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Susceptibilities of *Corynebacterium bovis* and *Corynebacterium amylocolatum* Isolates from Bovine Mammary Glands to 15 Antimicrobial Agents

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Coryneform bacteria are frequently isolated from bovine mastitis and are associated with economic losses. Generally, the MICs of the 15 antimicrobial agents tested at which 90% of the isolates tested are inhibited for 46 *Corynebacterium bovis* and 13 *Corynebacterium amylocolatum* strains were low. These are the first quantitative antimicrobial susceptibility data available for coryneforms from bovine mastitis. Data from this study suggest that comparable corynebacteria from humans have a much higher level of antimicrobial resistance to a variety of antimicrobial agents.

Coryneform bacteria are frequently isolated from bovine mastitis (2, 6, 7). In a previous study, the lipophilic species, *Corynebacterium bovis* and *Corynebacterium amylocolatum* were the most frequently isolated coryneform organisms (10). Antibiotic therapy is an essential component of mastitis control programs, and the accurate selection of the most effective antimicrobial agents depends upon the susceptibility of the organism to the agent. The purpose of this study was to determine the susceptibility of *C. bovis* and other coryneforms isolated from bovine mastitis to various antimicrobial agents.

Forty-six strains of *C. bovis* and 13 strains of *C. amylocolatum* from a previous study were used (10). Prior to the MIC determinations, all isolates were revived by subculture on Trypticase soy agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood and 1.0% Tween 80 and incubated for 24 h at 35 to 37°C. MICs were determined using a broth microdilution method (Sensititre, Westlake, Ohio), except that the Mueller-Hinton broth was supplemented with 1% Tween 80 (6, 10). The MIC panel contained the following antimicrobial agents: ampicillin, oxacillin, cephalothin, ceftiofur, penicillin plus novobiocin, erythromycin, clindamycin, pirlimycin, tilmicosin, florfenicol, tetracycline, enrofloxacin, sarafloxacin, danofloxacin, and premafloxacin. The NCCLS-recommended quality control strains were included with each batch of organisms tested.

No information is available on the in vitro activity of antimicrobial agents commonly used to treat bovine mastitis caused by strains of *C. bovis*. The lack of in vitro antimicrobial susceptibility data may be due to the difficulty in cultivating *C. bovis*, as it fails to grow without lipid supplementation of the basal medium (3, 10). Previous studies (4, 8) determining the antimicrobial susceptibility of lipophilic corynebacteria isolated from humans, such as *Corynebacterium jeikeium*, have recommended the addition of rabbit serum or 0.1% Tween 80. However, the lipid requirements for *C. jeikeium* appear to be lower than those for *C. bovis*, as the former organism will grow on blood-supplemented media, while *C. bovis* grows very poorly, if at all. Results of a previous study (10) indicated that acceptable growth of *C. bovis* could be achieved by supplementing the basal medium with 1% Tween 80. Thus, we chose to use Mueller-Hinton agar supplemented with 1% Tween 80 for the test medium. Although this supplementation has not been recommended by NCCLS, the test results for the individual antimicrobial agents with the quality control organisms fell within guidelines published by NCCLS (6).

The MICs obtained with 46 strains of *C. bovis* are summarized in Table 1. Soriano et al. (8) determined the activity of various antimicrobial agents against 43 strains of *C. jeikeium*, a lipophilic corynebacterium isolated from humans. Phylogenetic studies (as reviewed in reference 3) have also determined that *C. jeikeium* is the organism most closely related to *C. bovis*. All of the antimicrobial agents tested except tilmicosin were active against the strains of *C. bovis*. For example, the MICs at which 90% of the isolates tested are inhibited (MIC_{90}s) of ampicillin, oxacillin, cephalothin, and ceftiofur against *C. bovis* ranged from 0.25 to 4.0 μg/ml compared to 16.0 to >64.0 μg/ml for the type strain of *C. jeikeium*. In contrast, Soriano et al. (8) reported that the MIC_{90}s of ampicillin, oxacillin, cephalothin, and cefuroxime for *C. jeikeium* were >256.0 μg/ml.

The macrolides (erythromycin and tilmicosin) and lincosamides (clindamycin and pirlimycin) inhibit protein synthesis in bacterial cells by binding to the same site on the ribosome (1, 5, 11). In the present study, the MIC_{90}s for erythromycin, tilmicosin, clindamycin, and pirlimycin were ≤0.06, >32.0, 0.25, and 0.25 μg/ml, respectively. This is markedly different from the MIC_{90}s obtained for *C. jeikeium*, which were >256.0 μg/ml for both erythromycin and clindamycin (8). Tilmicosin appears to be less potent than erythromycin, with a higher MIC_{50} (1.0 μg/ml) and MIC_{90} (>32.0 μg/ml). With the exception of one strain for which the MIC of erythromycin was high, the MICs for all strains were 0.5 μg/ml or below for this compound. In contrast, the tilmicosin MICs for all strains of *C. bovis* were 0.5 μg/ml or higher. The reason for the differences is unknown, but they are most likely due to differences in tilmicosin's and erythromycin's abilities to penetrate the cell.

Tetracycline and florfenicol are approved for use in veterinary medicine but are not available as intramammary infusion products. Both compounds were much more active against strains of *C. bovis* than against human strains of *C. jeikeium* (8). For example, the MIC_{90} of tetracycline was 0.25 μg/ml for *C. bovis* compared to 64.0 μg/ml for *C. jeikeium* (8).

The fluoroquinolones are widely used in human and veter-

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TABLE 1. MICs for 46 strains of *C. bovis* isolated from bovine mammary glands

Antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
Ampicillin[a]	0.25	0.25	≤0.06–0.25
Oxacillin[a]	1.0	4.0	0.125–8.0
Cephalothin[a]	0.5	0.5	≤0.06–64.0
Ceftiofur	0.125	0.5	≤0.06–64.0
Penicillin plus novobiocin[a]	0.125	0.5	≤0.06–>64.0
Erythromycin[a]	≤0.06	≤0.06	≤0.06–>64.0
Tilmicosin	1.0	>32.0	0.5–>32.0
Clindamycin	0.125	0.25	0.125–0.5
Pirlimycin[a]	0.125	0.25	≤0.06–>64.0
Tetracycline	0.25	0.25	0.125–>32.0
Florfenicol	1.0	2.0	1.0–>32.0
Enrofloxacin	0.125	0.25	≤0.03–>32.0
Sarafloxacin	0.25	0.5	0.25–>32.0
Danofloxacin	0.125	0.25	0.06–>32.0
Premafloxacin	0.015	0.015	≤0.0078–>8.0

[a] Approved for use in treatment of bovine mastitis.

inary medicine to treat a variety of diseases (11). Of these, the fluoroquinolones enrofloxacin, sarafloxacin, and danofloxacin demonstrated good activity, with MIC_{90}s ranging from 0.25 to 0.5 μg/ml. In contrast, the expanded-spectrum fluoroquinolone (9) premafloxacin was much more active, with a MIC_{90} of 0.015 μg/ml.

C. amylocolatum is a normal resident of healthy human skin and is one of the corynebacteria most frequently isolated from this body site (3, 4). In the present study, we tested 13 strains isolated from bovine mastitis (Table 2). In contrast to the bovine strains, the human strains tend to be much more resistant to antimicrobial agents. For example, the MIC_{90}s of ampicillin, oxacillin, cephalothin, and ceftriaxone for the human strains were >64.0 μg/ml, while the MIC_{90}s for the bovine strains were 0.25, 2.0, 0.5, and 0.5 μg/ml (ceftiofur value), respectively (4).

TABLE 2. MICs for 13 strains of *C. amylocolatum* isolated from bovine mammary glands

Antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
Ampicillin[a]	0.125	0.25	≤0.06–0.25
Oxacillin[a]	0.5	2.0	0.5–4.0
Cephalothin[a]	0.25	0.5	0.13–0.5
Ceftiofur	0.125	0.5	≤0.06–64.0
Penicillin plus novobiocin[a]	≤0.06	0.125	≤0.06–0.125
Erythromycin[a]	≤0.06	0.13	≤0.06–>64.0
Tilmicosin	4.0	32.0	2.0–>32.0
Clindamycin	0.25	0.5	0.25–64.0
Pirlimycin[a]	0.25	0.25	0.125–>64.0
Tetracycline	0.25	16.0	0.125–32.0
Florfenicol	32.0	32.0	1.0–>32.0
Enrofloxacin	0.125	0.25	0.06–0.25
Sarafloxacin	0.25	0.5	0.125–>32.0
Danofloxacin	0.125	0.25	0.06–0.5
Premafloxacin	0.015	0.015	≤0.0078–>8.0

[a] Approved for use in treatment of bovine mastitis.

Similar results are also seen for *C. amylocolatum* with the macrolides and lincosamides, as the MIC_{90}s for the human strains were >64.0 μg/ml for both erythromycin and clindamycin, compared to 0.13 and 0.5 μg/ml for these same agents with the bovine strains (4). As was the case with *C. bovis*, tilmicosin was much less active (MIC_{90} = 32.0 μg/ml) than erythromycin, clindamycin, or pirlimycin. Both tetracycline and florfenicol were much less active against the *C. amylocolatum* strains tested than against *C. bovis*. The MIC_{90} of ciprofloxacin was also >64.0 μg/ml for the human strains (4) compared to 0.25 μg/ml for enrofloxacin (a ciprofloxacin analogue) for the bovine strains.

This study represents the first quantitative assessment of the in vitro activity of various antimicrobial agents against bovine strains of *C. bovis* and *C. amylocolatum*. Generally, all the agents tested demonstrated good in vitro activity against these organisms. Data from this study suggest that comparable corynebacteria from humans have a much higher level of antimicrobial resistance to a variety of antimicrobial agents.

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