JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2267–2269 Vol. 34, No. 9
0095-1137/96/$04.00+0

Comparison of Five Tests for Identification of *Staphylococcus aureus* from Clinical Samples

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Received 26 February 1996/Returned for modification 22 April 1996/Accepted 24 June 1996

Five different laboratory tests for the identification of *Staphylococcus aureus* were compared. Analyses of 271 presumptive *S. aureus* strains, supplemented with 59 well-defined methicillin-resistant *S. aureus* (MRSA) isolates, were performed. Only the Staphaurex Plus (Murex Diagnostics, Dartford, United Kingdom) and the Pastorex Staphplus (Sanofi, Marnes-La-Coquette, France) tests displayed 100% sensitivity. The observed difference with the free-coagulase test (Bacto coagulase plasma; Difco, Detroit, Mich.), a bound-coagulase (clumping factor) test, and the former Staphaurex test (Murex Diagnostics) was caused mainly by the inability of these three tests to identify some MRSA strains correctly. Among Polish MRSA isolates included in the analysis, a group of free-coagulase-negative *S. aureus* strains was detected. Genetic typing by random amplification of polymorphic DNA revealed that the strains showing aberrant behavior when the different test results were compared belonged to a limited number of *S. aureus* clones.

Staphylococcus aureus is one of the most frequently encountered pathogens in clinical specimens. To make the distinction between this species and other, less virulent, staphylococci it is of importance to have a reliable, fast, simple, and cheap identification test available. For many years the tube test, which detects the production of free coagulase, was considered the "gold standard" for this purpose (4). A more simple and widely used test is the detection of bound coagulase (clumping factor), for instance, by a slide agglutination reaction. This test, however, is less reliable and can produce both false-positive and false-negative results (4). In recent years, several commercial agglutination assays which are based on the detection of clumping factor and several other products specific for *S. aureus* (e.g., protein A) have become available. In a previous report it was shown that a significant number of clinical isolates of methicillin-resistant *S. aureus* (MRSA) gave negative results by one of these newer tests (Staphaurex; Murex Diagnostics Limited, Dartford, United Kingdom) (2). Therefore, the manufacturer made some modifications which were aimed at improving the test's sensitivity.

In this study we evaluated the former and current versions of Staphaurex and compared them with three other diagnostic tests. These assays are a free-coagulase test (Bacto coagulase plasma; Difco Laboratories, Detroit, Mich.), a bound-coagulase test, and the Pastorex Staphplus (Sanofi Diagnostics Pasteur, SA, Marnes-La-Coquette, France) test. Besides clinical specimens, an additional number of well-defined MRSA strains were included.

MATERIALS AND METHODS

***S. aureus* strains.** Consecutive clinical samples (n = 271) processed in the Department of Bacteriology of the University Hospital Rotterdam yielded colonies suspected to be *S. aureus* isolates. This observation was based on morphological criteria. In addition, 59 strains of MRSA were tested. These strains were derived from different patients in several European countries. A relatively large proportion of the MRSA strains came from Poland (n = 31; 52.5%). To determine methicillin susceptibility, a disk diffusion method on Mueller-Hinton agar (CM337; Oxoid, Amsterdam, The Netherlands) using a 5-μg methicillin disk (Oxoid) was used (1). Breakpoints were determined according to the criteria of the National Committee for Clinical Laboratory Standards (6). A total of 330 staphylococcal isolates were tested. In the end, the clinical samples yielded 241 *S. aureus* isolates derived from 158 patients. The number of isolates per patient ranged from 1 to 10.

Tests for identification of *S. aureus*. (i) Free-coagulase (tube) test. Bacto coagulase plasma (Difco) was used according to the manufacturer's instructions. Briefly, two to four colonies were suspended in 0.5 ml of coagulase plasma, mixed, and incubated at 37°C. The tubes were inspected for formation of a clot every hour until 4 h had passed and then were inspected after 24 h. Positive and negative control strains were included in every run.

(ii) Bound-coagulase (agar) test. For detection of clumping factor, an agar plate containing human fibrinogen was used (10). A colony of staphylococci was transferred from the original plate to the coagulase agar plate with a sterile loop. The plate was incubated for 18 h at 37°C and inspected immediately thereafter. If a zone of opaqueness in the agar surrounding the colony was visible the test was considered positive. In each run, positive and negative control strains were included.

(iii) Staphaurex and Staphaurex Plus (Murex Diagnostics Limited). Staphaurex is a latex agglutination test which detects clumping factor and staphylococcal protein A simultaneously. Staphaurex Plus is a newer version which has included the detection of antigens raised against difficult-to-detect MRSA strains (2). The procedures for both tests were identical except for the reaction time required, being 20 s for Staphaurex and 30 s for Staphaurex Plus. One drop of test latex was placed in one circle of the reaction card. The blunt head of a mixing stick was covered with bacteria, and these were emulsified in the drop of test latex. Then, the card was rocked slowly while agglutination was observed for. If agglutination occurred within the required reaction time, the same procedure was repeated with control latex to check for nonspecific agglutination. If this type of autoagglutination occurred, the result was considered noninterpretable.

(iv) Pastorex Staphplus (Sanofi). The Pastorex Staphplus test is based on the same principles as those of the Staphaurex tests, i.e., latex agglutination with detection of clumping factor, staphylococcal protein A, and capsular polysaccharides for the detection of certain MRSA strains (2). The test procedure was identical to the procedure of the Staphaurex test.

Additional testing of strains with discordant results. (i) Retesting. Strains with variable outcomes when the results of the different tests were compared were retested by all procedures mentioned previously and were subsequently studied further with the aid of the additional tests mentioned below. The Accuprobe assay and the coagulase gene PCR can be considered the gold standard; all strains ultimately tested were positively identified as *S. aureus* by both tests.

(ii) Free-coagulase tests. Three additional tube tests for detection of free coagulase were used: first, the Bacto coagulase EDTA (Difco) test; second, the Staphylocoagulase (Sanofi) test; and third, the rabbit plasma tube test developed in our laboratory. The procedure for each test was identical to that of the Bacto coagulase test described above.

(iii) Accuprobe (Gen-Probe Incorporated, San Diego, Calif.). The Accuprobe *S. aureus* culture identification test detects specific rRNA sequences that are unique to *S. aureus*. Therefore, it is considered to be independent from the production of coagulase, clumping factor, and other phenotypic characteristics. It uses a chemiluminescent DNA probe, and after hybridization a selection step eliminates any unhybridized probe. The procedure was performed according to the manufacturer's instructions. Briefly, a loopful of bacteria was suspended in 50

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TABLE 1. Survey of the results of the different *S. aureus* identification assays

Test	MSSA (n = 222)		MRSA (n = 78)		Total (n = 300)	
	No. of false-negative results	Test sensitivity (%)	No. of false-negative results	Test sensitivity (%)	No. of false-negative results	Test sensitivity (%)
Staphaurex Plus	0	100	0	100	0	100
Staphaurex	2	99.1	12	84.6	14	95.3
Pastorex Staphplus	0	100	0	100	0	100
Free coagulase	0	100	6	92.3	6	98.0
Bound coagulase	0	100	3	96.1	3	99.0

μl of lysis reagent and incubated for 5 min in a water bath at 37°C. Thereafter, 50 μl of hybridization mixture was added and incubated for 5 min in a water bath at 60°C. Finally, 300 μl of selection reagent was added and incubated for 5 min at 60°C in a water bath. The results were measured with a luminometer (Leader; Gen-Probe). A sample with a signal of equal to or more than 50,000 relative light units was considered positive. With a signal of less than 50,000 relative light units, the sample was considered negative.

(iv) Coagulase gene PCR. All discordant samples were also processed by PCR using a specific primer set to detect the coagulase gene as published previously (3). The PCR aims at the 3′ end of the coagulase gene by employing primers COAG2 and COAG3 (5′-CGAGACCAAGATTCAACAAG-3′ and 5′-AAAG AAAACCACTCACATCA-3′, respectively). PCR was performed in a mixture of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM (each) deoxynucleoside triphosphate, 50 pmol of the respective primers, and 0.2 U of *Tth* polymerase (Supertaq; HT Biotechnology, Cambridge, United Kingdom). The PCR machines were manufactured by Biomed (Theres, Germany). No postamplification restriction analysis was performed; the PCR was meant for detecting and not typing of the coagulase gene.

(v) Typing of *S. aureus* strains. To find out whether the discordant results were caused by a single subset of clonally related strains, typing was performed for all discordant strains and a random sample (n = 10) of strains showing concordant results. Typing was performed by arbitrarily primed PCR (AP-PCR) as described previously (5, 7–9). The buffer conditions were as described above, and the primers used were the same as those described in reference 5.

RESULTS

Table 1 shows the sensitivity of the various tests for methicillin-susceptible *S. aureus* (MSSA) and on MRSA. The sensitivity for MSSA was 100% for all but one test, Staphaurex (sensitivity, 99.1%). However, for MRSA only Staphaurex Plus and Pastorex Staphplus had a sensitivity of 100%. Again, Staphaurex had the lowest sensitivity, 84.6%.

Out of 330 staphylococcal isolates tested, 30 gave negative results in all tests and were considered not to be *S. aureus*. This included results by Accuprobe and coagulase PCR testing. Two hundred eighty gave positive results in all tests. The remaining 20 isolates (18 MRSA and 2 MSSA isolates [Table 2]) gave discordant results, meaning a negative result in one or two of the five identification assays. Strains 7 to 20 were negative in the Staphaurex assay, whereas strains 1 to 6 did not seem to produce free coagulase. Three of the latter strains did not produce bound coagulase as well. Interestingly, all strains appeared to contain the (silenced?) coagulase gene as determined by PCR, whereas also the Accuprobe assay identified the strains as *S. aureus*. The 20 strains mentioned in Table 2 were derived from 11 patients. Repeated analysis performed for these discordant isolates corroborated the initial data. The additional tests for detection of free coagulase were also negative on the six MRSA strains from Polish patients with negative outcomes by the Difco coagulase plasma test. All 20 discordant isolates gave positive results in the Accuprobe assay and the coagulase gene PCR. Therefore, these 20 isolates were still considered to be *S. aureus.*

TABLE 2. Diagnostic data obtained for discordant *S. aureus* strains

Strain no.	Patient no.	Test result[a]							AP-PCR pattern
		Staphaurex Plus	Staphaurex	Pastorex Staphplus	Free coagulase	Bound coagulase	Accuprobe	PCR for coagulase gene	
1	1	+	+	+	−	−	+	+	3
2	2	+	+	+	−	+	+	+	3
3	3	+	+	+	−	+	+	+	3
4	4	+	+	+	−	−	+	+	3
5	5	+	+	+	−	+	+	+	3
6	6	+	+	+	−	−	+	+	3
7	7	+	−	+	+	+	+	+	2
8	7	+	−	+	+	+	+	+	2
9	7	+	−	+	+	+	+	+	2
10	7	+	−	+	+	+	+	+	2
11	7	+	−	+	+	+	+	+	2
12	7	+	−	+	+	+	+	+	2
13	7	+	−	+	+	+	+	+	2
14	8	+	−	+	+	+	+	+	2
15	8	+	−	+	+	+	+	+	2
16	8	+	−	+	+	+	+	+	2
17	8	+	−	+	+	+	+	+	2
18	9	+	−	+	+	+	+	+	2
19	10	+	−	+	+	+	+	+	4
20	11	+	−	+	+	+	+	+	1

[a] +, positive; −, negative.

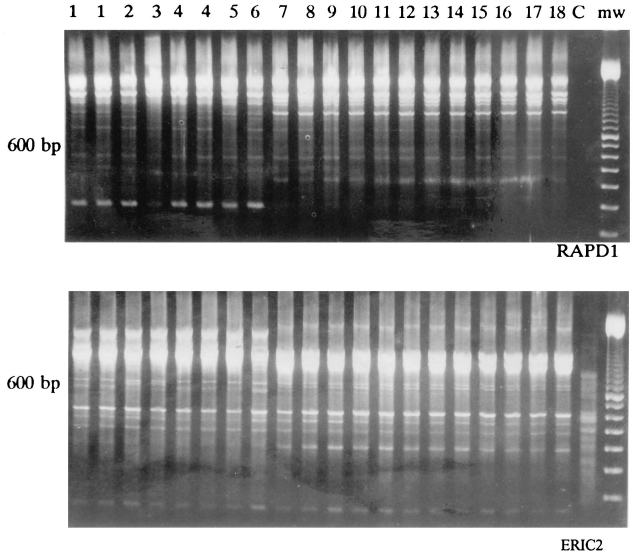

FIG. 1. Random amplification of polymorphic DNA analysis for the discordant *S. aureus* strains. Strain numbers are indicated above the lanes, and the positions of the molecular size markers (Pharmacia 100-bp ladder) are indicated (lane mw). The upper panel displays the results of application of the primer RAPD1; the lower panel summarizes the results of application of the primer ERIC2. For assessing reproducibility, multiple isolates of strains 1 and 4 were included. Strain numbering corresponds to that used in Table 2.

The results of AP-PCR revealed that the 20 discordant isolates from 11 patients derived from only four clones (Fig. 1 [shows examples of two of the clones] and Table 2). Ten randomly selected control strains showed 10 different patterns (results not shown; see also references 5, 7, 8, and 9). This proves the discriminatory power of the AP-PCR method used. Pattern 1 was found for only one patient with discordant results, pattern 2 was found for three patients involved in a small outbreak of MRSA in our hospital, and pattern 3 was found for six patients from Poland, probably involved in an outbreak of MRSA. Pattern 4 was an MSSA strain.

DISCUSSION

Both Staphaurex Plus and Pastorex Staphplus showed an optimal sensitivity for the identification of both MSSA and MRSA. The initial format of Staphaurex had the lowest sensitivity, especially with MRSA, as reported previously (2). A remarkable finding was the failure of the tests to detect free coagulase in six MRSA strains from Poland. Also, the test to detect clumping factor was negative in three of these six strains. This is worrisome, since detection of free coagulase is considered to be the gold standard (4). It should be noted that these six strains represented one clone. Both the PCR for the coagulase gene and the Accuprobe test were positive with these isolates. An explanation could be that the coagulase gene, though present, is not expressed or is insufficiently expressed in these strains. The specificity of all tests was 100%, although the number of non-*S. aureus* isolates (total, 30) is insufficient to draw definite conclusions with regard to the absolute specificity. The value of genetic typing methods in this kind of analysis is shown in Table 2. The 20 isolates with discordant results represented only four clonally related strains. In conclusion, Staphaurex Plus and Pastorex Staphplus have excellent sensitivity for identifying *S. aureus* in clinical samples, which makes them reliable screening tests in the clinical microbiology laboratory.

ACKNOWLEDGMENT

This study was financially supported by Murex Diagnostics Limited.

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