Sensitivity of *Brucella melitensis* to Chemotherapeutic Agents

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The sensitivity of 106 strains of *Brucella melitensis* to tetracycline, streptomycin, netilmicin, rifampicin, co-trimoxazole and ciprofloxacin was determined. Ninety percent of the strains were inhibited by tetracycline, netilmicin and ciprofloxacin at 1 µg/ml and by streptomycin and rifampicin at 4 µg/ml and by co-trimoxazole at 16 µg/ml. Three strains were resistant to tetracycline and one resistant to rifampicin. No resistance was found to other drugs.

Tetracycline and streptomycin are the most frequently used drugs for the treatment of human brucellosis. Relapses however are common, therefore there is a continuing search for alternative and more potent drugs to improve response and shorten the duration of therapy. Several studies on *Brucella* spp. susceptibility have reported effective in vitro activity of tetracycline, streptomycin, gentamicin, third-generation cephalosporins, rifampicin, and imipenem. 1-7 Ciprofloxacin, a quinolone with effective activity against intracellular organisms, has been reported to be effective against *Brucella* spp. 8 The purpose of this study was to determine the sensitivity of *Brucella melitensis* strains isolated at our hospital to tetracycline, streptomycin, rifampicin, co-trimoxazole, netilmicin and ciprofloxacin.

**Material and Methods**

This study included 106 strains of *B. melitensis* isolated from blood cultures of patients with brucellosis at King Saud University Hospital, Riyadh, during the period between July 1987 and August 1989. The strains were identified by standard methods. 9 Antimicrobial agents tested were tetracycline, co-trimoxazole (Adatab, Mast Laboratories, UK), streptomycin (Specia, France), rifampicin (Lepitit, Italy), netilmicin (Schering Corporation, USA), ciprofloxacin (Bayer, West Germany). Organisms were inoculated into trypticase soy agar (Oxoid Ltd, UK) and incubated at 37°C under 10% of CO₂ for 48 h. These then were subcultured in brain heart infusion broth (Oxoid Ltd, UK) and incubated for 48 h. A post-incubation dilution of 1:20 was made giving a final inoculation of approximately 10⁵ colony forming units. Minimal inhibitory concentrations (MIC) were determined by an agar dilution method. Isosensitest agar (Oxoid Ltd, UK) was used as the culture medium, with drug concentrations for tetracycline, streptomycin, netilmicin, rifampicin and ciprofloxacin ranging from 0.06-16 µg/ml. For co-trimoxazole the range was 0.06 to 128 µg/ml. The ratio of trimethoprim to sulphamethoxazole was 1:20. An antibiotic-free plate was used as a control. Plates were inoculated by a multipoint inoculator then incubated at 37°C for 48 h in a CO₂ incubator. Control strains were used for all antibiotics, and these were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 25922. The minimum inhibitory concentration (MIC) was defined as the least concentration that prevented any growth.

**Results**

The in vitro activity of the six antimicrobial agents against 106 isolates of *B. melitensis* are shown in Table 1. Ciprofloxacin and netilmicin were more active than other drugs as they inhibited all strains at a concentration of 1 µg/ml. Tetracycline inhibited 90% of the strains at a concentration of 1 µg/ml. Streptomycin and rifampicin had an MIC of 4 µg/ml, while co-trimoxazole inhibited
Table 1
Sensitivity of 106 strains of Brucella and melitensis to six antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>50%&lt;sup&gt;a&lt;/sup&gt;</th>
<th>90%&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>1</td>
<td>1</td>
<td>0.25–&gt;16</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2</td>
<td>1</td>
<td>1–4</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>0.5</td>
<td>1</td>
<td>0.25–1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2</td>
<td>1</td>
<td>0.25–16</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>16</td>
<td>16</td>
<td>1–&gt;32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>1</td>
<td>0.5–2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Minimum inhibitory concentration that inhibited the growth of 50% or 90% of the isolates.

90% of the strains at a concentration of 16 µg/ml (0.8/15.2). The ranges of inhibitory concentrations for each drug are shown in Table 1.

Three strains were resistant to tetracycline (MIC>16 µg/ml) while only one was resistant to rifampicin (MIC>16 µg/ml). None of the patients from whom those strains were isolated gave history of using these drugs before. There was no resistance to streptomycin, co-trimoxazole, netilmicin or ciprofloxacin.

Discussion

The results of our study confirm the findings of other studies on in vitro sensitivity of *B. melitensis* to tetracycline, streptomycin, rifampicin, co-trimoxazole and ciprofloxacin. Hall & Manion found that Brucella isolates were inhibited by tetracycline at a concentration of 0.15 to 10 µg/ml. Other investigators found that the MIC of tetracycline was between 0.5 and 1 µg/ml. The MIC 90 for streptomycin in this study was 4 µg/ml, and for netilmicin 1 µg/ml. Streptomycin was found in other studies to be the least potent of aminoglycosides against *Brucella* isolates. The reported MIC for streptomycin ranged between 1 and 5 µg/ml.

This study showed that the MIC 90 of rifampicin was 4 µg/ml. In other studies the MIC for rifampicin ranged between 0.5 and 10 µg/ml. Ciprofloxacin showed satisfactory activity with all *Brucella* isolates since they were inhibited at a concentration of 1 µg/ml. This finding was in agreement with Bosch et al.

Cotrimoxazole was found to inhibit 90% of the isolates at a concentration of 16 µg/ml. Other investigators found that its MIC 90 ranged between 3.25 and 10 µg/ml. Our isolates needed higher concentrations probably because of the frequent use of this drug in the treatment of brucellosis particularly in children.

Other studies have shown that penicillins have poor activity against *Brucella* spp. with the exception of ampicillin which had an MIC of 2.5 µg/ml. First generation cephalosporins and cefoxitin also demonstrated poor activity against *Brucella* spp. Third generation cephalosporin particularly ceftriaxone, cefotaxime and cefotaxime were reported to have good activity.

Presently the recommended treatment for human brucellosis is tetracycline for 3 weeks supplemented with streptomycin for the first 14 days. Relapses however are frequent and some authorities recommend prolongation of treatment to 6 weeks particularly in complicated brucellosis. There is a pressing need for more active drugs to reduce relapses and shorten the duration of therapy. As shown in this study netilmicin and ciprofloxacin have demonstrated good activity against all isolates. These drugs may be satisfactory alternatives to the presently recommended drugs for treatment of human brucellosis in adults. However, their effectiveness would have to be demonstrated in clinical trials.

The possible synergy of antibrucella drugs is an area which requires further study.

References