Minimum inhibitory concentrations of cephalosporin compounds and their active metabolites for selected mastitis pathogens

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Objective—To compare the minimum inhibitory concentration (MIC) of cephapirin and ceftiofur with MICs of their active metabolites (desacetylcephapirin and desfuroylceftiofur) for selected mastitis pathogens.

Sample—488 mastitis pathogen isolates from clinically and subclinically affected cows in commercial dairy herds in Wisconsin.

Procedures—Agar dilution was used to determine MICs for *Staphylococcus aureus* (n = 98), coagulase-negative staphylococci (99), *Streptococcus dysgalactiae* (97), *Streptococcus uberis* (96), and *Escherichia coli* (98).

Results—All *S aureus* isolates were susceptible to cephapirin and ceftiofur. Most coagulase-negative staphylococci were susceptible to cephapirin and ceftiofur. For *E coli*, 50 (51.0%; cephapirin) and 93 (94.95%; ceftiofur) isolates were susceptible to the parent compounds, but 88 (89.8%) were not inhibited at the maximum concentration of desacetylcephapirin. All *S dysgalactiae* isolates were susceptible to ceftiofur and cephapirin, and consistent MICs were obtained for all compounds. Most *S uberis* isolates were susceptible to cephapirin and ceftiofur. Of 98 *S aureus* isolates classified as susceptible to ceftiofur, 42 (42.9%) and 51 (52%) were categorized as intermediate or resistant to desfuroylceftiofur, respectively. For 99 coagulase-negative staphylococci classified as susceptible to ceftiofur, 45 (45.5%) and 17 (17.2%) isolates were categorized as intermediate or resistant to desfuroylceftiofur, respectively. For all staphylococci and streptococci, 100% agreement in cross-classified susceptibility outcomes was detected between cephapirin and desacetylcephapirin. No *E coli* isolates were classified as susceptible to desacetylcephapirin.

Conclusions and Clinical Relevance—Differences in inhibition between parent compounds and their active metabolites may be responsible for some of the variation between clinical outcomes and results of in vitro susceptibility tests. (*Am J Vet Res* 2013;74:683–690)

Mastitis is recognized as the most common and costly disease of dairy cattle, but treatment remains a challenge.[1] The ability to ensure effectiveness of mastitis treatments is diminished by the absence of complete antimicrobial treatment records for some farms, inconsistency of applying treatments in accordance with approved protocols, and limited amount of veterinary oversight.[2] In addition, the need to reserve many classes of antimicrobials only for therapeutic use in humans has resulted in a relatively limited number of antimicrobial classes available for mastitis treatments of dairy cattle in the United States.[3]

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Abbreviations

MIC	Minimum inhibitory concentration
MIC_{50}	Minimum inhibitory concentration required to inhibit growth of 50% of bacterial isolates tested
MIC_{90}	Minimum inhibitory concentration required to inhibit growth of 90% of bacterial isolates tested

Cephalosporins are one of the most important classes of semisynthetic antimicrobials used for the treatment of mastitis in dairy cattle. Cephalosporins are β-lactam antimicrobials that kill bacteria by disrupting bacterial cell wall synthesis. Cephalosporin antimicrobials are classified as first to fourth generation on the basis of their relative in vitro spectrum of activity (narrow, expanded, broad, or extended), structural similarities, and time of introduction into the market.[4] Cephapirin is a first-generation cephalosporin that is frequently used for intramammary treatment of mastitis and for treatment of cows during the nonlactating (dry) period.[2] Ceftiofur is the other cephalosporin that is approved for treatment of mastitis for cattle

in the United States. Ceftiofur is a broad-spectrum, third-generation cephalosporin that initially was developed for systemic treatment of bovine respiratory disease but is now used extensively for intramammary treatment of mastitis.[5,6]

After intramammary infusion, both cephapirin and ceftiofur are partially converted into active metabolites with bactericidal activity. Investigators in 1 study[7] administered cephapirin IV in humans and other animals and confirmed partial conversion of cephapirin into the metabolite desacetylcephapirin. On the basis of results of in vitro tests, and depending on the pathogen, desacetylcephapirin is reportedly 5% to 55% less active than cephapirin.[8] When cephapirin was administered to calves via IM injections in another study,[9] investigators detected almost complete conversion of cephapirin to desacetylcephapirin in tissues. After intramammary infusions of cephapirin, these researchers also reported prolonged persistence of desacetylcephapirin, compared with that of the parent compound, in milk samples obtained from cows with naturally acquired mastitis.[9] In a more recent study,[10] other investigators found equal or greater concentrations of cephapirin, compared with concentrations of desacetylcephapirin, in milk samples collected after intramammary infusion of cephapirin sodium. Some researchers[7,9–11] have described the pharmacokinetics of desacetylcephapirin, but little is known about the activity of desacetylcephapirin against common mastitis pathogens.[8]

The primary metabolite of ceftiofur is desfuroylceftiofur. Desfuroylceftiofur results from a cleavage of thioester and retains the same β-lactam ring of the ceftiofur group, which is essential for the biological activity of these compounds.[4] Agar diffusion was used in a study[5] that found both ceftiofur and desfuroylceftiofur caused in vitro inhibition of bacteria isolated from cattle, horses, poultry, and swine; however, few pathogens that cause mastitis in cattle were tested in that study.

In vitro susceptibility testing is used to determine the concentration of an antimicrobial that prevents growth of the bacteria of interest. In vitro susceptibility testing provides some indication of expected clinical efficacy but does not completely describe in vivo expectations.[12] Several studies[13–16] have found poor associations between results of in vitro susceptibility tests and in vivo responses after treatment of mastitis. Differences in the immunologic response of the host and the poor understanding of pharmacokinetics of drugs administered via the intramammary route are thought to contribute to the poor associations between in vitro outcomes and observed in vivo responses. However, determination of MICs remains an important tool to study and compare antimicrobial susceptibility of microorganisms against new drugs and to monitor changes in resistance over time. Similarly, little is known about the role of the active metabolites of intramammary drugs in achieving successful treatment outcomes. The objectives of the study reported here were to determine and compare the distribution of MICs of cephapirin and ceftiofur to the MICs of their active metabolites, desacetylcephapirin and desfuroylceftiofur, respectively, for selected mastitis pathogens.

Materials and Methods

Selection and bacteriologic culture of isolates— Isolates of mastitis pathogens (n = 488) were selected within target pathogen groups by use of random numbers to provide isolates from the cryopreserved stored collection of pathogens that cause clinical and subclinical mastitis; these pathogens had been previously isolated from cows in commercial dairy herds located in Wisconsin. Isolates were originally collected in 2005 (n = 233), 2006, (87), 2007 (89), 2008 (6), 2009 (4), and 2010 (69). The original studies[3,6,14–20] that involved collection of these isolates had all been previously approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Staphylococcus aureus isolates were recovered from cattle with clinical (48 isolates; 18 farms) and subclinical (50 isolates; 18 farms) mastitis. Coagulase-negative staphylococci isolates (99 isolates; 23 farms) were recovered from cattle with subclinical mastitis (25 *Staphylococcus epidermidis* isolates from 12 farms, 25 *Staphylococcus simulans* isolates from 16 farms, 25 *Staphylococcus haemolyticus* isolates from 12 farms, and 24 *Staphylococcus chromogenes* isolates from 8 farms). *Streptococcus dysgalactiae* isolates were recovered from cattle with clinical (47 isolates; 12 farms) and subclinical (50 isolates; 24 farms) mastitis. *Streptococcus uberis* isolates were recovered from cattle with clinical (48 isolates; 10 farms) and subclinical (48 isolates; 21 farms) mastitis. *Escherichia coli* isolates (98 isolates; 42 farms) were recovered from cattle with clinical mastitis. Microbiological procedures for the identification of the isolates were consistent and performed as described by the National Mastitis Council.[21] For gram-positive bacteria, initial identification was made to the genus level; species then were determined by use of appropriate commercial test kits[a] on the basis of biochemical reactions.

Preparation of stock solutions of antimicrobials—The preparation of stock solutions of antimicrobials and methods used for agar dilution testing were performed as described by the Clinical and Laboratory Standards Institute.[22] In accordance with Clinical and Laboratory Standards Institute guidelines for all isolates, cephapirin[b] was first dissolved in phosphate buffer solution (0.1 mol/mL [pH, 6.0]) and ceftiofur[c] was dissolved in water. In accordance with the manufacturer's recommendations for all isolates, desfuroylceftiofur[d] was dissolved in dimethyl sulfoxide[c] and a methanol solution as a solvent. In accordance with the manufacturer's recommendations, desacetylcephapirin[e] used for coagulase-negative staphylococci, *S dysgalactiae*, *S uberis*, and *E coli* isolates was dissolved in sterile distilled water. The suspension of desacetylcephapirin was then used to prepare a stock solution of each antimicrobial (concentration of 1,280 µg/mL; created by the addition of sterile distilled water). Stock solutions were stored (up to 2 months) at –70°C until used.

Antimicrobial susceptibility testing—Antimicrobial susceptibility tests were performed with agar dilution as described by the Clinical and Laboratory Standards Institute.[22] Dilutions of each antimicrobial were incorporated into Mueller-Hinton media[f] and cooled to

produce agar plates. For *S aureus*, *E coli*, and coagulase-negative staphylococci, 12 serial dilutions (concentrations ranging from 0.03 to 64.0 µg/mL) were used for each of the 4 antimicrobials. For *Streptococcus* spp, 14 serial dilutions (concentrations ranging from 0.008 to 64 µg/mL) were used for each antimicrobial. In accordance with Clinical and Laboratory Standards Institute guidelines,[22] 5% defibrinated sheep blood[g] was added to the Mueller-Hinton agar to ensure growth of *Streptococcus* spp.

Isolates maintained at –70°C in tryptic soy broth with 20% glycerol were retrieved, plated, and then plated again (passaged twice) onto tryptic soy agar supplemented with 5% sheep blood and incubated for 18 to 20 hours at a mean ± SD of 36 ± 1°C. Bacterial suspensions were prepared and standardized to 0.5 McFarlands with a nephelometer.[h] A multipoint inoculator[i] was used to apply 1 µL of 36 suspensions (33 test organisms, 2 positive quality-control organisms, and sterile water as a negative control sample) onto each agar plate. Inoculated plates were incubated under aerobic conditions for 18 to 20 hours at 35 ± 1°C. Quality control was performed in accordance with Clinical and Laboratory Standards Institute guidelines[22] and included *S aureus* ATCC29213, *E coli* ATCC25922, and *Streptococcus pneumonia* ATCC49619.

Interpretation of susceptibility test results—The MIC was defined as the lowest concentration of each antimicrobial that inhibited visible growth of the target pathogens. Breakpoints for resistance were based on Clinical and Laboratory Standards Institute guidelines.[22] Breakpoints were ≤ 8 µg/mL (susceptible), 16 µg/mL (intermediate), and ≥ 32 µg/mL (resistant) for cephapirin. Breakpoints were ≤ 2 µg/mL (susceptible), 4 µg/mL (intermediate), and ≥ 8 µg/mL (resistant) for ceftiofur. No breakpoints were available for desacetylcephapirin and desfuroylceftiofur; thus, breakpoints of the parent compounds were used to classify the isolates (cross-susceptible or cross-resistant).

Statistical analysis—For all statistical analyses, values of $P < 0.05$ were considered significant.[23] Results for cephapirin, ceftiofur, desacetylcephapirin, and desfuroylceftiofur were summarized by calculating the MIC_{50} and MIC_{90}. Survival analysis[j] was used to determine whether cephapirin and ceftiofur had different MICs, compared with the MICs for the active metabolites. The range of antimicrobial concentrations tested was used as the time variable in the survival analysis.[3] Inhibition of bacterial growth was used as the event, and isolates that had growth at the highest concentration tested were defined as not inhibited. For the Kaplan-Meier survival curves, time was defined as cephapirin, ceftiofur, desacetylcephapirin, and desfuroylceftiofur concentration, and the null hypothesis of no difference in the survival strata (antimicrobial concentration at inhibition) was tested via log-rank and Wilcoxon tests.

Results

The MICs of all quality-control isolates tested with ceftiofur and cephapirin were within expected ranges.[22] However, no data were available for acceptable ranges of quality-control organisms tested with desfuroylceftiofur and desacetylcephapirin. All *S aureus* were susceptible to both cephapirin and ceftiofur (**Table 1**). The lowest MIC_{90} for *S aureus* was for cephapirin (0.25 µg/mL) and was identical for isolates obtained from cattle with clinical or subclinical mastitis. The highest MIC_{90} for *S aureus* was for desfuroylceftiofur (16.0 µg/mL; cattle with subclinical mastitis). The MIC_{90} was 3 and 4 dilutions as great for desfuroylceftiofur, compared with that for ceftiofur, on isolates obtained from cattle with *S aureus*–induced clinical and subclinical mastitis, respectively. For both clinical and subclinical mastitis caused by *S aureus*, the MIC_{50} and MIC_{90} of cephapirin (0.25 µg/mL) were similar or within 1 dilution of the values for desacetylcephapirin. Heterogeneous survival curves based on clinical and subclinical mastitis were obtained for *S aureus* for desfuroylceftiofur ($P < 0.001$; log-rank and Wilcoxon tests) and desacetylcephapirin ($P = 0.037$ and 0.031; log-rank and Wilcoxon tests, respectively [data not shown]). Homogenous survival curves for clinical and subclinical mastitis were

Table 1—Percentages of *Staphylococcus aureus*, *Escherichia coli*, and coagulase-negative staphylococci mastitis pathogen isolates at each MIC for ceftiofur, desfuroylceftiofur, cephapirin, and desacetylcephapirin.

					MIC (µg/mL)														
Bacteria	Antimicrobial	Type of mastitis	No. of isolates	Susceptible isolates (%)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	NI	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
S aureus	Ceftiofur	Clinical	48	100	0	0	0	10.4	58.3	31.3	0	0	0	0	0	0	0	0.50	1.00
		Subclinical	50	100	0	0	0	12.0	42.0	46.0	0	0	0	0	0	0	0	0.50	1.00
	Desfuroylceftiofur	Clinical	48	—	0	0	0	0	0	0	8.3	58.3	25.0	8.3	0	0	0	4.00	8.00
		Subclinical	50	—	0	0	0	0	0	0	2.0	28.0	38.0	32.0	0	0	0	8.00	16.00
	Cephapirin	Clinical	48	100	0	4.2	64.6	29.2	2.1	0	0	0	0	0	0	0	0	0.12	0.25
		Subclinical	50	100	0	10.0	34.0	56.0	0	0	0	0	0	0	0	0	0	0.25	0.25
	Desacetylcephapirin	Clinical	48	—	0	0	25.0	45.8	29.2	0	0	0	0	0	0	0	0	0.25	0.50
		Subclinical	50	—	0	0	10.0	44.0	46.0	0	0	0	0	0	0	0	0	0.25	0.50
Coagulase-negative staphylococci	Ceftiofur	Subclinical	99	97	0	3.0	9.1	36.4	37.4	8.1	3.0	2.0	1.0	0	0	0	0	0.50	1.00
	Desfuroylceftiofur	Subclinical	99	—	0	0	0	0	0	20.2	14.1	45.5	13.1	6.1	0	0	1.0	4.00	8.00
	Cephapirin	Subclinical	99	100	1.0	46.5	43.4	8.1	1.0	0	0	0	0	0	0	0	0	0.12	0.12
	Desacetylcephapirin	Subclinical	99	—	5.1	32.3	40.4	18.2	4.0	0	0	0	0	0	1.0	0	0	0.12	0.25
E coli	Ceftiofur	Clinical	98	95	0	0	4.1	45.9	33.7	7.1	4.1	3.1	2.0	0	0	0	0	0.50	1.00
	Desfuroylceftiofur	Clinical	98	—	0	0	0	0	19.4	59.2	12.2	0	1.0	3.1	0	3.1	2.0	1.00	2.00
	Cephapirin	Clinical	98	51	0	0	0	0	0	0	3.1	15.3	32.7	29.6	8.2	2.0	9.2	8.00	64.0
	Desacetylcephapirin	Clinical	98	—	0	0	0	0	0	0	0	0	0	0	4.1	6.1	89.8	NI	NI

*Bacteria were classified as susceptible to ceftiofur at an MIC of ≤ 2 µg/mL and to cephapirin at an MIC of ≤ 8 µg/mL.[22]
NI = Bacterial growth not inhibited at highest antimicrobial concentration. — = Not determined.

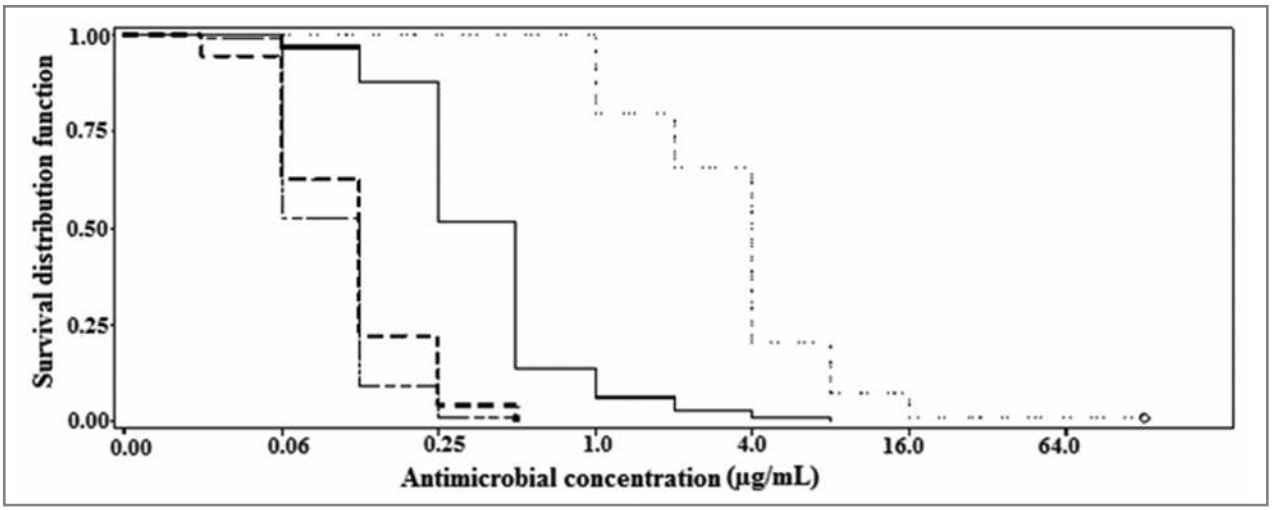

Figure 1—Kaplan-Meier survival curves for coagulase-negative staphylococci (n = 99) isolated from cattle with subclinical mastitis and stratified on the basis of the antimicrobial (ceftiofur [solid line], desfuroylceftiofur [dotted line], cephapirin [thin dashed line], and desacetylcephapirin [thick dashed line]) used for susceptibility testing. Censored data are indicated on the right (circle). A significant ($P < 0.001$; log-rank and Wilcoxon tests) difference in inhibition of isolates by ceftiofur versus desfuroylceftiofur and cephapirin versus desacetylcephapirin was detected.

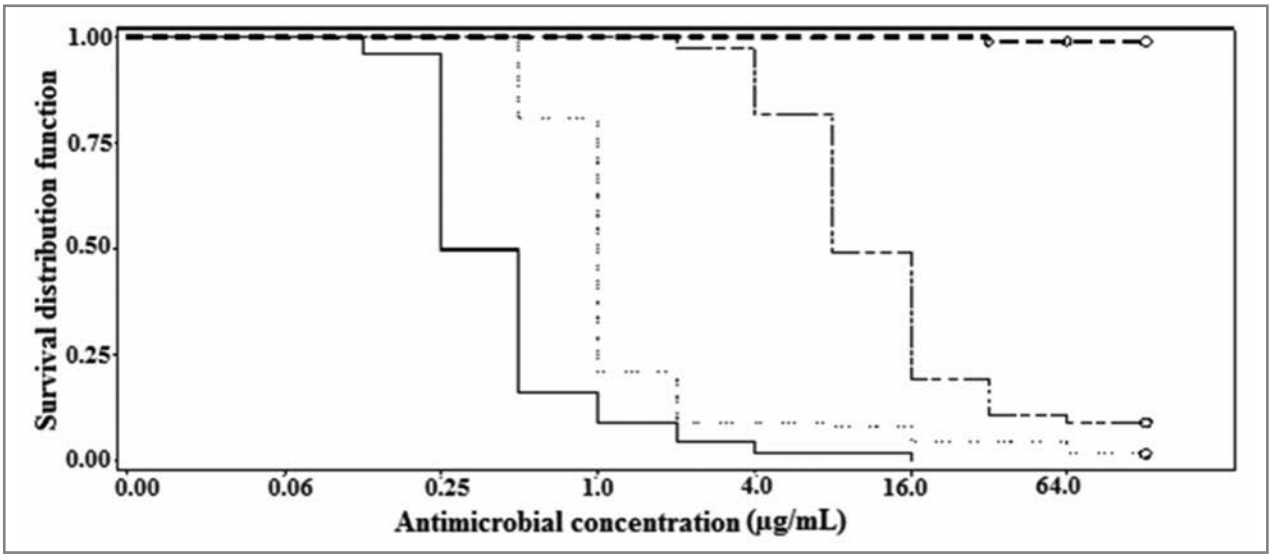

Figure 2—Kaplan-Meier survival curves for *Escherichia coli* (n = 98) isolated from cattle with clinical mastitis and stratified on the basis of the antimicrobial (ceftiofur [solid line], desfuroylceftiofur [dotted line], cephapirin [thin dashed line], and desacetylcephapirin [thick dashed line]) used for susceptibility testing. Censored data are indicated on the right (circle). A significant ($P < 0.001$; log-rank and Wilcoxon tests) difference in inhibition of isolates by ceftiofur versus desfuroylceftiofur and cephapirin versus desacetylcephapirin was detected.

obtained for *S aureus* for ceftiofur ($P = 0.227$ and 0.252; log-rank and Wilcoxon tests, respectively) and cephapirin ($P = 0.091$ and 0.078; log-rank and Wilcoxon tests, respectively).

All 99 coagulase-negative staphylococci isolates were considered susceptible to cephapirin, and 96 of 99 (97.0%) were susceptible to ceftiofur. Among tested antimicrobials, cephapirin had the lowest MIC_{90} (0.12 µg/mL) for coagulase-negative staphylococci (Table 1). Desfuroylceftiofur had the highest MIC_{90} for coagulase-negative staphylococci (8.0 µg/mL). The MIC_{90} for coagulase-negative staphylococci was 3 additional serial dilutions as great for desfuroylceftiofur (8 µg/mL) as for ceftiofur (1 µg/mL). For coagulase-negative staphylococci, the MIC_{90} was 1 serial dilution as great for desacetylcephapirin as for cephapirin. One coagulase-negative staphylococci isolate was not inhibited by the highest concentration of desfuroylceftiofur. For subclinical mastitis caused by coagulase-negative staphylococci, the MIC_{50} and MIC_{90} were all identical or within 1 serial dilution. Heterogeneous survival curves were obtained between ceftiofur and desfuroylceftiofur, cephapirin, and desacetylcephapirin ($P < 0.001$; log-rank and Wilcoxon tests) when tested on the basis of subclinical mastitis caused by coagulase-negative staphylococci infection (**Figure 1**).

Of the 98 *E coli* isolates, 50 (51.0%) and 93 (94.9%) were susceptible to the parent compounds of cephapirin and ceftiofur, respectively. The lowest MIC_{90} was for ceftiofur (1 µg/mL), and the highest MIC_{90} was

Table 2—Percentages of *Streptococcus dysgalactiae* and *Streptococcus uberis* mastitis pathogen isolates at each MIC for ceftiofur, desfuroylceftiofur, cephapirin, and desacetylcephapirin.

					MIC (μg/mL)															
Bacteria	Antimicrobial	Type of mastitis	No. of isolates	Susceptible isolates	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	NI	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
S dysgalactiae	Ceftiofur	Clinical	47	100	0	0	72.3	27.7	0	0	0	0	0	0	0	0	0	0	0	0.03	0.06
		Subclinical	50	100	0	0	72.0	28.0	0	0	0	0	0	0	0	0	0	0	0	0.03	0.06
	Desfuroyl-ceftiofur	Clinical	47	—	0	0	0	61.7	38.3	0	0	0	0	0	0	0	0	0	0	0.06	0.12
		Subclinical	50	—	0	0	0	66.0	34.0	0	0	0	0	0	0	0	0	0	0	0.06	0.12
	Cephapirin	Clinical	47	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0.03
		Subclinical	50	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0.03
	Desacetyl-cephapirin	Clinical	47	—	0	0	0	53.2	46.8	0	0	0	0	0	0	0	0	0	0	0.06	0.12
		Subclinical	50	—	0	0	0	40.0	60.0	0	0	0	0	0	0	0	0	0	0	0.12	0.12
S uberis	Ceftiofur	Clinical	48	93.8	0	0	0	20.8	8.3	0	4.2	39.6	20.8	2.1	0	4.2	0	0	0	1.00	2.00
		Subclinical	48	93.8	0	0	0	2.1	18.7	0	2.1	12.5	58.3	4.2	0	2.1	0	0	0	2.00	2.00
	Desfuroyl-ceftiofur	Clinical	48	—	0	0	0	0	22.9	6.2	2.1	18.8	43.7	2.1	4.2	0	0	0	0	2.00	2.00
		Subclinical	48	—	0	0	0	0	12.5	8.3	0	8.3	60.4	8.3	0	2.1	0	0	0	1.00	2.00
	Cephapirin	Clinical	48	100	0	0	10.4	18.7	4.2	417	22.9	2.1	0	0	0	0	0	0	0	0.25	0.50
		Subclinical	48	100	0	0	8.3	14.6	0	41.7	33.3	2.1	0	0	0	0	0	0	0	0.25	0.50
	Desacetyl-cephapirin	Clinical	48	—	0	2.1	20.8	6.2	6.2	6.2	43.7	12.5	2.1	0	0	0	0	0	0	0.50	1.00
		Subclinical	48	—	0	0	2.1	16.7	4.2	10.4	52.1	8.3	6.2	0	0	0	0	0	0	0.50	1.00

See Table 1 for key.

for cephapirin (64 μg/mL); 9 of 98 (9.2%) isolates were not inhibited at the highest concentration of cephapirin (Table 1). Of the 98 *E coli* isolates, 88 (89.8%) were not inhibited at the highest concentration of desacetylcephapirin (64 μg/mL). The MIC_{50} and MIC_{90} were within 1 serial dilution for desfuroylceftiofur and its parent compound of ceftiofur. In contrast, the MIC_{50} and MIC_{90} for cephapirin differed by 3 serial dilutions. For *E coli* isolates, heterogeneous survival curves were obtained when comparing ceftiofur with desfuroylceftiofur and cephapirin with desacetylcephapirin ($P < 0.001$; log-rank and Wilcoxon tests; **Figure 2**).

All 97 *S dysgalactiae* isolates were susceptible to both ceftiofur and cephapirin, and consistent inhibitory concentrations were obtained for all tested compounds (**Table 2**). The MIC_{90} values were identical for desfuroylceftiofur and desacetylcephapirin (0.12 μg/mL). For both ceftiofur and desfuroylceftiofur, the MIC_{50} was consistently 1 serial dilution less than the MIC_{90}. Heterogeneous survival curves were obtained for ceftiofur and cephapirin, compared with their parent compounds and desacetylcephapirin, for clinical or subclinical mastitis ($P < 0.001$; log-rank and Wilcoxon tests). Survival curves for ceftiofur and desfuroylceftiofur by clinical or subclinical mastitis were homogeneous ($P = 0.970$ and 0.195; log-rank and Wilcoxon tests, respectively [data not shown]).

All 96 *S uberis* isolates were susceptible to cephapirin, and 90 of 96 (93.8%) were susceptible to ceftiofur. The MIC_{90} was the lowest for cephapirin (0.5 μg/mL), and there was a difference of only 1 dilution between the MIC_{90} for cephapirin and desacetylcephapirin (Table 2). The MIC_{90} values for ceftiofur were 2.0 μg/mL for isolates obtained from cattle with clinical and subclinical mastitis and were similar or within 1 serial dilution of the MIC_{90} values for desfuroylceftiofur. Heterogeneous survival curves were obtained between cephapirin and desacetylcephapirin, regardless of whether the organism was from an animal with clinical or subclinical mastitis ($P < 0.001$; log-rank and Wilcoxon tests), and for ceftiofur by clinical or subclinical mastitis ($P = 0.008$ and 0.003; log-rank and Wilcoxon tests, respectively [data not shown]). Homogenous survival curves were obtained for *S uberis* when comparing ceftiofur with desfuroylceftiofur, cephapirin, desfuroylceftiofur, and desacetylcephapirin by clinical or subclinical mastitis (data not shown).

Although there were no interpretive criteria for determining the breakpoint for susceptibility of the active metabolites, there was an interesting relationship between the classifications of the isolates on the basis of the breakpoints of the parent compounds (**Tables 3 and 4**). Although all 98 *S aureus* were classified as susceptible to the parent compound (ceftiofur), only 5 of 98 (5.1%) *S aureus* isolates were considered susceptible to the metabolite (desfuroylceftiofur). Similarly, although 96 of 99 (97.0%) coagulase-negative staphylococci were classified as susceptible to ceftiofur, only 34 of 99 (34.3%) coagulase-negative staphylococci isolates were classified as susceptible to desfuroylceftiofur. In

Table 3—Cross-susceptibility and cross-resistance of desfuroylceftiofur and ceftiofur for isolates of *S aureus*, coagulase-negative staphylococci, *E coli*, *S dysgalactiae*, and *S uberis*.

	Ceftiofur		
Isolate	Susceptible	Intermediate	Resistant
Desfluroylceftiofur			
S aureus (n = 98)			
Susceptible	5.1	0	0
Intermediate	42.9	0	0
Resistant	52.0	0	0
Coagulase-negative staphylococci (n = 99)			
Susceptible	34.3	0	0
Intermediate	45.5	0	0
Resistant	17.2	2.0	1.0
E coli (n = 98)			
Susceptible	90.8	0	0
Intermediate	0	0	0
Resistant	4.1	3.1	2.0
S dysgalactiae (n = 97)			
Susceptible	100	0	0
Intermediate	0	0	0
Resistant	0	0	0
S uberis (n = 96)			
Susceptible	91.7	0	0
Intermediate	2.1	3.1	0
Resistant	0	0	3.1

Values reported are percentages.

Table 4—Cross-susceptibility and cross-resistance of desacetylcephapirin and cephapirin for *S aureus*, coagulase-negative staphylococci, *E coli*, *S dysgalactiae*, and *S uberis*.

	Cephapirin		
Isolate	**Susceptible**	**Intermediate**	**Resistant**
Desacetylcephapirin			
S aureus (n = 98)			
Susceptible	100	0	0
Intermediate	0	0	0
Resistant	0	0	0
Coagulase-negative staphylococci (n = 99)			
Susceptible	100	0	0
Intermediate	0	0	0
Resistant	0	0	0
E coli (n = 98)			
Susceptible	0	0	0
Intermediate	0	0	0
Resistant	29.6	19.4	51
S dysgalactiae (n = 97)			
Susceptible	100	0	0
Intermediate	0	0	0
Resistant	0	0	0
S uberis (n = 96)			
Susceptible	100	0	0
Intermediate	0	0	0
Resistant	0	0	0

Values reported are percentages.

contrast, 89 of 98 (90.8%) *E coli* isolates were classified as susceptible to both ceftiofur and desfuroylceftiofur, and only 4 of 98 (4.1%) *E coli* isolates classified as susceptible to the parent compound (ceftiofur) were classified as resistant to the metabolite (desfuroylceftiofur). There was agreement for all classifications of susceptibility between ceftiofur and desfuroylceftiofur for *S dysgalactiae* isolates, and only 2 of 96 (2.1%) *S uberis* isolates would have been reclassified as intermediate for desfuroylceftiofur.

On the basis of the MIC breakpoints of cephapirin, for all staphylococci and streptococci, there was total agreement in cross-classified susceptibility outcomes with desacetylcephapirin (Table 4). In contrast, 50 of 98 (51.0%) *E coli* were classified as resistant to both the parent compound (cephapirin) and the active metabolite (desacetylcephapirin). On the basis of the breakpoints for cephapirin, which were used for desacetylcephapirin, all of the *E coli* isolates classified as susceptible (29/98 [29.6%]) or intermediate (19/98 [19.4%]) to cephapirin were also classified as resistant to the metabolite (desacetylcephapirin).

Discussion

In the study reported here, we used isolates of mastitis pathogens collected from numerous cattle with clinical or subclinical mastitis at several farms. The tested isolates were representative of those that caused mastitis in cattle on commercial dairy farms in Wisconsin during the past 8 years. Results of this study are likely to be extrapolated for herds in regions with a similar distribution of mastitis pathogens.

The higher inhibitory activity for cephapirin, compared with that for desacetylcephapirin, was similar to results reported in another study.[8] In that study,[8] both *S aureus* and *E coli* were inhibited at lower concentrations of cephapirin than of desacetylcephapirin. Although the isolates used in that study were from clinically affected humans and the investigators used broth dilution, the geometric mean MICs reported for *S aureus* (0.23 and 0.42 µg/mL for cephapirin and desacetylcephapirin, respectively) were reasonably similar to the MIC_{90} values for isolates in the present study (0.25 and 0.50 µg/mL for cephapirin and desacetylcephapirin, respectively). In contrast, the geometric mean MIC of cephapirin (7.6 µg/mL) for *E coli* reported in that other study[8] was approximately 3 dilutions less than the MIC_{90} of cephapirin for *E coli* in the present study. This difference may have been a result of differences in the methods or pathogens over time or attributable to differences in the origin of the isolates. The MIC_{90} of desacetylcephapirin for *E coli* was not determined in the present study because approximately 90% of tested isolates were not inhibited at the highest concentration (64 µg/mL). Investigators in 1 study[24] also used agar dilution and reported similar MIC_{90} values of cephapirin for *S aureus*, *S dysgalactiae*, and *S uberis*, but they reported a 3-fold lower dilution for the MIC_{90} of cephapirin for *E coli*.

The MICs of ceftiofur and desfuroylceftiofur for *S aureus* isolated from bovine intramammary infections were determined via agar dilution in 1 study,[5] and the values were almost identical to the MIC_{90} values determined in the present study (1 µg/mL for ceftiofur and 8 µg/mL for desfuroylceftiofur). The MIC_{90} of ceftiofur reported here for all isolates (except *S uberis*) was similar to the MIC_{90} listed on the FDA-approved US product label of the commercially available intramammary ceftiofur product. The MIC_{90} of ceftiofur for *S uberis* in the present study was approximately 3 dilutions as great as the values listed on the product label. Differences of only 1 or 2 dilutions in the MIC_{90} were detected for desfuroylceftiofur when tested against *E coli* and both species of streptococci. In contrast, the MIC_{90} of desfuroylceftiofur for *S aureus* and coagulase-negative staphylococci was approximately 3 to 4 times as great as the MIC_{90} of the parent compound. This outcome was not unexpected, given that later-generation cephalosporin compounds are expected to have greater activity against gram-negative organisms but slightly reduced activity against gram-positive organisms.

Clinical outcomes after mastitis treatment are influenced by the pathogen, characteristics of the host, and adequacy of the antimicrobial treatment. Several studies[14–16] have indicated that results of in vitro susceptibility tests cannot completely predict clinical outcomes, and a better understanding of the role of the active metabolites may improve understanding of the best way to target mastitis treatment. The current guidelines by the Clinical and Laboratory Standards Institute define breakpoints of antimicrobials approved for mastitis treatment.[22] The guidelines do not specifically provide breakpoints for desfuroylceftiofur and desacetylcephapirin; however, results of studies[10,25] have suggested that the pharmacokinetics of both active metabolites is reasonably similar to that of their parent compounds, and it is probably acceptable to extrapolate those breakpoints when determining susceptibility for the metabolites.

When breakpoints of the parent compounds listed in the guidelines of the Clinical and Laboratory Stan-

dards Institute were used to cross-classify the isolates, differences in the categorized outcomes for the susceptibility tests were detected for combinations of several pathogens and compounds. The commercially available intramammary ceftiofur product has label indications for treatment of mastitis caused by coagulase-negative staphylococci, *S dysgalactiae*, and *E coli*. For *S dysgalactiae*, *S uberis*, and *E coli*, there was substantial agreement (91.7% to 100%) between the in vitro susceptibility of the parent compound and susceptibility for the active metabolite. In contrast, there were considerable differences in the interpretation of susceptibility results for ceftiofur and desfuroylceftiofur when tested against *S aureus* and coagulase-negative staphylococci. For 98 *S aureus* and 99 coagulase-negative staphylococci isolates, 98 of 98 (100%) and 96 of 99 (97%) were categorized as susceptible to the parent compound, respectively. In contrast, many isolates were characterized as intermediate or resistant to desfuroylceftiofur, and only 5 of 98 (5.1%) and 34 of 99 (34.3%) *S aureus* and coagulase-negative staphylococci isolates, respectively, were considered susceptible to the active metabolite. These results are somewhat consistent with expectations for third-generation cephalosporins, considering that this class of drugs was developed to have better activity against gram-negative bacteria but are less active against staphylococci because of enhanced hydrolytic stability.[4] The clinical implications of these differences should be determined in future studies.

The commercially available intramammary product containing cephapirin has label indications for the treatment of mastitis caused by *S agalactiae* and *S aureus*. The prevalence of mastitis caused by *S agalactiae* has greatly decreased,[26] and this organism was not included in the present study because of its minor clinical importance. On the basis of the Clinical and Laboratory Standards Institute[22] breakpoint of the parent compound, all tested isolates (except for *E coli*) were considered susceptible to both cephapirin and desacetylcephapirin. First-generation cephalosporins are not indicated for treatment of infections caused by gram-negative bacteria. Thus, the considerable divergence in susceptibility test results for cephapirin and desacetylcephapirin was not unexpected. Although approximately half of the *E coli* isolates had apparent susceptibility to cephapirin, approximately 90% of the same isolates were not inhibited at the highest concentration of desacetylcephapirin that was tested (64 µg/mL). Intramammary antimicrobial treatment of most cattle with mild or moderate clinical mastitis caused by *E coli* is not generally recommended because of the expected high rate of spontaneous cure.[27] Nevertheless, analysis of results of the present study indicated that *E coli* have a considerable amount of innate resistance to desacetylcephapirin.

Results of the study reported here indicated differences in MICs between both ceftiofur and cephapirin and their active metabolites for all tested pathogens. There were variations in the differences in MICs among pathogens and compounds and in the magnitude of differences relative to clinical breakpoints of the parent compounds. Considerable differences in inhibition were evident between ceftiofur and desfuroylceftiofur when tested against staphylococci, whereas few *E coli* isolates were inhibited by the active metabolite of cephapirin. Differences in inhibition between parent compounds and their active metabolites may be responsible for some of the variation in results of susceptibility tests relative to clinical outcomes of mastitis treatment, and future studies should be directed toward a better understanding of the clinical implications of these differences.

a. Biomerieux, Marcy l'Etoile, France.
b. Interchem Corp, Paramus, NJ.
c. Sigma Chemical Co, St Louis, Mo.
d. Rocky Mountain Labs, Golden, Colo.
e. Toronto Research Chemicals, North York, ON, Canada.
f. Becton Dickinson, Sparks, Md.
g. Remel, Lenexa, Kan.
h. Sensititer, Trek Diagnostics, Westlake, Ohio.
i. Mast Diagnostics Ltd, Bootle, Merseyside, England.
j. PROC LIFETEST, SAS, version 9.1, SAS Institute Inc, Cary, NC.

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