Identification of clinical coagulase-negative staphylococci, isolated in microbiology laboratories, by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and two automated systems

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Abstract

A study was performed to compare matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS), linked to a recently engineered microbial identification database, and two rapid identification (ID) automated systems, BD Phoenix (Becton Dickinson Diagnostic Systems, France) and VITEK-2 (bioMérieux, Marcy L'Etoile, France), for the ID of coagulase-negative staphylococci (CoNS). Two hundred and thirty-four clinical isolates of CoNS representing 20 species were analyzed. All CoNS isolates were characterized by *sodA* gene sequencing, allowing interpretation of the ID results obtained using the respective database of each apparatus. Overall correct ID results were obtained in 93.2%, 75.6% and 75.2% of the cases with the MALDI-TOF-MS, Phoenix and VITEK-2 systems, respectively. Mis-ID and absence of results occurred in 1.7% and 5.1% of the cases with MALDI-TOF-MS, in 23.1% and 1.3% with the Phoenix, and in 13.7% and 0.9% with the VITEK-2 systems, respectively. In addition, with the latter automate, 10.3% of the IDs were proposed with remote possibility. When excluding the CoNS species not included in the databases of at least one of the three systems, the final percentage of correct results, Mis-ID and absence of ID were 97.4%, 1.3% and 1.3% with MALDI-TOF-MS, 79%, 21% and 0% with the Phoenix, and 78.6%, 10.3% and 0.9% with the VITEK-2 system, respectively. The present study demonstrates the robustness and high sensitivity of our microbial identification database used with MALDI-TOF-MS technology. This approach represents a powerful tool for the fast ID of clinical CoNS isolates.

Keywords: Bacterial identification, coagulase-negative staphylococci, MALDI-TOF-MS, Phoenix, VITEK
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Introduction

Coagulase-negative staphylococci (CoNS) are among the most frequently isolated bacteria in clinical microbiology laboratories. CoNS species are the most commonly reported strains isolated from the majority of nosocomial bloodstream infections [1]. They are responsible for bacteraemia, endocarditis, mediastinitis, meningitis and progressive joint destruction [2–4]. CoNS are commensals of the skin, their virulence is low in healthy populations and they have long been considered to be contaminants. Most staphylococci isolated from humans belong to the *Staphylococci epidermidis* group or to the *Staphylococci saprophyticus* group. The first group includes the species: *S. epidermidis*, *Staphylococci capitis*, *Staphylococci hominis*, *Staphylococci haemolyticus*, *Staphylococci warneri*, *Staphylococci caprae*, *Staphylococci saccharolyticus*, *Staphylococci pasteuri* and *Staphylococci lugdunensis* [3]. It should be noted that some species (i.e. *S. haemolyticus*, *S. lugdunensis* and *Staphylococci schleiferi*) are more often isolated from severe human infections such as native valve endocarditis [4].

The increasing importance of CoNS in hospital-acquired infections emphasizes the need for an accurate identification of staphylococci at the species level. Bacterial identification is usually achieved using phenotype-based techniques with systems such as rapid identification (ID) automates, which detect more than 50 phenotypic characters. However, most commercial identification kits and automated systems remain unable to differentiate between several species of CoNS [5–7]. Molecular methods targeting the *sodA* or the *tuf* genes are currently favoured for diagnostic purposes. However, they can be time-consuming and often are expensive [8,9]. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been used to characterize bacteria [10–14]. MALDI-TOF-MS can examine the pattern of proteins detected directly from intact bacteria. For a given bacterial strain, MALDI-TOF-MS yields a reproducible spectrum within minutes, consisting of a series of peaks corresponding to mass-to-charge (*m*/*z*) ratios of ions released from bacterial proteins during laser desorption.

Recently, we engineered a database to identify bacteria belonging to the *Micrococcaceae* family [15]. In the present study, using this database, MS was evaluated for its ability to identify CoNS at the species level compared to two commercial automated systems. All strains were identified by sequencing the *sodA* gene. The data obtained demonstrate the superiority of the identification obtained by MS compared to that obtained using both the Phoenix Automated Microbiology (BD Diagnostic Systems, Le Pont de Claix, France) and the VITEK-2 (bioMérieux, Marcy L'Etoile, France) systems.

Materials and Methods

Study design

Mass spectrometry (MALDI-TOF-MS) and two commercial microbiological identification systems were evaluated and compared for their ability to identify CoNS isolated from clinical samples. The automated systems tested were the Phoenix Automated Microbiology system and the VITEK-2 system. The two automated systems are used daily in the microbiology laboratories at Raymond Poincaré hospital and Ambroise Paré hospital, respectively. The spectrometer used for the analysis at Necker-Enfants Malades hospital in routine practice was a MALDI-TOF-MS Autoflex with the flex control software (Bruker Daltonics, Bremen, Germany). All CoNS isolates were identified at the species level by molecular methods. It should be noted that the data were obtained independently and were compared only at the end of the study.

Bacterial isolates

A total of 234 CoNS isolates representative of 20 species were studied (Table 1). All isolates were recovered from cultures performed routinely in two clinical microbiology laboratories (Raymond Poincaré and Ambroise Paré hospitals).

Molecular identification

The identification of the CoNS isolates at the species level was obtained by sequencing an internal fragment of the *sodA* gene as previously described [8,9]. The partial *sodA* gene was amplified and the PCR product sequenced as previously described [9] using primers d1 (5′-CCITAYICITAYGAYGC IYTIGARCC-3′) and d2 (5′-ARRTARTAIGCRTGYTCCCAI ACRTC-3′). The nucleotide sequences were analyzed using a local database of $sodA_{int}$ sequences of *Staphylococcus* type strains and the GenBank database.

VITEK-2 system

The method used was described previously [16–18]. Frozen strains were grown on trypticase soy agar supplemented with 5% sheep blood agar (bioMérieux) for 18–24 h at 37°C and subcultured on a new agar plate for an additional 18–24 h at 37°C before testing. Bacterial suspensions were prepared extemporaneously by suspending bacterial isolates in 0.45% saline to the equivalent of a 0.5–0.63 McFarland turbidity standard with the VITEK-2 Densichek instrument. The ID-GP identification card is a 64-well plastic card that includes 43 tests. Data were analyzed using VITEK-2 database, version 4.03. The *Staphylococcus* species and subspecies that can be identified with the VITEK-2 system are listed in Table 2.

Phoenix system

The method used was described previously [19]. Briefly, bacterial isolates were subcultured on trypticase soy agar supplemented with 5% sheep blood agar (bioMérieux) as described above. The Phoenix ID broth was inoculated with several bacterial colonies from a pure culture and adjusted to a 0.5–0.6 McFarland standard using a Crystal Spec Nephelometer (Becton Dickinson Diagnostic Systems). The ID broth suspension was then poured into the ID side of the Combo panel (PMIC/ID 13 panel for Gram positive cocci). The *Staphylococcus* species or subspecies that can be identified with Phoenix system are listed in Table 2.

MALDI-TOF-MS

The method used was described previously [15,20]. The strains were grown on Mueller–Hinton agar and incubated for 24 h at 37°C. An isolated colony was harvested in 10 μL of sterile water. One microlitre of this mixture was deposited on a target plate in two replicates and allowed to dry at