ABSTRACT

The antimicrobial susceptibility of 88 isolates of Moraxella bovis of Argentine origin was evaluated for 12 antimicrobials by broth microdilution procedures. The isolates had a minimum inhibitory concentration (MIC$_{90}$) of $\leq 0.06$ µg/mL to enrofloxacin; $\leq 0.12$ µg/mL to ceftiofur; $\leq 0.25$ µg/mL to ampicillin; $\leq 0.5$ µg/mL to florfenicol and gentamicin; $\leq 1.0$ µg/mL to tilmicosin, erythromycin, and oxytetracycline; $\leq 4.0$ µg/mL to tylosin; $\leq 8.0$ µg/mL to spectinomycin; $\leq 0.25/4.75$ µg/mL to trimethoprim/sulfamethoxazole; and $\geq 32$ µg/mL to lincomycin. Modal MIC values for these antimicrobials were as follows: enrofloxacin, 0.03 µg/mL; ceftiofur, 0.06 µg/mL; ampicillin, 0.25 µg/mL; florfenicol, gentamicin, erythromycin, and oxytetracycline, 0.5 µg/mL; tilmicosin, 1.0 µg/mL; tylosin and spectinomycin, 4.0 µg/mL; lincomycin and erythromycin, 16 µg/mL; and trimethoprim/sulfamethoxazole, $\leq 0.25/4.75$ µg/mL. These data show that all antimicrobials except lincomycin have MICs suggestive of sensitivity in vitro, though confirmation of clinical efficacy can only be properly assessed based on pharmacologic and/or clinical data to support the MIC values.

INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is one of the most widespread diseases in cattle production operations, with the primary causal agent being Moraxella bovis. This pathology causes economic loss because of delayed growth in steers, antibiotic treatment costs, animal depreciation at sale, and labor costs for animal movement.1,2

Many drugs have been used to treat IBK—topically, intramuscularly, or subconjunctivally—because of the high antibiotic sensitivity M. bovis demonstrates. Among the more frequently used drugs are penicillin, furazolidone (mostly used topically), tetracyclines, and gentamicin.3
Drug efficacy for IBK treatment can be accurately measured only by means of controlled efficacy tests on sick animals compared with untreated animals because some drugs, like kanamycin, are active in vitro against the organism, but are not able to eliminate *M. bovis* from the conjunctiva of the eye 5 hours after parenteral injection of the effective dose (10 mg/kg in the case of kanamycin). Kanamycin's diffusion into the tears is low, as documented by one report. According to the same report, tetracycline at a dose of 20 mg/kg does not reach high lachrymal concentrations but is effective in vivo at the 1 µg/mL level that it does attain. This concentration is well below the in vitro minimum inhibitory concentration (MIC) of 4 µg/mL for tetracycline, as will be shown later. Although effective against *M. bovis* in vitro, penicillin G injected with or without dexamethasone (300,000 IU penicillin plus or minus 4 mg dexamethasone administered subconjunctivally once daily for 3 days when ulcers were first observed) did not show any therapeutic efficacy in a natural outbreak compared with an untreated group from the same outbreak.

Nevertheless, the measurement of the antibiotic activity of different drugs in vitro is an important step before performing efficacy tests in animals or recommending generalized use of any antimicrobial for the treatment of a particular disease. The objective of this study was to measure the sensitivity of a collection of 88 *M. bovis* isolates against a set of 12 antimicrobials.

**MATERIALS AND METHODS**

**Isolates**

A collection of 88 *M. bovis* strains, all isolated by swabbing the conjunctivae and corneas of cattle affected by IBK in Argentina, was used. In the samples there were strains from the same herd, from different herds in similar locations, and from different locations. These strains had been isolated from the 1970s until March 1999. The strains were maintained lyophilized since isolation. Also, the strains show different phenotypes: some express the piliated phenotype, and others express the nonpiliated phenotype. Some of them are classified by the pilar serotype. In addition, two international strains were added to the collection (i.e., strain EPP63-300, originally from the United States, a gift from Dr. R. Rosenbusch, Iowa State University, Ames, IA; and strain 5-Buch, originally from Germany, a gift from Dr. M. Fort, EEA Anguil, INTA, La Pampa, Argentina). The reference strain *Staphylococcus aureus* ATC 29213 was used as the standard quality control strain.

**Antimicrobials**

The antimicrobials, all of which are used parentally, were provided in 96-well microdilution plates specifically made for this occasion (Sensititre, Trek Diagnostic Systems, West Sussex, UK, distributed by AccuMed International Ltd, Westlake, OH). Within the penicillin group, ampicillin, representing the aminopenicillins, was tested. The list of antibiotics and their concentrations is shown in Table 1.

**Broth Microdilution Method**

*Moraxella bovis* cultures were spread on tryptose agar (Merck KgaA, 64271 Darmstadt, Germany) plates with 5% bovine defibrinated blood and incubated at 37°C for 18 to 22 hours. Once developed, colonies were taken with a flamed loop and resuspended in sterile phosphate buffered saline solution pH 7.4 until there was an approximate density of 0.5 on the McFarland scale. By means of a sterile-tipped micropipette, 10 µL of this suspension were transferred to a 10-mL Mueller and Hinton broth (Merck KgaA, 64271 Darmstadt, Germany), containing 23 mg/L calcium and 6 mg/L magnesium, adjusted to 10 mg/L mag-
nesium. This broth was supplemented with 10% bovine fetal serum (Lot 231, Serono Arg. Martinez, Prov. Bs As, Argentina), so *M. bovis* could develop freely. This suspension was shaken for homogenization. Then, 50 µL were inoculated with a sterile-tipped multichannel pipette in each plate's well. Plates were then sealed with self-adhesive tape provided by the manufacturer and incubated at 37˚C for no longer than 20 hours. After this time elapsed, plates were read with the help of a microtiter reader and the MIC was taken as the lowest antibiotic concentration with no bacterial growth.

### RESULTS

Quality control–based MIC results of *S. aureus* strain ATCC 29213 coincided with the standard values established by the rules of the National Committee for Clinical Laboratory Standards (NCCLS), 1997, except for tylosin and for lincomycin. For tylosin, NCCLS standards have not been established. For lincomycin, the standard recommended antibiotic representative of the lincosamide group is clindamycin. Clindamycin has a different MIC for the standard NCCLS bacterial strain than lincomycin; therefore the results may not be directly correlated.

The MIC frequency of different antibiotics with respect to the studied *M. bovis* strains are listed in Table 2. Modal values of the MICs obtained in this study are listed in Table 3. In addition, the criteria established by NCCLS for classifying the strains as susceptible, intermediate, or resistant to these antimicrobials are listed in Table 4.

### DISCUSSION

In general, *M. bovis* is susceptible to a variety of antibiotics, which would suggest that the use of these drugs could be of value for treatment of IBK. However, this is not completely accurate because not all drugs with MICs that suggest a good clinical response are completely effective for IBK treatment and vice versa. Therefore, the information conveyed by this report should not be interpreted as a direct treatment recommendation; it should be used only as a reference.

The results from this study generally agree with those reported previously, though some

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Maximum Concentration (µg/mL)</th>
<th>Minimum Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>8</td>
<td>0.06</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>32</td>
<td>0.25</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>32</td>
<td>0.25</td>
</tr>
<tr>
<td>Tylosin</td>
<td>64</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>16</td>
<td>0.12</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>32</td>
<td>0.25</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>128</td>
<td>1</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>16</td>
<td>0.12</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32</td>
<td>0.5</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>32</td>
<td>0.5</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>15/304</td>
<td>0.25/4.75</td>
</tr>
</tbody>
</table>
Although 10% volume per volume of fetal calf serum was added to the Mueller and Hinton broth, which is a change from the NCCLS 1997 standards, the tests were valid because the MIC values for *S. aureus ATCC 29213* were in the same range for both broths. In a previous study by Zielinski et al, *M. bovis* strains, some of which were used in this study, were susceptible...
and resistant to similar antibiotics when tested by the agar diffusion method with antibiotic discs. The exception was erythromycin, in which 100% of strains were sensitive. In the 1995 study, resistance of *M. bovis* to lincomycin was reported, which agrees with the data generated with the broth dilution method used in the study reported here.\(^7\) Moraxella bovis strains also were resistant to bacitracin,\(^7\) an antibiotic not evaluated in this study. The discrepancy regarding sensitivity of *M. bovis* to erythromycin could be caused by the method used. In Zielinski’s study the definition of susceptibility or resistance was given as a ≥13-mm (susceptible *M. bovis* strains) or ≤ 13-mm (resistant *M. bovis* strains) inhibition halo with no intermediate zone, according to the manual used at that time.\(^8\) It is possible that imprecise measurements of the inhibition halo produced erroneous results. However, the erythromycin results in this study using the broth microdilution method coincide in general with those previously published.\(^3\) Shryock et al reported that 31%, 67%, and 1.81% of *M. bovis* strains were susceptible, intermediately susceptible, and resistant to erythromycin, respectively.\(^3\)

Results from this work also differ from those previously reported with respect to the MIC modal values for tilmicosin, tylosin, and erythromycin.\(^3\) Shryock et al reported the existence of resistant strains to tilmicosin, erythromycin, and oxytetracycline, while this study found no resistant strains to these antibiotics (Table 4). It could be speculated that North American *M. bovis* strains are more resistant to antibiotics than Argentine strains. Perhaps this is because of the lower usage of antimicrobials in pasture-

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**TABLE 4. Antibiotic Concentration (µg/mL) from the Cutoff Point for the Interpretation of MIC Results, and Percentage of Isolates Obtained Using this Classification Criterion**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Cutoff Point</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
<td>Resistant</td>
</tr>
<tr>
<td><em>Enrofloxacin</em></td>
<td>≤0.5 (100)(^1)</td>
<td>1</td>
<td>≥2</td>
</tr>
<tr>
<td><em>Ceftiofur</em></td>
<td>≤2 (100)</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td><em>Ampicillin</em>(^2)</td>
<td>≤0.25 (100)</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td><em>Tilmicosin</em></td>
<td>≤8 (100)</td>
<td>16</td>
<td>≥32</td>
</tr>
<tr>
<td><em>Erythromycin</em></td>
<td>≤0.5 (15.9)</td>
<td>1 (84.1)</td>
<td>≥8</td>
</tr>
<tr>
<td><em>Lincomycin</em></td>
<td>≤0.5</td>
<td>1 (2.2)</td>
<td>≥4 (97.8)</td>
</tr>
<tr>
<td><em>Gentamicin</em></td>
<td>≤4 (100)</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td><em>Oxytetracycline</em></td>
<td>≤4 (100)</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td><em>Trimethoprim/Sulfamethoxazole</em></td>
<td>≤2/38 (100)</td>
<td>—</td>
<td>≥4/76</td>
</tr>
</tbody>
</table>

\(^1\)Tylosin, spectinomycin, and florfenicol were not included because they did not meet NCCLS antibiotic standards.

\(^2\)Indicates percentage of isolates classified according to this criterion.

\(^3\)Ampicillin MIC breakpoints (µg/mL) are as follows such as stated in NCCLS standards:\(^6\):

- **Enterobacteriaceae**
  - ≤8
  - 16
  - ≥32

- **Staphylococci**
  - ≤0.25
  - —
  - ≥0.5

- **Enterococci**
  - ≤8
  - —
  - ≥16

- **Streptococci (not*S. pneumoniae*)**
  - ≤0.25
  - 0.5
  - ≥8

- **Listeria monocytogenes**
  - ≤2
  - —
  - ≥4
fattened Argentine cattle than in American cattle, though this statement cannot be supported with scientific evidence. However, the discrepancy in MIC modal values for tilmicosin, tylosin, and erythromycin observed between Shryock’s study and the one reported here (2, 8, 1 µg/mL versus 1, 4, 0.5 µg/L, respectively) appears to support the first speculation. It is interesting to note the relative uniformity of the results obtained with the Argentine strains, though very diverse isolates with respect to place of isolation and age of the strain are represented. No important deviations have been detected in modal values of the international strains (i.e., from Germany and the US) that were included in this study with respect to the Argentine strains. Defined susceptibility profiles between strains of identical or distinct phenotypes or serotypes could not be identified either (data not shown).

CONCLUSION

Except for lincomycin, in which 98% of strains were resistant, and perhaps erythromycin, because of the high number of intermediate susceptible strains, the antibiotics tested in this study showed good activity in vitro against Moraxella bovis. However, as previously mentioned, pharmacologic studies should be performed to determine concentrations and persistence in lachrymal fluid after application topically or parenterally. In addition, clinical studies should be conducted to determine the ultimate efficacy of these drugs to improve the condition of severely affected ocular tissues.

REFERENCES