# MONITORING OF ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM BOVINE INTRAMAMMARY INFECTIONS

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### Introduction

Antimicrobial substances are frequently used to treat clinical cases of bovine mastitis and as dry cow therapy. Both measures are considered important items of mastitis control programs for dairy herds (BRADLEY & GREEN, 2004). The continued use of antimicrobials in dairy herds is of concern due to selection of resistant forms of bacteria (WHO, 1997). The antimicrobial resistance found in animal pathogens may be related to failures in the mastitis curing rates. It might also affect human health by influencing the generation or selection of drug-resistant foodborne pathogens. Staphylococci are the most prevalent mastitis pathogens in Brazilian herds. The aim of this work was to monitor the antimicrobial susceptibility of staphylococci isolated from dairy herds during three years.

## Material and Methods

Eleven herds were visited four times at approximately six-month intervals during 2009-2011. Composite milk samples were collected from all cows immediately before milking. The herds were selected according to the harmonization of national antimicrobial resistance surveillance and monitoring programs (OIE) and based on a prevalence of 35% penicillin resistance of *S. aureus*. Six herds often acquired lactating cows from other herds, while the other five replaced the stock with their own heifers. Sample collection, bacterial isolation and identification followed procedures of NMC (2004). All staphylococci were submitted to disk diffusion antimicrobial susceptibility tests according to CLSI M31-A3 (2008), using *Staphylococcus aureus* ATCC 29213 as reference strain. Antimicrobials (Oxoid) tested were: ampicillin 10μg, ceftiofur 30μg, cephalothin 30μg, clindamycin 2μg, erythromycin 5μg, enrofloxacin 5μg, gentamicin 10μg, oxacillin 1μg, penicillin 10 units, sulfa-trimethoprim 1.25/23.75 μg and tetracycline 30μg.

## Results and Discussion

A total of 254 S. aureus and 113 coagulase-negative staphylococci (CNS) were isolated. Respectively, the numbers of S. aureus isolated in samplings 1, 2, 3 and 4 were 40, 74, 56 and 84 and the numbers of CNS were 37, 22, 23 and 31. Considering all S. aureus isolates, resistance was found to ampicillin (68%), penicillin (69.7%) and tetracycline (27.6%). The rate of sensitivity was above 90% for the other antimicrobials tested. The percentages of resistance found in CNS were: ampicillin (44%), penicillin (50%) and tetracycline (13%), oxacillin (13%), clindamycin (13%) and erythromycin (15%). The rate of sensitivity was above 90% for the other

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antimicrobials tested. The sensitivity rates for the four period sampled are shown in Table 1. The lack of a strict biosecurity program concerning the introduction of animals in some herds may have influenced the variation found in susceptibility patterns. The resistance molecular markers will be evaluated to verify their distribution among the herds.

Table 1. Percentages of antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative staphylococci isolated from 11 Brazilian herds, in four consecutive periods (S1, S2, S3, S4), separated by approximately six-month intervals.

| Antimicrobials     | Staphylococcus aureus (n=254) |      |      |      | Coagulase-negative staphylococci (n=113) |      |      |      |
|--------------------|-------------------------------|------|------|------|--|------|------|------|
|                    | S1                            | S2   | S3   | S4   | S1                                       | S2   | S3   | S4   |
| Ampicillin         | 42,5                          | 37,8 | 26,8 | 25   | 51,4                                     | 63,6 | 60,9 | 51,6 |
| Penicillin         | 50                            | 40,5 | 16   | 22,6 | 45,9                                     | 54,5 | 52,2 | 48,9 |
| Oxacillin          | 100                           | 98,6 | 100  | 100  | 100                                      | 90,9 | 65,2 | 83,9 |
| Cephalothin        | 100                           | 98,6 | 100  | 100  | 100                                      | 100  | 100  | 100  |
| Ceftiofur          | 100                           | 100  | 100  | 100  | 100                                      | 100  | 95,7 | 96,8 |
| Clindamycin        | 97,5                          | 91,9 | 100  | 89,3 | 97,3                                     | 95,5 | 69,6 | 80,6 |
| Gentamicin         | 95                            | 95,9 | 96,4 | 100  | 100                                      | 95,5 | 82,6 | 100  |
| Erythromycin       | 97,5                          | 81   | 98,2 | 80,1 | 94,6                                     | 77,3 | 78,3 | 83,9 |
| Enrofloxacin       | 100                           | 98,6 | 98,2 | 98,8 | 100                                      | 100  | 100  | 100  |
| Sulfa-trimethoprim | 100                           | 100  | 100  | 100  | 100                                      | 100  | 95,7 | 100  |
| Tetracycline       | 72,5                          | 70,3 | 85,7 | 65,5 | 91,9                                     | 68,2 | 69,6 | 70,9 |

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