomonas pseudomallei). It is endemic in Southeast Asia and northern Australia. Most cases occur during the monsoonal wet season. The fatality rate of septicemic cases is high, up to 70% in northern Thailand (6, 24) and 47% in the tropical top end of the Northern Territory, where it is the commonest cause of community-acquired fatal bacteremic pneumonia (8). In acute melioidosis, death can occur within 24 to 48 h of the onset of symptoms. The clinical presentation of the disease is extremely variable, ranging from subclinical infections, cutaneous lesions, and subacute pneumonia to fulminant septicemia (5). Recrudescent disease can occur years after initial infection (18). Until recently, diagnosis was confirmed only by bacterial culture, which can take up to 1 week for species confirmation. Serology-based tests are not reliable because of cross-reaction with other organisms, high background levels in areas where melioidosis is endemic, and up to 20% negative results early in the illness (values from Royal Darwin Hospital). Considerable effort has lately gone into developing quicker tests based on PCR using primers from multiple organs abscesses (5, 6, 9, 25). Recrudescent disease and treated with Chelex-100 as described above. Thoracic and rectal swabs were vortexed in 1-mL portions of 0.9% NaCl solution for 2 min in order to elute the organisms. The swab was then removed, and the eluate was centrifuged. Sedimented organisms were resuspended in 0.9% NaCl and treated with Chelex-100 as described above. Pus and synovial fluid specimens were treated as described above for Chelex-100 and cerebrospinal fluid (CSF) specimens did not require any treatment. 

**PCRs.** 16S rRNA-based primers were used by the standard PCR protocol described by Dharakul et al. (11). A 100-µl reaction mixture contained 5 µl of processed specimen, 20 µmol of each primer, 200 µM deoxynucleoside triphosphates, and 1 U of Taq polymerase. Amplification conditions were 30 cycles of PCR, with 1 cycle consisting of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C. Part (1 µl) of this first reaction mixture was used for further amplification with nested primers under the same conditions. 23S rRNA primers (16) were used by the following protocol of the Perkin-Elmer Gene-Amp XL-PCR kit (7) with a hot start and 10-min extension step at 72°C at the end. Part (2 µl) of the first reaction mixture was added to a regular PCR mixture containing 50 µmol (each) of primer PMA and PM2 and 2 mM MgCl₂. Samples were preincubated at 95°C for 2 min and then subjected to 30 cycles of PCR, with 1 cycle consisting of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C. Sensitivity was initially determined by testing water inoculated with defined numbers of organisms and subsequently control specimens (blood and sputum specimens from healthy people) inoculated with serial dilutions of organisms. PCR products were analyzed by agarose gel electrophoresis on ethidium bromide-stained gels.

**RESULTS**

Comparison of published PCR approaches. The intergenic primer set (16) failed to detect B. pseudomallei from our collection of Australian isolates. The 23S rRNA primer set (16) with Taq polymerase was not sensitive enough to detect the organism in clinical specimens that gave positive results by culture. By adopting a method originally designed for long PCR (XL-PCR; Promega) (7) with a specifically thermostable enzyme, rTth, sensitivity was determined as follows: 1 organism/PCR mixture for bacterial suspensions in water, 10 to 30 for sputum specimens spiked with serial dilutions of bacteria in water, and 100 to 300 for spiked blood specimens. This level of

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**MATERIALS AND METHODS**

**Microbiological diagnosis.** The Microbiology Department of the Royal Darwin Hospital performed all bacterial isolations. B. pseudomallei was cultured from nonsterile sites by using Ashdown’s selective broth and Ashdown’s selective medium (1). Blood cultures were incubated at 35°C in tryptic soy broth bottles (Roche). The presence of B. pseudomallei was confirmed biochemically by using the API 20E system (API System SA, Lyon, France) or, more recently, MicroScan WalkAway (DuPont Diagnostics, West Sacramento, Calif.).

**Evaluation of PCR for Diagnosis of Melioidosis**

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Previously published PCR-based diagnostic tests for melioidosis were evaluated for clinical usefulness. A Burkholderia pseudomallei 16S rRNA-derived primer set had a sensitivity approaching 100% for clinical samples from 22 culture-confirmed cases of melioidosis and enabled diagnosis of 3 culture-negative cases. However, samples from 10 of 30 inpatients from Royal Darwin Hospital with other diagnoses were positive by PCR, giving a specificity of 67% and a positive predictive value of only 70%. Although there are a number of intriguing possible explanations for our results, concerns of inappropriate therapy resulting from a positive result by PCR have led us to forgo the advantage of rapid PCR diagnosis for melioidosis until a better system is validated.

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sensitivity was usually sufficient for culture-positive sputum samples but, in comparison to the 16S rRNA primer set (11), was found to be inadequate for blood samples that contain smaller numbers of organisms. Of 10 culture-positive clinical buffy coat samples tested with both primer sets in parallel, all 10 were positive with the 16S rRNA primer set, while only 2 were positive with the 23S rRNA primer set.

Furthermore, serial dilutions in water gave positive results for both primer sets. For spiked blood specimens, the 16S rRNA primer set was up to 100 times more sensitive than the 23S rRNA primer set.

**Specificity of the 16S rRNA primers.** Based on these results, further evaluation was performed using the 16S rRNA primers only. In order to identify potential false-positive results, DNA from a range of organisms was tested by using purified DNA from cultures. None was PCR positive. The organisms tested include *Chromobacterium violaceum*, *Chrysononas spp.*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *Neisseria meningitidis*, *Acinetobacter spp.*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*.

**Control specimens.** Blood specimens from 20 healthy control individuals and sputum and/or blood or urine specimens from 30 hospital patients with unrelated diseases were tested. None of the blood specimens from healthy control individuals were positive by PCR. However, specimens from 10 of 30 hospital patients with unrelated diseases were positive by PCR. Specimens from 2 of these 10 patients gave positive results for melioidosis by serological tests, suggesting that these two patients had been previously exposed to the organism. Specimens from eight patients were positive by PCR as follows: throat swabs and endotracheal fluid specimens (one patient), urine specimens (one patient), and blood specimens (eight patients). Infectious organisms isolated from these patients did not cross-react with our PCR primers, and these results remain unexplained. In none of these patients did subsequent cultures and follow-up suggest or confirm melioidosis. Contamination and carryover remain possible, although negative controls were run throughout the experiments, including during specimen preparation and PCR mixes were set up in a different room (away from any area that came into contact with template DNA).

**Application to clinical specimens from melioidosis patients.** We tested 51 specimens from 22 patients with culture-confirmed melioidosis. Specimens successfully used for PCR include buffy coat, sputum, throat swab, rectal swab, urine, endotracheal fluid, pus, joint aspirate, and tissue from autopsies. Nearly all culture-positive specimens were also positive by PCR. The two exceptions were a urine sample which was 5 days old on arrival due to transport from a remote area and a CSF sample which had been centrifuged for other purposes prior to PCR, resulting in loss of the organism. In three cases where *B. pseudomallei* was cultured from other sites, blood samples were negative by culture but positive by PCR.

In addition, blood specimens from three patients with serologically positive, clinically suspected but culture-negative melioidosis were positive by PCR (one patient with pneumonia, one with pericardial disease, and one with neurological disease). All three patients responded to appropriate antibiotics.

Blood specimens from one previously culture-positive patient were positive for melioidosis by PCR on several occasions, although the disease was considered cured after extensive antibiotic treatment, the patient remained well, and repeated cultures remained negative.

**DISCUSSION**

When appropriate specimen transport and preparation are used, we have shown that the sensitivity of the 16S rRNA-based PCR described by Dharakul et al. (11) approaches 100% compared with culture for *B. pseudomallei*. No cross-reaction was demonstrated with other organisms tested, and blood, sputum, joint aspirate, and urine specimens and throat and rectal swabs were adequate. Furthermore, buffy coat PCR was slightly more sensitive than blood culture and enabled a diagnosis in three culture-negative cases of melioidosis, including one case of neurological melioidosis where CSF is usually culture negative (25).

PCR of buffy coat specimens from 20 healthy controls gave negative results for melioidosis, suggesting a specificity of 100%. However, in 30 hospital inpatients without clinical melioidosis, 10 had PCR-positive specimens and subsequent follow-up and cultures did not confirm melioidosis in any of these patients, giving a specificity in patients at Royal Darwin Hospital of 67% and a positive predicted value of 70%.

There are a number of possible explanations for the false-positive results seen in hospital patients. Although negative controls were used in all assays, including at the specimen preparation stage, and we used a separate room for PCR, there is still the possibility of contamination and carryover. Contamination in PCR is likely to remain problematic, especially in many of the regions where melioidosis is endemic and where laboratory resources are limited.

While we have not shown that the 16S rRNA primers cross-react with other organisms, it is possible that at least some of the false-positive results reflect amplification of *B. pseudomallei* DNA, despite the patients having alternative diagnoses. Some of these patients were seropositive for *B. pseudomallei* exposure, and latency, which is probably related to survival in macrophages, is well recognized as a characteristic of the organism (14, 17, 19). Furthermore, the presence of circulating *B. pseudomallei* bacteria in overtly healthy people has been documented (21). Similarly, our finding of PCR-positive buffy coat in an apparently cured patient may reflect the persistence of organisms, whether viable or nonviable, in macrophages. Another possibility is that *B. pseudomallei* coexists with nonvultrulent strains of *B. pseudomallei*, recently described and termed *B. pseudomallei*-like (3).

However, even in the region of the Northern Territory where melioidosis is endemic, it seems unlikely that these possibilities could account for the apparently high proportion of PCR-positive results among hospital inpatients. Our experience is similar to earlier reports of difficulties adapting PCR for diagnosis of *Mycobacterium tuberculosis* (2, 12).

Despite difficulties in clinical applications, we found that PCR was very useful for environmental samples as previously documented by Brook et al. (4). Using the described 16S rRNA-based method, we were able to detect the organism in bore water and soil samples from various sites around Darwin and rural areas. These samples, particularly soil samples but also water samples, contain a large variety of organisms which may often overgrow *B. pseudomallei*, even in selective media. Usually multiple plating procedures are required before a sample can be confirmed as *B. pseudomallei* positive or negative. Hence, time and money can be saved by analyzing the initial broth culture supernatant by PCR and only processing the samples for culture that are positive by PCR. This has important applications for environmental risk factor assessments and epidemiological studies.

The apparent false-positive PCR results in inpatients may lead to the initiation of inappropriate therapy in patients with...
other bacterial sepsis. It is important to treat melioidosis early with ceftazidime to optimize the outcome (24). Because of the poor specificity of PCR in our experience, we have decided to forgo the advantage of rapid diagnosis using PCR in our hospital until a better system is validated. Our emphasis remains on maximizing culture of \textit{B. pseudomallei} using multiple samples, including throat and rectal swabs where appropriate, and selective culture media (10, 23). Because of delays in culture results we use empirical antibiotic therapy to cover \textit{B. pseudomallei} in septic patients with illness consistent with melioidosis, especially if the patient has one of the classical risk factors for melioidosis (alcoholism, diabetes, or renal disease) and especially for those presenting in the wet season (8).

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