In Vitro Susceptibility of *Brucella* to Various Antibiotics

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Received for publication 23 June 1970

A series of 27 strains of six species of *Brucella* was tested for susceptibility in vitro to a representative cross section of antibiotics in current use. The activity against each species was plotted, with the cumulative per cent of strains inhibited indicated for each concentration. As a class, the tetracycline antibiotics were the most effective. Erythromycin, gentamicin, streptomycin, and kanamycin, as well as rifampin, were quite active. The penicillin-cephalosporin group, with the exception of ampicillin, was comparatively ineffective, as were the polypeptides and the miscellaneous group of chloramphenicol, lincomycin, cycloderoxine, and sulfadiazine. Species differences were noticeable, with some strains of *B. canis* being considerably more resistant to streptomycin and the tetracyclines than *B. suis* and *B. abortus*. *B. melitensis*, *B. ovis*, and *B. neotomae* were intermediate in antibiotic susceptibility.

It has been known for some time now that *Brucella*, like many other gram-negative bacilli, will not grow in vitro in the presence of such antimicrobial agents as sulfonamides, chlorotetracycline, oxytetracycline, tetracycline, streptomycin, chloramphenicol, and ampicillin (4, 6). Most of the strains are highly resistant to the narrow-spectrum penicillins and cephalosporins. Comparatively little is known of the bactericidal activity of antibacterial agents against *Brucella* (7). Further, there is relatively little information available on the antimicrobial susceptibility of *Brucella* species other than *B. abortus* and *B. melitensis* (4, 6).

The purpose of the present study was to measure the concentration of various classes of antimicrobial agents required for bacteriostasis as well as bactericidal action on a collection of the available species and biotypes of *Brucella* by using a standard tube dilution method with subcultures to agar plates. Readings of end points were made after various periods of incubation to evaluate the stability of the antibacterial agents (7).

**MATERIALS AND METHODS**

The antibiotics and antibacterial agents were supplied as follows. Pfizer Laboratories provided methacycline hydrochloride; doxycycline hydrochloride; oxytetracycline hydrochloride; disodium carbenicillin; penicillin G, potassium; and streptomycin sulfate. Lederle Laboratories supplied tetracycline hydrochloride; demethylchlortetracycline hydrochloride; chlorotetracycline hydrochloride; minocycline; and sulfadia-

zine, sodium. Bristol Laboratories furnished kanamycin sulfate; oxacillin, sodium; methicillin, sodium; and ampicillin trihydrate. Lilly Laboratories provided cycloserine; cephalothin, sodium; cephaloridine; cephalaxin; cephaloglycin; and erythromycin. Naftilin, sodium, and sodium dicloxacillin monohydrate were obtained from Wyeth Laboratories; gentamicin sulfate was obtained from Schering Corp.; colistin sulfate was obtained from Warner-Chilcott Laboratories; polymyxin B sulfate was obtained from Burroughs Wellcome & Co.; lincomycin hydrochloride monohydrate was obtained from Upjohn Co.; rifampin was obtained from Pitman-Moore; and chloramphenicol was obtained from Parke, Davis & Co.

A total of 27 strains representing six species of *Brucella* was tested (Table 1). The six species consisted of nine strains of *B. suis*, seven strains of *B. abortus*, four strains of *B. canis*, and two each of *B. melitensis*, *B. neotomae*, and *B. ovis*. The medium used for dilutions was Alibi Brucella broth; the agar medium for subculture was Alibi Brucella agar.

As a general rule, the purified antibiotic powders were weighed and diluted to 1,000 μg/ml in sterile distilled water. Further dilutions to 200 μg/ml were made in Brucella broth. Stock solutions were either made fresh daily or stored for not more than 4 days at 4 C.

*Brucella* inocula were 1:1,000 dilutions of a 24-hr broth culture which had been inoculated from a stock Brucella agar slant stored at 4 C. Only in the case of the slow-growing *B. ovis* was a 48-hr broth culture used. An equal volume of diluted culture was added to each antibiotic dilution. Incubation was at 37 C, with 10% CO₂ atmosphere provided for *B. abortus* cultures. Readings were made at 48 hr and again at
TABLE 1. Species, strains and sources of Brucella tested

| Species   | Strains          | Sources                                                                 |
|-----------|------------------|-------------------------------------------------------------------------|
| B. suis   | Biotype I        | Veterinary Microbiology, Univ. of California, Davis (M. E. Meyer)       |
|           | Biotype II       | Same                                                                    |
|           | Biotype III      | Same                                                                    |
|           | Biotype IV       | Same                                                                    |
|           | Biotype V        | Same                                                                    |
|           | 1330             | Univ. of Minnesota, Minneapolis (W. W. Spink)                           |
|           | 1330 W           | FAO/WHO Reference, Central Veterinary Laboratory, Weybridge, England    |
|           | Biotype III, 2271| State Hygienic Laboratory, Univ. of Iowa, Iowa City (F. P. Koontz)     |
|           | Biotype III, 7834| Same                                                                    |
|           | Biotype III, 7835| Same                                                                    |
| B. abortus| Biotype I        | Veterinary Microbiology, Univ. of California, Davis (M. E. Meyer)       |
|           | Biotype II       | Same                                                                    |
|           | Biotype III      | Same                                                                    |
|           | Biotype IV       | Same                                                                    |
|           | 19               | VA Hospital, Minneapolis, Minn. (stock strain)                         |
|           | 544              | Univ. of Minnesota, Minneapolis (W. W. Spink)                           |
|           | 11098            | State Hygienic Laboratory, Univ. of Iowa, Iowa City (F. P. Koontz)     |
| B. neotomae| 7E164            | Rocky Mountain Laboratory, Hamilton, Mont. (H. G. Stoenner)            |
|           | 5K33             | FAO/WHO Reference, Central Veterinary Laboratory, Weybridge, England    |
| B. canis  | RM-5             | Veterinary Virus Research Institute, Cornell Univ. (L. E. Carmichael)  |
|           | BK-7             | Univ. of Minnesota, Minneapolis (W. W. Spink)                           |
|           | BK-24            | Same                                                                    |
|           | BK-48            | Same                                                                    |
| B. melitensis| 16               | Same                                                                    |
|           | 16M              | FAO/WHO Reference, Central Veterinary Laboratory, Weybridge, England    |
| B. ovis   | Reo 198          | Univ. of Wisconsin, Madison (L. M. Jones)                              |
|           | M                | Veterinary Microbiology, Univ. of California, Davis (M. E. Meyer)       |

7 days to determine the minimum bacteriostatic concentration (MIC). The 7-day readings were necessary for inclusion of the slow-growing B. ovis. At 7 days, subcultures to Brucella agar were done to determine the minimum bactericidal concentration (MBC).

RESULTS

As expected, the tetracycline antibiotics were consistently effective against all strains of all species (Fig. 1). The MIC at 7 days showed all of the tetracycline analogues except chlorotetracycline to be effective at a concentration of 1.25 µg/ml or less, the range being from 0.15 µg/ml for tetracycline to 10 µg/ml for chlorotetracycline. The MBC at 7 days ranged from 0.15 µg/ml for tetracycline to 10 µg/ml for chlorotetracycline. The MBC for the tetracyclines was usually identical to the MIC at 1 week.

With the exception of ampicillin, the penicillins as a group did not exhibit a comparable activity against Brucella (Fig. 2). Whereas the 50% inhibitory concentration (MIC) for the most active tetracycline was less than 0.005 µg/ml, the 50% inhibitory concentration for ampicillin was about 0.5 µg/ml, nearly a 100-fold difference. The remaining penicillins tested showed inhibition of 100% of the strains only at a concentration of...
required a concentration of more than 100 μg/ml for inhibition of 100% of the strains, with inhibition of 50% of the strains at about 12.5 to 25 μg/ml.

Of the three aminoglycosides tested, streptomycin was the least potent antibiotic. Kanamycin and gentamicin were much alike in their ability to prevent the growth of Brucella (Fig. 3); all strains were inhibited by 5.0 μg/ml, half were inhibited by 0.15 to 0.3 μg/ml. The MBC for the aminoglycosides was nearly identical to the MIC at 1 week. Two strains of B. canis were resistant to as much as 15,000 μg of streptomycin per ml but fully susceptible for gentamicin and kanamycin.

over 100 μg/ml. The most potent of the penicillins other than ampicillin were penicillin G and carbenicillin, with 50% of the strains inhibited at 10 to 12.5 μg/ml.

The cephalosporins (cephalothin, cephalexin, cephaloglycin, cephalaxin), also grouped in Fig. 2 with the penicillin because of the similarities in chemical structure, closely parallel the results obtained with penicillin G and carbenicillin. All required a concentration of more than 100 μg/ml for inhibition of 100% of the strains, with inhibition of 50% of the strains at about 12.5 to 25 μg/ml.

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Erythromycin, the only member of the macro- 
lide class to be tested, was somewhat more effec- 
tive than the aminoglycosides (Fig. 4). All strains 
were inhibited by 2.5 µg/ml, and half were in- 
hibited by 0.15 µg/ml.

Two members of the polypeptide group of 
antibiotics, colistin and polymyxin B, were in- 
hibitory at 100 µg/ml (Fig. 4). Of a miscellaneous 
group of antibacterial substances tested, rifampin 
and chloramphenicol were the most active, in- 
hibiting 50% of strains at a concentration of 
0.3 to 1.25 µg/ml (Fig. 5). Lincomycin, cyclo-
sorine, and sulfadiazine were bacteriostatic to all 
strains only in concentrations of more than 100 
µg/ml.

Species differences were apparent even with the 
limited number of strains in each classification. 
For example, all nine strains of B. suis tested 
(including one of canine origin) were uniformly 
sensitive to 5 µg of streptomycin per ml. Two of 
the four strains of B. canis, however, were highly 
resistant to streptomycin. This resistance was 
independent of the size of the bacterial popula-
tion, and the cultures did not appear to inactivate 
the streptomycin. We do not know whether the 
dogs from which these cultures were obtained had 
been treated with streptomycin. As a general rule, 
the B. suis strains were more sensitive to the tetra-
cyclines than the few strains of B. canis tested. 
The patterns for the seven strains of B. abortus 
were much like those of B. suis, except for the 
indication of a greater overall susceptibility of B. 
abortus to ampicillin. No distinctive species pat-
terns were discernible for the B. ovis, B. neotomae, 
and B. melitensis, all strains falling in the zone 
between the most susceptible and the most re-
sistant.

**DISCUSSION**

The susceptibility of B. abortus and B. suis was 
found to be about the same as reported in earlier 
studies (5, 7). The pH of the Albi media broth used 
herein is 7.0, obviating the reduction of strepto-
mycin activity found in an acidic medium. The 
use of a small population of *Brucella* would tend 
to minimize the influence of resistant variants 
which grow in the presence of exceptionally high 
centrations of streptomycin (7).

*Bruceia* is known to be quite susceptible to 
chlorotetracycline over short periods of time (7). 
The present studies indicate that demethylchlo-
tetracycline and tetracycline are even more ac-
tive, partly because they are more stable in solu-
tion than is chlorotetracycline (1, 2, 7). It is not-
worthy that the more stable tetracyclines proved 
to be more bactericidal for *Brucella* than is gen-
ernally appreciated.

The lesser activity of chloramphenicol against 
*Bruceia* has been documented (3, 7). The superi-
ority of ampicillin over penicillin G has also been 
demonstrated (3). Carbencillin, which is closely 
related in chemical structure to ampicillin, is 
surprisingly inactive. Cephalexin, also chemically 
similar to ampicillin, is ineffective against the 
*Brucella* strains tested.

Earlier studies have shown little difference in 
the susceptibility of different species of *Brucella* 
to streptomycin, chlorotetracycline, and chlor-
amphenicol (7). Studies of three common species 
of *Brucella* obtained from human and animal 
ources in India showed no consistent relation-
ship between the serotype and the pattern of suscep-
tibility to a broad panel of chemotherapeutic 
agents (6). A comparison of the antibiotic 
susceptibility of *B. abortus* from cattle in Germany 
showed nine different patterns, and *B. melitensis* 
and *B. suis* gave similar results (4). *B. suis* strains 
were more often susceptible to penicillin G than 
was the case for *B. abortus*. The greater resistance 
of *B. canis* to streptomycin and the tetracyclines 
has not been previously reported. Furthermore, 
there is very little published information on the 
susceptibility of *B. neotomae* and *B. ovis* to anti-
biotics. *B. melitensis* has been found to be about 
as sensitive as *B. abortus* (1, 2, 4).

**ACKNOWLEDGMENTS**

We thank Joan Paymar for valuable technical assistance.

This investigation was supported by research grants from Eli 
Lilly & Co. and the Veterans Administration (grant 618/01/3403).

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