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NOTES

Evaluation of the Vitek 2 System with a Variety of *Staphylococcus* Species[▽]

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The Vitek 2 gram-positive (GP) card was compared with an oligonucleotide array approach for the identification of 190 *Staphylococcus* strains, including 35 species, isolated from clinical and environmental specimens. The GP card provided a rapid and reliable identification of most species, whatever their origin.

Staphylococci are widespread in nature and can be isolated from humans and animals and from various food products (4, 5, 11, 13, 15). Some staphylococcal strains are associated with diseases in humans or animals, and others are used for technological purposes (4, 14, 15). *Staphylococcus aureus* is a well documented pathogen. Of the non-*S. aureus* staphylococci, *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus* are those most frequently implicated in disease in humans (4, 9, 15). Other staphylococcal species, such as *S. hominis* and *S. warneri*, can be found as contaminants of blood cultures but can also be associated with a variety of infections. In food, particularly in fermented meat products, staphylococci such as *S. xylosus*, *S. carnosus*, *S. simulans*, *S. warneri*, *S. epidermidis*, and *S. saprophyticus* may be found (1). *S. xylosus* and *S. carnosus* strains are used as starter cultures in fermented meat products because they contribute to their color and flavor (14). However, the presence of *S. aureus* in food is a potential public health hazard, since many strains of *S. aureus* produce enterotoxins (7, 10). Accurate identification of the *Staphylococcus* species is therefore of great importance in microbiological laboratories.

The Vitek 2 system used with the gram-positive (GP) identification card (bioMérieux, Marcy l'Etoile, France) is an automated machine designed to provide rapid and accurate phenotypic identifications for most clinical staphylococci (2, 6, 8, 16). The colorimetric GP card contains 43 tests. The database of the GP card particularly includes the environmental species *S. caprae*, *S. carnosus*, *S. equorum*, *S. gallinarum*, and *S. vitulinus*. However, there has been no documented assessment of this identification card for any collection of *Staphylococcus* strains, including environmental isolates. The purpose of this study was to assess the ability of the Vitek 2 GP card to identify *Staphylococcus* species of clinical and environmental origins.

A total of 190 *Staphylococcus* strains, including 38 type strains representing 35 species (Table 1) and 152 strains from our collection, were studied (Table 2). The latter 152 strains were isolated from clinical samples ($n = 60$) and from food and plant samples ($n = 92$). The GP card was used in accordance

TABLE 1. Identifications obtained with the GP card for 38 *Staphylococcus* reference strains

Species	Strain	Vitek 2 GP card identification
S. arlettae	CIP 103501T	*S. arlettae*
S. aureus subsp. *aureus*	CIP 65.8T	*S. aureus*
S. auricularis	DSM 20609T	*S. hominis*/*S. auricularis*
S. capitis subsp. *capitis*	CIP 81.53T	*S. equorum*
S. caprae	DSM 20608T	*S. caprae*
S. carnosus	DSM 20501T	*S. carnosus* subsp. *carnosus*
S. chromogenes	CIP 81.59T	*S. chromogenes*
S. cohnii subsp. *cohnii*	DSM 20260T	*S. cohnii* subsp. *cohnii*
S. cohnii subsp. *urealyticus*	CIP104024T	*S. cohnii* subsp. *urealyticus*
S. condimenti[a]	CIP 105760T	*S. carnosus* subsp. *carnosus*
S. delphini[a]	CIP 103732T	*S. intermedius*
S. epidermidis	DSM 20044T	*S. epidermidis*
S. equorum subsp. *equorum*	DSM 20674T	*S. xylosus*
S. equorum subsp. *linens*	CIP 107656T	*S. equorum*
S. felis[a]	ATCC 49168T	*S. chromogenes*
S. fleurettii[a]	CIP 106114T	*S. vitulinus*
S. gallinarum	CIP 103504T	*S. gallinarum*
S. haemolyticus	CIP 81.56T	*S. warneri*/*S. pasteuri*
S. hominis subsp. *hominis*	CIP 81.57T	*S. hominis*
S. hyicus	DSM 20459T	*S. hyicus*
S. intermedius	ATCC 29663T	*S. intermedius*
S. kloosii	DSM 20676T	*S. kloosii*
S. lentus	CIP 81.63T	*S. lentus*
S. lugdunensis	DSM 4804T	*S. lugdunensis*
S. lutrae[a]	CIP 105399T	*S. chromogenes*
S. muscae[a]	DSM 7068T	*S. warneri*
S. nepalensis[a]	CIP 108211T	*S. xylosus*
S. pasteuri[a]	CIP 103540T	*S. warneri*/*S. pasteuri*/*S. haemolyticus*
S. piscifermentans[a]	CIP 103958T	*S. hominis*
S. saprophyticus subsp. *saprophyticus*	CIP 76.125T	*S. hominis*/*S. saprophyticus*
S. schleiferi subsp. *schleiferi*	DSM 4807T	*S. schleiferi*
S. sciuri subsp. *sciuri*	CIP 81.62T	*S. sciuri*
S. simulans	DSM 20322T	*S. simulans*
S. succinus subsp. *succinus*[a]	CIP 107307T	*S. xylosus*
S. succinus subsp. *sasei*[a]	CIP 107658T	*S. xylosus*
S. vitulinus	CIP 104850T	*S. sciuri*
S. warneri	DSM 20316T	*S. warneri*
S. xylosus	DSM 20266T	*S. xylosus*

[a] Absent from the GP card database.

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TABLE 2. GP card identification of staphylococci in the laboratory collection

Species	No. of isolates							Present in GP card database
	Total	Clinical	Environmental	Identified as one choice	Identified with low discrimination	Misidentified	Not identified	
S. arlettae	1	0	1	1	0	0	0	Yes
S. aureus	6	5	1	6	0	0	0	Yes
S. capitis	7	5	2	6	0	0	1[a]	Yes
S. caprae	1	1	0	1	0	0	0	Yes
S. carnosus	7	0	7	5	0	2	0	Yes
S. cohnii spp ureal.	4	2	2	4	0	0	0	Yes
S. epidermidis	20	12	8	20	0	0	0	Yes
S. equorum	13	0	13	0	2	11	0	Yes
S. haemolyticus	6	5	1	6	0	0	0	Yes
S. hominis	6	5	1	6	0	0	0	Yes
S. lugdunensis	5	5	0	5	0	0	0	Yes
S. saprophyticus	16	6	10	14	1[a]	1[a]	0	Yes
S. schleiferi	2	2	0	2	0	0	0	Yes
S. sciuri	3	1	2	3	0	0	0	Yes
S. similans	4	4	0	2	1	1	0	Yes
S. vitulinus	5	0	5	4	0	1	0	Yes
S. warneri	12	6	6	11	1[a]	0	0	Yes
S. xylosus	14	0	14	14	0	0	0	Yes
Subtotal	132	59	73	110	5	16	1	
S. delphini	1	1	0	0	0	1	0	No
S. fleurettii	2	0	2	0	0	1	1	No
S. pasteuri	8	0	8	0	8	0	0	No
S. succinus	9	0	9	0	0	8	1	No
Subtotal	20	1	19	0	8	10	2	
Total	152	60	92	110	13	26	3	

[a] Isolate of clinical origin.

with the recommendations of the manufacturer. The results were compared with those of the oligonucleotide "Staph array," a method, which is based on the hybridization of the internal part of the *sodA* gene (3). The strain was retested with the GP card if the identification results differed between the two methods. In case of a persistent discrepancy, the Staph array identifications were confirmed by DNA sequencing of the *sodA* gene (12). Results from the GP card were separated into four groups: (i) the "one-choice identification" group corresponded to identical identifications for the GP card and Staph array; (ii) the "low level of discrimination" group corresponded to results for which the GP card indicated the possibility of two or three species, including the result of Staph array and proposed supplementary tests (pigmentation, hemolysis, or novobiocin resistance) to determine the correct identification; (iii) the "misidentification" group corresponded to different identifications for the GP card and Staph array; and (iv) the "no identification" group corresponded to strains for which the GP card gave no result. Correct identification was defined as the association of the first two groups.

Twenty-four reference strains out of 38 (63%) were correctly identified by the GP card (Table 1). Of the 14 misidentified reference strains, 10 were species not included in the database of the GP card. The four other strains were *S. capitis* subsp. *capitis*, identified as *S. equorum*; *S. equorum* subsp. *equorum*, identified as *S. xylosus*; *S. haemolyticus*, identified as *S. warneri*/*S. pasteuri*; and *S. vitulinus*, identified as *S. sciuri*. In a study by Layer et al. (6), 70% (20 out of 27) of reference strains were correctly identified. However, the misidentified strains belonged to different species.

Fifty-six of the 60 clinical strains (93.3%), belonging to 14 different species, were identified from the collection. Complementary tests were necessary for three strains. These results were in agreement with those reported by Layer et al. (6). Two other studies found higher identification accuracy (98.2 to 99.3%) (2, 16). In those studies, the numbers of taxa tested and their proportions were different; 66 and 74% of clinical strains belonged to the three species *S. aureus*, *S. epidermidis*, and *S. hominis*, versus only 37% in our study. Three strains belonging to the species *S. saprophyticus*, *S. simulans*, and *S. delphini* were misidentified, and one strain of *S. capitis* was not identified.

The GP card also correctly identified 67 of the 92 environmental strains (73%). Complementary tests were necessary for 10 strains. Twenty-three strains (25%), belonging to five species, were misidentified (Table 2). Nine misidentified strains belonged to *S. fleurettii* ($n = 1$) and *S. succinus* ($n = 8$), which were absent in the database of the GP card. Eleven of the 14 remaining misidentified strains were *S. equorum* strains, which were frequently reported as *S. xylosus* ($n = 10$). The last three misidentified strains belonged to *S. carnosus* and *S. vitulinus*. One strain each of *S. fleurettii* and *S. succinus* were not identified as expected. Except for *S. equorum* ($n = 13$) and *S. succinus* ($n = 9$), 65 of the 70 environmental strains were correctly identified (92.9%). The misidentifications of *S. equorum* as *S. xylosus* were suspected with the vibriostatic O129 test of the GP card. Indeed, all the strains identified as *S. xylosus*

TABLE 3. Routinely collected laboratory strains misidentified with the GP card

Staph array identification	Vitek 2 GP card result (no. of misidentified strains)
S. carnosus	*S. auricularis* (2)
S. delphini	*S. intermedius*
S. equorum	*S. xylosus* (10), *S. cohnii* (1)
S. fleurettii	*Aerococcus viridans*
S. saprophyticus	*Aerococcus viridans*/*S. hominis*
S. simulans	*S. haemolyticus*
S. succinus	*S. sciuri* (4), *S. xylosus* (4)
S. vitulinus	*S. sciuri*

which had a negative test with the vibriostatic compound were misidentified strains. According to our data, the identifications of *S. xylosus*, *S. sciuri*, and *S. auricularis* provided by the GP card required complementary tests for confirmation (Table 3).

In conclusion, the Vitek 2 GP card allowed the identification of 123 (93.2%; $n = 132$) *Staphylococcus* strains belonging to the species which are included in the database. The results obtained in this study highlight the interesting performance of the colorimetric Vitek 2 GP card, which can be used in clinical, agrochemical, and food laboratories. However, the GP card demonstrated low accuracy in identification of the species *S. equorum* and misidentified *S. succinus*, which is not included in the current Vitek 2 GP database.

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