Comparison of genomic and antimicrobial resistance features of latex agglutination test-positive and latex agglutination test-negative *Staphylococcus aureus* isolates causing bovine mastitis

A. Moser,* R. Stephan,* S. Corti,* and S. Johler*†¹

*Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland

†Skirball Institute of Biomolecular Medicine, New York University Medical Center, 540 First Avenue, New York 10016

ABSTRACT

The dairy industry suffers massive economic losses due to staphylococcal mastitis in cattle. The Staphaurex latex agglutination test (Oxoid, Basel, Switzerland) was reported to lead to negative results in 54% of bovine *Staphylococcus aureus* strains, and latex-negative strains are thought to be less virulent than Staphaurex latex-positive strains. However, comparative information on virulence and resistance profiles of these 2 groups of *Staph. aureus* is scarce. Our objective was to associate the latex agglutination phenotype of *Staph. aureus* strains isolated from bovine mastitis milk with data on clonal complexes, virulence genes, and antibiotic resistance to (1) determine the virulence profiles of the Staphaurex test positive and Staphaurex test negative groups, and (2) provide data needed to improve treatment of bovine mastitis and to identify potential vaccine targets. Seventy-eight *Staph. aureus* strains isolated from 78 cows on 57 Swiss farms were characterized. Latex agglutination was tested by Staphaurex kit, and resistance profiles were generated by disk diffusion. A DNA microarray was used to assign clonal complexes (CC) and to determine virulence and resistance gene profiles. By the Staphaurex test, 49% of the isolates were latex-positive and 51% were latex-negative. All latex-negative strains were assigned to CC151, whereas latex-positive strains were assigned to various clonal complexes, including CC97 (n = 16), CC8 (n = 10), CC479 (n = 5), CC20 (n = 4), CC7 (n = 1), CC9 (n = 1), and CC45 (n = 1). Although the latex-negative isolates were susceptible to all antimicrobial agents tested, 24% of latex-positive isolates were classified as intermediate with regard to cefalexin-kanamycin and 13% were resistant to both ampicillin and penicillin. Microarray profiles of latex-negative isolates were highly similar, but differed largely from

those of latex-positive isolates. Although the latex-negative group lacked several enterotoxin genes and *sak*, it exhibited significantly higher prevalence rates of genes encoding enterotoxin C, toxic shock syndrome toxin, and leukocidins (*lukM/lukF-P83*, *lukD*). Our findings suggest that latex-negative isolates represent a group of closely related strains with specific resistance and virulence gene patterns.

Key words: *Staphylococcus aureus*, bovine mastitis, Staphaurex latex agglutination test, virulence

INTRODUCTION

The dairy industry suffers from massive economic losses due to staphylococcal mastitis in cattle (Wells et al., 1998). The Staphaurex latex agglutination test (Oxoid, Basel, Switzerland) is a diagnostic instrument widely used to confirm putative *Staphylococcus aureus* isolates through detection of characteristic *Staph. aureus* surface proteins. Latex particles coated with human IgG and fibrinogen interact with the bacterial target proteins SpA (staphylococcal protein A), ClfA/B (clumping factor A/B), and FnbA/B (fibronectin-binding protein A/B), mediating a rapid agglutination reaction that is visible to the naked eye. Although the Staphaurex latex agglutination test exhibits high specificity (99.5%) and sensitivity (99.8%) when applied to *Staph. aureus* strains obtained from humans, Stutz et al. (2011) reported that 54% of *Staph. aureus* isolates obtained from cases of bovine mastitis yield negative test results. These false-negative results are due to sequence polymorphisms leading to impaired functionality of one or several of the targeted virulence factors (SpA, ClfA/B, or FnbA/B). Therefore, Staphaurex latex agglutination test (SLAT)-negative [SLAT(–)] strains are thought to be less virulent than SLAT-positive [SLAT(+)] strains (Stutz et al., 2011). Although assessing the virulence potential of SLAT(–) strains is of crucial importance to the dairy industry, data on the genomic background and antimicrobial resistance of bovine SLAT(–) isolates are scarce.

Received July 15, 2012.

Accepted September 18, 2012.

¹Corresponding author: sophia.johler@uzh.ch

Although antibiotic treatment is widely used to fight bovine mastitis, its merits are controversial. Use of antimicrobial agents is not only economically questionable and favors the development of antibiotic resistance, but it is also unsuitable in addressing intracellular persistence of the organism (Steenefeld et al., 2011; Fluit, 2012; Saini et al., 2012). Therefore, increased efforts are now focused on the development of vaccines. Recent studies postulate extended characterization of the genetic background of bovine mastitis isolates to enable identification of proteins crucial for colonization and infection that could serve as biomarkers in the identification of vaccine targets (Fluit, 2012; Klein et al., 2012).

The objective of this study was to link the latex agglutination phenotype of *Staph. aureus* strains isolated from bovine mastitis milk with data on clonal complexes, virulence genes, and antibiotic resistance to (1) determine the virulence profiles of the SLAT(+) and SLAT(−) groups, and (2) provide data needed to improve treatment of bovine mastitis and to identify potential vaccine targets.

MATERIALS AND METHODS

Bacterial Isolates, DNA Extraction, and Presumptive Species Identification

Seventy-eight *Staph. aureus* strains were isolated from bovine mastitis milk samples collected from different cows on 57 Swiss farms between March 2011 and February 2012. Putative *Staph. aureus* isolates were identified by streaking samples onto rabbit plasma fibrinogen plates (Oxoid), which were subsequently incubated at 37°C and examined for coagulase activity after 48 h. A single typical *Staph. aureus* colony from each plate was transferred to blood agar and incubated overnight at 37°C. Then, DNA was isolated using kits supplied by Qiagen (Hilden, Germany), according to the manufacturer's instructions. The concentration of nucleic acids was measured by using a Nanodrop ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Staphaurex Latex Agglutination Test and Genotyping

The Staphaurex test kit (Oxoid) was used according to the manufacturer's instructions to determine latex agglutination.

The presence of 284 genes and allelic variants was assessed using StaphyType ArrayStrips (Clondiag Chip Technologies, Jena, Germany) following the manufacturer's instructions. Multiplex linear DNA amplification and microarray hybridization allowed for identi-

cation of species markers, genes conferring resistance to antimicrobial agents, and virulence determinants such as genes encoding enterotoxins, toxic shock syndrome toxin, leukocidins, hemolysins, and adhesins. The microarray also enables assignment of strains to clonal complexes and *agr* types. The DNA microarray profiles were converted to sequence-like strings, as described elsewhere, to allow for visualization by SplitsTree4 (<http://www.splittree.org/>), a software package designed to compute unrooted phylogenetic networks from molecular sequence data (Wattinger et al., 2012).

Susceptibility Testing

Disk diffusion was used to classify isolates as susceptible, intermediate, or resistant depending on respective zone diameters following Clinical and Laboratory Standards Institute (CLSI) standard protocols (CLSI, 2008). All antimicrobial agents were chosen with regard to their relevance in mastitis therapy. Antibiotic agents tested included ampicillin (30 µg), amoxicillin (20 µg) with clavulanic acid (10 µg), cephalothin (30 µg), ceftiofur (30 µg), erythromycin (15 µg), cefoxitin (30 µg), gentamicin 10 (µg), kanamycin (30 µg), kanamycin-cefalexin (30 µg-15 µg), penicillin (10 IU), and penicillin-novobiocin (10 IU-30 µg). Mueller-Hinton agar and disks containing ceftiofur and penicillin-novobiocin were provided by Oxoid, and disks containing cefalexin-kanamycin (Ubrolexin) were provided by Boehringer Ingelheim (Basel, Switzerland). All other disks containing antibiotic agents were obtained from Becton Dickinson (Basel, Switzerland). Reference strain *Staph. aureus* ATCC 25923 was used as a quality control.

Statistical Analysis

The distribution of specific genes among latex-positive and latex-negative isolates was compared based on the hybridization results of the DNA microarray. SPSS Statistics 19 (SPSS Inc., Chicago, IL) was used to run the Pearson χ^2 test, identifying significant associations between the latex phenotype and the presence of the examined genes. Results were considered to be statistically significant for *P*-values <0.05.

RESULTS

Species Confirmation and Exclusion Criteria

All isolates were confirmed to represent *Staph. aureus* using the species markers of the DNA microarray. To avoid bias, the sample collection was screened for identical isolates by comparison of all features tested, including microarray profiles and resistance patterns, and all isolates were found to be unique.

Figure 1. A SplitsTree (<http://www.splitsree.org>; Huson and Bryant, 2006) phylogenetic tree depicting similarity of resistance and virulence gene profiles among isolates testing positive [SLAT(+)] and negative [SLAT(-)] by the Staphaurex latex agglutination test (Oxoid, Basel, Switzerland). A unique identifier was assigned to each *Staphylococcus aureus* isolate (e.g., SA_01). The latex phenotype (+ or -) of all isolates clustered in one clonal complex is indicated in parentheses; clonal cluster (CC) 151 represents the only cluster comprising SLAT(-) isolates.

Latex Agglutination and Clonal Complexes

Latex agglutination was tested for all 78 confirmed bovine *Staph. aureus* strains. Although 38 isolates (49%) were latex-positive and would have been correctly identified as *Staph. aureus*, 40 isolates (51%) were latex-negative (i.e., false negatives). As depicted in the phylogenetic tree in Figure 1, all latex-negative strains exhibited very similar resistance and virulence gene profiles. They formed a single cluster of isolates assigned to clonal complex (CC) 151, whereas latex-positive strains were assigned to various clusters, including CC97 (n = 16), CC8 (n = 10), CC479 (n = 5), CC20 (n = 4), CC7 (n = 1), CC9 (n = 1), and CC45 (n = 1).

Resistance Phenotypes (Disk Diffusion)

Resistance phenotypes determined by disk diffusion are listed in Table 1. The SLAT(-) isolates were susceptible to all antimicrobial agents tested. Among the SLAT(+) group, 24% of isolates were classified as intermediate with regard to cefalexin-kanamycin, and 13% of isolates were classified as resistant to both ampicillin and penicillin. No methicillin-resistant *Staph. aureus* (MRSA) isolates were detected among the bovine mastitis strains investigated in this study.

Resistance and Virulence Gene Profiles

Selected DNA microarray results on resistance and virulence genes are depicted in Table 2. Although no

Table 1. Antimicrobial resistance phenotypes determined by disk diffusion among isolates testing positive [SLAT(+)] or negative [SLAT(–)] by the Staphaurex latex agglutination test (SLAT; Oxoid, Basel, Switzerland)

SLAT status/ clonal complex	Antimicrobial agent ¹										
	AM	AMC	CF	EFT	ERY	FOX	GM	K	K/CFX	P	P/NB
SLAT(–)											
CC151 (n = 40)	0	0	0	0	0	0	0	0	0	0	0
SLAT(+)											
CC7 (n = 1)	1	0	0	0	0	0	0	0	0	1	0
CC8 (n = 10)	1	0	0	0	0	0	0	0	(1) ²	1	0
CC9 (n = 1)	1	0	0	0	0	0	0	0	(1) ²	1	0
CC20 (n = 4)	0	0	0	0	0	0	0	0	(3) ²	0	0
CC45 (n = 1)	1	0	0	0	0	0	0	0	0	1	0
CC97 (n = 16)	1	0	0	0	0	0	0	0	(4) ²	1	0
CC479 (n = 5)	0	0	0	0	0	0	0	0	0	0	0
Total (n = 78)	5	0	0	0	0	0	0	0	(9) ²	5	0

¹AM = ampicillin, AMC = amoxicillin with clavulanic acid, CF = cephalothin, EFT = ceftiofur, ERY = erythromycin, FOX = cefoxitin, GM = gentamicin, K = kanamycin, K/CFX = kanamycin-cefalexin, P = penicillin, P/NB = penicillin-novobiocin.

²The number of isolates that exhibited intermediate sensitivity to the respective antimicrobial agent is presented in parentheses.

resistance genes were detected among SLAT(–) isolates, some SLAT(+) isolates exhibited genes involved in resistance to antibiotic agents, including *blaI/R/Z* (26%), *ermC* (3%), *fosB* (39%), and *vanB* (3%). Several SLAT(+) strains also displayed the enterotoxin genes *entA* (21%), *entD* (26%), and *entJ* (16%), as well as *sak*, encoding staphylokinase (26%), virulence factors that were not found among SLAT(–) isolates. In contrast, the SLAT(–) group exhibited significantly higher prevalence rates of both tested allelic variants of *entC*, as well as the *egc* enterotoxin gene cluster, genes encoding toxic shock syndrome toxin, and the leukocidins *lukM/lukF-P83* and *lukD*. Neither *pvl*, encoding panton-valentine leukocidin, nor *etA/B/C*, encoding exfoliative toxins, was detected in this study. Microarray data on the presence of selected adhesin genes coding for target proteins of the Staphaurex test are presented in Table 2. Although almost all strains were positive for one or more allelic variants of the adhesin genes tested, latex-positive and latex-negative isolates differed largely in the allelic variants found. A comprehensive overview of the prevalence rates of all genes tested is provided as a supplemental file (available at <http://www.journalofdairyscience.org/>) that includes *P*-values used to determine significant differences in the prevalence of respective genes among SLAT(+) and SLAT(–) isolates.

DISCUSSION

Although the Staphaurex test kit exhibits high specificity (99.5%) and sensitivity (99.8%) when applied to *Staph. aureus* strains obtained from humans, it frequently leads to false-negative results when applied to *Staph. aureus* isolates obtained from bovine masti-

tis milk. In this study, 51% of isolates were SLAT(–), consistent with the rate of 54% reported by Stutz et al. (2011). In both Stutz et al. (2011) and the current study, SLAT(+) isolates were associated with a wide range of clonal complexes, whereas the SLAT(–) isolates were assigned to CC151 only. Clonal complex 151 is reported to represent the most prevalent clonal complex among *Staph. aureus* isolated from bovine milk obtained from both seemingly healthy and clinically infected udders (Sakwinska et al., 2011; Stutz et al., 2011; Schlotter et al., 2012). The most frequent clonal complexes among SLAT(+) strains in our study included CC97, CC8, CC497, and CC20, whereas CC7, CC9, and CC45 were assigned to only one isolate each. Interestingly, no isolates were assigned to CC133, a dominant clonal complex among *Staph. aureus* isolated from the milk of seemingly healthy cows in Germany (Schlotter et al., 2012).

When antimicrobial susceptibility was determined, the resistance proportions among clonal complexes differed, with CC151, CC479, and CC20 representing the only clonal complexes that included no resistant isolates. These findings are consistent with an extensive study on antimicrobial resistance among Swiss and French bovine *Staph. aureus* isolates, in which penicillin resistance rates among CC151 and CC20 were found to be far lower than those among CC97 (Sakwinska et al., 2011). Overall, 6% of bovine *Staph. aureus* isolates tested in our study were resistant to both ampicillin and penicillin, and 12% exhibited intermediate susceptibility to kanamycin/cefalexin. Although the emergence of livestock-associated MRSA represents an increasing problem in Switzerland (Huber et al., 2010) and worldwide, no MRSA isolates were detected among the isolates investigated in our study. The single strain

Table 2. Prevalence (%) of selected virulence and resistance genes detected by DNA microarray among bovine mastitis isolates testing positive [SLAT(+)] and negative [SLAT(–)] by the Staphaurex latex agglutination test (SLAT; Oxoid, Basel, Switzerland)

Group	Gene/Probe	Function	SLAT(+) (n = 38)	SLAT(–) (n = 40)
agr typing	agrI	Accessory gene regulator, type I	84	0*
	agrII	Accessory gene regulator, type II	16	100*
Capsule	capsule-5	Capsule type 5	79	0*
	capsule-8	Capsule type 8	21	100*
Resistance	blaI, blaR, blaZ	β-Lactamase resistance	26	0*
	fosB	Putative marker for fosfomycin, bleomycin	39	0*
Enterotoxins	entA	Enterotoxin A	21	0*
	entC	Enterotoxin C	3	28*
	entCM14	Enterotoxin-like protein	3	100*
	entD	Enterotoxin D	26	0*
	entJ	Enterotoxin J	16	0*
	egc-cluster	Enterotoxin gene cluster	29	100*
Toxic shock	tst-1	Toxic shock syndrome toxin	0	13*
	tst-RF122	Allelic variant of toxic shock syndrome toxin from RF122	0	15*
Leukocidins	lukM/lukF-P83	Bovine leukocidin	42	100*
	lukD	Leukocidin D component	87	100*
Staphylokinase	sak	Staphylokinase	26	0*
SpA	spa	Staphylococcal protein A	100	100
ClfA/B	clfA-all	Clumping factor A (ClfA)	97.4	100
	clfA-COL+RF122	ClfA, allele from COL/ RF122	71	100*
	clfB-COL+Mu50	ClfB, allele from COL/ Mu50	26	0*
	clfB-MW2	ClfB, allele from MW2	5	100*
	clfB-RF122	ClfB, allele from RF122	18	100*
FnbA/B	fnbA	Fibronectin-binding protein A (FnbA)	89	100*
	fnbA-COL	FnbA, allele from COL	29	0*
	fnbA-MRSA252	FnbA, allele from MRSA252	16	0*
	fnbA-RF122	FnbA, allele from RF122	0	100*
	fnbB-COL+Mu50+MW2	Fibronectin-binding protein B (FnbB)	100	0*

*Prevalence differed significantly between the SLAT(+) and SLAT(–) bovine *Staph. aureus* isolates tested in this study ($P < 0.05$).

exhibiting *vanB*, one of the genes involved in vancomycin resistance, was found to be sensitive to vancomycin by Etest (bioMérieux, La Balme les Grottes, France).

The DNA microarray results indicated that the resistance and virulence gene profiles of the SLAT(–) strains in our study were very similar, but they differed largely from those of SLAT(+) strains (Figure 1). Epidemiological studies suggest that a subset of *Staph. aureus* strains exhibits a distinctive genetic background that renders these strains highly successful in causing bovine mastitis (Herron-Olson et al., 2007). The SLAT(–) isolates characterized in our study lacked several virulence genes frequently found among SLAT(+) isolates, including genes coding for resistance factors, staphylokinase, and enterotoxins A, D, and J. This particular combination of enterotoxin genes (*entA*, *entD*, and *entJ*) was recently described to represent one of the main criteria in the identification of *Staph. aureus* strains classified as genotype B (Boss et al., 2011). Strains of this genotype were reported to be exclusively associated with very high (up to 65%) within-herd prevalence of mastitis (Graber et al., 2009). In our study, strains exhibiting the combination of *entA*, *entD*, and *entJ* were assigned to CC8 only. A wide variety of enterotoxin genes can be found among

Staph. aureus isolates obtained from cases of bovine mastitis (Monecke et al., 2007). Still, the exact role of enterotoxins in the pathogenesis of bovine mastitis remains poorly understood (Haveri et al., 2007).

Among SLAT(–) strains, we found significantly higher numbers of several other virulence genes, including genes coding for toxic shock syndrome toxins (*tst-1*, *tst-RF122*), enterotoxins (*entC*, *egc-cluster*), and leukocidins (*lukM/lukF-P83*, *lukD*). Recent studies suggest that *lukM/lukF-P83* plays a role in the pathogenesis of bovine mastitis (Barrio et al., 2006; Schlotter et al., 2012).

As false-negative results in the Staphaurex test are caused by impaired functionality of one or several of the targeted virulence factors SpA, ClfA/B, and FnbA/B, SLAT(–) strains are thought to be less virulent than SLAT(+) strains (Stutz et al., 2011). The DNA microarray results revealed considerable heterogeneity regarding *clfA/B* and *fnbA/B*, and we found that SLAT(–) and SLAT(+) isolates exhibited different alleles of the respective genes. Virulence factors ClfA/B and FnbA/B represent a group of bacterial surface proteins designated “microbial surface components recognizing adhesive matrix molecules” (MSCRAMM). These proteins are of particular interest in the develop-

ment of vaccines because they mediate adherence of *Staph. aureus* to components of the host's extracellular matrix. Attachment of the organism to the epithelial cells of the udder, as well as subsequent invasion of epithelial and endothelial cells was shown to depend on MSCRAMM (Lammers et al., 1999; Sinha et al., 1999), which also play a role in the evasion of host immune response (Higgins et al., 2006).

CONCLUSIONS

The genomic background of SLAT(−) and SLAT(+) strains differed significantly with respect to a vast range of genes encoding crucial virulence and resistance factors. In consideration of the high heterogeneity among MSCRAMM genes detected by DNA microarray, we conclude that a combination of diverse antigens is crucial to the development of highly functional adhesin-based diagnostic tools and vaccines. Based on the comparison of microarray profiles and resistance phenotypes of SLAT(+) and SLAT(−) bovine mastitis isolates investigated in this study, we hypothesize that SLAT(+) strains exceed SLAT(−) strains in virulence potential.

ACKNOWLEDGMENTS

This study was financed in part by a fellowship from the Swiss National Science Foundation (PBZHP3-138695).

REFERENCES

- Barrio, M. B., P. Rainard, and G. Prévost. 2006. LukM/LukF⁺-PV is the most active *Staphylococcus aureus* leukotoxin on bovine neutrophils. *Microbes Infect.* 8:2068–2074.
- Boss, R., J. Naskova, A. Steiner, and H. U. Graber. 2011. Mastitis diagnostics: Quantitative PCR for *Staphylococcus aureus* genotype B in bulk tank milk. *J. Dairy Sci.* 94:128–137.
- CLSI (Clinical and Laboratory Standards Institute). 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard M31–A3. 3rd ed. CLSI, Wayne, PA.
- Fluit, A. C. 2012. Livestock-associated *Staphylococcus aureus*. *Clin. Microbiol. Infect.* 18:735–744. <http://dx.doi.org/10.1111/j.1469-0691.2012.03846.x>.
- Graber, H. U., J. Naskova, E. Studer, T. Kaufmann, M. Kirchhofer, M. Brechbühl, W. Schären, A. Steiner, and C. Fournier. 2009. Mastitis-related subtypes of bovine *Staphylococcus aureus* are characterized by different clinical properties. *J. Dairy Sci.* 92:1442–1451.
- Haveri, M., A. Roslöf, L. Rantala, and S. Pyörälä. 2007. Virulence genes of bovine *Staphylococcus aureus* from persistent and nonpersistent intramammary infections with different clinical characteristics. *J. Appl. Microbiol.* 103:993–1000.
- Herron-Olson, L., J. R. Fitzgerald, J. M. Musser, and V. Kapur. 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. *PLoS ONE* 2:e1120.
- Higgins, J., A. Loughman, K. P. M. van Kessel, J. A. G. van Strijp, and T. J. Foster. 2006. Clumping factor A of *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. *FEMS Microbiol. Lett.* 258:290–296.
- Huber, H., S. Koller, N. Giezendanner, R. Stephan, and C. Zweifel. 2010. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *EuroSurveillance* 15(16):pii:19542.
- Huson, D. H., and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* 23:254–267.
- Klein, R. C., M. H. Fabres-Klein, M. A. Brito, L. G. Fietto, and A. D. Ribon. 2012. *Staphylococcus aureus* of bovine origin: Genetic diversity, prevalence and the expression of adhesin-encoding genes. *Vet. Microbiol.* 160:183–188. <http://dx.doi.org/10.1016/j.vetmic.2012.05.025>.
- Lammers, A., P. J. M. Nuijten, and H. E. Smith. 1999. The fibronectin binding proteins of *Staphylococcus aureus* are required for adhesion to and invasion of bovine mammary gland cells. *FEMS Microbiol. Lett.* 180:103–109.
- Monecke, S., P. Kuhnert, H. Hotzel, P. Slickers, and R. Ehrlich. 2007. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Vet. Microbiol.* 125:128–140.
- Saini, V., J. T. McClure, D. T. Scholl, T. J. DeVries, and H. W. Barkema. 2012. Herd-level association between antimicrobial use and antimicrobial resistance in bovine mastitis *Staphylococcus aureus* isolates on Canadian dairy farms. *J. Dairy Sci.* 95:1921–1929.
- Sakwinska, O., D. Morisset, J.-Y. Madec, A. Waldvogel, P. Moreillon, and M. Haenni. 2011. Link between genotype and antimicrobial resistance in bovine mastitis-related *Staphylococcus aureus* strains, determined by comparing Swiss and French isolates from the Rhône Valley. *Appl. Environ. Microbiol.* 77:3428–3432.
- Schlötter, K., R. Ehrlich, H. Hotzel, S. Monecke, M. Pfeffer, and K. Donat. 2012. Leukocidin genes *lukf-P83* and *lukM* are associated with *Staphylococcus aureus* clonal complexes 151, 479 and 133 isolated from bovine udder infections in Thuringia, Germany. *Vet. Res.* 43:42 <http://dx.doi.org/10.1186/1297-9716-43-42>.
- Sinha, B., P. P. François, O. Nüsse, M. Foti, O. M. Hartford, P. Vaudaux, T. J. Foster, D. P. Lew, M. Herrmann, and K. H. Krause. 1999. Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin $\alpha 5 \beta 1$. *Cell. Microbiol.* 1:101–117.
- Steenefeld, W., T. van Werven, H. W. Barkema, and H. Hogeveen. 2011. Cow-specific treatment of clinical mastitis: An economic approach. *J. Dairy Sci.* 94:174–188.
- Stutz, K., R. Stephan, and T. Tasara. 2011. SpA, ClfA, and FnbA genetic variations lead to Staphaurex test-negative phenotypes in bovine mastitis *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* 49:638–646.
- Wattinger, L., R. Stephan, F. Layer, and S. Johler. 2012. Comparison of *Staphylococcus aureus* isolates associated with food intoxication with isolates from human nasal carriers and human infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 31:455–464.
- Wells, S. J., S. L. Ott, and A. H. Seitzinger. 1998. Key health issues for dairy cattle—New and old. *J. Dairy Sci.* 81:3029–3035.