

# CNS in bovine mastitis: Rapid identification and molecular characterization of antibiotic resistance genes

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## Key words

Bovine mastitis, coagulase-negative staphylococci, antibiotic resistance, methicillin-resistance, genotyping

## Aim of the study

To establish a rapid identification method of coagulase-negative staphylococci (CNS) species, determine their occurrence in bovine mastitis milk, and characterize their antimicrobial resistance mechanisms.

## Material and methods

CNS (n=417) were isolated from bovine mastitis milk samples, and from control milk samples in Switzerland. CNS were identified by MALDI-TOF and tested for antimicrobial resistance by broth dilution and microarray. Multiresistant and *mecA*-positive CNS were characterized by PFGE and SCC*mec* typing. *S. epidermidis* were examined by MLST, for biofilm operon, and for arginine catabolic mobile elements ACME (Frey et al. 2013). PCR/ESI-MS was evaluated for rapid molecular diagnosis of bovine mastitis (Perreten et al. 2013).

## Results and significance

Nineteen different CNS species were identified with *S. xylosus*, *S. chromogenes*, *S. haemolyticus* and *S. sciuri* being the most frequent. Resistances to oxacillin (47.0%), fusidic acid (33.8%), tiamulin (31.9%), penicillin (23.3%), tetracycline (15.8%), streptomycin (9.6%), erythromycin (7.0%), sulfonamides (5%), trimethoprim (4.3%), clindamycin (3.4%), kanamycin (2.4%) and gentamicin (2.4%) were detected. Resistance to oxacillin was attributed to the *mecA* gene in 9.7% of the oxacillin-resistant isolates. *MecA* was detected in *S. fleurettii*, *S. epidermidis*, *S. haemolyticus*, and *S. xylosus*. Resistance to tetracycline was attributed to *tet(K)* and *tet(L)*, penicillin resistance to *mecA* and *blaZ*, streptomycin resistance to *str* and *ant(6)-Ia*, erythromycin resistance to *erm(C)*, *erm(B)*, and *msr*. In total, 15.1% of the CNS isolates exhibited resistance to two or more antimicrobials. Otherwise, CNS were susceptible to antibiotics commonly used in mastitis treatment. High genetic diversity was observed among multiresistant CNS. ACME types 1 and 3 were detected in *S. epidermidis* (ST 59 and ST111). This study revealed the presence of multidrug-resistant CNS among a heterogeneous CNS population, recommending the consultation of an antibiogram prior treatment with antimicrobials for persisting infections (Frey et al. 2013). We also showed that PCR/ESI-MS has significant potential to serve as a rapid screening method in the diagnosis of mastitis and can be used to track resistance genes in milk (Perreten et al. 2013).

## Publications, posters and presentations

- Frey, Y. (2013) Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis. Dissertation, Vetsuisse Faculty, University of Bern.
- Frey, Y.; Rodriguez, J.P.; Thomann, A.; Schwendener, S.; Perreten, V. (2013) Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk. J. Dairy Sci. 96(4): 2247-57.
- Perreten, V.; A. Endimiani; A. Thomann; J.R. Wipf; A. Rossano; M. Bodmer; A. Raemy; K.A. Sannes-Lowery; D.J. Ecker; R. Sampath; R.A. Bonomo (2013) Evaluation of PCR electrospray-ionization mass spectrometry for rapid molecular diagnosis of bovine mastitis. J. Dairy Sci. 96(6): 3611-3620.
- Frey, Y. (2012) Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis. Oral presentation, workshop "Bovine mastitis research in Switzerland", Bern.
- Perreten V. Molecular diagnosis of bovine mastitis by PCR/ESI-MS (2012) Oral presentation, Joint annual meeting of the SSI, SSHH, SSM and SSTMP, St. Gallen, Switzerland.
- Perreten, V.; A. Endimiani; R. Sampath; D.J. Ecker; R. Bonomo. (2012) Molecular diagnosis and characterisation of bovine mastitis by PCR/ESI-MS. Poster presentation, 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) Meeting, London, UK.
- Frey, Y.; Rodriguez, J.P.; Thomann, A.; Schwendener, S.; Perreten, V. (2012) Antibiotic resistance in coagulase-negative staphylococci from bovine mastitis. Poster presentation, Joint annual meeting of the SSI, SSHH, SSM and SSTMP, St. Gallen, Switzerland.
- Frey, Y.; Schwendener, S.; Perreten, V. (2012) Genetic properties of multidrug-resistant *Staphylococcus epidermidis* isolates from bovine mastitis. Poster presentation, International Symposium on Staphylococci and Staphylococcal Infections, Lyon, France.

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