Arch Lebensmittelhyg 65,
137–140 (2014)
DOI 10.2376/0003-925X-65-137

ISSN 0003-925X

Korrespondenzadresse:
sophia.johler@uzh.ch

[1]) Institute for Food Safety and Hygiene, University of Zurich, [2]) Mabritec AG

Coagulase-negative staphylcococci lead to false-positive results on chromID *S. aureus* and chromID MRSA agar

Koagulase-negative Staphylokokken führen zu falsch-positiven Ergebnissen auf chromID S. aureus und chromID MRSA Agar

Axel Merz[1]), Valentin Pflüger[2]), Sophia Johler[1])

Summary

While *Staphylococcus (S.) aureus* is a frequent cause of infections and intoxications and causes severe losses for the dairy industry, coagulase-negative staphylococci (CNS) are commonly considered benign commensals. Correct identification of *S. aureus* and differentiation from CNS is crucial for diagnostic purposes. SAID and chromID MRSA agar plates (bioMérieux, FR) are selective chromogenic agar plates that were reported to allow for highly specific and sensitive identification of *S. aureus* and MRSA (methicillin-resistant *S. aureus*). However, when using these media in our laboratory, we noticed incongruent identification results, prompting us to question the specificity of SAID and chromID MRSA agar. In this study, we aimed to evaluate the performance of SAID and chromID MRSA agar by identifying organisms that may lead to false-positive results. To this end, we used the isolates that had yielded inconsistent identification results (n = 3), as well as 15 CNS strains representing *S. chromogenes, S. cohnii, S. xylosus, S. sciuri, S. haemolyticus, S. epidermidis, S. simulans, S. warneri, S. equorum, S. hyicus, S. succinus, S. fleurettii,* and *S. lentus*. Species identification was performed using matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. The phenotype of all isolates on SAID agar and of cefoxitin-resistant *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* isolates on chromID MRSA agar was evaluated. For SAID, we detected false-positive results for *S. fleurettii, S. sciuri, S. succinus, S. lentus, S. vitulinus, Arthrobacter nicotianae,* and *Micrococcus luteus*. For chromID MRSA, cefoxitin-resistant *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* led to false-positive results. In the light of our findings, screening for MRSA using chromID MRSA agar only may lead to false-positive results, potentially leading to severe therapeutic mistakes. We therefore strongly advise combination of the chromID MRSA agar with additional tests.

Keywords: SAID agar, MRSA, misidentification, *Staphylococcus sciuri, Staphylococcus succinus*

Zusammenfassung

Während *Staphylococcus (S.) aureus* eine häufige Ursache von Infektionen und Intoxikationen darstellt und schwere Verluste für die Milchwirtschaft verursacht, werden Koagulase-negative Staphylokokken (CNS) gemeinhin als Kommensalen angesehen. Die korrekte Identifikation von *S. aureus* und deren Abgrenzung von CNS sind in der Diagnostik äußerst wichtig. SAID und chromID MRSA Agar (bioMérieux, FR) sind chromogene Selektivmedien, von denen berichtet wurde, dass sie einen hochspezifischen und hochsensitiven Nachweis von *S. aureus* und MRSA (Methicillin-resistenten *S. aureus*) ermöglichen. Beim Einsatz der Medien in unserem Labor fielen uns hingegen widersprüchliche Identifikationsergebnisse auf, die uns veranlassten die Spezifität des SAID und chromID MRSA zu hinterfragen. In der vorliegenden Studie zielten wir darauf ab, die Zuverlässigkeit der SAID und MRSA Agare zu überprüfen, indem wir Organismen identifizierten, die zu falsch-positiven Resultaten führen. Dahingehend verwendeten wir sowohl die Isolate, die zu widersprüchlichen Identifikationsergebnissen geführt hatten (n = 3), als auch 15 CNS Stämme der Spezies *S. chromogenes, S. cohnii, S. xylosus, S. sciuri, S. haemolyticus, S. epidermidis, S. simulans, S. warneri, S. equorum, S. hyicus, S. succinus, S. fleurettii* und *S. lentus*. Zur Speziesidentifikation wurde die Matrix-unterstützte Laser Desorption/Ionisation Time-of-Flight Massenspektrometrie herangezogen. Der Phänotyp aller Isolate auf SAID Agar und der Phänotyp Cefoxitin-resistenter *S. fleurettii, S. sciuri, S. succinus* und *S. lentus* Isolate auf chromID MRSA Agar wurde überprüft. Der SAID Agar ergab falsch-positive Ergebnisse für *S. fleurettii, S. sciuri, S. succinus, S. lentus, S. vitulinus, Arthrobacter nicotianae* und *Micrococcus luteus*. Beim chromID MRSA Agar führten Cefoxitin-resistente *S. fleurettii, S. sciuri, S. succinus* und *S. lentus* zu falsch-positiven Ergebnissen. In Anbetracht unserer Ergebnisse führt die alleinige Verwendung von chromID MRSA Agar beim MRSA Screening zu falsch-positiven Resultaten, die schwerwiegende therapeutische Fehler nach sich ziehen können. Wir empfehlen daher den chromID MRSA Agar ausschließlich in Kombination mit zusätzlichen Tests zu verwenden.

Schlüsselwörter: SAID Agar, MRSA, Fehlidentifikation, *Staphylococcus sciuri, Staphylococcus succinus*

Journal of Food Safety and Food Quality 65, Heft 6 (2014), Seiten 133–164

Introduction

Staphylococcus (S.) aureus causes a wide variety of severe infections, toxinoses, and life-threatening illnesses in both humans and animals. The dairy industry is particularly affected by major losses due to bovine mastitis caused by *S. aureus.* In contrast, coagulase-negative staphylococci (CNS) are commonly considered benign commensals or "minor pathogens" (Supré et al., 2011; Otto, 2013). Correct and rapid identification of *S. aureus* and differentiation of the organisms from CNS is therefore crucial for diagnostic purposes.

In recent years, there has been an increase in the use of selective chromogenic agar plates as screening tools. SAID (*S. aureus* chromID®, bioMérieux, FR) agar represents a popular chromogenic medium for isolation and identification of *S. aureus.* SAID is a selective medium that contains not only two chromogenic substrates, but also inhibits growth of most bacteria other than staphylococci. *S. aureus* forms characteristic light or dark green colonies on SAID agar, due to production of alpha-glucosidase. SAID agar was reported to exhibit high sensitivity (99 %) and specificity (93 %) (Perry et al., 2003). Still, we were able to show in a previous study that polymorphisms in the alpha-glucosidase gene of *S. aureus* can lead to growth of yellow colonies and thus false-negative results (Johler et al., 2012).

With the emergence of MRSA (methicillin-resistant *S. aureus*) infections that lead to high morbidity and mortality worldwide, MRSA screening using selective plates has gained in popularity. The chromID® MRSA agar (bioMérieux) is a chromogenic medium containing cefoxitin that allows for isolation and identification of MRSA strains. MRSA colonies exhibit a characteristic green coloration on chromID MRSA agar, which results from alpha-glucuronidase formation.

Our laboratory used SAID agar to screen bovine mastitis milk, as well as swab samples from an automated milking system for *S. aureus.* For some isolates, positive identification results of the chromogenic plates were disproven by additional tests, prompting us to question the specificity of SAID agar and the similar chromID MRSA agar. In this study, we therefore report the phenotype of both these isolates and a representative collection of CNS strains on SAID and chromID MRSA agar, with the aim of identifying organisms that may lead to false-positive results.

Material and methods

Bacterial strains used. An overview of all isolates is provided in Table 1. We included the three isolates that had prompted the study, because they had yielded contradictory results in routine diagnostic testing. The isolates had exhibited a phenotype characteristic for *S. aureus* on SAID agar, but had failed to show hemolysis on sheep blood agar (Oxoid, CH), had yielded negative results in the Staphaurex latex agglutination test (Oxoid), and had lacked coagulase production (rabbit plasma fibrinogen agar, Oxoid). In addition, we included strains representing the following 13 CNS species in the study: *S. chromogenes, S. cohnii, S. xylosus, S. sciuri, S. haemolyticus, S. epidermidis, S. simulans, S. warneri, S. equorum, S. hyicus, S. succinus, S. fleurettii,* and *S. lentus.* With the exception of the *S. lentus* isolate that originated from poultry (Huber et al., 2011), all CNS strains originated from bovine milk (Huber et al., 2011; Moser et al., 2013).

Evaluation of phenotype on SAID agar and chromID MRSA. We determined the phenotype of Isolate_1, Isolate_2, Isolate_3, and 13 CNS species on SAID agar, as well as the phenotype of cefoxitin-resistant *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* isolates on chromID MRSA agar. All strains were resuscitated from cryo cultures stocked at –80 °C by plating on sheep blood agar (Oxoid) and incubation at 37 °C over night. A single colony was used for subculture on the respective chromogenic agar and each strain was streaked next to a positive control (PC_34 for *S. aureus,* PC_MN01 for MRSA) to allow for

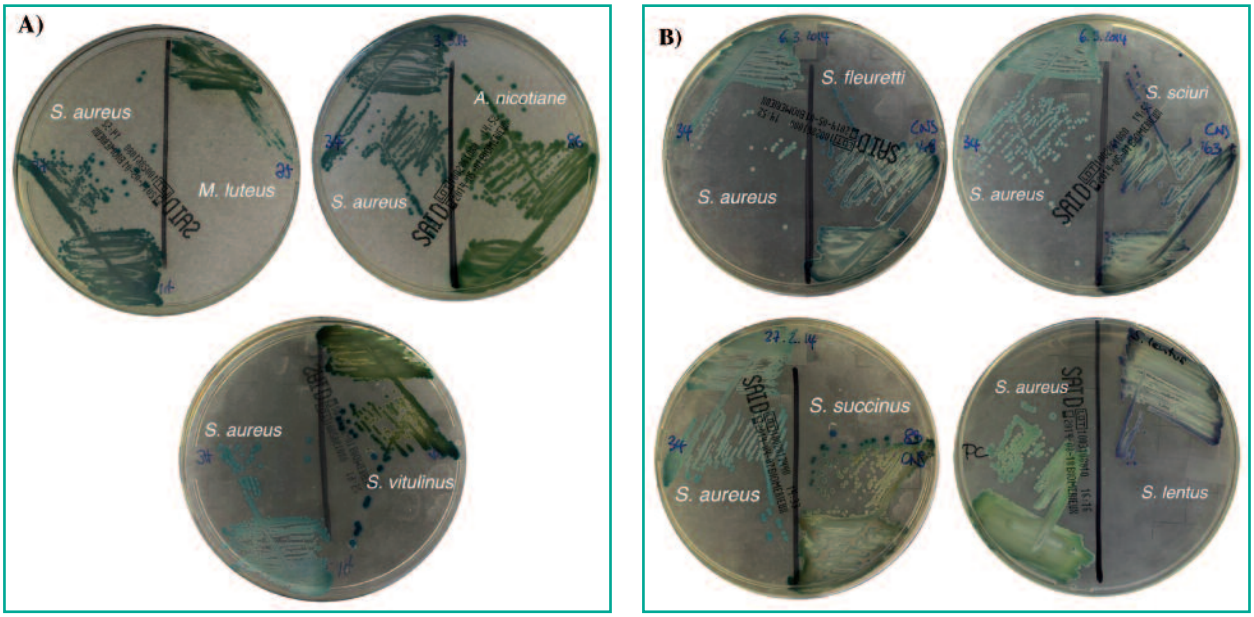

FIGURE 1: *Phenotypes of organisms that formed green colonies on SAID (after 24 h) and would therefore be misidentified as S. aureus. All isolates were streaked directly next to a positive control (S. aureus) to allow for direct comparison. A) Phenotypes of the three Micrococcus luteus, Arthrobacter nicotiane, and Staphylococcus vitulinus strains on SAID agar. B) Phenotypes of S. fleuretti, S. sciuri, S. succinus, and S. lentus on SAID agar after incubation at 37 °C for 24 h.*

direct comparison of phenotypes. Morphology and coloration of colonies on SAID agar plates was assessed and photographed after 24 h.

Species identification. Species identification was performed by MALDI-TOF MS (matrix-assisted laser desorption and ionization time-of-flight mass spectrometry) in cooperation with Mabritec AG (Riehen, Switzerland). MALDI-TOF MS allows for identification of microorganisms including *S. aureus* and CNS through generation of mass spectral fingerprints (Wieser et al., 2012; Moser et al., 2013).

TABLE 1: *Bacterial strains used in this study.*

Isolate	Source	Species	Reference
Isolate_1	bovine mastitis milk	*Arthrobacter nicotianae*[1])	this study
Isolate_2	bovine mastitis milk	*Micrococcus luteus*[1])	this study
Isolate_3	swab of automated milking system (dairy cattle farm)	*Staphylococcus vitulinus*[1])	this study
CNS strain collection			
CNS_1	bovine mastitis milk	*Staphylococcus chromogenes*	(Moser et al., 2013)
CNS_41	bovine mastitis milk	*Staphylococcus cohnii*	(Moser et al., 2013)
CNS_42	bovine mastitis milk	*Staphylococcus epidermidis*	(Moser et al., 2013)
CNS_47	bovine mastitis milk	*Staphylococcus equorum*	(Moser et al., 2013)
CNS_49	bovine mastitis milk	*Staphylococcus fleuretti*	(Moser et al., 2013)
CNS_50	bovine mastitis milk	*Staphylococcus haemolyticus*	(Moser et al., 2013)
CNS_61	bovine mastitis milk	*Staphylococcus hyicus*	(Moser et al., 2013)
CNS_63	bovine mastitis milk	*Staphylococcus sciuri*	(Moser et al., 2013)
CNS_64	bovine mastitis milk	*Staphylococcus sciuri*	(Moser et al., 2013)
CNS_78	bovine mastitis milk	*Staphylococcus simulans*	(Moser et al., 2013)
CNS_83	bovine mastitis milk	*Staphylococcus succinus*	(Moser et al., 2013)
CNS_84	bovine mastitis milk	*Staphylococcus warneri*	(Moser et al., 2013)
CNS_88	bovine mastitis milk	*Staphylococcus xylosus*	(Moser et al., 2013)
L15	bulk tank milk	*Staphylococcus fleurettii*	(Huber et al., 2011)
G67	poultry	*Staphylococcus lentus*	(Huber et al., 2011)
Strains used as positive controls			
PC_34	bovine mastitis milk	*Staphylococcus aureus*	this study
PC_MN01	MRSA skin infection	*Staphylococcus aureus*	(Nüesch-Inderbinen et al., 2014)

[1]): Isolate had yielded false-positive identification result on SAID agar. Correct species assignment was performed by MALDI-TOF MS during this study.

Results

According to the manufacturer, very light green to dark green colonies are typical for *S. aureus* on SAID agar. However, in our experience and consistent with previous publications (Perry et al., 2003), most colonies of *S. aureus* on SAID agar exhibit a green coloration that ranges very close to turquoise or light blue. We therefore evaluated the phenotype of all isolates next to a *S. aureus* positive control on the same plate that allowed for direct comparison (Fig. 1 and Fig. 2).

This study was initiated upon detection of contradictory identification results for three isolates collected from cows with mastitis milk and from an automated milking system. All three isolates grew on SAID agar and exhibited a green phenotype (see Fig. 1A). Using MALDI-TOF MS, we were able to identify the three isolates (Isolate_1, Isolate_2, Isolate_3) as *Arthrobacter (A.) nicotianae, Micrococcus (M.) luteus,* and *S. vitulinus.* In addition, four of the tested 13 CNS species also yielded false-positive results (see Fig. 1B): *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* produced green colonies on SAID that could not be differentiated from *S. aureus* by colony size. An overview of the phenotypes of all tested strains on SAID agar after 24h incubation at 37 °C is provided in Table 2.

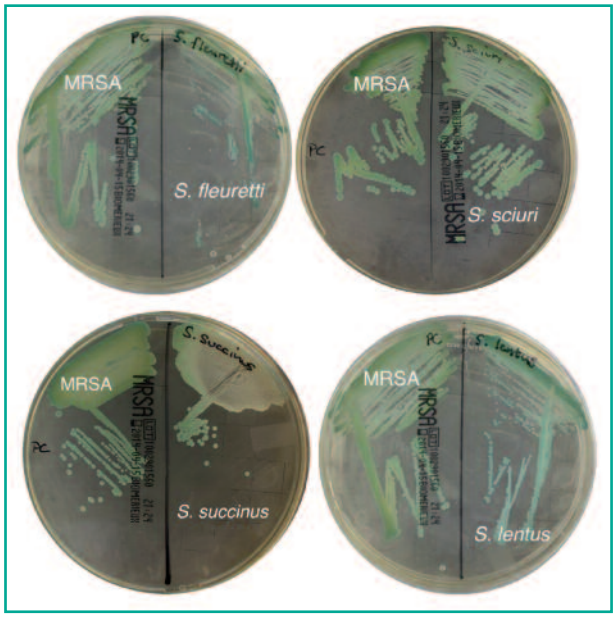

FIGURE 2: *Phenotypes of S. fleuretti, S. sciuri, S. succinus, and S. lentus on chromID MRSA. The organisms were able to form green colonies after 24h and are therefore be misidentified as MRSA. All isolates were streaked next to a positive control to allow for direct comparison.*

As for the chromID MRSA agar, we found that cefoxitin-resistant *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* led to false-positive results (see Table 2 and Fig. 2). The isolates were able to form green colonies after 24 h at 37 °C that were in accordance with the colony morphology expected for MRSA.

Discussion

In our study, *A. nicotianae, M. luteus,* as well as the CNS species *S. vitulinus, S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* led to false-positive results on SAID agar. Except for *M. luteus,* the green colonies formed by isolates of these species could not be differentiated from *S. aureus* by colony size. According to product information provided by the manufacturer, false-positive results can occur for coagulase-positive staphylococci other than *S. aureus,* as well as for Micrococcus, *S. saprophyticus, S. haemolyticus,* and *S. warneri.* Interestingly, the *S. haemolyticus* and *S. warneri* strains used in our study did not yield false-positive results. The manufacturer recommends additional testing using the Staphaurex latex agglutination test (Remel, Oxoid, CH). However, we were able to show in previous studies that 51–54 % of bovine *S. aureus* yield false-negative results (Stutz et al., 2011, Moser et al., 2013). Thus, screening of bovine isolates using either SAID agar only, or SAID agar in combination with the Staphaurex latex agglutination test is highly unreliable.

We used cefoxitin-resistant isolates of *S. fleurettii, S. sciuri, S. succinus,* and *S. lentus* to evaluate the performance of

Journal of Food Safety and Food Quality 65, Heft 6 (2014), Seiten 133–164

TABLE 2: *MALDI-TOF MS identification results and phenotype of the different organisms on SAID agar or chromID MRSA agar, respectively.*

Species	Isolate	Colour	Test result
A. nicotianae	Isolate_1	green	false-positive on SAID
M. luteus	Isolate_2	green	false-positive on SAID
S. vitulinus	Isolate_3	green	false-positive on SAID
S. fleurettii	CNS_49, L15	green	false-positive on SAID
S. sciuri	CNS_63	green	false-positive on SAID
S. succinus	CNS_83	green	false-positive on SAID
S. lentus	G67	green	false-positive on SAID
S. warneri	CNS_84	white	negative on SAID
S. simulans	CNS_78	white	negative on SAID
S. cohnii	CNS_41	white	negative on SAID
S. epidermidis	CNS_42	white	negative on SAID
S. equorum	CNS_47	blue/violet	negative on SAID
S. haemolyticus	CNS_50	yellow	negative on SAID
S. hyicus	CNS_61	beige	negative on SAID
S. chromogenes	CNS_1	orange	negative on SAID
S. xylosus	CNS_88	orange	negative on SAID
S. fleurettii	L15	green	false-positive on chromID MRSA
S. sciuri	CNS_64	green	false-positive on chromID MRSA
S. succinus	CNS_83	green	false-positive on chromID MRSA
S. lentus	G67	green	false-positive on chromID MRSA

the chromID MRSA agar and found that all four species led to false-positive results. MRSA strains exhibit the *mecA* gene that confers resistance to methicillin by encoding an alternative penicillin binding protein with reduced binding affinity to methicillin (Chambers et al., 1985). Our results are consistent with studies implicating several CNS species such as *S. sciuri* and *S. fleurettii* as origin and potential reservoir of *mecA* (Couto et al., 1996; Kloos et al., 1997; Tsubakishita et al., 2010; Otto, 2013). A recent study by Huber et al. showed that methicillin-resistant CNS (MR-CNS) occur in 62 % of bulk tank milk samples in Switzerland, with *S. fleurettii* and *S. sciuri* representing the most commonly detected MR-CNS species (74 % and 22 %, respectively) (Huber et al., 2011). As both species yielded false-positive results on chromID MRSA agar in our study, this would suggest that up to 96 % of MR-CNS present in bulk tank milk could be misidentified as MRSA when using this chromogenic agar.

In contrast to our results, a study by Nahimana et al. evaluating performance of chromID MRSA agar found no false-positive results after 16–18 h of incubation, suggesting a specificity of 100 % (Nahimana et al., 2006). The authors concluded that the manufacturer's recommendation of no additional testing for MRSA identification is justified (Nahimana et al., 2006). However, in the light of our findings, screening for MRSA using chromID MRSA agar only may lead to false-positive results, potentially leading to severe therapeutic mistakes. We therefore strongly advise combination of the chromID MRSA agar with additional tests.

References

Chambers HF, Hartman BJ, Tomasz A (1985): Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant *Staphylococcus aureus* exposed to nafcillin. J Clin Invest 76: 325–331.

Couto I., de Lencastre H, Severina E, Kloos W, Webster JA, Hubner RJ, Santos Sanches I, Tomasz A (1996): Ubiquitous presence of a mecA homologue in natural isolates of *Staphylococcus sciuri.* Microb Drug Resist 2: 377–391.

Huber H, Ziegler D, Pflüger V, Vogel G, Zweifel C, Stephan R (2011): Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet Res 7: 6.

Johler S, Moser M, Engl C, Tasara T, Corti S, Chen J, Stephan R (2012): A coagulase- and α-glucosidase-negative variant of *Staphylococcus aureus:* a challenge for routine microbiological diagnostics. J Clin Microbiol 50: 1827–1828.

Kloos WE, Ballard DN, Webster JA, Hubner RJ, Tomasz A, Couto I, Sloan GL, Dehart HP, Fiedler F, Schubert K, de Lencastre H, Santos Sanches I, Heath HE, Leblanc PA, Ljungh A (1997): Ribotype delineation and description of *Staphylococcus sciuri* subspecies and their potential as reservoirs of methicillin resistance and staphylolytic enzyme genes. Int J Syst Bacteriol 47: 313–323.

Moser A, Stephan R, Corti S, Johler S (2013): Comparison of genomic and antimicrobial resistance features of latex agglutination test-positive and latex agglutination test-negative *Staphylococcus aureus* isolates causing bovine mastitis. J Dairy Sci 96: 329–334.

Moser A, Stephan R, Ziegler D, Johler S (2013): Species distribution and resistance profiles of coagulase-negative staphylococci isolated from bovine mastitis in Switzerland. Schweiz Arch Tierheilkd 155: 333–338.

Nahimana I, Francioli P, Blanc DS (2006): Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus.* Clin Microbiol Infect 12: 1168–1174.

Nüesch-Inderbinen MT, Stalder U, Johler S, Hächler H, Stephan R, Nüesch H-J (2014): Intrafamilial spread of a Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* belonging to the pediatric clone ST5 SSCmecIV. JMM Case Reports: submitted.

Otto M (2013): Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: Staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. Bioessays 35: 4–11.

Perry JD, Rennison C, Butterworth LA, Hopley AL, Gould FK (2003): Evaluation of *S. aureus* ID, a new chromogenic agar medium for detection of *Staphylococcus aureus.* J Clin Microbiol 41: 5695–5698.

Supré K, Haesebrouck F, Zadoks RN, Vaneechoutte M, Piepers S, De Vliegher S (2011): Some coagulase-negative *Staphylococcus* species affect udder health more than others. J Dairy Sci 94: 2329–2340.

Stutz, K, Stephan R, Tasara T (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.

Tsubakishita S, Kuwahara-Arai K, Sasaki T, Hiramatsu K (2010): Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. Antimicrob Agents Chemother 54: 4352–4359.

Wieser A, Schneider L, Jung J, Schubert S (2012): MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl Microbiol Biotechnol 93: 965–974.

Address of corresponding author:
Dr. Sophia Johler
Institute for Food Safety and Hygiene
University of Zurich
Winterthurerstr. 272
8057 Zurich
Switzerland
sophia.johler@uzh.ch