

Antibiotic Susceptibility of *Mycoplasma bovis* Strains Recovered From Mycoplasmal Pneumonia and Arthritis in Feedlot Cattle

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Summary

Mycoplasmal pneumonia and arthritis is a problem of increasing significance in Midwestern feedlots. The disease presentation cannot be prevented by vaccination or successfully treated with antimicrobials. Due to the reported difficulty in treating these outbreaks, in-vitro antimicrobial susceptibility was tested on isolates of *Mycoplasma bovis* recovered from cases of pneumonia or pneumonia and arthritis where the mycoplasma was involved as a causative agent. Using a broth microdilution method, 36 *M. bovis* isolates from cases of pneumonia and 9 from cases of pneumonia and arthritis were tested for susceptibility to antimicrobials currently used in cattle with respiratory disease (ampicillin, tilmycosin, spectinomycin, tylosin, lincomycin, tetracycline, ceftiofur, and erythromycin). Among the isolates from cases with pneumonia, resistance to more antimicrobials was shown in recent isolates than in isolates from earlier years. Tetracycline and lincomycin were the drugs of choice for these isolates, although 3 of 36 isolates were resistant to all drugs tested. Isolates from cases of pneumonia and arthritis were from recent accessions. A majority of these isolates (5/9) were resistant to all antimicrobials tested. Lincomycin, spectinomycin, and tetracycline were antibiotics usable with 4/9 of the isolates. Overall, the results indicate that antimicrobial therapy in cases of mycoplasmal feedlot pneumonia and arthritis may be unrewarding.

Introduction

Mycoplasmal pneumonia and arthritis is an emerging disease in the US. Although reported occasionally in the historical record, it now appears frequently during fall and winter months in feedlots, particularly those of Midwestern states. No preventive vaccination is available, and response is very poor to antibiotic therapy. We have been investigating cases of pneumonia and pneumonia and arthritis where the major pathogen was *Mycoplasma bovis*,

as evidenced by isolation of the microorganism and immunohistochemistry of typical lesions. In most cases, tissues submitted were from calves that had been subjected to multiple antibiotic therapies without clinical improvement. Isolates of *M. bovis* were tested for antibiotic sensitivity against currently approved antimicrobials.

Materials and Methods

Forty-five isolates of *M. bovis* were tested for antimicrobial sensitivity. Of these, 36 were from cases of pneumonia and 9 were from cases of pneumonia and arthritis. Each isolate was from a separate diagnostic accession, usually a separate farm, or at least separated by several months if from the same farm. Isolates were clonally purified, and stocks were stored frozen in aliquots at -70°C . They were passaged a minimal number of times in culture, usually 4 to 12 times. After titration of frozen stocks, aliquots were thawed and diluted to 200 color changing units (CCU)/100 μl and delivered to a 96-well U-bottom microtitration plate. The plate was previously prepared with two-fold dilutions of antibiotics so that each antibiotic was tested at 20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, 0.07, and 0.03 $\mu\text{g/ml}$ of drug. All dilutions were made in mycoplasma broth (modified Friis broth) containing 5% Alamar Blue® as redox indicator. Readings were made after 44 hours of incubation at 37°C , and shift from blue to red was taken as indication of growth of the mycoplasma in that well. Antibiotics tested were ampicillin, tilmycosin, spectinomycin, tylosin, lincomycin, tetracycline, ceftiofur, and erythromycin.

Results and Discussion

There were clear-cut differences between resistant and sensitive isolates with some antimicrobials. With lincomycin, resistant isolates grew in the presence of 20 $\mu\text{g/ml}$ of drug, while sensitive isolates were inhibited by ≤ 0.3 $\mu\text{g/ml}$. Similar results were obtained with tylosin. With tilmycosin, spectinomycin, and tetracycline, isolates were found that were resistant to 5 and 2.5 $\mu\text{g/ml}$, and resistance to ≥ 2.5 $\mu\text{g/ml}$ was defined arbitrarily as resistance to these drugs. All *M. bovis* isolates tested were resistant to ampicillin, ceftiofur, and erythromycin at ≥ 20 $\mu\text{g/ml}$.

The pneumonia isolates were obtained from accessions received from 1979 to 1996, with the majority received in 1994 (Table 1). Among 1979 isolates, 2/5 were sensitive to two or fewer of the antimicrobials, with sensitivity to lincomycin (5/5) and tetracycline (3/5) prominent. More

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recent isolates were sensitive to fewer antimicrobials, with three isolates of 1994 resistant to all drugs tested. Sensitivity to tetracycline (25/36) and lincomycin (12/36) was frequent among these isolates.

Isolates from arthritis cases (with pneumonia and arthritis) were received in 1993 and 1994 (Table 2). Of two isolates of 1993, one was sensitive to three antimicrobials and the other was resistant to all. Among isolates received in 1994, three were sensitive to two or fewer antimicrobials (lincomycin and spectinomycin or tetracycline) while four were resistant to all drugs.

A shift to resistance to more antimicrobials is commonly observed with bacterial strains recovered from animal populations that are treated with antimicrobials. This shift was also observed when comparing sensitivities of 1979 isolates of *M. bovis* with those from later years. Unexpectedly, isolates from cases involving arthritis were resistant to more antimicrobials than those from pneumonia, or they were resistant to all drugs tested. These in-vitro observations correlate with the poor response to therapy commonly reported from feedlot cases of *M. bovis* pneumonia and

arthritis. Possible explanations for this difference could be that the arthritis isolates were recovered from calves that had been recently medicated with a larger variety of antimicrobials. Alternatively, strains producing pneumonia and arthritis are more pathogenic than those producing pneumonia only, and broad antimicrobial resistance is selected for over time, upon dissemination of these strains among herds. In either case, producers and practitioners should expect poor response to antimicrobial therapy in feedlot cases of pneumonia and arthritis.

Implications

Cases of mycoplasmal pneumonia in feedlots may be very hard to treat with conventional antibiotic therapy. In-vitro susceptibility data suggests that tetracycline and lincomycin may provide good responses. Similar cases involving arthritis are likely to be unresponsive to therapy.

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Table 1. Antimicrobial susceptibility of *M. bovis* isolates from cases of pneumonia

| Year | No. of isolates | Isolates sensitive to > 3 drugs | Isolates sensitive to 1-2 drugs | Resistant isolates |
|------|-----------------|---------------------------------|---------------------------------|--------------------|
| 1979 | 5 | 3 | 2 | 0 |
| 1994 | 23 | 3 | 17 | 3 |
| 1995 | 5 | 1 | 4 | 0 |
| 1996 | 3 | 1 | 2 | 0 |

Table 2. Antimicrobial susceptibility of *M. bovis* isolates from cases of pneumonia and arthritis.

| Year | No. of isolates | Isolates sensitive to > 3 drugs | Isolates sensitive to 1-2 drugs | Resistant isolates |
|------|-----------------|---------------------------------|---------------------------------|--------------------|
| 1993 | 2 | 1 | 0 | 1 |
| 1994 | 7 | 0 | 3 | 4 |