Genus identification and antibiotic susceptibility patterns of bacterial isolates from cows with acute mastitis in a practice population

David M. Bezek, DVM, PhD

**Objective**—To determine frequency and antibiotic susceptibility patterns of bacterial pathogens from cows with mastitis treated at a private practice during a 2-year period.

**Design**—Observational study.

**Animals**—Lactating dairy cows from 47 herds of 40 to 600 cows each.

**Procedure**—Bacteria isolated from milk samples were identified as coifers, *Staphylococcus* spp, or *Streptococcus* spp, using selective media. Antibiotic susceptibility testing was performed, using the disk diffusion method with the following antibiotics: gentamicin, amikacin sulfate, penicillin G, penicillin G-novobiocin, ampicillin, cephalothin sodium, ticarcillin, cefotaxime, benzylpenicillin, lincomycin, erythromycin, andrifloxin hydrochloride, sulfonamide, tetracycline, and polymyxin B.

**Results**—Of 354 samples tested, 82 (23.2%) yielded no growth. Of bacteria isolated, 54 (15.3%) were coifers, 96 (27.1%) were *Staphylococcus* spp, and 94 (26.6%) were *Streptococcus* spp. Antibiotic susceptibility testing was performed on 62.4% of all samples cultured. For *Staphylococcus* isolates, cephapilin was the most effective antibiotic in vitro for which a commercially available preparation exists. Penicillin G-novobiocin was the most effective antibiotic in vitro for *Streptococcus* isolates. Commercial antibiotic preparations approved for intramammary use were not effective in vitro against coifers that were found to cause mastitis.

**Clinical Implications**—Mastitis caused by coifers does not respond to commercial preparations intended for intramammary use, however, it may respond to parenterally administered antibiotics. Mastitis caused by *Staphylococcus* spp or *Streptococcus* spp should be treated first with a cephapilin or penicillin G-novobiocin preparation. ([J Am Vet Med Assoc 1998;212:404–406](https://www.jamvanimalhealth.com/doi/abs/10.2460/javma.1998.212.404))

Identification of bacteria that cause mastitis in cows and their antibiotic susceptibility patterns is necessary to select appropriate antibiotics for treatment. With variability in antibiotic susceptibility patterns for bacteria isolated from human and other animal populations, it is prudent to periodically monitor bacterial isolates from cows with mastitis in a study population for the range of antibiotic susceptibility.

Treatment of mastitis in cows is directed against a limited number of possible pathogens. The only organism that causes mastitis in cows that can be treated in a cost-effective manner is generally agreed to be *Streptococcus agalactiae*, because this organism responds well to antibiotic treatment. Spontaneous resolution of mastitis has been relied on in the past as the primary course of action in treating *Streptococcus* infections, but recent studies have questioned this practice. Studies have not found differences in cure rates with or without antibiotic treatment, but effectiveness of treatment was not verified by bacterial culture methods before or after treatment. Environmental *Streptococcus* organisms are emerging as important causes of mastitis in cows, whereas infections caused by *S. agalactiae* are controlled through treatment of nonlactating cows and teat-dipping practices.

Previous studies have shown meaningful differences in pathogen profiles and antibiotic susceptibility patterns among farms. The purpose of the study reported here was to quantify the major pathogens (ie, coifers, *Staphylococcus* spp, and *Streptococcus* spp) causing mastitis in cows that are part of a private practice population. Some bacterial isolates were tested for antibiotic susceptibility to eliminate ineffective antibiotics from the treatment regimen, thus resulting in more successful antibiotic treatments.

**Materials and Methods**

Forty-seven farms with 40 to 600 cows each were studied during a 2-year period. Cows with acute mastitis were identified, and a sample from each quarter was tested to identify affected glands. Selection was influenced by the magnitude of the mastitis problem on the farm, interest of the producer in addressing the problem, value and condition of the cow at diagnosis, and results of previous bacterial cultures.

Fresh or once frozen samples (0.01 ml) of milk were inoculated onto 5% sheep blood/MacConkey's agar plates. For subsequent tests, colonies were selected from these plates. Coifers (eg, *Escherichia* spp, Klebsiella spp, Enterobacter spp) were identified by growth on MacConkey's agar. *Streptococcus* spp and *Staphylococcus* spp were identified by failure to grow on MacConkey's agar. Further identification of colonies as *Streptococcus* spp relied on failure of isolates to subsequently grow on mannitol salt agar. An esculin test was not performed. Colonies with unusual morphology were sent to a reference laboratory for definitive identification.

Antibiotic susceptibility testing was performed, using the Kirby-Bauer disk diffusion method with 150-mm Mueller-Hinton plates. Disks were impregnated with 1 of the following antibiotics: gentamicin (10 μg), amikacin sulfate (30 μg), penicillin G (10 U), penicillin G-novobiocin (10 U/20 μg), ampicillin (10 μg), cephalothin sodium (30 μg), ticarcillin (75 μg), cefofoxin (30 μg), lincomycin (2 μg), erythromycin (15 μg), pirlimycin hydrochloride (2 μg), sulfonamide (0.25 μg), tetracycline (30 μg), or polymyxin B (300 μg). Zones of susceptibility were determined on the basis of manufacturer recommendations as follows: gentamicin, 13 mm; amikacin, 17 mm; penicillin G, 29 mm; penicillin G-novobiocin, 17 mm; ampicillin, 29 mm; cephalothin, 18 mm; ticarcillin, 20 mm; cefofoxin, 16 mm; lincomycin, 15 mm; erythromycin, 18 mm; pirlimycin, 15 mm.
13 mm; sulfonamide, 17 mm; tetracycline, 19 mm; and polymyxin B, 12 mm. During the second year of the study, use of polymyxin B-impregnated disks replaced lincomycin-impregnated disks, and penicillin G-novobiocin-impregnated disks replaced amikacin-impregnated disks.

Results
Coliforms were isolated from 15.3% of all milk samples. Nearly all coliform isolates fermented lactose. Staphylococcus spp and Streptococcus spp were isolated from 27.1 and 26.6% of all samples, respectively.

Forty-three of 54 (79.6%) coliform isolates were tested for antibiotic susceptibility, compared with 45 of 96 (46.9%) Staphylococcus and 51 of 94 (54.2%) Streptococcus isolates. Coliform isolates were highly resistant in vitro to antibiotics contained in commercially available mastitis preparations; 95% were susceptible to ceftriaxone and gentamicin (Table 1). Eighty-four percent were susceptible to polymyxin B. Staphylococcus isolates were also most susceptible to ceftriaxone and gentamicin (95 and 96%, respectively), followed by cephalothin (87%). Streptococcus spp were most often susceptible to penicillin G-novobiocin (94%) and ceftriaxone (90%), followed by cephalothin (86%). The number of isolates per antibiotic vary, because intermittently, an antibiotic zone was not clear or a specific disk was not available at the time of testing for each isolate.

Staphylococcus isolates were susceptible at least half of the time to antibiotics contained in all commercially available mastitis preparations for intramammary use, with the highest percentage being susceptible to cephalothrin. Streptococcus spp were susceptible in vitro most often to penicillin G-novobiocin (94%). Coliform isolates were not susceptible in vitro to antibiotics in any commercial preparation more than half of the time. Data indicate systemic sulfonamide or ceftriaxone may be effective as parenteral treatment for coliform mastitis, because 74 and 95% of isolates, respectively, were susceptible in vitro.

Table 1—Results of antibiotic susceptibility testing on bacteria isolated from milk samples obtained from cows with mastitis

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Coliforms</th>
<th>Staphylococcus spp</th>
<th>Streptococcus spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>41/43 95</td>
<td>42/45 96</td>
<td>41/51 80</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>41/43 95</td>
<td>40/42 95</td>
<td>44/49 90</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>18/18 84</td>
<td>2/9 25</td>
<td>10/26 38</td>
</tr>
<tr>
<td>Amikacin</td>
<td>22/27 81</td>
<td>32/39 82</td>
<td>19/31 61</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>31/42 74</td>
<td>23/42 55</td>
<td>12/47 26</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>25/40 63</td>
<td>32/41 78</td>
<td>43/50 86</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>19/42 44</td>
<td>33/45 73</td>
<td>26/50 56</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>11/43 26</td>
<td>38/44 87</td>
<td>43/50 86</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>1/40 2</td>
<td>24/43 56</td>
<td>22/48 46</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1/43 2</td>
<td>32/44 73</td>
<td>37/50 74</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1/43 2</td>
<td>2/44 21</td>
<td>49/43 43</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0/43 0</td>
<td>29/43 67</td>
<td>37/50 74</td>
</tr>
<tr>
<td>- novobiocin</td>
<td>0/16 0</td>
<td>4/5 80</td>
<td>17/18 94</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0/24 0</td>
<td>11/20 37</td>
<td>1/13 8</td>
</tr>
</tbody>
</table>

No. = No. of isolates susceptible/total No. of isolates; % = percentage of susceptible isolates.

Discussion
Growth and fermentation of mannitol on mannitol salt agar have been used for identification of Staphylococcus aureus, regardless of results of coagulase testing, and these growth characteristics were used in this study as well. Most coagulase-positive Staphylococcus isolates in cows' milk are S. aureus, and the decision whether to treat was made on the basis of isolation of a single colony type.

Streptococcus spp and Staphylococcus spp were the most common bacterial isolates in this study population. With S. agalactiae becoming a less common pathogen causing mastitis in cows, prevention and treatment efforts should be redirected toward elimination of Staphylococcus spp and Streptococcus spp other than S. agalactiae.

When considering a potential antibiotic for treatment of nonlactating cows or cows with clinical mastitis, quantified data relevant to the common pathogens of the population under study are important. Bacterial isolates can be tested in vitro, using any antibiotic-impregnated disk available; however, cows must be treated with a drug approved for such use in cattle. Use of aminoglycoside antibiotics, for example, requires a potential 18-month meat withdrawal time, making them less attractive choices. Other antibiotics (eg, lincomycin, ticarcillin, polymyxin B) are not approved for use in cattle and should not be used at all. Ceftriaxone does not reach therapeutic concentrations in mammary tissue if administered intramuscularly and would be ineffective if used for treatment of mastitis, even though it is approved for use in lactating cattle.

Because mastitis caused by S. aureus is generally regarded as untreatable and because of the lack of commercially available antibiotic preparations that are effective against coliform mastitis, some treatment recommendations must be inferred. Cephalothin or penicillin G-novobiocin would have been the best choice for treatment of mastitis caused by gram-positive bacteria in nonlactating or lactating cows in this study population. Commercial preparations do not contain cephalothin but, rather, contain a closely related first-generation cephalexin (ie, cephradin). The second choice for treatment of mastitis caused by Streptococcus spp or Staphylococcus spp would be pirlimycin or erythromycin, of which commercial mastitis preparations are readily available.

Results of this study support previous work indicating that commercially available antibiotic preparations would not be effective in vivo if administered intramammarily for treatment of mastitis caused by coliforms. Clinical signs of mastitis caused by coliforms are primarily attributable to endotoxin. Antibiotics administered parenterally may be beneficial if bacteria is found. On the basis of these data, likely drug candidates for such treatment would be ceftriaxone (95% effectiveness in vitro) or sulfonamide (74% effectiveness in vitro). Currently, sulfadimethoxine is the only sulfonamide approved for use in lactating cattle.

Parenteral administration of antibiotics has been suggested as an alternative treatment for chronic infections with S. aureus. In principle, high concentrations of drug in mammary tissue may improve cure rates.
However, 1 protocol cannot be applied to all situations, and more data on long-term antibiotic use in such infections must be collected before formulating any recommendations.

Although the present study examined cows from a practice population, it may be useful to compare these results with those from university studies. These results disagree with those of Bottner et al.26 which indicated isolates were not susceptible to cephalixin. Results of other studies12 agree with findings of the present study. Erythromycin was highly effective in vitro against gram-positive organisms in this study, which agrees with some reports12 but not others. Support for in vitro ceftiofur data can be found.22,28

References


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