Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and tuf gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis

A. Capurro, K. Artursson, K. Persson Waller, B. Bengtsson, H. Ericsson-Unnerstad, A. Aspán

To cite this version:
A. Capurro, K. Artursson, K. Persson Waller, B. Bengtsson, H. Ericsson-Unnerstad, et al.. Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and tuf gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis. Veterinary Microbiology, Elsevier, 2009, 134 (3-4), pp.327. 10.1016/j.vetmic.2008.08.028 . hal-00532472

HAL Id: hal-00532472
https://hal.archives-ouvertes.fr/hal-00532472
Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Accepted Manuscript

Title: Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and tuf gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis

Authors: A. Capurro, K. Artursson, K. Persson Waller, B. Bengtsson, H. Ericsson-Unnerstad, A. Aspán

PII: S0378-1135(08)00354-4
Reference: VETMIC 4139

To appear in: VETMIC

Received date: 15-4-2008
Revised date: 20-8-2008
Accepted date: 21-8-2008

Please cite this article as: Capurro, A., Artursson, K., Waller, K.P., Bengtsson, B., Ericsson-Unnerstad, H., Aspán, A., Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and tuf gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis, Veterinary Microbiology (2007), doi:10.1016/j.vetmic.2008.08.028

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Comparison of a commercialized phenotyping system, antimicrobial susceptibility testing, and *tuf* gene sequence-based genotyping for species-level identification of coagulase-negative staphylococci isolated from cases of bovine mastitis

A. Capurro a,b,*, K. Artursson a, K. Persson Waller a,b, B. Bengtsson a, H. Ericsson-Unnerstad a and A. Aspán a

a National Veterinary Institute, SE-75189 Uppsala, Sweden

b Department of Clinical Sciences, Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden

*Corresponding author. Aldo Capurro

Department of Bacteriology
National Veterinary Institute
SE-75189 Uppsala, Sweden

Tel.: + 46 18 674000; Fax: + 46 18 309162.

E-mail: aldo.capurro@sva.se
Abstract

In order to evaluate the usefulness of some phenotypic and genotypic methods for species identification of coagulase negative staphylococci (CNS), isolates were obtained from bovine cases of clinical and sub-clinical mastitis from different geographical areas in Sweden. By using the Staph-Zym™ test, antimicrobial susceptibility testing, and sequencing of part of the CNS tuf gene and, when needed, part of the 16S rRNA gene we characterized 82 clinical isolates and 24 reference strains of 18 different species of staphylococci. The genotypic methods identified nine different species of CNS among the 82 milk isolates. A comparison with results obtained by tuf gene sequencing showed that Staph-Zym™ correctly identified CNS reference strains to species level more often than bovine milk CNS isolates (83% and 61%, respectively). In addition, tests supplementary to the Staph-Zym™ were frequently needed in both groups of isolates (50% of reference strains and 33% of milk isolates) to obtain an identification of the strain. It is notable that Staph-Zym™ judged two isolates as CNS, although they belonged to other species, could not give a species name in 11% of the bovine CNS isolates, and gave 28% of the isolates an incorrect species name. The present study indicates that the studied phenotypic methods are unreliable for identification of CNS from bovine intra-mammary infections.

Keywords: CNS; mastitis; Staph-Zym™; tuf gene sequencing
1. Introduction

Bovine mastitis is often associated with staphylococcal intra-mammary infections. In Sweden, as in many other countries, \textit{Staphylococcus aureus} (\textit{S. aureus}) is a common finding in bovine mastitis, but coagulase-negative staphylococci (CNS) are also frequently found (Bengtsson et al., 2005). CNS have been considered to mainly cause mild sub-clinical mastitis with a slight raise in milk somatic cell count (SCC) (Honkanen-Buzalski et al., 1994), but have recently been associated with both clinical and sub-clinical mastitis (Taponen et al., 2006).

The understanding and control of CNS mastitis is complicated by the heterogeneity of this group of bacteria. So far, 16 CNS species have been isolated from bovine mastitis, and despite some variation between herds and countries, \textit{S. simulans}, \textit{S. chromogenes}, \textit{S. hyicus} and \textit{S. epidermidis} seem to be the most common (Birgersson et al., 1992; Luthje and Schwarz, 2006; Taponen et al., 2006). Little information is available about species differences in virulence, but \textit{S. chromogenes} has been reported to cause more severe mastitis than other CNS (Zhang and Maddox, 2000). Some CNS species are also capable of persisting in the udder for months, or even throughout the lactation period (Taponen et al., 2007).

In spite of the increasing importance of CNS, these bacteria are not routinely identified to the species level. Conventional procedures are labour-intensive, and most commercial identification systems vary in accuracy when used on animal strains (Langlois et al., 1983; Thorberg and Brändström, 2000). However, Staph-Zym™ (Rosco, Taastrup, Denmark) was considered useful when evaluated on strains from bovine mastitis (Thorberg and Brändström, 2000). Lately, genotypic methods have contributed substantially in the classification of the genus \textit{Staphylococcus}. Among such methods, sequencing of a part of the \textit{tuf} gene indicates a high degree of intra-species stability, and excellent sensitivity and specificity among the
genus *Staphylococcus* from human isolates (Martineau et al., 2001; Heikens et al., 2005). Differences in antimicrobial susceptibility could also vary between species, and thus be used for typing of strains (Devriese et al., 2002).

To identify better diagnostic tools for bovine CNS, the objective of the study was to compare two phenotypic and one genotypic methods for species identification of CNS isolated from clinical and sub-clinical cases of bovine mastitis, by using the Staph-Zym™ test, antimicrobial susceptibility testing, sequencing of part of the CNS *tuf* gene and, when needed, the 16S rRNA gene.

2. **Materials and methods**

2.1. **Reference strains and milk isolates**

Twenty-four reference strains of 18 different species of staphylococci (Table 1), and 84 presumptive CNS isolates from clinical (n=64) and sub-clinical (n=20) cases of bovine mastitis were used in the study. Clinical isolates originated from a national Swedish survey on prevalence of udder pathogens in cases of acute clinical mastitis, conducted in 2002-2003 (Bengtsson et al., 2005). The sub-clinical mastitis isolates were from milk samples sent to the Mastitis laboratory (National Veterinary Institute, Uppsala, Sweden) during 2003, and emanated from the central part of Sweden. All milk samples were collected aseptically according to the method described by Jonsson et al. (1996).

Milk samples taken from udder quarters with clinical mastitis were directly cultured (10 µl on 5% bovine blood agar plates (Oxoid Ltd, Cambridge, UK)). The agar plates were incubated at 37 ºC for 16-24 h, evaluated in accordance with the routine of each field veterinarian, and sent to the National Veterinary Institute for bacteriological verification. Milk samples taken from
udder quarters with sub-clinical mastitis were sent directly to the National Veterinary Institute for analysis, and 10 µl were cultured on 5% bovine blood agar (Oxoid Ltd) with 0.05% esculine (Merck, Darmstadt, Germany). The agar plates were incubated at 37 ºC for 24 h. The isolates were identified as CNS by phenotypic appearance and negative coagulase reaction according to recommendations by the National Mastitis Council (Hogan et al., 1999). Isolates were stored at –20 ºC in trypticase soy broth (Oxoid Ltd) containing 15% glycerol. After thawing, the isolates were sub-cultured (over-night at 37 ºC) twice on 5% bovine blood agar (Oxoid Ltd) with 0.05% esculine before use in further studies.

2.2. Phenotypic identification of CNS species

Phenotypic identification of CNS to species level was performed using the Staph-Zym™ system (A/S Rosco, Taastrup, Denmark) according to the manufacturer’s instructions. Supplementary tests were performed as described by Devriese and Oeding (1975) and Devriese et al. (1985).

Isolates were tested for antimicrobial susceptibility by determination of minimum inhibitory concentrations using a microdilution method (Bengtsson et al., 2008). All isolates were also examined for β-lactamase production by the "clover-leaf" method (Bryan and Godfrey, 1991).

2.3. Genotypic identification of CNS species

Bacterial cells were grown overnight on blood agar plates at 37 ºC, and the cultures were checked for purity. A 1 µl loop of cells was suspended in 50 µl lysostaphin (100 µg/ml; Sigma-Aldrich Corporation, Saint Louis, MO, US) in water, and incubated at 37 ºC for 10 min. Then, 50 µl proteinase K (20 mg/ml; Roche, Basel, Switzerland) was added together with 150 µl 10mM Tris HCl, pH 7.5, and the mixture was incubated at 54 ºC for 10 min. The
cell lysate was boiled for five min, cooled on ice for 10 min, and briefly centrifuged at 14 000 g to remove cell debris. The supernatant was used as template in the PCR reaction, or was frozen at –20 °C until further use.

To prevent contamination, DNA extraction, preparation of reaction mixtures, amplification, and detection of PCR products were performed in different rooms. Also, aerosol-resistant filter pipette tips were used throughout all experiments. For the *tuf* gene sequencing, the primers *tuf*-F and *tuf*-R were used both for PCR amplification and as sequencing primers (Heikens et al., 2005). The primers used for 16S rRNA PCR amplification and sequencing were according to Drancourt et al. (1997) and Weisburg et al. (1991). Amplification of part of the 16S rRNA was carried out in a 50µl reaction mixture of 50 mM KCl, 10mM Tris-HCl (pH 8.3), 4 mM MgCl$_2$, 0.2 mM of each deoxynucleoside triphosphates, 0.25 mM of each primer and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems; Foster City, CA, USA) and 5 µl of extracted DNA as a template. The amplification reaction was started by a heating step at 95 °C for 8 min. A touch-down protocol followed where each cycle involved heating to 95 °C for 15 s, cooling to 68 °C for 30 s and heating again to 72 °C for 1 min, the annealing temperature was decreased to 58 °C over 20 cycles. After the touch-down cycles, 40 additional cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min were performed. Negative controls with the DNA template substituted to water in the reaction mixture were included in each PCR run. Amplicons were visualised on 1.5% agarose gels.

For DNA sequencing, cycle sequencing reactions were carried out with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer’s recommendations. Sequencing reaction products were separated and data were collected using an ABI 3100 automated DNA sequencer (Applied Biosystems). The sequence was fully
determined for both strands of each DNA template to ensure maximum accuracy of the data. Sequences were edited, assembled and analysed using Vector NTI Suite (InforMax, Inc., Bethesda, Maryland, US). Sequences were deposited in GenBank (accession numbers EU570987-EU571092). The \textit{tuf} gene sequences were translated to amino acid sequences and aligned with ClustalW Multiple Sequence Alignment tool and a rooted phylogenetic tree was constructed by the use of ClustalW Multiple Sequence Alignment, using default settings. This was performed via Biology WorkBench 3.2 (http://workbench.sdsc.edu/).

PCR amplification of the \textit{nuc} gene was used to confirm that two strains (ID 021011-023-391 and 021127-019-534) were \textit{S. aureus}, and was performed according to Brakstad et al. (1992).

3. Results

3.1. Staph-Zym

Of the 24 reference strains, Staph-Zym™ identified 20 (83%) strains correctly (Table 1). Supplementary tests were needed for identification of twelve (50%) of the 24 strains. Two strains could not be identified, as the codes were not found in the Staph-Zym™ code list. The \textit{S. pulvereri} reference strain was mis-identified as \textit{S. sciuri}. One of the species of the reference strains (\textit{S. pseudintermedius} CCUG 32915) was not listed in the Staph-Zym™ species list, and was misjudged as \textit{S. chromogenes}.

Two of the milk isolates were found to be \textit{Corynebacterium} spp and \textit{Micrococcus luteus} by 16S rRNA gene sequencing were both suggested to be \textit{S. capitis} by Staph-Zym™. These isolates were excluded from further studies. Of the remaining 82 CNS milk isolates, Staph-Zym™ suggested a species name for 73 (89%) isolates (Table 2). For nine isolates a “No match” result was obtained. Supplementary tests were needed for identification of 27 (33%) isolates.
of the 82 isolates. Two isolates previously identified as CNS were suggested to be *S. aureus* using *tuf* gene sequencing, and were confirmed as *S. aureus* by a *S. aureus* specific PCR. Those two strains exhibited a negative coagulase reaction, and Staph-Zym™ identified them as *S. chromogenes*.

3.2. *tuf* gene sequencing

A 371 or a 374-nucleotide long fragment of the *tuf* gene was sequenced for all reference strains and milk isolates. *S. chromogenes* displayed the most diverging *tuf* nucleotide sequences of the species analyzed, having nucleotide differences at four positions, rendering all in all seven genogroups. When using the deduced amino acid sequence, it was found that only one base substitution was non-synonymous. Thus, the deduced amino acid sequence was used to construct a rooted phylogenetic tree (Figure 1). For 79 milk strains the species was identified by comparing their partial *tuf* amino acid sequence to sequences obtained for the reference strains (Table 2). Identification of three milk isolates failed, as their sequences did not match any of the reference strains, or any sequences deposited in GenBank. For two other strains no *tuf* gene amplicon was obtained. Therefore, for these five isolates an approximately 1400-nucleotide long fragment of the 16S rRNA gene was sequenced, and sequences were compared to sequences deposited in GenBank. For two isolates (ID 020902-022-273 and 020703-008-145) the closest match (99.3%) was *S. haemolyticus* (GenBank ID AP006716), and for one isolate (ID 021107-018-437) the closest match (99.8%) was *S. lentus* (GenBank ID EF528296). Two isolates were not CNS as judged by their 16S rRNA sequence, but their closest match was *Corynebacterium* spp and *Micrococcus luteus*, respectively.

Genotyping identified nine different species of CNS among the 82 strains isolated from cases of bovine mastitis (Table 2). Overall, *S. chromogenes* and *S. simulans* were the most
common. The dominating isolates among clinical and sub-clinical isolates were S. chromogenes (29%) and S. epidermidis (30%), respectively (data not shown).

3.3. Comparison between *tuf* gene sequencing and Staph-Zym™

In comparison with results obtained by *tuf* gene sequencing, only 50 (61%) of the isolates were correctly identified using Staph-Zym™, and 17 (34%) of these 50 identifications needed supplementary tests (Table 2). Among the remaining isolates 9 (11%) had no code listed in the Staph-Zym™ code list (No match), and 23 (28%) were misjudged. Of those 23 cases 10 (43%) needed supplementary tests.

3.4. Antimicrobial susceptibility

Antimicrobial resistance was identified in 15 (18%) of 82 milk isolates, and β-lactamase production was found in 13 of those isolates (1 *S. aureus*, 1 *S. chromogenes*, 5 *S. epidermidis*, 5 *S. haemolyticus*, 1 *S. xylosus*). One *S. epidermidis* isolate was multi-resistant, i.e. resistant to more than three antimicrobials. This isolate, as well as one additional *S. epidermidis* isolate, were *mecA* gene positive by PCR. Two *S. chromogenes* isolates did not produce β-lactamase, but were resistant to trimetoprim/sulfametoxazole. Anti-microbial resistance was not associated with specific CNS species or *tuf* gene sequences.

4. Discussion

One conclusion of this study is that Staph-Zym™ was better at identifying CNS reference strains to species level than at identifying bovine milk CNS isolates. Supplementary tests were, however, frequently needed in both groups of isolates. Moreover, Staph-Zym™ could only identify 61% of the milk isolates correctly, as judged by genotyping methods. The use of supplementary tests in combination with Staph-Zym™ was not a guarantee for correct
identification of bovine milk isolates. Another finding was that variation in antimicrobial
susceptibility was not observed between CNS species, and such testing is therefore not
suitable for CNS strain typing.

In the literature, limited and diverging information is given on the performance of Staph-
Zym™ and the need for supplementary tests in relation to reference strains (Watts and
Washburn, 1991; Ieven et al., 1995; Burriel and Scott, 1997). We used the same \textit{S.}

cromogenes reference strain as Burriel and Scott (1997), and in both studies Staph-Zym™
was unable to give a name (No match) to that strain. In our study, 50\% of the reference strains
needed supplementary tests to be identified. One of the strains needing supplementary tests,
the \textit{S. hyicus} reference strain, was also used by Watts and Washburn (1991), but in that study
the strain was identified without the need of supplementary tests. In the study by Thorberg
and Brändström (2000), this reference strain was misidentified by Staph-Zym™ as \textit{S.}
epidermidis. Discrepancies between studies in identification of \textit{S. hyicus} may be due to
difficulties in evaluation of the ß-glucoronidase and ß-galactosidase tests in which \textit{S. hyicus} is
positive and negative, respectively (Bannerman and Peacock, 2007). According to Thorberg
and Brändström (2000), these two tests should be considered positive even when only weakly
coloured. Misidentification of a \textit{S. sciuri} subsp. \textit{sciuri} reference strain as \textit{S. cohnii} subsp.
\textit{cohnii} by Staph-Zym™ has also been reported in human cases (De Paulis et al., 2003). In our
study, the \textit{S. sciuri} reference strain needed supplementary tests for correct identification. The
\textit{S. pulvereri} strain was misidentified as \textit{S. sciuri} by Staph-Zym™. According to the test
instructions \textit{S. pulvereri} (synonymous with \textit{S. vitulus} and \textit{S. vitulinus}) is always mannose
negative, but the reference strain was mannose positive. According to other studies, \textit{S.}
\textit{pulvereri} strains may have variable mannose acidification results (Svec et al., 2004).
Previous studies on bovine milk isolates, comparing Staph-Zym™ to conventional phenotypic methods, have reported good agreement (Watts and Washburn, 1991; Thorberg and Brändström, 2000), but Burriel and Scott (1997) found relatively poor agreement when using ovine isolates, in spite that the reference method used as gold standard was similar to that used by Watts and Washburn (1991). In our study, the agreement between the tuf gene identification and Staph-Zym™ was better for *S. simulans* and *S. epidermidis* than for *S. chromogenes*. All *S. chromogenes* correctly identified by Staph-Zym™ needed supplementary tests. This finding concur largely with those of previous studies (Thorberg and Brändström, 2000; Watts and Washburn, 1991; Burriel and Scott, 1997).

In the present study, two CNS strains misjudged by Staph-Zym™ as *S. chromogenes* using supplementary tests were identified as *S. aureus* by tuf gene sequencing and PCR amplification of the nuc gene. One of the four supplementary tests performed was the coagulase reaction in which these two isolates failed to coagulate rabbit plasma. Such *S. aureus* strains have been described previously (Fox et al., 1996). Correct identification of *S. chromogenes* and *S. aureus* is important especially as several studies report *S. chromogenes* to be the major CNS pathogen isolated in both heifers and primiparous cows (Luthje and Schwarz, 2006; Taponen et al., 2006).

Recently, Ghebremedhin et al. (2008) compared phylogenetic analysis of staphylococcal species based on partial sequencing of different genes, as 16S rRNA sequence comparison, the most traditional target for bacterial phylogeny and taxonomy, is not considered to have enough discriminatory power within these genera. They suggested the glyceraldehydes-3-phosphate dehydrogenase (*gap*) gene to be a suitable target for taxonomical analysis of *Staphylococcus* species, as compared to hsp60, sodA, rpoB and tuf gene sequence analysis.
However, they needed to sequence a 931 bp fragment of this gene, as the variable regions were dispersed over the gene fragment. The method was not applied to clinical staphylococcal isolates, thus the intra-species variation was not evaluated. We used partial \textit{tuf} gene sequencing (ca 370bp), after translation to amino acid sequences to identify CNS. In this way, we obtained complete match between the reference strains used and the bovine mastitis isolates for \textit{S. simulans}, \textit{S. epidermidis}, \textit{S. hyicus}, and \textit{S. warneri}. The two isolates of \textit{S. xylosus} displayed one amino acid sequence difference; this intra-species sequence variation of \textit{S. xylosus} is in agreement with the findings of Heikens et al. (2005). \textit{S. chromogenes} also divided into two groups differing by one amino acid, with five and 17 isolates in each group. Two isolates that clustered most closely to \textit{S. haemolyticus} (two amino acid differences) also displayed the closest match by 16S rRNA sequencing to \textit{S. haemolyticus}. For \textit{S. lentus} we did not have a reference strain to compare with, but this isolate was identified to species by 16S rRNA sequencing.

5. Conclusion

Our results support the notion that micromethods such as Staph-Zym\textsuperscript{TM} based on phenotypic identification may be unable to reliably distinguish between different CNS species, and that genotypic methods are more reliable for diagnostic purposes in the identification of CNS from bovine IMI. It is notable that the accuracy of Staph-Zym\textsuperscript{TM} varied widely between CNS species, and that the test had low specificity in identification of important udder pathogens such as \textit{S. chromogenes}. There was also a tendency to give an incorrect species name rather than a ‘No match’. Additionally, a drawback of Staph-Zym\textsuperscript{TM} was that coagulase negative \textit{S. aureus} was not identified. Further studies using genotypic methods on field isolates of CNS species are needed.
Acknowledgements

We are very grateful to Sara Frosth for her excellent laboratory support. This project has been supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas).

References


Table 1

Reference strains (n=24) of the genus *Staphylococcus* used in this study, and their respective species identification by Staph-Zym™ (A/S Rosco, Taastrup, Denmark)

<table>
<thead>
<tr>
<th>Strain</th>
<th>CCUG / ATCC</th>
<th>Staph-Zym™</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>CCUG 46176</td>
<td><em>S. aureus</em>²</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>CCUG 41582 / NCTC 8325</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td><em>S. capitis</em></td>
<td>CCUG 7326T / ATCC 27840</td>
<td><em>S. capitis</em>²</td>
</tr>
<tr>
<td><em>S. chromogenes</em></td>
<td>CCUG 4319T</td>
<td>No match³,⁶</td>
</tr>
<tr>
<td><em>S. cohnii</em></td>
<td>CCUG 35144T</td>
<td><em>S. cohnii</em></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>CCUG 46178</td>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>CCUG 21989</td>
<td>No match⁴,⁶</td>
</tr>
<tr>
<td><em>S. gallinarum</em></td>
<td>CCUG 28809</td>
<td><em>S. gallinarum</em>²</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>CCUG 7323T</td>
<td><em>S. haemolyticus</em></td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>CCUG 35516T</td>
<td><em>S. hominis</em></td>
</tr>
<tr>
<td><em>S. hyicus</em></td>
<td>CCUG 6509T</td>
<td><em>S. hyicus</em></td>
</tr>
<tr>
<td><em>S. hyicus</em></td>
<td>CCUG 15602T / ATCC 11249</td>
<td><em>S. hyicus</em>²</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>CCUG 6520T</td>
<td><em>S. intermedius</em>²</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>ATCC 51874</td>
<td><em>S. intermedius</em>²</td>
</tr>
<tr>
<td><em>S. pseudintermedius</em>⁵</td>
<td>CCUG 32915</td>
<td><em>S. chromogenes</em>²</td>
</tr>
<tr>
<td><em>S. pulvereri</em></td>
<td>CCUG 41685</td>
<td><em>S. sciuri</em>²</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>CCUG 3706T</td>
<td><em>S. saprophyticus</em></td>
</tr>
<tr>
<td><em>S. schleiferi</em></td>
<td>ATCC 49545</td>
<td><em>S. schleiferi</em>²</td>
</tr>
<tr>
<td><em>S. schleiferi</em></td>
<td>CCUG 37248T</td>
<td><em>S. schleiferi</em>²</td>
</tr>
<tr>
<td><em>S. sciuri</em></td>
<td>CCUG 15598T</td>
<td><em>S. sciuri</em>²</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>CCUG 46177</td>
<td><em>S. simulans</em></td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>CCUG 7327Aᵀ</td>
<td><em>S. simulans</em></td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>CCUG 44859</td>
<td><em>S. warneri</em></td>
</tr>
<tr>
<td><em>S. xylosus</em></td>
<td>CCUG 7324ᵀ</td>
<td><em>S. xylosus</em>²</td>
</tr>
</tbody>
</table>

¹CCUG = Culture Collection, University of Göteborg, Göteborg, Sweden; ATCC = American Type Culture Collection, Manassa, USA. ²Supplementary tests were needed for identification. ³The closest Staph-Zym™ codes were *S. hyicus* or *S. chromogenes*. ⁴The closest Staph-Zym™ code was *S. epidermidis*. ⁵Species not listed in the Staph-Zym™ species list. ⁶Code not listed in the Staph-Zym™ code list.
Table 2

Species identification, using *tuf* gene sequencing, of 82 CNS strains isolated from cases of bovine mastitis, and number of those isolates correctly identified using Staph-Zym™

<table>
<thead>
<tr>
<th>Species</th>
<th>Identified using <em>tuf</em> gene</th>
<th>Identified by Staph-Zym™ as No match(^2)</th>
<th>Correctly identified using Staph-Zym™</th>
<th>Misjudged as</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n (%))</td>
<td>No (n (%))</td>
<td>Yes (n (%))</td>
<td>No (n (%))</td>
<td></td>
</tr>
<tr>
<td><em>S. chromogenes</em></td>
<td>22</td>
<td>4 (18)</td>
<td>13 (59)</td>
<td>5 (23)</td>
<td><em>S. cohnii</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. haemolyticus</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. simulans</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. warneri</em> 2</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>20</td>
<td>2 (10)</td>
<td>17 (85)</td>
<td>1 (5)</td>
<td><em>S. chromogenes</em> 1</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>12 (2(^1))</td>
<td>1 (8)</td>
<td>4 (33)</td>
<td>7 (58)</td>
<td><em>S. capitis</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. chromogenes</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. warneri</em> 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. xylosus</em> 1</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>10</td>
<td>1 (10)</td>
<td>8 (80)</td>
<td>1 (10)</td>
<td><em>S. chromogenes</em> 1</td>
</tr>
<tr>
<td><em>S. hyicus</em></td>
<td>9</td>
<td>0 (0)</td>
<td>4 (44)</td>
<td>5 (56)</td>
<td><em>S. chromogenes</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. haemolyticus</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. warneri</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. xylosus</em> 2</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>4</td>
<td>0 (0)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td><em>S. hyicus</em> 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>S. simulans</em> 1</td>
</tr>
<tr>
<td><em>S. xylosus</em></td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>2</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td><em>S. chromogenes</em> 2</td>
</tr>
<tr>
<td><em>S. lentus</em></td>
<td>1(^1)</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>82</td>
<td>9 (11)</td>
<td>50 (61)</td>
<td>23 (28)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Identified by 16S rRNA sequencing. \(^2\)Code not listed in the Staph-Zym™ code list.

Figure caption

Fig. 1. A rooted phylogenetic tree was constructed from the *tuf* gene amino acid sequences of the 24 reference strains (black letters) and 82 strains of CNS (blue letters) isolated from cases of clinical (CM) or sub-clinical (SCM) mastitis. Information on Staph-Zym™ results is given within brackets (blue=correct ID; azure blue=species not listed; orange=code not listed; red=misjudged). Species identification by 16S rRNA is given in grey letters. Green ● = supplementary tests were needed for Staph-Zym™ identification.