Comparative study using phenotypic, genotypic and proteomics methods for identification of coagulase-negative staphylococci

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Abstract

Five methods were compared to determine the most accurate identification of coagulase-negative staphylococci (CoNS) (n=142 strains). MALDI-TOF MS showed the best results for rapid and accurate CoNS differentiation (correct identity in 99.3%). An alternative to this approach could be Vitek2 combined with partial tuf gene sequencing (100% correct identity when both methods are performed simultaneously).
Coagulase negative staphylococci (CoNS) have emerged as significant pathogens and are mainly found in hospitalized immunocompromised patients often with indwelling or implanted medical devices (10, 13, 15, 18, 22). Most infections are hospital-acquired and CoNS infections can result in several diseases including blood stream infection, endocarditis, mediastinitis, meningitis, urinary tract infections (S. saprophyticus) and medical device related infections (1, 8, 12, 18, 19, 22). Accurate identification of CoNS is important when CoNS are isolated from multiple blood cultures of 1 patient. Many CoNS are described to be resistant to multiple antibiotics (11, 14, 17, 19). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a relatively new technique that allows examination of protein profiles from bacteria, and has been shown to work in a fast and accurate manner (4, 6, 7, 21).

In the present comparative study, five differentiation methods for CoNS were compared, i.e. the Vitek2 (Gram Positive card REF 21342, bioMérieux), the ID 32 Staph strip (bioMérieux), partial 16S rDNA gene sequencing (MicroSeq, Applied Biosystems), partial tuf gene sequencing (in-house), and MALDI-TOF MS (Bruker Daltonics) on the same set of CoNS strains, in order to find the most suitable method for identification of staphylococci available today.

A total of 142 CoNS strains were included in this study. Isolates were derived from clinical cultures (n=117) and 25 reference strains were included from the ATCC (American Type Culture Collection), and the National Institute for Health and Environment (RIVM, Bilthoven, the Netherlands). The clinical isolates were selected from positive blood cultures (two or more positive cultures from 1 patient), urinary tract infections (S. saprophyticus), or deep seeded infections. The CoNS species name that was found with ≥3 of the 5 used methods was chosen as the true CoNS species name (true ID). When only 2 methods resulted in a similar true ID name (4/142 cases, 2.8%), tuf sequencing was selected as most reliable, as 16S rDNA sequencing resulted in the non-informative Staphylococcus spp. as a result.

CoNS strains were cultured overnight at 35°C on bloodplates (made in-house). The phenotypical methods Vitek2 (bioMérieux, Marcy l’Etoile, France) and ID 32 Staph (bioMérieux, Marcy l’Etoile, France) were both performed as described by the manufacturer. DNA for sequencing was isolated from 2 McFarland bacterial suspensions by heating (10 min 95°C). The 16S rDNA primers were used from the MicroSeq kit and amplify approximately the first 500 bp of the gene (Applied Biosystems, Foster City, CA). The tuf forward primer (tuf_32_FW) was described previously (16) and the tuf reverse primer (tuf_512_RV: 5’-CAGCTTCAGCGTAGTCTAATAATTTACG-3’) was designed for this study.
Positions of the tuf primers were derived from the S. aureus tuf gene sequence (GenBank accession no.AF298796), and amplify a 480bp fragment. Tuf primers were obtained from Eurogentec (Liège, Belgium). PCR was performed according to standard procedures. Sequencing was performed using the Big Dye X Terminator sequencing mix 3.1 (Applied Biosystems, Foster City, CA) and ran on the ABI 310 PRISM (Applied Biosystems, Foster City, CA). The 16S DNA sequences were analysed by using MicroSeqID Analysis software (Applied Biosystems, Foster City, CA). The tuf DNA sequences were analysed by using the Basic Local Assignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

For MALDI-TOF MS (Bruker Daltonics) analysis 1 colony of each CoNS strain was spotted with a sterile wooden stick, in duplicate, on the polished steel target plate. Samples that could not be identified directly by MALDI-TOF MS were retested after pre-treatment (n=5 clinical CoNS strains; S.hominis, S.epidermidis, S.cohnii, S.schleiferi, and S.saprophyticus) as described by Van Veen et al.,,(21). However, in this study formic acid (70%) and acetonitrile were added according to pellet size in a 1:1 ratio. The BioTyper database version V3 1.1.0_3476-3740 was used as reference.

The results (table 1) of the phenotypic identification methods used in this study indicate that the Vitek2 performs best with a correct identification rate of 92.3% compared to 85.9% for ID 32 Staph. Acceptance criteria for all results are mentioned in the footnote of table 1.

By using 16S rDNA and tuf sequencing, 70.4% and 93% of the CoNS strains were correctly identified, respectively. When the first hit with the MicroSeqID database was selected as true finding (disregarding analysis rules) than 137 out of 142 strains were correctly identified (96.5%). Application of the more strict CLSI guidelines (23) will lead to even more non-informative results (Staphylococcus spp.) and thus incorrect identifications with 16S sequencing in this study. Whole 16S gene sequencing will improve the Staphylococcus spp. differentiation ability of this specific gene, but, will also increase the number of required sequence reactions and consequently slightly increases costs and in some cases time-to-results. Becker et al.,(2) showed that by using the quality controlled Ribosomal Sequence Database (RIDOM) identification of staphylococci improved from 83.6% to 98.2% compared to the NCBI database. By using the RIDOM database results obtained in this study might positively change as well. However, tuf gene sequencing resulted in more informative data (species name) and was also in concordance with MALDI-TOF MS results. Although, all S.warneri strains (n=10) resulted in incorrect identification with tuf gene sequencing, due to high homology to S.pasteuri, extensive analysis of S.pasteuri and S.warneri strains might
solves the current problems. Therefore, the *tuf* gene seems more suitable for identification of staphylococci, as described by others (5, 9).

MALDI-TOF MS data obtained in this evaluation indicates superiority in identification of CoNS, 99.3% of the strains were correctly identified. Strains were spotted in duplicate and resulted in the same identification (strain name). The database (V3 1.1.0_3476-3740) used for this study contained the following number of reference spectra per tested CoNS species: *S. capitis* (6), *S. caprae* (1), *S. cohnii* (5), *S. epidermidis* (9), *S. haemolyticus* (8), *S. hominis* (6), *S. lentus* (2), *S. lugdunensis* (5), *S. pasteurii* (2), *S. pettenkoferi* (4), *S. saprophyticus* (8), *S. schleiferi* (6), *S. sciuri* (4), *S. simulans* (5), and *S. warneri* (4). For *S. caprae* only 1 reference spectrum is available, duplicate measurement resulted in *S. caprae* (hit 1, score 1.853). The second hit for this CoNS species was twice *S. epidermidis* (<1.5). To be able to use the proposed result acceptance criteria for MALDI-TOF MS, the number of reference spectra per CoNS strain ideally need to be ≥2. However, as hit 2 provided an unreliable score for *S. epidermidis* we considered the *S. caprae* result as a correct identification. On average there was a 4.82% difference in score value between hit 1 and 2. The combination of MALDI-TOF with *tuf* gene sequencing resulted in a 100% correct identification rate.

The 25 reference strains used in this study showed that different misidentifications occurred with the investigated methods. With the 16S sequencing protocol 9/25 reference strains could not be identified to the species level (36%). For the Vitek2, ID32 Staph, and *tuf* sequencing 2/25 reference strains were misidentified (8%). MALDI-TOF MS resulted in correct identification of all reference strains. MALDI-TOF MS has been investigated by others in relation to *Staphylococcus spp* identification directly from agar plates (3, 6, 7, 20) and results vary between 74.2%-99.3% correct identification rates. Data obtained with this study are comparable with data from Spanu *et al.* (20) and Dubois *et al.* (6), in which a sensitivity of 99.3% was found. In this study, only one *S. hominis* strain could not be identified with MALDI-TOF MS (table 1), for unknown reasons.

Sequencing of the partial 16S gene resulted in most clinical relevant misidentifications, as both *S. lugdunensis* and *S. saprophyticus* were not correctly identified.

In conclusion, this is one of the most extensive comparative studies for CoNS identification. The results obtained in this study demonstrate the good performance of MALDI-TOF MS for identification of CoNS, a correct identification rate of 99.3% was achieved. *Tuf* gene sequencing is the most suitable substitute for MALDI-TOF MS. When cheap, fast and accurate identification of CoNS is needed, the usage of MALDI-TOF MS is the method of choice and when necessary the *tuf* gene can be sequenced. As an alternative method, due
to the fact that not every diagnostic microbiology laboratory has access to a MALDI-TOF MS system, Vitek2 combined with tuf sequencing are suggested as an accurate approach for CoNS differentiation.

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Conflict of interest

The authors declare that they have no conflict of interest.
155 References


Table 1. Summary of performance of used differentiation methods for CoNS.

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<th>True ID</th>
<th>ID 32 Staph Correct</th>
<th>ID 32 Staph Incorrect ND</th>
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<th>16S sequencing Correct</th>
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<th>TUF sequencing Correct</th>
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Result acceptance criteria: ID 32 Staph when a score of ≥70% was obtained; Vitek2 when a score of ≥70% was obtained (in-house validation); TUF sequencing when the provided species name by BLAST showed ≥99% sequence match (identity) and at least 1 nucleotide mismatch with hit 2; 16S sequencing when the first hit showed ≥99% sequence match (identity) and the second hit had at least 1 nucleotide mismatch (MicroSeq software); MALDI-TOF MS hits ≥1.7, however, <2 were considered reliable to the species level if database match number 1 and 2 resulted in the same strain name, ideally at least ≥2 reference spectra need to be available in the database, and scores ≥2 were considered reliable to the species level.