Use of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis to predict response to treatment with cephapirin and oxytetracycline

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Objective—To determine whether results of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis were associated with duration of clinical signs or bacteriologic cure rate following treatment with cephapirin and oxytetracycline.

Design—Observational study on a convenience sample.

Animals—58 dairy cows with 121 episodes of clinical mastitis.

Procedure—Cows that only had abnormal glandular secretions were treated with cephapirin alone. Cows with an inflamed gland and abnormal glandular secretions were treated with oxytetracycline and cephapirin. Cows with systemic signs of illness, an inflamed gland, and abnormal glandular secretions were treated with oxytetracycline and flunixin meglumine and frequent stripping of the affected glands. The Kirby-Bauer method was used for antimicrobial susceptibility testing, and current guidelines were used to categorize causative bacteria as susceptible or resistant to the treatment regimen.

Results—Median durations of episodes of clinical mastitis caused by susceptible (n = 97) and resistant (24) bacteria were not significantly different. Bacteriologic cure rates at 14 and 28 days were similar for episodes caused by susceptible and resistant bacteria; however, for 56 episodes of clinical mastitis caused by gram-positive bacteria and treated with cephapirin alone, bacteriologic cure rate at 28 days was significantly higher for susceptible than for resistant bacteria.

Conclusions and Clinical Relevance—Results suggest that antimicrobial susceptibility testing was of no value in predicting duration of clinical signs or bacteriologic cure rate in dairy cows with mastitis, except for episodes caused by gram-positive organisms treated with intramammary administration of cephapirin alone. (J Am Vet Med Assoc 2002;221:103–108)

Mastitis is a common disease in dairy cows and an important source of economic loss in the dairy industry. Despite recent advances in our understanding of mastitis prevention, it is unreasonable to believe that the incidence of clinical mastitis will dramatically decrease. Thus, veterinarians and producers will continue to treat cows with clinical mastitis. An important determinant of the efficacy of treatment in cows with clinical mastitis is, presumably, whether an effective concentration of an antimicrobial can be attained and maintained at the site of infection. However, antimicrobial susceptibility of pathogens involved in clinical mastitis has traditionally been determined with the Kirby-Bauer disk diffusion method, which was designed to reflect antimicrobial concentrations in serum and interstitial fluid of human patients following oral or IV antimicrobial administration. The validity of applying Kirby-Bauer antimicrobial susceptibility breakpoints derived from humans to the treatment of cattle with mastitis has not been established, and such a practice is questionable because the pH, growth factor composition, pharmacokinetic profiles, and electrolyte, fat, protein, and leukocyte concentrations of bovine milk are different from those for human plasma. In addition, human bacterial pathogens are often different from those causing mastitis in cows. Importantly, antimicrobials may be distributed unevenly in inflamed mammary glands, and high antimicrobial concentrations have been shown to alter neutrophil morphology and function in vitro, and high concentrations associated with uneven antimicrobial distribution may inhibit bacterial clearance in vivo. The purpose of the study reported here, therefore, was to determine whether results of in vitro Kirby-Bauer disk diffusion antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis were associated with duration of clinical signs of mastitis or bacteriologic cure rates following intramammary administration of cephapirin alone or intramammary administration of cephapirin and IV administration of oxytetracycline.

Materials and Methods

Animals and treatment—Data for the present study represent a portion of a 2-year study documenting the efficacy of antimicrobials in treating clinical mastitis. Farm personnel at the University of Illinois Dairy Research Farm examined all lactating dairy cows for signs of clinical mastitis by observing and palpating the udder before each milking and by examining the glandular secretions for abnormalities in color and consistency. Clinical mastitis was defined as a visually abnormal glandular secretion. Duplicate samples of the glandular secretions were aseptically obtained from each affected quarter of cows with clinical mastitis within 30 minutes after milking and submitted for bacterial culture. Methods for sample collection and bacterial culture and identification have been described and were in accordance with National Mastitis Council guidelines.

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Clinical mastitis episodes caused by important contagious bacterial pathogens (Staphylococcus aureus and Streptococcus agalactiae) and episodes during which bacteria could not be isolated were excluded from the present study. Thus, data analysis was confined to clinical mastitis episodes caused by environmental bacteria or Corynebacterium spp. Each episode of mastitis was considered a new infection. For individual cows, multiple episodes of mastitis were included if episodes were separated by at least 60 days during which time the affected quarters appeared clinically normal.

Individual episodes of mastitis were included in the present study if the same bacterial species was isolated from the 2 duplicate samples of glandular secretions collected from cows with clinical mastitis, if ≥ 50 bacterial colony-forming units/ml were isolated from at least 1 of the samples, and if antimicrobials were administered because of the mastitis episode. Cows were assigned a severity score of 1, 2, or 3 at each milking; the treatment protocol was then determined on the basis of this severity score. Cows that only had abnormal glandular secretions (severity score 1) were treated with cephapirin sodium (200 mg, intramammary) at 12-hour intervals. Cows with an inflamed gland (large, red, firm, or hot) and abnormal glandular secretions (severity score 2) were treated with oxytetracycline (16.6 mg/kg [7.5 mg/lb], IM, q 24 h) in addition to intramammary administration of cephapirin sodium. This treatment protocol was chosen on the basis of proven success in this herd in a previous study. Cows with systemic signs of illness, an inflamed gland, and abnormal glandular secretions (severity score 3) were treated with oxytetracycline, IV, and flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IM, q 12 h). Affected glands were stripped 2, 3, or 8 times/d following IM administration of oxytetracycline (20 U). Intramammary administration of cephalothin was not performed while cows had a severity score of 3 because of the frequent stripping of the affected gland. Crystalloid fluids were administered IV or PO, when indicated.

For cows with a severity score of 1 or 2, intramammary administration of cephapirin was continued for 24 hours after resolution of clinical signs of mastitis (or a minimum of 4 milkings) or until lactation ceased or the cow was culled. For cows with a severity score of 2 or 3, IV administration of oxytetracycline was continued until severity score was 1 or clinical signs of mastitis resolved (or a maximum of 5 days) or until lactation ceased or the cow was culled. In a previous study, this antimicrobial treatment regimen was associated with a higher clinical cure rate by the tenth milking, compared with supportive treatment measures alone, in cows with mastitis caused by Streptococcus spp or coliform bacteria.

Antimicrobial susceptibility testing—The disk diffusion method and direct saline inoculum standardization procedure (Kirby-Bauer test) were used for antimicrobial susceptibility testing. Organisms were classified as susceptible or resistant to cephapirin and oxytetracycline on the basis of diameter of the zone of inhibition, following recommendations from the National Committee for Clinical Laboratory Standards. Organisms that would typically have been classified as being of intermediate susceptibility under these recommendations were classified as resistant for purposes of the present study. Mueller-Hinton medium was used for susceptibility testing of gram-negative bacteria and Staphylococcus spp, and Mueller-Hinton medium with 5% bovine blood was used for susceptibility testing of Streptococcus spp and Corynebacterium spp. A 30-µg cephalothin sodium disk was used to determine susceptibility to cephapirin, and a 30-µg oxytetracycline disk was used to determine susceptibility to oxytetracycline. Cephalothin is a first generation cephalosporin that is closely related to cephapirin. Organisms were classified as susceptible to cephapirin if diameter of the zone of inhibition around the cephalothin disk was ≥ 18 mm (equivalent to a concentration ≤ 8 µg/ml for humans). Organisms were classified as susceptible to oxytetracycline if diameter of the zone of inhibition around the oxytetracycline disk was ≥ 19 mm (equivalent to a concentration ≤ 4 µg/ml for humans).

Statistical analyses—For mastitis episodes with a maximum severity score of 1, causative bacteria were considered to be susceptible to the antimicrobial regimen if antimicrobial susceptibility testing indicated that they were susceptible to cephapirin and resistant to oxytetracycline or C. album or C. pyogenes. For mastitis episodes with a maximum severity score of 2 or 3, causative bacteria were considered to be susceptible to the antimicrobial regimen if antimicrobial susceptibility testing indicated they were susceptible to either cephapirin or oxytetracycline and resistant if antimicrobial susceptibility testing indicated they were resistant to both cephapirin and oxytetracycline.

The Kaplan-Meier product-limit method for censored survival time data was used to determine median duration of clinical mastitis (return to visually normal glandular secretion), and survival curves were developed for mastitis episodes associated with susceptible and resistant bacteria. Associations between duration of clinical mastitis and antimicrobial susceptibility, gram staining reaction, and maximal severity score were evaluated at Cox proportional hazards regression models for censored survival time data.

Bacteriologic cure 14 and 28 days after the onset of an episode of clinical mastitis was defined as failure to isolate the same pathogen from 1 or both of the duplicate milk samples. 

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\begin{align*}
\text{Bacteriologic cure rate} & = \frac{\text{Number of cows cured}}{\text{Number of cows studied}} \\
& \times 100
\end{align*}
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Tests were used to compare bacteriologic cure rates 14 and 28 days after the onset of an episode of clinical mastitis between episodes associated with susceptible versus resistant bacteria. Fisher exact tests were used when expected counts in 1 or more cells were < 5. A P value < 0.05 was considered significant.

Power analysis—The minimum group size required to detect a 50% reduction in bacteriologic cure rate 14 or 28 days after the onset of clinical mastitis was calculated with epidemiology software assuming α = 0.05, β = 0.20 (power, 80%), and a 75% bacteriologic cure rate for episodes associated with susceptible bacteria. The minimum sample size to detect a 50% reduction in bacteriologic cure rate was calculated to be 20.

Results

One hundred twenty-one episodes of clinical mastitis in 58 cows met the criteria for inclusion in the present study. These episodes were caused by Streptococcus spp (40; 33%), Corynebacterium spp (30; 25%), other gram-positive bacteria (10; 8%), and other gram-negative bacteria (10; 8%). Of the 121 episodes of clinical mastitis, 79 were assigned a maximum severity score of 1 and were treated with intramammary administration of cephapirin alone, 4 were assigned a maximum severity score of 2 and were treated with intramammary administration of cephapirin and IV administration of oxytetracycline, and 38 were assigned a maximum severity score of 3 and were treated with IV administration of oxytetracycline and frequent stripping of the affected gland. Cows with mastitis episodes assigned a maximum severity score of 3 had severity scores of 1 or 2 for various periods after initiation of treatment. No antimicrobials other than cephapirin and oxytetracycline were administered.

Of the 121 episodes of clinical mastitis, 97 (80%)
were associated with susceptible bacteria and 24 (20%) were associated with resistant bacteria. All 11 (100%) Streptococcus uberis isolates, all 9 (100%) Streptococcus dysgalactiae isolates, 29 of 30 (97%) Corynebacterium isolates, 10 of 11 (91%) Streptococcus bovis isolates, 4 of 8 (50%) Streptococcus fecalis isolates, and all 11 (100%) other gram-positive isolates were classified as susceptible to the antimicrobial regimen. All 10 (100%) Klebsiella pneumoniae isolates, 10 of 18 (56%) E coli isolates, and 3 of 13 (31%) other gram-negative isolates were classified as susceptible to the antimicrobial regimen. Overall, bacteria isolated during 93 of the 121 (77%) mastitis episodes were susceptible to cephapirin, and bacteria isolated during 76 (63%) episodes were susceptible to oxytetracycline.

Susceptibility to a variety of other antimicrobials was also tested; 85% of the bacteria isolated during mastitis episodes were susceptible to ceftiofur, 89% were susceptible to trimethoprim-sulfonamide, 82% were susceptible to gentamicin, 81% were susceptible to enrofloxacin, 72% were susceptible to neomycin, 64% were susceptible to penicillin-novobiocin, 63% were susceptible to ampicillin, 58% were susceptible to pirlimycin, 58% were susceptible to sulfonamide, 56% were susceptible to polymyxin B, 54% were susceptible to penicillin, 53% were susceptible to erythromycin, 52% were susceptible to tilmicosin, 52% were susceptible to spectinomycin, 40% were susceptible to methicillin, 18% were susceptible to lincomycin, and 16% were susceptible to cloxacillin.

**Duration of clinical mastitis**—Kaplan-Meier survival analysis indicated that median duration of episodes of clinical mastitis caused by susceptible bacteria (132 hours; n = 97) was not significantly different from duration of episodes caused by resistant bacteria (126; 24; Fig 1). Univariate Cox proportional hazard analysis indicated that antimicrobial susceptibility was not significantly associated with duration of clinical mastitis (hazard ratio, 1.13; P = 0.61). The multivariate Cox proportional hazard ratio (1.31; P = 0.28), obtained by adjusting for gram staining reaction and maximum severity score, was similar to the univariate estimate, indicating that neither gram staining reaction nor severity score was a serious confounder.

Of the 79 clinical mastitis episodes treated with intramammary administration of cephapirin alone (severity score of 1), 23 were caused by gram-negative bacteria and 56 were caused by gram-positive bacteria. For the 23 episodes caused by gram-negative bacteria, median duration of episodes of clinical mastitis caused by susceptible bacteria (132 hours; n = 15) was not significantly different (P = 0.26) different from median duration of episodes caused by resistant bacteria (114; 8). Similarly, for the 56 episodes caused by gram-positive bacteria, median duration of episodes caused by susceptible bacteria (120 hours; n = 50) was not significantly different (P = 0.89) different from median duration of episodes caused by resistant bacteria (120; 6).

**Bacteriologic cure**—Bacteriologic cure rate at 14 days for episodes of clinical mastitis caused by susceptible bacteria (80%) was not significantly different (P = 0.75) different from cure rate at 14 days for episodes caused by resistant bacteria (76%). Similarly, bacteriologic cure rate at 28 days for episodes caused by susceptible bacteria (82%) was not significantly different (P = 0.54) different from cure rate at 28 days for episodes caused by resistant bacteria (75%). Because 24 episodes caused by resistant bacteria were included, the study had adequate power to detect a 50% reduction in bacteriologic cure rate.

For the 23 episodes of clinical mastitis caused by gram-negative bacteria that were treated with intramammary administration of cephapirin alone, bacteriologic cure rates at 14 days were similar (P = 0.61) for episodes caused by susceptible (69%) and resistant (88%) bacteria. Likewise, bacteriologic cure rates at 28 days were similar (P = 0.26) for susceptible (77%) and resistant (100%) bacteria. In contrast, for the 56 episodes of clinical mastitis caused by gram-positive bacteria that were treated with intramammary administration of cephapirin alone, although bacteriologic cure rates at 14 days were similar (P = 0.064) for episodes caused by susceptible (82%) and resistant (40%) bacteria, the bacteriologic cure rate at 28 days for episodes caused by susceptible bacteria (85%) was significantly (P = 0.008) higher than the rate for episodes caused by resistant bacteria (20%).

**Discussion**

Results of the present study suggest that results of Kirby-Bauer antimicrobial susceptibility testing were of no value in predicting the clinical response to treatment (ie, duration of clinical signs) in dairy cows with clinical mastitis treated with intramammary administration of cephapirin and IV administration of oxytetracycline. Results of antimicrobial susceptibility testing were of value in predicting the bacteriologic response (ie, bacteriologic cure rate) to intramammary administration of cephapirin among cows with mild clinical mastitis (severity score of 1) caused by gram-positive bacteria. However, results of antimicrobial sus-
susceptibility testing were not associated with duration of clinical signs in cows with mastitis caused by gram-negative or gram-positive bacteria treated with intramammary administration of cephapirin alone.

We elected to use intramammary administration of cephapirin and IV administration of oxytetracycline for treatment of cows in the present study because of the likelihood that coliform bacteria and Streptococcus spp, the most frequent causes of clinical mastitis in the herd before the study began, would be susceptible to 1 or both of these antimicrobials. We previously reported that this regimen was efficacious in the treatment of clinical mastitis in this population, and our objective in the present study was to determine whether results of antimicrobial susceptibility testing could be used to further predict the response to treatment.

Cephapirin is a first generation cephalosporin that is approved for treatment of mastitis and is the second most commonly prescribed intramammary treatment for clinical mastitis in dairy cows. Cephapirin was selected for use in this study because of its widespread use and suitable antimicrobial susceptibility pattern against all gram-positive and strain-specific gram-negative mastitis pathogens, with minimum inhibitory concentrations of < 0.5 \( \mu g/ml \) for 90% of gram-positive isolates associated with mastitis, 2 to 4 \( \mu g/ml \) for \( K \) pneumoniae, and 6 \( \mu g/ml \) for \( E \) coli. When bactericidal \( \beta \)-lactam antimicrobials such as cephapirin are used, it is generally agreed that time above the minimum inhibitory concentration is the important determinant of treatment success. Intramammary administration of cephapirin sodium (200 mg) results in milk antimicrobial concentrations of 21 to 280 \( \mu g/ml \) for 8 to 12 hours in cows with mastitis. It is therefore likely that cephapirin concentration in glandular secretions among cows in the present study exceeded the Kirby-Bauer antimicrobial susceptibility breakpoint (≤ 8 \( \mu g/ml \), on the basis of human data) for the duration of treatment.

Oxytetracycline is the most commonly prescribed nonapproved antimicrobial for parenteral treatment in lactating dairy cows and was selected for use in the present study because of its widespread use, low cost, and antimicrobial susceptibility pattern for gram-positive and gram-negative organisms commonly associated with mastitis and because the label for 1 oxytetracycline formulation (200 mg of oxytetracycline/ml) suggests that it is useful in the treatment of pneumonia, pinkeye, foot rot, wooden tongue, leptospirosis, and metritis in lactating dairy cows. The oxytetracycline dosage was chosen on the basis of the relatively high concentrations achieved in milk and the low protein binding. Intravenous administration was selected because of limited bioavailability following IM injection. When bacteriostatic drugs such as oxytetracycline are used, it is generally agreed that the antimicrobial concentration should be maintained above the minimum inhibitory concentration for the duration of treatment. Intravenous administration of oxytetracycline (16.5 mg/kg) to healthy dairy cows results in milk antimicrobial concentrations > 1 \( \mu g/ml \) for 24 hours, with a milk concentration of 4 \( \mu g/ml \) 12 hours after administration. Because clinical mastitis increases the permeability of the blood-milk barrier, thereby increasing antimicrobial concentration relative to that in healthy glands, it is likely that the oxytetracycline concentration in glandular secretions among cows in the present study exceeded the Kirby-Bauer antimicrobial susceptibility breakpoint (≤ 4 \( \mu g/ml \), on the basis of human data) for at least 12 hours of each 24-hour treatment period.

Antimicrobial susceptibility test results must be correlated with clinical outcome and bacteriologic cure rate to confirm the validity of the assigned breakpoints, but this does not appear to have been done for most antimicrobials used to treat mastitis in dairy cows. In fact, in vitro antimicrobial susceptibility test results have been suspected to be poorly correlated with outcome of treatment for clinical mastitis since 1955, and the value of using disk diffusion antimicrobial susceptibility testing to guide mastitis treatment decisions has been questioned. With the exception of pirlimycin and penicillin-novobiocin, diameters of zones of inhibition in the disk diffusion test have not been related to antimicrobial concentrations achieved in the bovine mammary gland with dosage regimens used by veterinarians and dairy producers. Moreover, milk can markedly decrease in vitro antimicrobial activity, relative to Mueller-Hinton medium, particularly with antimicrobials that are highly lipid or protein bound and when milk contains a high leukocyte concentration. Casein is suspected to be the major constituent of milk that decreases antimicrobial activity, and milk markedly decreases in vitro oxytetracycline activity. Aside from the effects of milk, mastitis has variable effects on in vitro antimicrobial activity, with enrofloxacin, gentamicin, and trimethoprim-sulfadiazine having increased in vitro activity in mastitic milk, compared with normal milk, and ampicillin and tetracycline having similar in vitro activities in mastitic and normal milk.

Despite widespread reservations regarding the validity of Kirby-Bauer antimicrobial susceptibility testing in mastitis, results of antimicrobial susceptibility testing could be used to predict the likelihood of a bacteriologic cure in cows with subclinical or clinical mastitis. Staphylococcus aureus mastitis treated with procaine penicillin G (20,000 U/kg [9,100 U/lb], IM, q 24 h) for 2 to 5 days, the degree of inflammation following antimicrobial treatment of clinical \( S \) aureus mastitis, and the clinical response to antimicrobial treatment in herds with clinical staphylococcal and streptococcal mastitis, but could not be used to predict the likelihood of a bacteriologic cure in cows with subclinical or mild clinical mastitis caused by environmental streptococci. On the basis of results for contagious mastitis pathogens, antimicrobial susceptibility testing has been promoted as a method of developing a “herd profile” of mastitis pathogens, thereby facilitating future treatment decisions. While this is a useful approach for detecting \( \beta \)-lactamase production by \( S \) aureus isolates, the herd profile approach appears to have little merit in herds with environmental mastitis, as contamination arises from a variety of diverse sources.

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Results of the study reported here contrast with those obtained by Shpigel et al, who evaluated cows with coliform mastitis and systemic signs of illness. In that study, which involved 228 mastitis episodes treated with IM administration of trimethoprim-sulphonamide and nonsteroidal antiinflammatory agents, the clinical cure rate was significantly ($P = 0.006$) higher in cattle with coliform bacteria susceptible to the antimicrobial (89%; $n = 165$) than in cattle with coliform bacteria resistant to the antimicrobial (75%; 63). The difference in results between our study and the study by Shpigel et al may be attributable to differences in antimicrobials used, geographic location of the herds, and proportion of episodes of coliform mastitis. It is unlikely that the difference in results was a result of inclusion of mild episodes of clinical mastitis or of episodes of gram-positive mastitis, as the multivariate Cox proportional hazards analysis in the present study indicated that adjustment for these 2 factors did not change the conclusion that results of antimicrobial susceptibility testing were not predictive of treatment outcome. We also do not believe that combined administration of a bacteriostatic antimicrobial (oxytetracycline) and bactericidal antimicrobial (cephapirin) confounded the results of this study, as the clinical importance of antimicrobial antagonism is not well defined, and well-documented clinical examples of this phenomenon are rare, with the exception of bacterial meningitis in humans and rabbits.

The current cost of antimicrobial susceptibility testing is $5 to $8/test. Because the dairy industry is economically driven, any diagnostic test should have appropriate sensitivity and specificity and an appropriate economic return on the cost of testing before it can be routinely recommended. Results of the present study indicate that Kirby-Bauer antimicrobial susceptibility testing does not accurately predict clinical outcome in cows with clinical mastitis treated with intramammary administration of cephalirin and IV administration of oxytetracycline. Apart from the documented value in predicting the response to treatment of subclinical and clinical S aureus mastitis and the association with bacteriologic cure rate among cows with clinical mastitis caused by gram-positive bacteria in the present study, additional research is needed to further define the role, if any, that antimicrobial susceptibility testing should play in the treatment of clinical mastitis episodes caused by noncontagous mastitis pathogens.

References


