Evaluation of Sensititre Plates for Identification of Clinically Relevant Coagulase-Negative Staphylococci[▽]

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Coagulase-negative staphylococcus isolates were identified using Sensititre GPID plates and API strips ($n = 156$). For selected isolates, partial sequencing of the 16S rRNA, *sodA*, and *tuf* genes was performed. The Sensititre plates correctly identified 68.9% of isolates, with a concordance of 86% for *Staphylococcus haemolyticus* and 73% for *Staphylococcus epidermidis*.

Infections caused by coagulase-negative staphylococci (CoNS) are increasing (2–4, 6, 8, 12, 14). None of the commercially available options for the identification of staphylococcal species is 100% accurate for all CoNS (1, 5, 7, 9). Sensititre identification GPID plates (TREK Diagnostic Systems Inc., Cleveland, OH) identify the most common staphylococci and include in its taxa *Staphylococcus aureus*, *Staphylococcus capitis* subsp. *capitis*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *hominis*, *Staphylococcus lugdunensis*, *Staphylococcus saprophyticus* subsp. *saprophyticus*, and *Staphylococcus warneri*. Our objective was to evaluate the Sensititre system for the identification of CoNS species for routine diagnostic use.

Clinically relevant CoNS isolates ($n = 156$) were collected from May 2006 to May 2009 from the Hospital Civil de Guadalajara and the Hospital Universitario in Monterrey. Only one isolate per patient was included. Pure bacterial isolates were stored in *Brucella* broth containing 15% glycerol at -70°C .

All isolates were identified using both Sensititre GPID plates and API Staph strips following manufacturer's instructions. Sensititre plates were read with a Sensititre AutoReader, and API STAPH V4.1 identification software was used for the API strips. For both phenotypic methods, when the result was inconclusive, manual tests recommended by the manufacturer were performed to resolve the identity of the test isolate. If no satisfactory identification was obtained, the identification was repeated.

For all isolates with discordant results between the two phenotypic methods and for randomly selected clinical isolates with the same result from both phenotypic methods, partial sequencing of the 16S rRNA gene was performed. For these

isolates, 3 to 5 colonies were suspended in sterile water, and the suspension was boiled for 5 min and then centrifuged; the supernatant was used as the DNA template for PCR assays. PCR was performed as described previously (10), and PCR products were purified using the commercial Wizard PCR Preps DNA purification system (Promega, Madison, WI). Sequencing of the $\sim 1,200$ -bp products was performed in both directions at the Instituto de Biotecnología, Universidad Nacional Autónoma de México. When no definitive identification could be made based on 16S rRNA analysis, partial sequencing of the *sodA* and *tuf* genes was performed as described previously (5, 11).

DNA sequence fragments were compared to NCBI GenBank sequence entries using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>). Homology values above 97% were considered reliable (13). If more than one species fulfilled this criterion, definitive species identification was archived based on the species with the higher percentage homology.

The quality control strains *S. haemolyticus* ATCC 29970, *S. capitis* ATCC 35661, *Staphylococcus xylosus* ATCC 700404, *S. lugdunensis* ATCC 49576, *S. hominis* ATCC 27844, *S. saprophyticus* ATCC 15305, *S. epidermidis* ATCC 14990, *Staphylococcus sciuri* ATCC 29060, and *Staphylococcus intermedius* ATCC 29663 were tested with both the API strips and the Sensititre plates.

The quality control strains were correctly identified by both phenotypic methods for *S. saprophyticus*, *S. epidermidis*, *S. haemolyticus*, *S. capitis*, and *S. hominis*. The API strips correctly identified *S. sciuri*, *S. xylosus*, and *S. lugdunensis*, but the Sensititre system did not identify any of these species. *S. intermedius* was not identified by either of the two methods.

Ninety-six (62%) isolates showed agreement in the phenotypic test results (Table 1), and identification was confirmed by partial sequencing of the 16S rRNA gene in 32/36 (89%) isolates.

For isolates with discordant results by both phenotypic methods, partial sequencing of the 16S rRNA confirmed the identification with the API strips in 40/60 isolates (67%) and

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TABLE 1. Identification results for CoNS isolates

Agreement	No. of isolates	Identification according to:					Definitive identification
		API strips	Sensititre plates	16S rRNA	<i>sodA</i>	<i>tuf</i>	
API and Sensititre	44	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND ^b	ND	ND	<i>S. epidermidis</i>
	15	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	ND	ND	ND	<i>S. haemolyticus</i>
	1	<i>S. saprophyticus</i>	<i>S. saprophyticus</i>	ND	ND	ND	<i>S. saprophyticus</i>
API, Sensititre, and 16S rRNA	14	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	ND	ND	<i>S. haemolyticus</i>
	13	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	4	<i>S. hominis</i>	<i>S. hominis</i>	<i>S. hominis</i>	ND	ND	<i>S. hominis</i>
	1	<i>S. capitis</i>	<i>S. capitis</i>	<i>S. capitis</i>	ND	ND	<i>S. capitis</i>
API and 16S rRNA	4	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	4	<i>S. epidermidis</i>	<i>S. hominis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	3	<i>S. epidermidis</i>	<i>S. warneri</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. epidermidis</i>	None	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. epidermidis</i>	<i>S. capitis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. epidermidis</i>	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	5	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	ND	ND	<i>S. haemolyticus</i>
	1	<i>S. haemolyticus</i>	<i>S. capitis</i>	<i>S. haemolyticus</i>	ND	ND	<i>S. haemolyticus</i>
	1	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. haemolyticus</i>	ND	ND	<i>S. haemolyticus</i>
	1	<i>S. haemolyticus</i>	<i>S. warneri</i>	<i>S. haemolyticus</i>	ND	ND	<i>S. haemolyticus</i>
	1	<i>S. hominis</i>	<i>S. warneri</i>	<i>S. hominis</i>	ND	ND	<i>S. hominis</i>
	1	<i>S. saprophyticus</i>	None	<i>S. saprophyticus</i>	ND	ND	<i>S. saprophyticus</i>
	3	<i>S. sciuri</i>	<i>S. epidermidis</i>	<i>S. sciuri</i>	ND	ND	<i>S. sciuri</i>
	2	<i>S. sciuri</i>	None	<i>S. sciuri</i>	ND	ND	<i>S. sciuri</i>
	11	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. cohnii</i>	ND	ND	<i>S. cohnii</i>
Sensititre and 16S rRNA	2	<i>S. lugdunensis</i>	<i>S. hominis</i>	<i>S. hominis</i>	ND	ND	<i>S. hominis</i>
	1	<i>S. lugdunensis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. xylosum</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. chromogenes</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	ND	ND	<i>S. epidermidis</i>
	1	<i>S. hominis</i>	<i>S. epidermidis</i>	<i>S. hominis</i>	ND	ND	<i>S. hominis</i>
	1	<i>S. epidermidis</i>	<i>S. hominis</i>	<i>S. hominis</i>	ND	ND	<i>S. hominis</i>
16S rRNA, <i>sodA</i> , and <i>tuf</i>	1	<i>Kocuria varians</i>	None	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>
	1	<i>Kocuria varians</i>	<i>S. epidermidis</i>	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>	<i>S. pettenkoferi</i>
	1	<i>S. warneri</i> ^a	<i>S. haemolyticus</i>	<i>S. warneri</i>	<i>S. warneri</i>	<i>S. warneri</i>	<i>S. warneri</i>
	1	<i>S. warneri</i> ^a	None	<i>S. warneri</i>	<i>S. warneri</i>	<i>S. warneri</i>	<i>S. warneri</i>
	1	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>
	1	<i>S. lentus</i>	<i>S. hominis</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>
	1	<i>S. sciuri</i>	None	<i>S. cohnii</i>	<i>S. cohnii</i>	<i>S. cohnii</i>	<i>S. cohnii</i>
	1	<i>S. chromogenes</i>	<i>S. warneri</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>
	1	Non-identified	None	<i>S. gallinarum</i>	<i>S. gallinarum</i>	<i>S. gallinarum</i>	<i>S. gallinarum</i>
	1	<i>S. hominis</i>	<i>S. warneri</i>	<i>S. pasteurii</i>	<i>S. pasteurii</i>	<i>S. pasteurii</i>	<i>S. pasteurii</i>
API, Sensititre, <i>tuf</i> , and <i>sodA</i>	1	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. cohnii</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>
	1	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>
	1	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. cohnii</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>
API, <i>tuf</i> , and <i>sodA</i>	1	<i>S. haemolyticus</i>	<i>S. epidermidis</i>	<i>S. sciuri</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>	<i>S. haemolyticus</i>
	1	<i>S. cohnii</i>	<i>S. warneri</i>	<i>S. saprophyticus</i>	<i>S. cohnii</i>	<i>S. cohnii</i>	<i>S. cohnii</i>
	1	<i>S. epidermidis</i>	<i>S. capitis</i>	<i>S. hominis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>

^a Identification was considered unacceptable (85%). After sequencing of all three genes, molecular identification confirmed the API result.

^b ND, not determined.

with the Sensititre plates in 8/60 isolates (13%). Identification with the 16S rRNA gene was confirmed for 10 isolates out of 16 sequenced for the *tuf* and *sodA* gene.

The percentage of correct results for all isolates was recorded. The overall percentage of isolates correctly identified by Sensititre was 68.9% (102/148), and that correctly identified by the API strips was 91% (141/155) (Table 2).

Sensititre panels are widely used for antimicrobial susceptibility assays, and some laboratories may prefer the use of a single type of panel for both identification and susceptibility

testing. This allows data to be collected using a single reader and might also have administrative advantages. However, there are some limitations of the Sensititre identification plates: the plates cannot be read manually, they allow identification only of Gram-positive taxa in the Sensititre database, which does not include species such as *S. cohnii* and *S. sciuri* (8.5% and 3.8%, respectively, in this population), and they display low reliability for some species, as demonstrated by this study.

The overall performance of the molecular identification by the partial sequencing of the 16S rRNA gene proved to be

TABLE 2. Concordance of Sensititre plates and API Staph strips

Species ID ^a	Sensititre plates				API strips			
	No. of isolates			Concordance (%) ^c	No. of isolates			Concordance (%) ^c
	Total	With correct ID	With incorrect ID		Total	With correct ID	With incorrect ID	
<i>S. epidermidis</i>	86	63	23	73	76	74	2	97
<i>S. haemolyticus</i>	35	30	5	86	39	39	0	100
<i>S. hominis</i>	13	7	6	54	7	6	1	86
<i>S. warneri</i>	8	0	8	0	2	2	0	100
<i>S. saprophyticus</i>	2	1	1	50	2	2	0	100
<i>S. capitis</i>	4	1	3	25	1	1	0	100
<i>S. cohnii</i> ^b	0				13	12	1	92
<i>S. sciuri</i> ^b	0				6	5	1	83
<i>S. lentus</i> ^b	0				1	0	1	0
<i>S. lugdunensis</i> ^b	0				3	0	3	0
<i>S. xylosum</i> ^b	0				1	0	1	0
<i>S. chromogenes</i> ^b	0				2	0	2	0
<i>Kocuria varians</i> ^b	0				2	0	2	0
Total identified	148	102	46		155	141	14	
Total not identified	8				1			

^a ID, identification.^b Not in Sensititre database.^c Concordance of API and Sensititre results was determined by assigning a definitive identification based on molecular methods (16S rRNA, *sodA*, and *tuf* genes).

quite good, with only 6 misidentifications out of 96 (94%). Misidentification using this strategy has also been reported by Heikens et al. (5) and Brigante et al. (1). An explanation for these misidentifications may be that the deposited sequences in GenBank are incorrectly assigned to the various *Staphylococcus* species, which has been suggested by Heikens et al. (5). For the sequencing of 16S rRNA, it is accepted that homology values above 97.4% are reliable if a 1.45-kb fragment is sequenced (13). In this study, we sequenced only ~1,200 bp, and we decided to use a 97% cutoff value.

In this study, the *sodA* and *tuf* genes were effective for the identification of CoNS with the advantage of requiring a small fragment of DNA sequence for effective discrimination (236 bp for *sodA* and 412 bp for *tuf*) (5, 11).

In only 10 isolates, the API strips gave a result discordant with the decisive molecular data, and among these isolates were *Staphylococcus pasteuri*, *Staphylococcus gallinarum*, and *Staphylococcus pettenkoferi*, which are not in the database of the API system, explaining the misidentification.

In conclusion, the Sensititre system provided moderate reliability for the identification of the most commonly identified CoNS, but reliability notably decreased for other less commonly encountered species.

Nucleotide sequence accession numbers. Partial sequences of the 16S rRNA genes of *S. pettenkoferi*, *S. gallinarum*, and *S. pasteuri* were deposited in GenBank under accession numbers GQ145596 and GQ979971 (*S. pettenkoferi*) and GQ979967 (*S. gallinarum*) and GQ979969 (*S. pasteuri*).

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