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Performance of API Staph ID 32 and Staph-Zym for identification of coagulase-negative staphylococci isolated from bovine milk samples

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Abstract

In this study, the accuracy of two phenotypic tests, API Staph ID 32 and Staph-Zym, was determined for identification of coagulase-negative staphylococci (CNS) from bovine milk samples in comparison with identification based on DNA-sequencing. A total of 172 CNS isolated from bovine milk were classified into 17 species. The most frequently isolated species based on \textit{rpoB} sequencing were \textit{Staphylococcus chromogenes} and \textit{Staphylococcus}}
epidermidis, followed by Staphylococcus xylosus, and Staphylococcus equorum (37, 13, 9 and 6% of isolates, respectively). The API Staph ID 32 correctly identified 41% of the CNS isolates. Best agreement with rpoB sequence based species identification was found for S. epidermidis, Staphylococcus hyicus and S. xylosus (100, 89 and 87%, respectively). The positive predictive value was 89, 100 and 52%, respectively. Poor sensitivity was observed for 3 of the 5 most frequently found species, S. chromogenes (37%), Staphylococcus warneri (15%) and S. equorum (0%) albeit with specificity of 100%. The Staph-Zym needed additional tests for 66% of the isolates and identified 31% of the CNS isolates correctly. Good sensitivity was found for S. epidermidis, S. simulans and S. xyloxs (100, 78 and 73%, respectively). The positive predictive value was 89, 78 and 98%, respectively. Poor sensitivity was observed for S. chromogenes, S. warneri and S. equorum (0, 54 and 0%, respectively) but with a specificity of 100, 99 and 100%, respectively. Both phenotypic tests misidentified a large proportion of CNS isolates and were thus unsuitable for identification of CNS species from bovine milk samples.

Keywords: bovine; mastitis; coagulase-negative staphylococci; genotyping; phenotyping

1. Introduction

Coagulase-negative staphylococci (CNS) are commonly isolated from cases of subclinical and clinical mastitis, and also from teat canals, teat skin and teat ducts (De Vliegher et al., 2003; Taponen et al., 2006; Sampimon et al., 2007). In several recent prevalence studies, CNS were the most frequently isolated udder pathogens from bovine milk samples with high somatic cell count (SCC) (Pitkälä et al., 2004; Bradley et al., 2007; Piepers et al., 2007). Coagulase-negative staphylococci are a heterogeneous group of organisms. Currently, the genus Staphylococcus consists of more than 40 named species (Bes et al., 2000; Devriese et al., 2002). Coagulase-negative staphylococcal species may
differ in antimicrobial susceptibility, virulence factors, host response to infection and transmissibility. To assess the pathogenic significance of individual CNS species and to develop species-specific management practices, accurate species identification is needed.

Several schemes for the identification of CNS based on phenotypic characteristics have been developed (Devriese et al., 1994). These phenotypic schemes require numerous media and are labour intensive. Also, they require extended incubation periods that limit usefulness for routine diagnostics. The ideal test for routine diagnostic laboratory identification of CNS species in milk samples of cows would be reliable, time and cost-effective and easy to use. Commercial test kits, like the API Staph ID 32 (API Test, bioMérieux, France) and the Staph-Zym test (Rosco, Taastrup, Denmark) are used for phenotypic identification of CNS in diagnostic laboratories. However, they may lack accuracy because these tests and their accompanying databases were mainly developed for human isolates (Watts and Washburn, 1991; Thorberg and Brändström, 2000; Taponen et al., 2006; Zadoks and Watts, 2008). Because of the large and increasing diversity of microorganisms and the prevalence of organisms with rare, inconsistent or poorly defined phenotypic characteristics, conventional methods often cannot fully characterize bacterial isolates, and laboratories are increasingly relying on DNA sequencing for microorganism identification (CLSI, 2007; Zadoks and Watts, 2008). For identification of CNS species, sequence data of housekeeping genes such as rpoB, cpn60, dnaJ or tuf can be used (Capurro et al., 2008; Drancourt and Raoult, 2002; Mellmann et al., 2006; Shah et al., 2007; Zadoks and Watts, 2008).

The aim of the present study was to measure the sensitivity, specificity and positive predictive value of two frequently used phenotypic methods, the API Staph ID 32 and the Staph-Zym test, for identification of CNS isolated from bovine milk samples through comparison with species identification based on DNA-sequence data of housekeeping genes.
2. Materials and methods

2.1. Cultures

A total of 175 CNS isolates were selected at random from quarter milk samples of clinical and subclinical mastitis cases submitted for bacteriological examination to the GD Animal Health Service (GD; Deventer, The Netherlands). Only isolates from a pure culture on agar plates were included, with a maximum of one isolate per herd.

Bacteriological culturing was carried out according to NMC protocols (Harmon et al., 1990). Briefly, an inoculum of 0.01 ml of milk was spread on a sheep blood agar plate. All plates were incubated at 37ºC and examined after 24 and 48 h. A milk sample was considered CNS-positive when ≥ 500 cfu/ml of CNS were cultured. Coagulase-negative staphylococci were differentiated from coagulase positive staphylococci using Slidex Staph Plus (bioMérieux, France). Isolates with a negative test result on the Slidex Staph Plus were further tested with the tube coagulase test. Coagulase-negative staphylococci isolates were frozen and stored at -70ºC.

2.2. Reference method

The identification of the CNS isolates with the API Staph ID 32 and the Staph-Zym test was compared with species identification based on DNA-sequence data. Isolates were grown on Colombia agar with 5% sheep blood (Gibco Technologies, Paisley, Scotland) for 24 h at 37ºC. To prepare DNA extracts, one calibrated loop (1 µl) of cells was suspended in 20 µl lysis buffer (0.25% SDS, 0.05 N NaOH), heated for 5 min at 95ºC, and diluted with 180 µl distilled water. Cell debris was spun down by centrifugation at 16000g for 5 min and supernatants were used as PCR template. PCR of a 751 bp fragment of the rpoB gene was performed using published primers and run parameters (Drancourt en Raoult, 2002). PCR-products were purified with the PureLink DNA kit (Invitrogen Corporation, Carlsbad, CA). DNA-sequencing was carried out in two directions by the BioResource Center (Cornell University, Ithaca, NY) using the PCR primers and Big Dye Terminator chemistry on an ABI
PRISM 3700 DNA analyzer. Sequence data were assembled and proofread using SeqMan (version 5.08, Lasergene, DNASTAR Inc., Madison, WI). Assembled sequence data were compared with sequence data in GenBank using the nucleotide-nucleotide BLAST algorithm of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Although 94% DNA sequence homology is considered sufficient for identification of CNS species using rpoB (Mellman et al., 2006), additional methods were used to confirm or determine the identity of isolates with < 98% homology to reference data. Additional identification was performed using the amplification and sequencing of a 600 bp fragment of the housekeeping gene cpn60 (Zadoks et al., 2005) using degenerate primers that amplify this target gene in multiple gram-positive bacterial genera (Goh et al., 1997) or, if species identity was still unresolved, based on sequencing of 16S rDNA (Durak et al., 2005).

2.3. API Staph ID 32

The API Staph ID 32 test strip consists of 32 capsules, 26 of which contain dehydrated biochemical media for colorimetric tests. Tests were performed and interpreted according to the manufacturer’s recommendations (API Test, bioMérieux, Lyon, France). Briefly, the frozen CNS isolates were recultured on agar plates containing 6% sheep blood (Biotrading, Mijdrecht, The Netherlands). After 24 hours of incubation, bacterial suspensions were prepared to a density of 0.5 McFarland in 6 ml of sterile distilled water and distributed into the wells of the API test strip. The strips were incubated at 37°C and examined visually after 24 h. The Apiweb software was used to calculate the probability of the identification result in a range of 10 to 100% (https://apiweb.biomerieux.com). In this study, only identification with a probability ≥ 90% was considered acceptable (Thorberg and Brändström, 2000; Taponen et al., 2006). If the probability of a result was < 90%, isolates were considered unidentified.

2.4. Staph-Zym test
The Staph-Zym test consists of a rigid, transparent plastic strip with 10 upright minitubes divided into four groups of tests. The manufacturer’s instructions (Rosco, Taastrup, Denmark) were followed to conduct the test and interpret the results. Briefly, the frozen CNS isolates were recultured on sheep blood agar plates. Bacterial suspensions were prepared from cultures after 24 hours of incubation and standardized to a 2 McFarland standard in 3 ml physiological saline (0.9% NaCl). Approximately 0.25 ml of the bacterial suspension was dispensed into each of the 10 tubes. After an incubation of 24 h at 37ºC, positive reactions were recorded and converted to five-digit numbers. The 5-digit number provided a numerical profile of known CNS species and suggested additional tests, i.e. enzyme reactions, Pyrrolidonyl Aminopeptidase 2h, Vogues Proskauer 4h, anaerobic growth, susceptibility to fosfomycin and nalidixic acid, and acid formation from sucrose, cellobiose, raffinose, and xylose for species delineation. Isolates not recognized by numerical profile and additional tests, were considered unidentified.

2.5. Data analysis

Isolates were considered to be correctly identified by API Staph ID 32 and Staph-Zym if the same species was found as with the reference method. Misidentified was defined as a different species found compared to reference method. For both phenotypic methods, sensitivity, specificity and predictive value positive (PVP) were calculated in comparison with DNA-based species identification. Sensitivity was calculated as the proportion of the true positive isolates that are correctly identified with the phenotypic tests, e.g. the proportion of *S. chromogenes* isolates based on sequence data that was identified as such by phenotypic testing. Specificity was calculated as the proportion of the true negatives that are correctly identified with the phenotypic tests, e.g. the proportion of isolates other than *S. chromogenes* based on sequence data that were identified as something other than *S. chromogenes* by phenotypic testing. Finally, PVP was calculated as the proportion of isolates identified as a specific species based on phenotypic testing that truly represented that particular species,
e.g. the proportion of isolates that were identified as *S. chromogenes* by phenotypic testing that had been identified as *S. chromogenes* based on DNA sequence data.

3. Results

3.1. Isolates

A total of 175 isolates were analyzed by DNA sequencing. One isolate was identified as *Streptococcus uberis* and one isolate was identified as *Moraxella osloensis* based on cpn60 sequencing. PCR with *rpoB* primers had been negative for both isolates, which is explained by the fact that the primers are specific to the genus *Staphylococcus* and neither isolate belonged to that genus. Of the remaining 173 CNS isolates, all of which yielded an amplicon with *rpoB* PCR, 172 were identified at the species level. Based on *rpoB*, *cpn60* or 16S sequence data, one isolate could be identified at the genus level but not at the species level, suggesting that it is an unknown species (≤92% homology to reference sequences for *rpoB* and *cpn60*; 99% homology to *S. pasteuri* as well as *S. lugdunensis* based on partial 16S sequence data). This isolate was not identified to the species level by API Staph ID 32 or Staph-Zym typing either. Further analyses were performed on the 172 isolates that were identified to species level. The most frequently isolated species based on gene sequencing were *S. chromogenes* and *S. epidermidis*, followed by *S. xylosus*, *S. warneri* and *S. equorum* (Table 1). For 7 isolates with 94% to 97% homology of the *rpoB* sequence to reference data, species identity was also determined based on *cpn60* sequence data (4x *S. hyicus*, and 3x *S. warneri*). For all 7 isolates, *rpoB* based species identity was confirmed, with *cpn60* sequences showing 97% to 100% homology to reference data. In addition, species identity was determined based on *cpn60* data for 8 isolate that had yielded noisy or deteriorated *rpoB* sequence data (3x *S. equorum*, 2x *S. succinus*, and 3x *S. xylosus*). For one isolate, *rpoB* and *cpn60* data were noisy, and isolate identity was determined based on 16S sequence data (S. equorum). Sequence data for *rpoB* of isolates showing 94% to 97%
homology with previously published sequence data were deposited in GenBank under accession number FJ 404467, and FJ 404468 for S. hyicus, and S. warneri, respectively.

3.2. API Staph ID 32

The API Staph ID 32 identified 71 of 172 (41%) isolates correctly (Table 1). The best sensitivity in comparison with rpoB-sequence based identification was found for S. epidermidis, S. hyicus and S. xylosus, (100, 89 and 87%, respectively). The specificity of detection for these species was 98, 100 and 92%, respectively and PVP was 89, 100 and 52%, respectively (Table 2). Poor to very poor sensitivity was observed for 3 of the 5 most commonly found species, i.e. for S. chromogenes, S. warneri, and S. equorum (37, 15 and 0% sensitivity, respectively) (Table 1). The specificity of detection of these species and PVP were high (100%), with the exception of the PVP for S. equorum (Table 2). Of the 101 (59%) isolates in this set of samples that were not identified correctly, 80 were unidentified and 21 were misidentified (Table 1), i.e. the species identification by the Apiweb system (with probability ≥ 90%) was not in agreement with the reference method. Of 9 S. equorum isolates 8 were identified as S. xylosus and one as S. sciuri. Two S. chromogenes isolates were misidentified as S. caprae and S. xylosus, respectively; one S. haemolyticus isolate as S. auricularis; one S. nepalensis isolate as S. xylosus; one S. saprophyticus isolate as S. epidermidis; one S. simulans isolate as S. epidermidis; two S. succinus isolates as S. xylosus; two S. warneri as S. capitis and S. simulans, respectively and finally two S. xylosus isolates as S. epidermidis and as S. simulans, respectively.

3.3. Staph-Zym test

Using the Staph-Zym test, 53 of 172 isolates (31%) were identified correctly, based on a single suggestion for identification. Sensitivity of identification was highest for S. epidermidis (100%), S. simulans (78%) and S. xylosus (73%) (Table 2). The specificity of identification of those species was 98, 99 and 98%, respectively and PVP was 89, 78 and 98%, respectively (Table 2). Poor to very poor sensitivity was observed for three of the five
most commonly found species: *S. chromogenes*, *S. warneri* and *S. equorum* (0, 54 and 0%, respectively) (Table 1). The specificity of identification of these species was 100, 99 and 100%, respectively. Because sensitivity of detection was 0% for *S. chromogenes* and *S. equorum*, PVP could not be calculated. For *S. warneri*, PVP was 78% (Table 2). No identification was obtained for 101 (59%) isolates. For 86 isolates, the Staph-Zym test yielded more than one species name as a result. For 15 isolates the Staph-Zym test could not suggest any species identification. Misidentification was observed for 18 of 172 (10%) isolates. One *S. capitis* isolate was misidentified as *S. saprophyticus*; two *S. chromogenes* isolates were misidentified as *S. haemolyticus*; one *S. cohnii* subsp. *cohnii* as *S. xylosus*; two *S. equorum* isolates as *S. gallinarum* and *S. xylosus*, respectively, three *S. fleuretti* isolates as *S. sciuri*; three *S. haemolyticus* isolates as two *S. warneri* and one *S. simulans*; one *S. hominis* isolate as *S. epidermidis*; one *S. simulans* isolate as *S. epidermidis*; one *S. succinus* as *S. xylosus* and three *S. xylosus* isolates as *S. epidermidis*, *S. equorum* and *S. simulans*, respectively. For 113 of 172 (66%) isolates additional tests were needed.

4. Discussion

Coagulase-negative staphylococci as a group are increasingly seen as important mastitis pathogens (Taponen et al., 2007). The group is comprised of many different staphylococcal species. In a recent study of Taponen et al. (2007), CNS IMI were persistent throughout lactation, significantly increased quarter SCC, and because of their relatively high prevalence contributed to a higher average BMSCC. Because of their impact on milk quality, control measures may need to be developed for CNS. Also for epidemiological investigations and assessment of their pathogenic significance, accurate species identification of CNS is needed.

Several commercial test kits are available for CNS identification, all of which are based on phenotypic methods. Genotypic test methods are not available in commercial test
kits. Genotypic methods have higher typeability and accuracy than phenotypic methods, and identify over 99% of CNS isolates from bovine milk (Zadoks and Watts, 2008). The high proportion of species identification based on DNA sequence data is likely due to the large reference database (GenBank) which is continuously updated with new strains or species including species common in animals but not in humans (Zadoks and Watts, 2008). Routine updates to methodology or databases are not economically feasible for commercial phenotypic test kits. Because of the superior identification obtained with genotypic methods, the implementation of such methods by diagnostic laboratories is encouraged (CLSI, 2007).

Among 172 CNS isolates from bovine milk, 17 CNS species were identified with \( rpoB \) sequencing, and 11 and 15 species with API Staph ID 32 and Staph-Zym, respectively. The API Staph ID 32 and Staph-Zym identified 41 and 31%, respectively, of the CNS isolates correctly, which was lower than reported in other studies (Watts and Washburn, 1991; Thorberg and Brändström, 2000; Taponen et al., 2006; Carpurro et al., 2008). An explanation could be the different study designs and associated species distribution. Both phenotypic databases were developed to identify CNS isolates from human origin which may explain the relatively high sensitivity for identification of species such as \( S. \) epidermidis and \( S. \) xylosus, which are commonly found in humans. However, the PVP of the API Staph ID 32 for \( S. \) xylosus was 52% which indicates that only about half of the isolates from bovine milk that are identified as \( S. \) xylosus by API Staph ID 32 really belong to this species. With the Staph-Zym, the PVP for \( S. \) xylosus was much higher, i.e. 98%, implying that almost all isolates that are identified as \( S. \) xylosus by Staph-Zym testing are indeed \( S. \) xylosus. However, only about three-quarters of all \( S. \) xylosus isolates are recognized as such by Staph-Zym testing. The low sensitivity of the Staph-Zym for most species in our study was mainly caused by the large number of isolates for which identification could not be narrowed down to a single species. Another drawback of the Staph-Zym system was the large number of additional tests needed to obtain final results. Additional tests were needed for 66% of the isolates in our study, compared to 45% of isolates in the study by Thorberg and Brändström (2000) and 33% in the study by Carpurro et al. (2008). Additional tests increase cost, labor and time.
delay of final outcomes of the test. Despite additional testing, many isolates could not be identified to the species level by the Staph-Zym test, in agreement with results from Capurro et al. (2008).

*Staphylococcus chromogenes* was the most frequently isolated species in our study. Approximately two-thirds of these isolates were misidentified by the API Staph ID 32. However, the specificity and PVP were both 100% which means that the API Staph ID 32 had a low number of false-positives in detection of *S. chromogenes*. The Staph-Zym did not identify a single *S. chromogenes* isolate correctly, which is a big concern considering that *S. chromogenes* is among the most common CNS species in bovine milk. Identification of coagulase negative *S. aureus* as *S. chromogenes* by the Staph-Zym has been reported (Capurro et al., 2008) but was not observed in our study. Differences between countries seem to exist in predominance of CNS species. In Sweden, USA (Tennessee), and Zimbabwe, *S. chromogenes* was the predominant species, while in Norway, Finland and Belgium *S. simulans* was the most common CNS species (Matthews et al., 1990; Devriese et al., 1994; Waage et al., 1999; Thorberg and Brändström, 2000; Kudinha and Simango, 2002; Taponen et al., 2006; Capurro et al., 2008). These may be true differences, but differences can also be caused by differences in identification methods. Our data suggest that prevalence of *S. chromogenes* is more likely to be underestimated with the Staph-Zym method than with the API Staph ID 32, whereas the reverse is true for *S. simulans*.

*Staphylococcus hyicus* was commonly identified in the 1980s (Watts and Owens, 1989), but is not among the most common species in more recent studies. This is probably explained by the fact that *S. chromogenes* was considered a subspecies of *S. hyicus* in the 1980s, whereas it is recognized as a separate species nowadays. True misidentification of isolates may also have occurred (Bes et al., 2000; Zadoks and Watts, 2008).

In addition to well known species, we found some CNS species that have thus far rarely been associated with bovine mastitis, i.e. *S. equorum*, *S. nepalensis* and *S. fleuretti*. *Staphylococcus equorum* constituted 6% of our isolates, making it the fifth-most frequently isolated species. The API 20 Staph test does not recognize *S. equorum*, but both phenotypic
tests used in our study should have the ability to identify the species (bioMérieux, France; Rosco, Taastrup, Denmark). However, none of the nine *S. equorum* were identified correctly. In a study in Finland, only one isolate of *S. equorum* was found (Taponen et al., 2006). We also found *S. nepalensis* and *S. fleuretti* in pure culture from bovine samples. *Staphylococcus nepalensis* was first isolated from the respiratory tract of goats kept in the Himalayan region in 2003 (Spergser et al., 2003). *Staphylococcus fleuretti* was isolated from goat’s milk cheeses for the first time in 2000 (Vernozy-Rozand et al., 2000). These examples illustrate that new CNS species continue to be discovered, and explain why phenotypic test methods may not be as up to date as reference databases based on DNA-sequence data. In our study, more CNS species were found with a genotypic method than expected from earlier studies with phenotypic tests. The prevalence and impact of these particular CNS species on udder health is unknown. Further studies would be necessary to determine their impact and relevance.

We conclude that, in our study, the API Staph ID 32 and the Staph-Zym had a high number of unidentified or misidentified CNS isolates. Both tests were insufficient to identify CNS species from bovine milk samples as demonstrated by poor sensitivity and positive predictive value for identification of several of the most commonly isolated CNS species. Misidentification or ambiguous test results can lead to erroneous interpretation of data and to false conclusions in epidemiological or monitoring studies. Comparing test results between studies that used different techniques is not meaningful if test results are not accurate. For most routine laboratories it is more appropriate to report “coagulase-negative *Staphylococcus* species” based on coagulase testing, than reporting of more detailed but potentially inaccurate results. In research and to identify new species, genotypic methods for species identification are to be preferred over phenotypic methods.

**Conflict of interest**
None of the authors (O.C. Sampimon, R.N. Zadoks, S. De Vliegher, K. Supré, F. Haesebrouck, H.W. Barkema, J. Sol and T.J.G.M. Lam) has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Performance of API Staph ID 32 and Staph-Zym for identification of coagulase-negative staphylococci isolated from bovine milk samples”.

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infections caused by coagulase-negative staphylococci may persist throughout
lactation according to amplified fragment length polymorphism-based analysis. J.
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Table 1

Identification of coagulase-negative staphylococci with the API Staph ID 32 and the Staph-Zym test, compared with rpoB sequencing as the reference method.

<table>
<thead>
<tr>
<th>CNS species</th>
<th>rpoB sequencing</th>
<th>API Staph ID 32</th>
<th>Staph-Zym</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>MI</td>
</tr>
<tr>
<td>S. arlettae</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. capitis</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>63</td>
<td>23</td>
<td>36.5</td>
</tr>
<tr>
<td>S. cohnii subsp. cohnii</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. epidermidis</td>
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<td>23</td>
<td>100.0</td>
</tr>
<tr>
<td>S. equorum</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. fleuretti</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<td>S. hyicus</td>
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<td>88.9</td>
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<td>0</td>
</tr>
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<td>S. saprophyticus</td>
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<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. warneri</td>
<td>13</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>15</td>
<td>13</td>
<td>86.7</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>71</td>
<td>41.3</td>
</tr>
</tbody>
</table>

\(^{1}\text{MI=misidentified, } ^{2}\text{UI=unidentified.}\)
Table 2

Sensitivity, specificity and positive predictive value of the API Staph ID 32 and the Staph-Zym test of most common CNS species in this study, using rpoB sequencing as reference method.

<table>
<thead>
<tr>
<th>CNS species</th>
<th>API Staph ID 32</th>
<th>Staph-Zym</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>36.5</td>
<td>100.0</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>100.0</td>
<td>98.0</td>
</tr>
<tr>
<td>S. equorum</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>88.9</td>
<td>100.0</td>
</tr>
<tr>
<td>S. simulans</td>
<td>22.2</td>
<td>98.8</td>
</tr>
<tr>
<td>S. warneri</td>
<td>15.4</td>
<td>100.0</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>86.7</td>
<td>92.4</td>
</tr>
</tbody>
</table>

¹ PV+ = positive predictive value.
² n/a = not applicable.