In Vitro Activity of N-Formimidoyl Thienamycin Against 98 Clinical Isolates of *Brucella melitensis* Compared with Those of Cefoxitin, Rifampin, Tetracycline, and Co-Trimoxazole

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In vitro susceptibilities of 98 isolates of *Brucella melitensis* to N-formimidoyl thienamycin, tetracycline, co-trimoxazole, rifampin, and cefoxitin were determined. N-Formimidoyl thienamycin showed good activity which was similar to those of tetracycline and rifampin and different from that of the other beta-lactam antibiotic tested (cefoxitin), which showed poor activity. Co-trimoxazole showed good activity.

N-Formimidoyl thienamycin (N-F-thienamycin) is a novel beta-lactam antibiotic which has been shown to be highly effective against a broad spectrum of microorganisms (8, 10, 11). The purpose of this study was to determine the activity of this new agent against *Brucella melitensis* in comparison with those of three other drugs known to be active against this organism (rifampin, co-trimoxazole, and tetracycline) and with that of another beta-lactam antibiotic (cefoxitin).

N-F-thienamycin, cefoxitin, tetracycline chlorhydrate, rifampin, and trimethoprim-sulfamethoxazole (co-trimoxazole) were supplied in pure form by Merck Sharp & Dohme, S.A.E., Antibioticos S.A., Lepetit S.A., and Almirall S.A., respectively. The 98 strains of *B. melitensis* were obtained from the blood of 98 patients, and the organisms were identified by the methods of Alton et al. (1) and Corbel and Brinley Morgan (1). The study material was preserved by being frozen in milk at −70°C. The inoculum was prepared from Trypticase soy agar slants (BBL Microbiology Systems) in brain heart infusion broth (Difco Laboratories) and incubated for 48 h at 37°C and a postincubation dilution in brain heart infusion broth of 1/20, which resulted in the inoculum having, once it was delivered onto the agar surface, approximately 10^5 to 10^6 colony-forming units. Susceptibility tests were performed by the agar dilution method (5) with a Steers replicator (9). Iso-Sensitest Agar CM47L (Oxoid Ltd.) was the medium used. Incubation was carried out at 37°C for 48 h. The concentrations used ranged from 16 to 0.06 μg/ml for N-F-thienamycin, tetracycline, and rifampin; from 128 to 2 μg/ml for cefoxitin; and from 200 to 0.19 μg/ml for co-trimoxazole. In the co-trimoxazole preparation, the ratio of trimethoprim to sulfamethoxazole was 1:20. The minimal inhibitory concentration (MIC) was defined as the lowest concentration which either prevented any growth or which allowed the growth of a maximum of 3 to 4 isolated colonies (5). The experiment was controlled by using strains of *B. melitensis* 16M (Central Veterinary Laboratory, Weybridge, Surrey, England), which are inhibited by the following MICs: N-F-thienamycin, 0.5 μg/ml; tetracycline, 0.25 μg/ml; rifampin, 0.5 μg/ml; cefoxitin, 32 μg/ml; and co-trimoxazole, 1.56 μg/ml. Also used as a control organism was *Staphylococcus aureus* ATCC 25923, which is inhibited by the following MICs: N-F-thienamycin, ≤0.06 μg/ml; tetracycline, ≤0.5 μg/ml; rifampin, ≤0.06 μg/ml; and co-trimoxazole, ≤1.56 μg/ml. Both organisms were included in each determination for reproducibility.

Figure 1 shows the cumulative percentages of strains inhibited by tetracycline, rifampin, N-F-thienamycin, cefoxitin, and co-trimoxazole. The first three agents exhibited good in vitro activity and gave very similar results. Inhibition of all strains was achieved by tetracycline at 0.5 μg/ml or less, by rifampin at 1 μg/ml or less, and by N-F-thienamycin at 2 μg/ml or less. On the other hand, cefoxitin showed poor activity, inhibiting only 15.3% of all strains at a concentration of 16 μg/ml or less; total inhibition required a concentration of 128 μg/ml. Co-trimoxazole (1:20 trimethoprim/sulfamethoxazole ratio) at a concentration of 6.25 μg/ml or less inhibited 98.9% of all strains, and the strain not inhibited by this concentration was effectively inhibited when the concentration was increased to 12.5 μg/ml.

These results confirm the in vitro studies reported by other investigators; the slightly lower MICs that we found may be explained by our
Brucella strains 
using a medium different from that of other 
investigators. Hall and Manion (7) found that in 
27 Brucella strains studied, among which figured 
the six known species, the MIC of tetracycline is 
between 0.15 and 10 μg/ml. These researchers 
also found that 50% of the strains are inhibited 
by rifampin at concentrations of 0.3 to 1.25 μg/ 
ml. Farrell et al. (6), in a study involving 100 
Brucella strains (including B. melitensis, B. 
abortus, and B. suis), found that the MIC of 
tetracycline is between 0.1 and 1.4 μg/ml. Cor- 
bel (3), in a study of 107 Brucella strains 
representing all species and biotypes of the genus, 
found that the MIC of rifampin is between 0.018 
and 10 μg/ml. The findings of our study suggest 
that the MIC of tetracycline is between 0.1 and 
0.5 μg/ml, and the MIC of rifampin is between 
0.06 and 1 μg/ml. In regard to co-trimoxazole, 
Bushby (2), in a study of 160 strains of B. 
abortus isolated from human and animal 
sources, found that 3.15 μg/ml inhibits 158 of the 
strains tested, and the 2 strains not inhibited by 
this concentration are effectively inhibited by a 
concentration of 10.5 μg/ml. In our study, the 
MIC of co-trimoxazole was between 0.39 to 6.25 
μg/ml, although one strain required a concentra-
tion of 12.5 μg/ml to be inhibited. This shows 
that the in vitro susceptibility of B. melitensis 
to co-trimoxazole is quite similar to that of B. 
abortus. The results obtained for older cephalo-
sporins are very poor. In their study, Hall and 
Manion were obliged to use concentrations of 
12.5 to 25 μg/ml to inhibit just 50% of the strains, 
and they obtained total inhibition only at a 
concentration of 100 μg/ml or more (7). Our 
results for cefoxitin show a similar pattern: we 
observed inhibition of only 15.3% of the strains 
at concentrations of 8 to 16 μg/ml, and a con- 

![Cumulative percent of strains inhibited](image)

**FIG. 1.** Susceptibilities of 98 strains of *B. melitensis* to *N*-F-thi enamycin (NFT), tetracycline (T), cefoxitin (CXT), rifampin (R), and co-trimoxazole (TMP-SMZ) at a trimethoprim/sulfamethoxazole ratio of 1:20.

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