



# Dairy Technical Bulletin

**ToDAY<sup>®</sup>** (cephapirin sodium)    **ToMORROW<sup>®</sup>** (cephapirin benzathine)    **Cefa-Lak<sup>®</sup>** (cephapirin sodium)    **Cefa-Dri<sup>®</sup>** (cephapirin benzathine)    **Hetacin-K<sup>®</sup>** (hetacillin potassium)    **Dry-Clox<sup>®</sup>** (cloxacillin benzathine)  
**PYRAMID<sup>®</sup>** Modified-Live vaccines    **Triangle<sup>®</sup>** Killed vaccines    **PRISM<sup>®</sup>** Modified-Live+Killed vaccines    **Polyflex<sup>®</sup>** (injectable ampicillin)    **Factrel<sup>®</sup>** (gonadorelin hydrochloride)    **CYDECTIN<sup>®</sup>** (moxidectin)

## Select Cephapirin Cefa-Lak<sup>®</sup>/ToDAY<sup>®</sup> and Cefa-Dri<sup>®</sup>/ToMORROW<sup>®</sup>

### Introduction

Cows infected with mastitis are usually treated with antibiotics. The use of antibiotics in food producing animals is increasingly being scrutinized because of a theoretical possibility that selection of antibiotic resistant strains of bacteria may develop over time. Monitoring antimicrobial resistance patterns has been shown to be important in selecting effective products for treatment of cows diagnosed with mastitis.

Cephapirin (Cefa-Lak/ToDAY, Cefa-Dri/ToMORROW) is a Cephalosporin class drug. It is bacteriocidal, and has a broad spectrum of proven activity against penicillin resistant, gram-positive organisms.

Since its development as an antimicrobial, numerous studies have been published to evaluate if resistance against Cephapirin has developed over time in the most common mastitis-causing organisms.

Year(s)	Samples tested	% samples resistant		Reference:
		<i>Staph. spp.</i>	<i>Strep. spp.</i>	
1974	637 isolates	1.1	7.5	VMSAC, April 1976
1975-1979	3013 isolates	2.6	8	Proceedings, Nat'l Mastitis Council, 1980
1999	468 isolates	0	0	Proceedings, Nat'l Mastitis Council, 2000

Cephalothin, a first generation Cephalosporin whose therapeutic spectrum includes  $\beta$ -lactamase susceptible organisms, is used to represent the Cephalosporin class of antibiotics in laboratory testing.

### Laboratory Testing of Milk Samples for Antimicrobial Resistance

The Kirby-Bauer disc diffusion assay is a laboratory procedure used to evaluate the susceptibility or resistance of bacteria to a specific drug or series of drugs. In this test, a specialized bacterial culture medium (Mueller-Hinton agar) plate is covered with a solution of bacteria. Discs, which have been saturated with a specified concentration of drug, are placed on this plate after incubation, a measurement of the diameter around the disc "zone of inhibition" is interpreted as indicating the ability of the drug to inhibit growth of the bacteria on the culture plate.



The validity of the results obtained by this test, as a guide to clinical response in mastitis cure rates, is questionable. The diameters of the zones of inhibition do not always correlate with drug diffusion in the mammary gland. Milk can also markedly decrease the in vitro activity of antibacterial drugs that are protein or lipid bound. Mastitic milk, in particular, has a different pH, fat and protein content, and casein distribution than 'normal' milk. This can affect the response to treatment with drugs when tested by the Kirby-Bauer method.

Despite some reservations regarding the scientific accuracy of this test, both clinical and herd profiles of mastitis can use the results of the Kirby-Bauer test to predict the outcome of treatment and the likelihood of a mastitis cure with a specific drug. In vitro antimicrobial susceptibility values "cannot be used to predict clinical efficacy" (J. Watts and R. Yancey, *Clinical Microbiological Review*, 7:346-356, 1994). It is, however, generally accepted that in vitro resistance is indicative of clinical resistance.

## Studies Evaluating Susceptibility Testing

In numerous studies, Cephapirin has been demonstrated to be a broad spectrum and clinically effective antibiotic for the control of common environmental mastitis pathogens.

In "**Antibiotic Susceptibility Patterns for Environmental Streptococci Isolated from Bovine Mastitis in Central California Dairies**," P.V. Rossitto, L. Ruiz, et al, (*J. Dairy Sci.* 85:132-138) demonstrated that 95.5 percent of 362 *Streptococcus* isolates in their laboratory were sensitive to Cephalosporin antibiotics.

In "**Comparison of seven antibiotic treatments with no treatment for bacteriological efficacy against bovine mastitis pathogens**" Wilson, D. J.; Gonzalez, R. N., et al, (*J. Dairy Sci.* 82: 1664-1670) demonstrated an overall 75 percent efficacy for Cephalosporin antibiotics against 9007 isolates of mastitis pathogens, including gram-positive and gram-negative organisms.

Drs. Peter Constable and Dawn Morin from the Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, published a scientific report in *JAVMA*, Vol. 221, No. 1, July 1, 2002, pp. 103-108.

### **"Use of antimicrobial susceptibility testing of bacterial pathogens isolated from milk of dairy cows with clinical mastitis to predict response to treatment with cephalirin and oxytetracycline"**

The authors evaluated 58 dairy cows with 121 episodes of clinical mastitis. Animals were evaluated by palpating the udder prior to milking, forestripping the milk to evaluate abnormalities, and evaluating the body temperature and demeanor of animals with visible signs of mastitis.

Mastitis was defined as abnormal glandular secretions (score 1), inflamed gland plus abnormal secretions (score 2), and systemic signs of illness (fever, off-feed, depression, etc), inflamed gland and abnormal secretions (score 3).

Cows with a score of 1 were treated in the gland with cephalirin (Cefa Lak). Cows with a score of 2 were treated with oxytetracycline IV (extra-label use) and with cephalirin in the gland. Cows with a score of 3 were treated with oxytetracycline IV and flunixin meglumine IM (extra-label use), and affected glands were stripped two, three, or eight times per day following administration of Oxytocin.

Milk samples were obtained prior to treatment and were submitted for bacterial culture and sensitivity testing by the Kirby-Bauer method in accordance with National Mastitis Council guidelines.

Of the 121 episodes of mastitis evaluated in this study, 97 were associated with bacteria susceptible to cephalirin by the Kirby-Bauer test, and 24 cases were associated with resistant bacteria. All intermediate results were considered resistant in this study.

Kirby-Bauer Results for Cephapirin (Cefa-Lak/ToMORROW)

Gram-Positive Bacteria	% Susceptible	% Resistant
<i>Strep uberis</i>	100	0
<i>Strep dysgalactiae</i>	100	0
<i>Corynebacterium</i>	97	3
<i>Strep bovis</i>	91	9
<i>Strep fecalis</i>	50	50
<i>Coagulase Negative Staph</i>	100	0
Gram-Negative Bacteria	% Susceptible	% Resistant
<i>Klebsiella pneumoniae</i>	100	0
<i>E. coli</i>	56	44
Misc.	31	69
<b>TOTAL</b>	<b>77</b>	<b>23</b>

Susceptibility to other drugs used to treat mastitis was also evaluated

	% Susceptible	% Resistant
Cephapirin (Cefa-Lak/ToDAY)	77	23
Penicillin- Novobiocin	64	36
Pirlimycin	58	42
Penicillin	54	46
Erythromycin	53	47
Cloxicillin	16	84

The authors conclude that the results of the Kirby-Bauer test were of value in predicting the bacteriologic response to intramammary administration of cephapirin ONLY among cows with ‘mild’ clinical mastitis (score 1), but were NOT of value in predicting the clinical outcome of more severe mastitis cases (score 2,3). In more severe mastitis cases, there was no significant correlation between treatment and cure rate.

The authors state:

**“Cephapirin (Cefa-Lak/ToDAY) 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. When bactericidal b-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.”**

In a retrospective study, **“Trends in Antibacterial Susceptibility of Mastitis Pathogens during a Seven-Year Period,”** Drs. R. J. Erskine, R. D. Walker, C. A. Bolin, and P. C. Bartlett, of Michigan State University and D. G. White of the Food and Drug Administration, evaluated trends in susceptibility of common mastitis-causing bacteria from clinical samples to determine if antimicrobial resistance developed over time.

The authors reviewed results from a total of 2778 isolates received in the diagnostic laboratory from 1994 to 2000. Samples were isolated, identified and subjected to in vitro antimicrobial susceptibility testing.

The results of the study demonstrate:

**“That the proportion of bacterial isolates determined to be susceptible did not change during the seven-year period for the majority of bacterial-antibacterial interactions tested. Overall, there was no indication of increased resistance of mastitis isolates to antibacterials that are commonly used in dairy cattle.”**

A study “**Antimicrobial Resistance of Bacteria Isolated from Dairy Cow Milk Samples Submitted for Bacterial Culture: 8,905 samples (1994-2001)**” was published in JAVMA, Vol. 222, No.11, June 1, 2003, pp.1582-1589.

The authors, J.A. Makovec and P. L. Ruegg, evaluated 8,905 milk samples received in the Milk Mastitis Laboratory from January 1994 through June 2001. They evaluated whether patterns of bacterial resistance changed appreciably over time.

Their results demonstrate that for  $\beta$ -lactam antibiotics (example: Cephalosporin) the percentage of antibiotic resistant Gram positive bacteria did not increase over time. The percentage of Streptococcus isolates resistant to Cephalothin decreased significantly ( $P = 0.048$ ) from four percent in 1994 to one percent in 2001.

In contrast there was an increase in the percentage of organisms resistant to Macrolide antibiotics (example: Pirlimycin) over time. The percentage of Staphylococcus isolates resistant to Pirlimycin increased significantly ( $P < 0.001$ ) from six percent in 1994 to 19 percent in 2001.

% Strains Resistant to Antibiotic

	<i>Staph. aureus</i>	<i>Staph. spp.</i>	<i>Strep. spp</i>	<i>E. coli</i>	<i>Klebsiella</i>
Ampicillin	34.9	30.1	1.8	21.9	89.1
<b>Cephalothin</b>	<b>0.1</b>	<b>0.6</b>	<b>3.0</b>	<b>27.9</b>	<b>12.1</b>
Cloxicillin	1.8	6.8	42.3	99.4	99.1
Penicillin	35.4	32.6	5.1	99.6	99.5
<b>Pirlimycin</b>	<b>4.8</b>	<b>13.6</b>	<b>20.9</b>	<b>NT</b>	<b>NT</b>

The authors also evaluated the percentage of resistant strains seen in their laboratory for each of the commonly used antibiotics.

### Some Important Points to Consider:

- 1) Gram-positive environmental organisms (Staph and Strep) are the most common source of intramammary infection especially in the periods around dry-off and early in lactation.
- 2) Intramammary infections with these organisms precede the visual detection of clinical mastitis in the animal.
- 3) Antimicrobial therapies are designed to eliminate infections by microbial organisms and do not have a specific effect on inflammation, which is the result of the intramammary infection.

### Take Home Messages

- 1) Early detection of intramammary infections and prompt treatment with Cefa-Lak was related to increased bacterial cure rates as opposed to late detection (waiting until animals have severe mastitis) or delayed treatment in the University of Illinois study.
- 2) There are NO INDICATIONS that bacterial resistance against Cefa- Lak is increasing. Studies conducted from 1974 to present have shown Cephapirin to be a broad spectrum and effective product. Dr. Ruegg points out resistance has decreased significantly in the 8,900 samples she evaluated at the University of Wisconsin Mastitis Laboratory. Dr. Ron Erskine at the University of Michigan evaluated 2,778 milk samples and also found no indications of increased bacterial resistance over time.
- 3) Cefa-Lak/ToDAY and Cefa-Dri/ToMORROW continue to be broad spectrum, safe and effective antibiotics for use in dry and fresh cows.
- 4) There is no evidence to recommend “rotation” between different antibiotics to avoid the development of bacterial resistance.

