Susceptibility testing of fastidious organisms


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Sir,

The NCCLS has published guidelines for the susceptibility testing of fastidious organisms.1 Following these guidelines for the assessment of a new trinem, sanfetrinem (GV 104326), against Neisseria spp. we obtained unexpectedly high MICs and therefore undertook a study to compare MICs obtained on a variety of media.

The media compared were as follows: medium A, Iso-Sensitest agar (Unipath, Basingstoke, UK) supplemented with 20 mg/L NAD (Sigma, Poole, UK) and 5% whole horse blood (E and O, Bonnybridge, UK); medium B, Mueller–Hinton agar (Unipath) supplemented with 20 mg/L NAD and 5% whole horse blood; medium C, Mueller–Hinton agar supplemented with 20 mg/L NAD and 2% (w/v) haemoglobin (Unipath); medium D, GC agar base (Unipath) supplemented according to NCCLS recommendations excluding L-cysteine; medium E, GC agar base supplemented with 2% (w/v) haemoglobin and Vitox (Unipath); medium F, GC agar base supplemented according to NCCLS recommendations including L-cysteine; medium G, Mueller–Hinton agar supplemented with 5% whole horse blood and Vitox.

The antibiotics studied were sanfetrinem (Glaxo-Wellcome, Greenford, UK), spectinomycin (Upjohn, Crawley, UK), meropenem (Zeneca, Macclesfield, UK), cefixime (Cyramid, Gosport, UK) and benzylpenicillin (Smith-Kline Beecham, Welwyn Garden City, UK). The organisms investigated were ten clinical strains of Neisseria gonorrhoeae and control strain NCTC 12700 (ATCC 49226) and five clinical strains of Neisseria meningitidis.

The methodology was that described in the NCCLS guidelines1 and in the Working Party Report of the BSAC.2 Briefly, the final inoculum was 10⁴ cfu/spot, incubation was at 35–37°C in 4–6% CO₂ in air for 20–24 h and the MIC was defined as the lowest concentration of antibiotic inhibiting growth, up to three colonies being ignored.

In the Figure are plotted the mode MICs for the 11 N. gonorrhoeae strains tested for each of the media investigated. The graph clearly illustrates a variation in results obtained depending on the medium used. The NCCLS recommends that a medium free of L-cysteine should be used when testing clavulanate and carbapenem antibiotics and the results were in agreement with this recommendation. MICs obtained were significantly raised with meropenem and sanfetrinem and to a lesser extent with benzylpenicillin when using media E, F and G (all containing cysteine).

When sanfetrinem MIC data for media with and without the addition of L-cysteine were compared statistically (paired t-test) a P value of <0.0001 was obtained, indicating that there was a significant statistical difference. When medium D (NCCLS reference medium) and medium A (BSAC Working Party recommendation) were compared there was no statistical difference in results (P = 0.4294). A similar pattern of results was obtained for N. meningitidis. It is also worthy of note that all strains grew on medium A, whereas one clinical isolate of N. gonorrhoeae failed to grow on the NCCLS recommended media (F and G).

These data suggest that Iso-Sensitest agar supplemented with 20 mg/L NAD and 5% whole horse blood would be appropriate for sensitivity testing compounds affected by the presence of L-cysteine. Previous data published from this department have also shown that this medium is suitable for testing other fastidious organisms such as Streptococcus pneumoniae and Haemophilus influenzae.

The results from a recent BSAC questionnaire have shown that there is a general consensus that there is a need to standardize the testing of fastidious organisms. These data suggest that supplemented Iso-Sensitest agar, the medium chosen by the BSAC Working Party on antibiotic sensitivity testing, would be appropriate and that MIC results obtained with this medium would be comparable to those obtained on the NCCLS recommended media.

References


**Pharmacokinetics and pharmacodynamics of ceftizoxime in abscess fluid**

*J. Antimicrob Chemother* 1997; **39**: 437–438

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Sir,

Antimicrobial therapy alone is generally ineffective in the treatment of abscesses, especially for large collections of pus. The failure of antibiotics has been suggested to be based upon (i) slower growth of bacteria within the abscess environment, (ii) low pH of pus which inactivates certain antibiotics or (iii) inability of the drug to penetrate abscess fluid. The pharmacokinetics and pharmacodynamics of antimicrobials in abscess fluid have not been well studied in humans. The degree and rapidity of antimicrobial penetration into abscess fluid may influence the identification of microbial pathogens and the optimal treatment of these infections. The following study characterizes the penetration of a third-generation cephalosporin, ceftizoxime, into abscess fluids in patients, including those with brain abscesses. The study was approved by the Committee on Human Research at the University of California San Francisco. Patients receiving intravenous ceftizoxime requiring either percutaneous or surgical drainage of abscess fluid were identified. Samples of abscess fluid were taken, and antimicrobial history (patients A–F all received concomitant metronidazole with ceftizoxime; patients G and H were treated with ceftizoxime monotherapy), blood chemistries and microbiology reports were documented. Once sampled, abscess fluid was stored at −70°C until performance of the assay. Determination of ceftizoxime concentrations in abscess fluid was performed via reversed-phase high pressure liquid chromatography, as previously described. Aerobic and anaerobic bacteriological cultures were performed according to routine laboratory methods by the clinical microbiology laboratory.

Ceftizoxime was found to penetrate readily into abscess fluid, with levels ranging from 2 to 30 mg/L (Table). The highest concentrations were noted in patients receiving 2.0 g doses (patients G and H) or associated with more long-term therapy (patients C, E, F and H). Penetration into abscess fluid was confirmed as early as 10–15 min after administration of a single iv dose. Immediate penetration similarly was seen in two patients with brain abscesses. One patient (patient G) had a level of 2.8 mg/L 0.5 h after a single dose, which increased to 30.1 mg/L 0.83 h after a single dose.

**Table. Penetration of ceftizoxime into abscess fluid**

<table>
<thead>
<tr>
<th>Location of patient abscess</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Serum creatinine (mg/dL)</th>
<th>Intravenous dose</th>
<th>No. of doses</th>
<th>Time after dose (h)</th>
<th>Cefitzoxime concentration in abscess (mg/L)</th>
<th>Microbiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A intraabdominal</td>
<td>61</td>
<td>81</td>
<td>1.4</td>
<td>1 g × 1</td>
<td>1</td>
<td>0.17</td>
<td>4.4</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>B intraabdominal</td>
<td>50</td>
<td>62</td>
<td>1.4</td>
<td>1 g q12h</td>
<td>3</td>
<td>0.25</td>
<td>5.7</td>
<td><em>S. aureus</em> (methicillin-resistant)</td>
</tr>
<tr>
<td>C intraabdominal</td>
<td>67</td>
<td>49</td>
<td>1.0</td>
<td>1 g q12h</td>
<td>20</td>
<td>5.0</td>
<td>28.1</td>
<td><em>Bacteroides</em></td>
</tr>
<tr>
<td>D intraabdominal</td>
<td>29</td>
<td>56</td>
<td>0.7</td>
<td>1 g q8h</td>
<td>3</td>
<td>7.0</td>
<td>2.2</td>
<td><em>Bacteroides</em></td>
</tr>
<tr>
<td>E gall bladder</td>
<td>83</td>
<td>68</td>
<td>1.5</td>
<td>1 g q8h</td>
<td>12</td>
<td>1.0</td>
<td>11.9</td>
<td><em>S. viridans</em> Enterococcus</td>
</tr>
<tr>
<td>F subhepatic</td>
<td>41</td>
<td>68</td>
<td>0.9</td>
<td>1 g q8h</td>
<td>10</td>
<td>4.5</td>
<td>6.2</td>
<td><em>N. asteroides</em></td>
</tr>
<tr>
<td>G brain</td>
<td>48</td>
<td>61</td>
<td>1.3</td>
<td>2 g × 1</td>
<td>1</td>
<td>0.5, 0.83</td>
<td>2.8, 30.1</td>
<td><em>S. aureus</em> (methicillin-resistant)</td>
</tr>
<tr>
<td>H brain</td>
<td>14</td>
<td>45</td>
<td>0.8</td>
<td>2 g q6h</td>
<td>9</td>
<td>3.5</td>
<td>19.7</td>
<td><em>S. aureus</em> (methicillin-susceptible)</td>
</tr>
</tbody>
</table>

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