In Vitro Susceptibility of Brucella to Various Antibiotics

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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, cycloserine, 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 sulfadiazine, sodium. Bristol Laboratories furnished kanamycin sulfate; oxacillin, sodium; methicillin, sodium; and ampicillin trihydrate. Lilly Laboratories provided cycloserine; cephalothin; sodium; cephaloridine; cephalaxin; cephaloglycin; and erythromycin. Nafcilin, sodium, and sodium dicloxacinil 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 Alibimi Brucella broth; the agar medium for subculture was Alibimi 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
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 chlortetracycline 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 chlortetracycline. The

MBC at 7 days ranged from 0.15 μg/ml for tetracycline to 10 μg/ml for chlortetracycline. 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
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, cephaloridine, cephalexin, cephalosporin), 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.

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, and 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.

FIG. 1. Susceptibility of 27 strains of Brucella to seven tetracycline antibiotics. Abbreviations: TC, tetracycline hydrochloride; DMTC, demethylchlortetracycline hydrochloride; MINO, minocycline; MOTC, methacycline hydrochloride; DOXY, doxycycline hydrochloride; OTC, oxytetracycline hydrochloride; CTC, chlortetracycline hydrochloride.

FIG. 2. Susceptibility of 27 strains of Brucella to seven penicillins and four cephalosporin antibiotics. Abbreviations: AMP, ampicillin trihydrate; PEN, penicillin G, potassium; CARB, disodium carbenicillin; LOR, cephalexin; LEX, ceftimox; GLX, cephalexin; KEF, cefalexin, sodium; METH, methicillin sodium; NAF, nafcillin, sodium; DICLOX, sodium dicloxacillin monohydrate; OXA, oxacillin, sodium.

FIG. 3. Susceptibility of 27 strains of Brucella to three aminoglycoside antibiotics. Abbreviations: GENTA, gentamicin sulfate; SM, streptomycin sulfate; KANA, kanamycin sulfate.

FIG. 4. Susceptibility of 27 strains of Brucella to one macrolide (erythromycin (ERY)) and two polypeptide antibiotics (colistin (COLY) and polymyxin B (POLY B)).
Erythromycin, the only member of the macro-lide class to be tested, was somewhat more effective than the aminoglycosides (Fig. 4). All strains were inhibited by 2.5 μg/ml, and half were inhibited by 0.15 μg/ml.

Two members of the polypeptide group of antibiotics, colistin and polymyxin B, were inhibitory at 100 μg/ml (Fig. 4). Of a miscellaneous group of antibacterial substances tested, rifampin and chloramphenicol were the most active, inhibiting 50% of strains at a concentration of 0.3 to 1.25 μg/ml (Fig. 5). Lincomycin, cycloserine, 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 population, 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 tetracyclines 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 patterns were discernible for the B. ovis, B. neotomae, and B. melitensis, all strains falling in the zone between the most susceptible and the most resistant.

**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 Albimi broth used herein is 7.0, obviating the reduction of streptomycin 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 concentrations of streptomycin (7).

*Brucella* is known to be quite susceptible to chlorotetracycline over short periods of time (7). The present studies indicate that demethylchlorotetracycline and tetracycline are even more active, partly because they are more stable in solution than is chlorotetracycline (1, 2, 7). It is noteworthy that the more stable tetracyclines proved to be more bactericidal for *Brucella* than is generally appreciated.

The lesser activity of chloramphenicol against *Brucella* has been documented (3, 7). The superiority of ampicillin over penicillin G has also been demonstrated (3). Carbenicillin, 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 chloramphenicol (7). Studies of three common species of *Brucella* obtained from human and animal sources in India showed no consistent relationship between the serotype and the pattern of susceptibility 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 antibiotics. *B. melitensis* has been found to be about as sensitive as *B. abortus* (1, 2, 4).

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