

Identification of *Staphylococcus hyicus* with the API Staph Strip

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The API Staph Strip system (API System S. A., Montalieu-Vercieu, France) was compared with conventional methods for identification of *Staphylococcus hyicus* isolated from cases of exudative epidermitis in swine. The API Staph Strip was found to provide unique profile numbers, namely, 6-514-151, 6-514-153, and 6-516-153. These profile numbers are not listed in the API Staph Strip data base. It was found that the use of this miniaturized system is preferable to conventional methods for the identification of the causal agent of swine exudative epidermitis.

Exudative epidermitis, commonly referred to as greasy pig disease, is a dermatitis affecting young pigs (15). This syndrome was originally described by Sompolinsky (14), who found the organism to be a gram-positive coccus. Taxonomic problems followed, but the organism was eventually named *Staphylococcus hyicus* by Devriese et al. (4). These workers also described two subspecies: *Staphylococcus hyicus* subsp. *hyicus* and *Staphylococcus hyicus* subsp. *chromogenes*. More recently, *S. hyicus* has also been implicated as an agent of septic polyarthrits (12) in swine and from mange lesions in cattle (3).

Staphylococci have been characterized by use of the API 20E system (8). A more specific delineation of this group of organisms can be achieved by employing the API Staph Strip, which is a biochemical typing scheme based on the classification of Kloos and Schleifer (11). The API Staph Strip combines 19 biochemical tests. The results of these tests form a data base of 95 profile numbers which represent varying biochemical reactions for 10 species of staphylococci (1, 5, 6).

A mixed staphylococcal flora often occurs in the lesions of exudative epidermitis of swine (2). The purpose of the present study was to investigate the usefulness of the API Staph Strip in distinguishing between *S. hyicus* and other staphylococci in the mixed flora of these lesions.

MATERIALS AND METHODS

Cultures. Eleven reference cultures of *S. hyicus* were obtained from L. A. Devriese, University of Gent, Gent, Belgium, and nine cultures were obtained from W. E. Kloos, North Carolina State University, Raleigh. One isolate obtained from W. E. Phillips, Mississippi Veterinary Diagnostic Laboratory, Jackson, was also used in the present study. Ten isolates of *S. hyicus* were submitted from field cases of porcine

exudative epidermitis at the Murray State University Veterinary Diagnostic and Research Center. W. E. Kloos also supplied 10 cultures of *Staphylococcus intermedius*, 10 cultures of *Staphylococcus simulans*, and 5 cultures of *Staphylococcus aureus*. A sixth culture of *S. aureus* (ATCC 25923) was obtained from the American Type Culture Collection.

Conventional biochemical tests. The clinical isolates were confirmed as staphylococci by testing their bacteriolytic activities in TP2 base medium (16). The coagulase test (17) was performed in a tube containing 0.5 ml of rabbit plasma with EDTA (Difco Laboratories). Other conventional biochemical tests included hemolysis on 5% sheep blood agar; colony pigmentation; production of DNase (17), hyaluronidase (2), phosphatase (13), and acetyl methyl carbinol; and precipitation of polysorbate (2). Fermentation of mannitol, lactose, and maltose was tested by incorporating these carbohydrates into an oxidative-fermentative base as described by E. O. King (9). In addition, all isolates were tested on purple agar base plates supplemented with 1% maltose for aerobic acid production (12).

API Staph Strip. Following recommendations of the manufacturer, inoculum for the API Staph Strip gallery was a bacterial suspension adjusted to tube no. 2 on the McFarland scale. The gallery includes the following tests: fermentation of glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, xylitol, melibiose, raffinose, xylose, saccharose, alpha-methylglucoside, and *N*-acetylglucosamine; reduction of nitrate to nitrite; and production of acetyl methyl carbinol, alkaline phosphatase, arginine dehydratase, and urease. The strips were developed after 24 h of incubation at 37°C. Positive reactions were converted to a seven-digit profile number. These profiles were then compared with the index supplied by the manufacturer (API System S. A.; publication no. 2050) and with the computer data base for the API Staph Strip system.

RESULTS

Conventional biochemical tests. Strains of *S. hyicus* included in this study were variable in

TABLE 1. Percentage of biochemical reactions of 57 strains of staphylococcal spp.

Method used and biochemical test	% Reaction of following strain (no.):			
	<i>S. hyicus</i> (31)	<i>S. intermedius</i> (10)	<i>S. simulans</i> (10)	<i>S. aureus</i> (6)
API Staph Strip				
Glucose	100	100	100	100
Fructose	100	100	100	100
Mannose	100	100	90	100
Maltose	0	90	0	100
Lactose	90	90	100	100
Trehalose	100	90	70	100
Mannitol	0	90	70	100
Xylitol	0	0	0	0
Melibiose	0	0	0	0
Nitrate to nitrite	26	100	100	100
Alkaline phosphatase production	100	100	20	100
Acetyl methyl carbinol production	100	100	50	100
Raffinose	0	0	0	0
Xylose	0	0	0	0
Saccharose	100	100	100	100
Acid from alpha-methylglucoside	0	0	0	0
Acid from <i>N</i> -acetylglucosamine	100	100	90	100
Arginine dehydrolase	100	100	100	100
Urease	61	100	100	83
Conventional				
Coagulase	71	100	0	100
DNase	100	100	80	100
Acetyl methyl carbinol production	0	0	0	100
Hyaluronidase production	100	10	0	100
Acid from maltose ^a	0	90	0	100
Acid from mannitol ^a	0	80	100	100
Acid from lactose ^a	94	90	100	100
Phosphatase production	100	100	100	100
Precipitation zone on Tween 80	100	60	100	50
Acid, aerobically from maltose ^b	0	60	0	100
Bacteriolytic activity	100	100	100	100

^a In oxidative-fermentative medium.

^b In purple agar base plates.

their production of coagulase, pigment, and precipitation of polysorbate. All strains were non-hemolytic on 5% sheep blood agar. Results of biochemical tests are tabulated in Table 1.

API Staph Strip. All of the strains of *S. hyicus* included in this study fermented glucose, fructose, mannose, trehalose, saccharose, and *N*-acetylglucosamine, whereas maltose, mannitol, xylitol, melibiose, raffinose, xylose, and alpha-methylglucoside were not fermented (Table 1). The test for production of acetyl methyl carbinol required a full 10 min for development after addition of reagents; a very faint pink was interpreted as a positive result. All profile numbers found with the 31 strains of *S. hyicus* are shown in Table 2.

DISCUSSION

Diagnosis of porcine exudative dermatitis relies on the identification of *S. hyicus* from clinical samples. Over a 12-month period, only 10

clinical isolates of *S. hyicus* were recovered from swine submitted to our veterinary diagnostic laboratory. The infrequent need to identify *S. hyicus* to species level led to problems of logistics. Media to detect DNase and phosphatase production, polysorbate hydrolysis, and maltose fermentation must be prepared fresh each time a suspect *S. hyicus* strain is isolated. The test recommended by Devriese (2) for hyaluronidase detection requires a mucoid *Pasteurella multocida* or *Streptococcus equi* strain. However, after initial isolation from clinical material, the mucoid consistency of these organisms rapidly disappears by frequent subculturing. Therefore, this test may not be practical in a routine diagnostic setting.

Comparison of the API Staph Strip and conventional methods revealed only one possible discrepancy. By conventional testing, *S. hyicus* did not produce acetyl methyl carbinol. However, in the API Staph Strip system, a weak positive was detected for all strains. This dis-

TABLE 2. Observed API profiles of 31 strains of *S. hyicus*

Profile no.	No. (%) of strains
6-116-151	1 (3.2)
6-116-153 ^a	2 (6.5)
6-514-151	10 (32.3)
6-514-152	1 (3.2)
6-514-153	12 (38.7)
6-516-151	1 (3.2)
6-516-153	4 (12.9)

^a This profile number is identical to one of the 10 strains of *S. intermedius* tested.

crepancy was probably due to varying degrees of sensitivity of these two methods.

Findings in the present study indicate that the API Staph Strip can be used to distinguish between *S. hyicus* and other staphylococci, namely, *S. intermedius* (7) and *S. simulans* (10). These two *Staphylococcus* spp. produced significantly different reactions in both conventional tests and in the API Staph Strip system.

With the API Staph Strip, seven profile numbers were observed with 31 strains of *S. hyicus* (Table 2). These profile numbers are not listed in the API Staph Strip data base. To include the four less frequent profile numbers, namely, 6-116-151, 6-116-153, 6-514-152, and 6-516-151, as characteristic of *S. hyicus*, a greater number of isolates from different sources should be tested with the API Staph Strip.

Although this system has been used to characterize staphylococci of human origin (1, 6), it is not yet marketed in the United States. The present study suggests that the API Staph Strip could be extremely useful in identifying the causal agent of porcine exudative epidermitis.

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