Trimethoprim/sulfamethoxazole resistance in clinical isolates of *Burkholderia pseudomallei*

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Objectives: Trimethoprim/sulfamethoxazole is commonly used to treat melioidosis. Antimicrobial susceptibility testing using the disc diffusion method is commonly used in melioidosis-endemic areas, but may overestimate resistance to trimethoprim/sulfamethoxazole.

Patients and methods: We performed disc diffusion and Etest on isolates from the first positive culture for all patients presenting to Sappasithiprasong Hospital, Ubon Ratchathani, Thailand, with culture-confirmed melioidosis between 1992 and 2003.

Results: The estimated resistance rate for 1976 clinical *Burkholderia pseudomallei* isolates was 13% by Etest and 71% by disc diffusion. All isolates classed as either susceptible (*n* = 358) or as having intermediate resistance (*n* = 218) on disc diffusion were susceptible by Etest. Only 258 of the 1400 (18%) isolates classed as resistant on disc diffusion were resistant by Etest.

Conclusions: Disc diffusion testing of *B. pseudomallei* may be useful as a limited screening tool in resource poor settings. Isolates assigned as ‘susceptible’ or ‘intermediate’ by disc diffusion may be viewed as ‘susceptible’; those assigned as ‘resistant’ require further evaluation by MIC methodology.

Keywords: *Burkholderia pseudomallei*, melioidosis, susceptibility, Etest

Introduction

Melioidosis, the disease caused by *Burkholderia pseudomallei*, is endemic in southeast Asia and northern Australia. Trimethoprim/sulfamethoxazole is widely used both in the intensive and eradication phases of treatment.¹ Antimicrobial susceptibility testing using the disc diffusion method is commonly used in melioidosis-endemic areas, but may overestimate resistance to trimethoprim/sulfamethoxazole.²³ We have recently introduced the Etest into routine clinical practice to determine MICs of this drug. In this study, we compared estimates of resistance by disc diffusion and Etest for a very large collection of *B. pseudomallei* isolates associated with invasive disease.

Patients and methods

We evaluated *B. pseudomallei* isolates obtained from all patients with culture-proven melioidosis presenting to Sappasithiprasong Hospital, Ubon Ratchathani, Thailand, between 1992 and 2003. The first positive culture was examined for each patient, thereby avoiding replications from a given individual. Sample sites were blood culture (*n* = 893), pus (544), respiratory secretions (301), urine (121),...
throat swab (89), wound swab (19), CSF (5) and intravenous catheter tip (4).

Isolates were stored from time of isolation to testing in trypticase soy broth with 15% glycerol at 70°C. The freezer vial was scraped with a sterile loop and the organism streaked onto Columbia agar and incubated for 48 h at 37°C. A subculture was diluted in sterile normal saline to obtain a final concentration of between 1×10^5 and 5×10^6 cfu/mL using spectrophotometric methods and plated onto Mueller–Hinton agar (Oxoid).

Agar dilution and disc diffusion tests were performed as described previously.4 Co-trimoxazole discs (Oxoid) contained 1.25 μg of trimethoprim and 23.75 μg of sulfamethoxazole. Trimethoprim/sulfamethoxazole Etests (AB Biodisk, Solna, Sweden) were used according to the manufacturer’s instructions, and the cultures read after incubation in air at 37°C for 16–20 h. Bacteriostatic drugs give diffuse end-points and tests were read at the 80% inhibition point, taken as the first point of significant inhibition as judged by the naked eye. Interpretive standards for disc diffusion were based on NCCLS guidelines for Pseudomonas aeruginosa, listing resistant as ≤10 mm, intermediate 11–15 mm and susceptible ≥16 mm.5 Interpretative standards for agar dilution and Etest were based on NCCLS guidelines for MIC testing of B. pseudomallei by broth microdilution, which lists susceptible organisms as ≤2/3 mg/L and resistant organisms as ≥4/76 mg/L.5 Escherichia coli ATCC 25922 was used as a susceptible control. Isolates with an estimated trimethoprim/sulfamethoxazole MIC by Etest of 3/57 mg/L were re-tested by agar dilution MIC. If the same result was obtained, these isolates were classed as resistant.

### Table 1. Comparison of trimethoprim/sulfamethoxazole susceptibility by Etest and disc diffusion

<table>
<thead>
<tr>
<th>Testing by disc diffusion</th>
<th>Testing by Etest</th>
<th>susceptible</th>
<th>intermediate</th>
<th>resistant</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>358</td>
<td>218</td>
<td>1142</td>
<td>1718 (87)</td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>0</td>
<td>0</td>
<td>258</td>
<td>258 (13)</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>358 (18)</td>
<td>218 (11)</td>
<td>1400 (71)</td>
<td>1976 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Results and discussion

A total of 1991 patients presented to Sappasithiprasong Hospital with culture-confirmed melioidosis during the study period. Of these, isolates from 1976 patients were tested and 15 were not available. All isolates were tested by disc diffusion and Etest. By disc diffusion, 18% of isolates were assessed as susceptible, 11% of isolates were intermediate and 71% were resistant (Table 1). The resistance rate was much lower when based on the results of Etest, with 87% of isolates assessed as susceptible and 13% as resistant. By Etest, the MIC50 was 1/19 mg/L and the MIC90 was 3/57 mg/L. Most resistance was low-level; of the 258 resistant isolates, 174 (68%) had an MIC of either 3/57 or 4/76 mg/L, and only 26 (10%) had an MIC of 32/608 mg/L. The rate of resistance defined in this study is higher than that described in Australia (0–2.5%),3,6 but similar to a previous report from Thailand (16%).2 The discrepancy between the two techniques confirms previous results reported for 80 isolates that tested as susceptible for 41.3% and 97.5% by disc diffusion and Etest, respectively. The greater proportion of susceptible isolates in the previous study compared with the findings presented here may be due to the higher interpretive standard for resistance used previously (MIC of ≥8/152 mg/L compared with ≥4/76 mg/L in this work).

To further verify the rate of resistance to trimethoprim/sulfamethoxazole, agar dilution MICs were determined for 250 of the 258 isolates assessed by Etest as resistant, and 300 isolates that were susceptible by Etest (of which 145 were resistant by disc diffusion and 75 had intermediate resistance). Only three discrepancies were found that would have led to a change of classification from ‘susceptible’ to ‘resistant’, or vice versa. One isolate was reported to be susceptible by agar dilution (MIC 2 mg/L) but by Etest had an MIC of 3 mg/L; and two isolates that were susceptible by Etest (MIC 0.75 and 2 mg/L, respectively) were assessed as having an MIC of 3 mg/L by agar dilution. Thus, there is a high degree of correlation between MIC as defined by Etest and agar dilution assays.

There was no relationship between clinical specimen type and Etest susceptibility results (P > 0.05). Resistance rates as based on Etest varied over time (P < 0.001), and ranged from a low of 0.6% in 2001 to a high of 24% in 2003 (Figure 1). Geographical localization suggestive of a disease outbreak with a resistant

Figure 1. Variation of trimethoprim/sulfamethoxazole MIC (by Etest) by year.

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strain was not seen (data not shown). We are unaware of any agricultural practices that may have impacted on trimethoprim/sulfamethoxazole resistance, and cannot explain this variation in resistance over time.

The burden of melioidosis falls largely in the tropical setting, where diagnostic microbiology is often under-resourced. Disc diffusion methodology is inexpensive, and represents the mainstay of susceptibility testing where microbiology laboratories exist in rural tropical regions. For these reasons, we explored further the relationship between Etest and disc diffusion results.

The correlation between disc diffusion and Etest results is depicted in Figure 2. All isolates classed as either susceptible \((n = 358)\) or as having intermediate resistance \((n = 218)\) on disc diffusion were susceptible by Etest. Based on these results, disc diffusion could be used as a limited first-line screen in the tropical setting, whereby isolates assessed as either susceptible or have intermediate resistance by disc diffusion could be classified as susceptible without further testing. However, this accounts for only one-third of isolates in this study. Of the 1400 isolates classed as resistant on disc diffusion, only 258 (18%) were resistant by Etest. All isolates that did not grow right up to the disc were susceptible by Etest; these could be classified as ‘probably susceptible’ in a resource-poor setting, although this should be tentative and verified wherever possible. Only 20% of the 1280 isolates that grew up to the disc were resistant by Etest; this group requires further susceptibility testing.

Affordable solutions to problem areas in susceptibility testing are required in the tropics. Further investigation of the utility of disc diffusion for trimethoprim/sulfamethoxazole susceptibility testing is required, and evaluation of the utility of a higher strength disc is warranted.

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**References**