PROFESSIONAL SERVICES
Boehringer Ingelheim Vetmedica, Inc.

TECHNICAL BULLETIN

# Minimum Inhibitory Concentrations (MIC) for cephalosporin compounds and their active metabolites for selected mastitis pathogens.

This technical bulletin is a summary of a study published in the AVJR: AJVR, Vol 74, No. 5, May 2013

## Introduction

Ceftiofur and cephapirin are cephalosporin class antibiotics currently labeled for use as lactating and dry cow intramammary tubes in the USA. However, when these antibiotics are infused into the mammary quarter they are partially converted to active metabolites. The major metabolite for ceftiofur (CEF) is desfuroylceftiofur (DFC) and for cephapirin (CEPH) the metabolite is deacetylcephapirin (DAC). After the IMM administration of 125 mg of ceftiofur hydrochloride, residue analysis at 12 hrs and 24 hrs showed that 50% and 20% respectively of total residues were from the parent.[1] In a recently published paper that measured residues after the IMM administration of 200mg Cephapirin, the ratio of parent to metabolite was approximately one at 24 hrs post last treatment.[2] These metabolites still have antimicrobial activity, but it was suspected they are not as active as the parent compound when tested against mastitis pathogens.

MIC is the minimum concentration of a particular antibiotic that will inhibit growth of a particular bacterium. A solution of bacteria at a predetermined concentration is cultured from a single isolate and then samples of that solution are tested against a range of dilutions of the test antibiotic. Each dilution change usually represents a doubling of the antibiotic concentration. This testing may be done in tubes containing broth or on agar plates containing the serial antibiotic concentrations. The process is repeated many times for different isolates of the same bacterium and the $MIC_{50}$ and $MIC_{90}$ are the lowest antibiotic concentrations that will inhibit 50% and 90% respectively of the isolates tested.

## Study Design

This study compared the MICs of CEF and CEPH to their respective metabolites DFC and DAC when tested against common mastitis pathogens. Agar dilution MIC methodology was utilized in the study according to Clinical and Laboratory Standards Institute (CSLI) guidelines.

The 488 mastitis isolates tested were all collected on Wisconsin dairy farms from 2005 to 2010. Isolates were from both subclinical and clinical intramammary infections.

With the *Staphylococcus aureus*, *Escherichia coli*, and coagulase-negative staphylococci isolates, 12 serial dilutions of the tested antibiotics were used with concentration range being from 0.03 to 64 µg/mL.

For the *Streptococcus dysgalactiae* and *Streptococcus uberis* isolates, 14 serial dilutions of the tested antibiotics were used with concentration range being from 0.008 to 64µg/mL.

Breakpoints for susceptibility and resistance were based on CLSI guidelines and are as follows:

| Antibiotic | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Ceftiofur | ≤ 2 µg/mL | 4 µg/mL | ≥ 8 µg/mL |
| Cephapirin | ≤ 8 µg/mL | 16 µg/mL | ≥ 32 µg/mL |

There are no guidelines for the metabolites and in the paper the authors assumed the same break points as for the parent antibiotics.

## Results

Tables 1 and 2 display a summary of the results of the MIC testing.

Boehringer Ingelheim

## Summary

This study demonstrated there can be a significant difference in the MIC between the parent and metabolite of cephalosporin antibiotics for mastitis pathogens. This difference in activity is only really important and relevant when it significantly changes the proportion of isolates that are classified as susceptible to the antibiotic parent compared to its metabolite. Table 1 and Table 2 show the susceptibility to the parent and the metabolite of each mastitis pathogen by clinical or subclinical type.

The breakpoints for the metabolite are assumed to be the same as the parent.

There was a very marked difference in susceptibility of *Staph aureus* to the parent ceftiofur (100% susceptible) compared to its metabolite desfuroylceftiofur (5.1% susceptible). In contrast 100% of *Staph aureus* isolates were susceptible to both cephapirin and deacetylcephapirin. Another interesting observation is that there is a 3 (clinical) or 4 (subclinical) dilution difference (8 and 16 times increase in concentration) between the $MIC_{90}$ of ceftiofur and desfuroylceftiofur. There was only a one dilution change (doubling in concentration) in the $MIC_{90}$ between cephapirin and deacetylcephapirin (both clinical and subclinical).

For the CNS isolates, 97% were susceptible to ceftiofur but only 34.3% were susceptible to its metabolite desfuroylceftiofur. In contrast, 100% of CNS isolates were susceptible to both cephapirin and deacetylcephapirin. There was a 3 dilution difference (8 times increase in concentration) between the $MIC_{90}$ of ceftiofur and desfuroylceftiofur. There was only a 1 dilution change (doubling of antibiotic concentration) between the $MIC_{90}$ of cephapirin and deacetylcephapirin.

*E coli* isolates were highly susceptible to both the parent ceftiofur and the metabolite desfuroylceftiofur (95% and 91% respectively). There was a marked difference between the parent cephapirin (51% susceptibility) and the metabolite deacetylcephapirin where no isolates were sensitive at the highest concentration tested. There was a single dilution difference (doubling in antibiotic concentration) between the $MIC_{90}$ of ceftiofur and desfuroylceftiofur. The $MIC_{90}$ of deacetylcephapirin could not be determined because it was beyond the highest antibiotic concentration tested (64 µg/mL).

**Table 1**— Percentage of *Staphylococcus aureus*, *Escherichia coli*, and coagulase-negative staphylococci isolates classified as sensitive, intermediate or resistant for ceftiofur, desfuroylceftiofur, cephapirin, and desacetylcephapirin.*

| Bacteria | Antimicrobial | Type of mastitis | No. of isolates | Susceptible isolates (%)* | Intermediate isolates (%) | Resistant isolates (%) | $MIC_{50}$ (mg/mL) | $MIC_{90}$ (mg/mL) |
|---|---|---|---|---|---|---|---|---|
| *S aureus* | Ceftiofur | Clinical | 48 | 100 | 0 | 0 | 0.50 | 1.00 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.50 | 1.00 |
| | Desfuroylceftiofur | Clinical | 48 | 8.3 | 42.9 | 33.3 | 4.00 | 8.00 |
| | | Subclinical | 50 | 2.0 | 28 | 70.0 | 8.00 | 16.00 |
| | Cephapirin | Clinical | 48 | 100 | 0 | 0 | 0.12 | 0.25 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.25 | 0.25 |
| | Desacetylcephapirin | Clinical | 48 | 100 | 0 | 0 | 0.25 | 0.50 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.25 | 0.50 |
| | | | | | | | | |
| Coagulase-negative staphylococci | Ceftiofur | Subclinical | 99 | 97 | 0 | 3.0 | 0.50 | 1.00 |
| | Desfuroylceftiofur | Subclinical | 99 | 34.3 | 45.5 | 19.2 | 4.00 | 8.00 |
| | Cephapirin | Subclinical | 99 | 100 | 0 | 0 | 0.12 | 0.12 |
| | Desacetylcephapirin | Subclinical | 99 | 100 | 0 | 0 | 0.12 | 0.25 |
| | | | | | | | | |
| *E coli* | Ceftiofur | Clinical | 98 | 94.9 | 3.1 | 2 | 0.50 | 1.00 |
| | Desfuroylceftiofur | Clinical | 98 | 90.8 | 0 | 9.2 | 1.00 | 2.00 |
| | Cephapirin | Clinical | 98 | 51 | 29.6 | 19.4 | 8.00 | 64.0 |
| | Desacetylcephapirin | Clinical | 98 | — | 0 | 100 | NI | NI |

* Bacteria were classified as susceptible to ceftiofur at an MIC of ≤ 2 µg/mL intermediate at 4 µg/mL and resistant at ≥ 8 µg/mL

Bacteria were classified as susceptible to desfuroylceftiofur at the same break points as for ceftiofur

Bacteria were classified as susceptible to cephapirin at an MIC of ≤ 8 µg/mL mL intermediate at 16 µg/mL and resistant at ≥ 32 µg/mL

Bacteria were classified as susceptible to desacetylcephapirin at the same breakpoints as for cephapirin

The MIC results from this study clearly demonstrate the 1st generation cephalosporins are more effective *in vitro* against Gram positive pathogens, whereas the 3rd generation cephalosporins have superior Gram negative activity. It is also interesting to note that where cephapirin and deacetylcephapirin had superior activity (gram positive pathogens) there was only a 1 dilution difference between their $MIC_{90}$ and where ceftiofur and desfuroylceftiofur had superior activity (*E coli*) there was also only a 1 dilution difference in their $MIC_{90}$.

There was a very large difference in the $MIC_{90}$ for ceftiofur and desfuroyl ceftiofur for *Staph aureus* and the coagulase negative staphylocci which could become a concern with therapy. The *Strep spp* tested were highly susceptible to parent and metabolite for both ceftiofur and cephapirin.

The efficacy of intramammary therapy for a particular pathogen may depend on how much of the parent antibiotic has been metabolized. Routine laboratory susceptibility testing only reports MIC results for the parent compound. Several papers cited by the authors of this paper have commented on the lack of correlation between MIC results and clinical outcome in the treatment of mastitis. These results strongly suggest the MIC difference between parent and metabolite may be a very important factor responsible for this lack of correlation. The most appropriate antibiotic for intramammary therapy is likely to be dependent on the proportion of parent-to-metabolite in the udder and the ramifications of the MIC change between the two.

**Table 2**—Percentage of *Streptococcus dysgalactiae* and *Streptococcus uberis* isolates classified as sensitive, intermediate or resistant for ceftiofur, desfuroylceftiofur, cephapirin, and desacetylcephapirin.*

| Bacteria | Antimicrobial | Type of mastitis | No. of isolates | Susceptible isolates (%)* | Intermediate isolates (%) | Resistant isolates (%) | $MIC_{50}$ (mg/mL) | $MIC_{90}$ (mg/mL) |
|---|---|---|---|---|---|---|---|---|
| *S dysgalactiae* | Ceftiofur | Clinical | 47 | 100 | 0 | 0 | 0.03 | 0.06 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.03 | 0.06 |
| | Desfuroylceftiofur | Clinical | 47 | 100 | 0 | 0 | 0.06 | 0.12 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.06 | 0.12 |
| | Cephapirin | Clinical | 47 | 100 | 0 | 0 | 0.03 | 0.03 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.03 | 0.03 |
| | Desacetylcephapirin | Clinical | 47 | 100 | 0 | 0 | 0.06 | 0.12 |
| | | Subclinical | 50 | 100 | 0 | 0 | 0.12 | 0.12 |
| | | | | | | | | |
| *S uberis* | Ceftiofur | Clinical | 48 | 93.8 | 2.1 | 4.2 | 1.00 | 2.00 |
| | | Subclinical | 48 | 93.8 | 4.2 | 2.1 | 2.00 | 2.00 |
| | Desfuroylceftiofur | Clinical | 48 | 93.8 | 2.1 | 4.2 | 2.00 | 2.00 |
| | | Subclinical | 48 | 89.6 | 8.3 | 2.1 | 1.00 | 2.00 |
| | Cephapirin | Clinical | 48 | 100 | 0 | 0 | 0.25 | 0.50 |
| | | Subclinical | 48 | 100 | 0 | 0 | 0.25 | 0.50 |
| | Desacetylcephapirin | Clinical | 48 | 100 | 0 | 0 | 0.50 | 1.00 |
| | | Subclinical | 48 | 100 | 0 | 0 | 0.50 | 1.00 |

* Bacteria were classified as susceptible to ceftiofur at an MIC of ≤ 2 µg/mL intermediate at 4 µg/mL and resistant at ≥ 8 µg/mL

Bacteria were classified as susceptible to desfuroylceftiofur at the same break points as for ceftiofur

Bacteria were classified as susceptible to cephapirin at an MIC of ≤ 8 µg/mL intermediate at 16 µg/mL and resistant at ≥32 µg/mL

Bacteria were classified as susceptible to desacetylcephapirin at the same breakpoints as for cephapirin

## Statistical analysis:

Survival analysis was used to determine whether the metabolites DFC and DAC had different MICs compared with the MICs of the parent compounds (CEF and CEPH respectively). Log rank and Wilcoxin tests were employed and values of $P < 0.05$ were considered significant.

A summary of the results for each mastitis pathogen tested is given on the following page.

Abbreviations used: Ceftiofur (CEF), Desfuroylceftiofur (DFC), Cephapirin (CEPH), Deacetylcephapirin (DAC), Clinical Isolates (CI), Subclinical Isolates (SCI)

***Staph aureus:* (48 clinical, 50 subclinical isolates tested)**

The lowest $MIC_{90}$ was for CEPH at 0.25 µg/mL (CI and SCI).

The highest $MIC_{90}$ was for DFC at 16 µg/mL (SCI).

The % of *Staph aureus* sensitive to CEF was 100% ($MIC_{90}$ 1.0 µg/mL).

The % of *Staph aureus* sensitive to DFC was 5.1%.

There was a 3 dilution (SCI) and 4 (CI) dilution difference between the $MIC_{90}$ of CEF and DFC.

The % of *Staph aureus* sensitive to CEPH was 100%.

The % of *Staph aureus* sensitive to DAC was 100% ($MIC_{90}$ 0.5 µg/mL).

There was a 1 dilution difference (CI & SCI) between the $MIC_{90}$ of CEPH and DAC.

Heterogeneous survival curves (significant difference) were obtained between CEF and DFC and CEPH and DAC. (P < .001; log-rank and Wilcoxon tests).

**Coagulase negative Staphylococcus (CNS): (99 Subclinical isolates tested)**

The lowest $MIC_{90}$ was for CEPH at 0.12 µg/mL.

The highest $MIC_{90}$ was for DFC (SC) at 8 µg/mL.

The % of CNS sensitive to CEF was 97 % ($MIC_{90}$ 1.0 µg/mL).

The % of CNS sensitive to DFC was 34.3%.

There was a 3 dilution difference between the $MIC_{90}$ of CEF and DFC.

The % of CNS sensitive to CEPH was 100%.

The % of CNS sensitive to DAC was 100% ($MIC_{90}$ 0.25 µg/mL).

There was a one dilution difference between the $MIC_{90}$ of CEPH and DAC.

Heterogeneous survival curves (significant difference) were obtained between CEF and DFC and CEPH and DAC (P < .001; log-rank and Wilcoxon tests).

***E coli:* (98 Clinical isolates tested)**

The lowest $MIC_{90}$ was for CEF at 1.0 µg/mL.

The highest $MIC_{90}$ would have been for DAC at > 64 µg/mL. The $MIC_{90}$ could not be determined as 64 µg/mL was the highest concentration tested.

The % of *E coli* sensitive to CEF was 95%.

The % of *E coli* sensitive to DFC was 91% ($MIC_{90}$ 2.0 µg/mL).

There was a 1 dilution difference between the $MIC_{90}$ of CEF and DFC.

The % of *E coli* sensitive to CEPH was 51% ($MIC_{90}$ 64.0 µg/mL).

The % of *E coli* sensitive to DAC was 0%.

The dilution difference between the $MIC_{90}$ of CEPH and DAC could not be determined.

Heterogenous (statistically different) survival curves were obtained between CEF and DFC and CEPH and DAC (P < .001; log-rank and Wilcoxon tests).

***S dysgalactiae:* (47 Clinical, 50 Subclinical isolates tested)**

The lowest $MIC_{90}$ was for CEPH at 0.03 µg/ml (CI & SCI).

The highest $MIC_{90}$ was for DFC and DAC at 0.12 µg/mL (CI and SCI).

The % of *S dysgalactiae* sensitive to CEF was 100 % ($MIC_{90}$ 0.06 µg/mL).

The % of *S dysgalactiae* sensitive to DFC was 100 %.

There was a one dilution difference between the $MIC_{90}$ of CEF and DFC (SCI).

The % of *S dysgalactiae* sensitive to CEPH was 100%.

The % of *S dysgalactiae* sensitive to DAC was 100%.

There was a two dilution change (CIs & SCIs) between the $MIC_{90}$ of CEPH and DAC.

Heterogenous survival curves were obtained between CEPH and DAC (CIs & SCIs) (P < .001; log-rank and Wilcoxon tests).

Survival curves for CEF and DFC (CIs & SCIs) were homogenous (no significant statistical difference).

***S uberis:* (48 Clinical, 48 Subclinical isolates tested)**

The lowest $MIC_{90}$ was for CEPH at 0.5 µg/mL (CI & SCI).

The highest $MIC_{90}$ was for CEF & DFC (SCI) at 2 µg/mL.

The % of *S uberis* sensitive to CEF was 94%.

The % of *S uberis* sensitive to DFC was 92%.

There was a no dilution (CIs & SCIs) difference between the $MIC_{90}$ of CEF and DFC.

The % of *S uberis* sensitive to CEPH was 100%.

The % of *S uberis* sensitive to DAC was 100% ($MIC_{90}$ 1.0 µg/mL).

There was a one dilution difference (CIs & SCIs) between the $MIC_{90}$ of CEPH and DAC.

Heterogenous survival curves were obtained between CEPH and DAC (CIs & SCIs) (P < .001; log-rank and Wilcoxon tests).

Survival curves for CEF and DFC (CIs & SCIs) were homogenous (no significant statistical difference).

**References**

1 Freedom of Information Summary, NADA 141-238, Spectramast LC

2 Gordon et al



Boehringer Ingelheim

2621 North Belt Highway • St. Joseph, MO 64506-2002

Printed in the U.S.A.