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Simplified Lyogroup System, a New Method for Routine Identification of Staphylococci: Description and Comparison with Three Other Methods

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A simplified system for routine identification of the six lyogroups of human staphylococci is described. The method is based on the determination of the lytic activity on five different test media and of the phosphatase activity of each isolate. A total of 689 staphylococci isolated from clinical material over a 6-month period were identified according to this simplified system. The same strains were identified in parallel using three other methods designed for routine identification of staphylococci: the Kloos and Schleifer abbreviated scheme (J. Clin. Microbiol. 1:82-88, 1975), the scheme proposed by the Subcommittee on the Taxonomy of Staphylococci and Micrococci (Int. J. Syst. Bacteriol. 26:332-334, 1976), and the API STAPH micromethod. Isolates whose identification was in disagreement were identified on a taxonomic level according to the extensive classification scheme of Schleifer and Kloos. In addition, 42 reference staphylococci of known identity were reidentified using the simplified lyogroup system as well as the three other routine methods. The results of this comparative study are detailed and discussed. A primary advantage of the simplified lyogroup system is that it also provides separation of staphylococci from micrococci, which must be performed separately when the other methods are used. Other significant advantages concerned with reliability, speed, and practicality are shown.

In recent studies conducted in our laboratory, the taxonomy of the *Micrococcaceae* has been treated by a new approach based on the analysis of the bacteriolytic activity (BA) produced by the strains (8, 9, 14-16). In particular, six different groups of human staphylococci, called lyogroups, have been recognized on the basis of their BA patterns analyzed with an appropriate assay system. BA proved very stable, and the BA pattern was found to be a very reproducible staphylococcal characteristic (15). Subsequent investigations have shown very close relationships between staphylococcal lyogroups and species (14). Recent deoxyribonucleic acid homology studies conducted in another laboratory suggest that some staphylococcal species are more closely related than others and form some species groups (11); these species groups appear to largely overlap with our lyogroups.

Given its ease and accuracy, we decided to investigate the application of lyogroup determination to the routine identification of staphylococci. The present study describes a simplified system for the identification of the six lyogroups of human staphylococci. This simplified scheme proves to be particularly suitable for routine use, yet is as reliable as the more extensive system originally applied to taxonomy (15). A unique merit of this method is that it also provides separation of staphylococci from micrococci, which would otherwise have to be determined separately. Other significant advantages become apparent in the comparison of the simplified lyogroup method with three other methods proposed for the routine identification of staphylococci: the abbreviated scheme proposed by Kloos and Schleifer (5), the scheme recommended by the Subcommittee on the Taxonomy of Staphylococci and Micrococci (12), and the API STAPH micromethod.

MATERIALS AND METHODS

Simplified lyogroup identification. To make it more suitable for routine use, the BA assay system, originally applied to taxonomy and composed of eight test media (15), was modified as follows.

Modification (i). Only five BA test media were used, all requiring the same BA substrate (heat-killed cells of *Micrococcus luteus* ATCC 4698). Besides the reduction of the number of media, the exclusion of those media that required special BA substrates represented a useful simplification. The five BA test media—namely, TP1, TP2, T0, T1, and T3—were prepared as described previously (15) and distributed into petri dishes. Isolated colonies of strains to be tested were picked up by a needle and stabbed into the test media. Up to 18 strains were tested on one

plate. The inoculated media were incubated for 24 h at 37°C.

Modification (ii). BA was read and scored according to a simpler scheme (− through ++, instead of − through +++): ++, large zone of transparency around the spot of bacterial growth, the distance between the edge of the spot and the edge of the zone of transparency being more than 3 mm; +, moderate zone of transparency, exceeding the spot by 1 to 3 mm; ±, small zone of transparency, exceeding the spot by less than 1 mm; −, no detectable zone of transparency. The lower score was used, as a rule, when a particularly indistinct outline of the zone of transparency made the determination of the above-mentioned distance doubtful.

Modification (iii). Determination of phosphatase production was included. In this simplified scheme, such a test was necessary to separate lyogroup I from lyogroup II, and was also useful to confirm the identification of most lyogroup V strains. Phosphatase activity was tested by the recently described methyl green-phenolphthalein diphosphate (MG-PDP) method (7), which is recommended since, after medium inoculation, no additional step is needed to read the test. Both methyl green and phenolphthalein diphosphate were added from Seitz-filtered master solutions to the base medium (tryptose phosphate broth [Difco] supplemented with 1.5% agar; melted and kept at 46°C) to obtain final concentrations of 25 and 200 μg/ml, respectively. The plates containing the MG-PDP medium were inoculated in the same way and incubated for the same time as those containing the BA test media. The strains that displayed green-stained spots of growth were then recorded as phosphatase positive, and those that displayed unstained spots were recorded as phosphatase negative.

Lyogroup identification of each isolate was achieved on the basis of its BA on the five different test media and its phosphatase activity, according to the scheme reported in Table 1. Previously established relationships between staphylococcal lyogroups and species (14) are also reported in this table.

Two hundred known staphylococci from the various lyogroups (15), tested by the above-described simplified scheme to check its reliability, were all reidentified as belonging to the originally determined lyogroups.

The Kloos and Schleifer abbreviated method. This method, through a 13-test dichotomic scheme, leads to distinguishing 11 positions, each virtually corresponding to a staphylococcal species (5). The 13 key characters (coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from fructose, xylose, arabinose, ribose, maltose, lactose, sucrose, trehalose, mannitol, and xylitol) were tested according to the procedures described, and the 11 species positions were distinguished according to the dichotomic scheme proposed (5).

Subcommittee method. The method recommended by the Subcommittee on the Taxonomy of Staphylococci and Micrococci provides, based on five tests, identification of three species regarded as particularly relevant to human pathology (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*), and by two additional tests also allows separation of two other species (*Staphylococcus cohnii* and *Staphylococcus xylosus*) from *S. saprophyticus* (12). Six characters (coagulase activity, novobiocin susceptibility, and aerobic acid production from sucrose, trehalose, mannitol, and xylose) were tested according to the procedures described by Kloos and Schleifer (5); the remaining one (phosphatase activity) was tested by the above-mentioned MG-PDP method (7).

API STAPH micromethod. This micromethod, resulting from a study of Brun et al. (1), combines 19 tests for the identification of staphylococcal species following the Schleifer and Kloos classification (4, 10). The API STAPH strips were inoculated, incubated, and read according to the directions of the manufacturer (API System S.A., La Balme Les Grottes, Montalieu Vercieu, France). Strain identification was achieved by comparing the seven-digit number resulting from the reactions (according to the coding scheme described by the manufacturer) with known numerical profiles. Less often, identification was obtained from the reactions considered as a whole, with the aid of a differential chart. Both the numerical profile index and the differential chart were included in the API STAPH kit.

Taxonomic recognition of species. Strain identification on a taxonomic level was achieved according to the extensive classificatory scheme proposed by Schleifer and Kloos (4, 10). Character determinations were as described by the authors. Cell wall composition and the type of lactic acid produced were not determined.

TABLE 1. *Simplified scheme for lyogroup identification of staphylococci and relationships between staphylococcal lyogroups and species*

Lyogroup	BA displayed on test media:					Phosphatase activity	Species[a]
	TP1	TP2	T0	T1	T3		
I	++	+	±	++	−	+	*S. aureus*
II	++	+	+	++	−	−	*S. simulans*
III	+	±	++	+	−	−	*S. capitis*
IV	++	++	±	++	+	−[b]	*S. saprophyticus, S. xylosus, S. cohnii*
V	+	++	−	±	++	+[c]	*S. epidermidis*
VI	−	+	−	−	±	−	*S. hominis, S. haemolyticus, S. warneri*

[a] Relationships between staphylococcal lyogroups and species are reported as established previously (14).
[b] Phosphatase was produced by a minority of strains.
[c] Phosphatase was not produced by a minority of strains.

Bacterial strains. Clinical isolates of *Staphylococcus* examined in this study totaled 689. These strains were isolated from clinical material in the Clinical Bacteriology Laboratory, Institute of Microbiology of the University of Genoa Medical School, over a 6-month period (January through June 1979). In addition, 42 reference staphylococci, obtained from the American Type Culture Collection (ATCC), Rockville, Md., or from the Czechoslovak Collection of Microorganisms (CCM), Brno, Czechoslovakia, were examined. These strains included representatives from all recognized staphylococcal species: 17 from *S. aureus* (ATCC 10832, 12598, 12599, 12600, 12604, 12610, and 25904; CCM 299, 364, 681, 1770, 2286, 2287, 2469, 2504, 2570, and 2571); 4 from *S. epidermidis* (ATCC 14852 and 14990; CCM 50 and 2343); 4 from *S. saprophyticus* (CCM 2602, 2635, 2682, and 2728); 3 from *Staphylococcus haemolyticus* (CCM 1798, 2574, and 2737); 3 from *Staphylococcus warneri* (ATCC 155; CCM 2445 and 2730); 3 from *S. xylosus* (CCM 1400, 2210, and 2725); 2 from *S. cohnii* (CCM 2726 and 2736); 2 from *Staphylococcus hominis* (CCM 2732 and 2733); 2 from *Staphylococcus capitis* (CCM 2734 and 2735); and 2 from *Staphylococcus simulans* (CCM 2705 and 2724).

RESULTS

Comparative identification of 689 strains isolated from clinical material. All of the 689 staphylococcal strains isolated during the 6-month study period were identified according to the simplified lyogroup method. The same strains were identified in parallel using three other methods designed for routine identification of staphylococci: the 13-test dichotomic scheme proposed by Kloos and Schleifer (5), the Subcommittee method (12), and the API STAPH micromethod.

No further identification was carried out for 555 isolates, whose identification coincided by all four methods. In this respect, identifications at the level of lyogroup and species were regarded as coinciding when they were consistent with those lyogroup-to-species relationships established previously (14) and summarized above (see Table 1). Also, the strains that turned out to belong to lyogroups II, III, and VI and to the respective related species, according to both the 13-test dichotomic scheme and the API STAPH micromethod, were not investigated further, although they could not be identified by the Subcommittee scheme: the latter, in fact, intentionally disregards such species.

The remaining 134 isolates whose identification was in disagreement were further investigated and identified according to the extensive taxonomic scheme regarded as a reference (4, 10).

Table 2 presents a synthesis of the comparative identification of the 689 clinical isolates examined. The more salient points of this comparison between the simplified lyogroup method and the three other methods are briefly outlined below.

Lyogroup I included 331 isolates, all belonging to the species *S. aureus*. Four coagulase-negative strains were misidentified as *S. hominis* or *S. haemolyticus* by the 13-test dichotomic scheme, and were missed by the Subcommittee scheme. Additional strains with other uncommon features (e.g., mannitol negative or novobiocin resistant) were missed by the API STAPH micromethod (seven strains) and did not fit well into

TABLE 2. *Comparative identification of 689 staphylococci isolated from clinical material, as performed by the simplified lyogroup system and three other routine methods*

Reference identification		Strain identification by different methods[a]											
Species	No.[b]	13-test dichotomic scheme			Subcommittee scheme			API STAPH micromethod			Simplified lyogroup system		
		C	M	U	C	M	U	C	M	U	C	M	U
S. aureus	331	327	4		319		12	324		7	331 (I)[c]		
S. simulans	15	13	2				15			15	15 (II)		
S. capitis	9	6	2	1			9	4		5	8 (III)		1
S. saprophyticus	28	20	6	2	23		5	15		13	26 (IV)		2
S. xylosus	7	5	2		2		5	1		6	6 (IV)		1
S. cohnii	9	5	2	2	3		6	4		5	8 (IV)		1
S. epidermidis	192	187	5		169		23	183		9	192 (V)		
S. hominis	51	44	7				51	28		23	51 (VI)		
S. haemolyticus	42	35	6	1			42	21		21	42 (VI)		
S. warneri	5	2	3				5	2		3	4 (VI)	1 (IV)	
Total	689	644	39	6	516		173	582		107	683	1	5

[a] Data are given as number of strains. C, Correctly identified; M, misidentified; U, unidentified.

[b] Including both the isolates whose identification coincided using all four methods and those that were identified, in case of disagreement, according to the taxonomic reference scheme.

[c] Parentheses indicate the lyogroup.

the Subcommittee scheme (eight strains).

Lyogroup II included 15 isolates, all belonging to the species *S. simulans*. Two strains (one nitrate negative, the other strongly hemolytic) were misidentified as *S. saprophyticus* and *S. haemolyticus*, respectively, by the 13-test dichotomic scheme. All strains remained unidentified by the API STAPH micromethod as well as by the Subcommittee scheme (the latter, however, disregards the species *S. simulans*).

Lyogroup III included eight isolates, all belonging to the species *S. capitis*. Because this species is disregarded by the Subcommittee scheme, all strains were missed by this method. Four strains were missed by the API STAPH micromethod. Two strains with less usual sugar reactions (trehalose or maltose positive) were misidentified by the 13-test dichotomic scheme (one as *S. cohnii*, the other as *S. epidermidis*).

Lyogroup IV included 41 isolates, all but one belonging to a cluster of three species: *S. saprophyticus*, *S. cohnii*, and *S. xylosus*. The exception was a strain classifiable as *S. warneri*, which was identified as *S. saprophyticus* by the 13-test dichotomic scheme and remained unidentified by the two other methods. Ten isolates were misidentified (most as *S. warneri* or *S. hominis*) and two were unidentified by the 13-test dichotomic scheme; 14 and 21 isolates were missed using the Subcommittee scheme and the API STAPH micromethod, respectively.

Lyogroup V included 192 isolates, all belonging to the species *S. epidermidis*. Five trehalose-positive isolates were misidentified as *S. hominis* by the 13-test dichotomic scheme and were missed by the two other methods. Additional strains with other uncommon features (e.g., phosphatase negative or novobiocin resistant) were missed by the Subcommittee scheme (18 strains) and by the API STAPH micromethod (4 strains).

Lyogroup VI included 97 isolates, all belonging to a cluster of three species: *S. hominis*, *S. haemolyticus*, and *S. warneri*. Because these species are disregarded by the Subcommittee scheme, all strains were missed by this method. Unidentified strains totaled 46 by the API STAPH micromethod. Fifteen strains were misidentified (most as *S. epidermidis*), and one was unidentified, using the 13-test dichotomic scheme.

Five isolates, classifiable as *S. saprophyticus* (two strains), *S. xylosus*, *S. cohnii*, and *S. capitis* by the extensive taxonomic scheme regarded as reference, could not be included in any of the six lyogroups. All five strains remained unidentified by the API STAPH micromethod, four were unidentified by the Subcommittee scheme, and three were unidentified by the 13-test dichotomic scheme; another strain was misidentified by the latter method. The *S. xylosus* isolate was devoid of BA and produced phosphatase, a character combination that has already been encountered in a small minority of strains of this species (9, 13).

Comparative reidentification of reference strains. Forty-two reference staphylococci were subjected to reidentification according to the simplified lyogroup scheme, the 13-test dichotomic scheme, the Subcommittee scheme, and the API STAPH micromethod (Table 3).

All strains but one displayed lyogroup-to-species relationships consistent with those referred

TABLE 3. *Comparative reidentification of 42 reference staphylococci, as performed by the simplified lyogroup system and three other routine methods*

Reference strains		Strain reidentification by different methods[a]											
Species	No.	13-test dichotomic scheme			Subcommittee scheme			API STAPH micromethod			Simplified lyogroup system		
		C	M	U	C	M	U	C	M	U	C	M	U
S. aureus	17	16	1		14		3	15		2	17 (I)[b]		
S. simulans	2	2					2			2	2 (II)		
S. capitis	2	2					2	2			2 (III)		
S. saprophyticus	4	3	1		4			3		1	4 (IV)		
S. xylosus	3	3			2		1	1		2	3 (IV)		
S. cohnii	2	1		1	1		1	2			2 (IV)		
S. epidermidis	4	4			3		1	4			4 (V)		
S. hominis	2	1	1				2			2	2 (VI)		
S. haemolyticus	3	2	1				3	1		2	3 (VI)		
S. warneri	3	2	1				3	2		1	2 (VI)	1 (IV)	
Total	42	36	5	1	24		18	30		12	41	1	

[a] Data are given as numbers of strains. C, Correctly identified; M, misidentified; U, unidentified.
[b] Parentheses indicate the lyogroup.

to above (see Table 1). The exception was strain ATCC 155, classified in the species *S. warneri* by Kloos and Schleifer (4), which fell in lyogroup IV. This strain was identified as *S. saprophyticus* by the 13-test dichotomic scheme and was unidentifiable both by the Subcommittee scheme and by the API STAPH micromethod.

By the 13-test dichotomic scheme, five strains were misidentified and one remained unidentified. The identity of several of the reference strains could not be established by the Subcommittee scheme (18 strains) or by the API STAPH micromethod (12 strains); no misidentification was obtained by either method.

DISCUSSION

The simplified lyogroup identification scheme dealt with in this study proves particularly workable and suitable for routine use, and combines good resolving power and reliability with simplicity, speed, and economy.

A major advantage of this method is that a preliminary separation of staphylococci from micrococci is not required: unlike all other methods, the lyogroup method itself provides this information. It has recently been demonstrated that micrococci, as opposed to staphylococci, fail to produce BA (9, 13). Exceptional strains can be recognized by checking for phosphatase activity and yellow pigment (13). Since BA and phosphatase activity are integrated into the simplified lyogroup assay system, and yellow pigment can easily be observed on the BA test media, there is no need for any further tests to separate staphylococci from micrococci.

Other advantages of the simplified lyogroup method are shown by its comparison with the three other systems for routine identification of staphylococci.

Practicality, time, and cost. The lyogroup system requires only that a colony be picked up by a needle and stabbed into the six test media. All of the latter (i.e., the five BA test media as well as the MG-PDP phosphatase test medium) are inoculated in the same way and read at the same time. These media are simple to prepare, do not require any sophisticated or expensive chemicals, and can be stored for several weeks at 4°C before use. Up to 18 isolates can easily be tested on one plate. Test reading and resulting lyogroup identification are easy to carry out and achieved within 24 h. With a little experience, the lyogroup can be recognized at first sight, by concentrating on the following essentials: (i) comparison between the zones of transparency on TP1 and TP2 media; (ii) comparison of the zones of transparency on T0, T1, and T3; and (iii) phosphatase activity. The recently attempted use of multi-parted dishes, so that an isolate can be examined on six test media contained in the same plate, should further facilitate the lyogroup on-sight identification.

The most complicated of the methods examined seems to be the 13-test dichotomic scheme. This agrees with the findings of others, who have estimated this method to be too complicated for routine use (2). Both this and the Subcommittee scheme require different procedures for inoculation and different times for reading; several tests, such as sugar tests or hemolysis, cannot usually be read before 72 h. Identification by the API STAPH micromethod consistently requires no less than 48 to 72 h from isolation. The latter is also the most expensive of the methods considered. All schemes other than the lyogroup method require preliminary separation of staphylococci from micrococci, which necessitates more work, time, and money.

Resolving power and reliability. Strain identification by the simplified lyogroup method, which resolves six lyogroups very closely related to species, correlates very well (about 99% agreement) with the taxonomic scheme regarded as reference. Such a high degree of reliability might be accounted for by a special correlation between BA and cell wall composition (14). Moreover, the simplified lyogroup method might also benefit by the fact that, unlike the other methods, it does not make use of sugar reactions, which are among the most misleading tests owing to their variability, their inconstant reproducibility, and the frequent problems with their interpretation.

The 13-test dichotomic scheme has a high resolving power. By this method, however, over 1% of *S. aureus* isolates and 10% of the other isolates were misidentified. Another 2% of the latter strains remained unidentified. Additional tests besides the 13 key tests have been suggested by the authors to recognize misplaced isolates (5). However, these additional tests may only partially improve reliability while greatly complicating and delaying identification. The lack of complete understanding of the pathogenicity of each particular staphylococcal species does not justify the a priori limited resolving power of the Subcommittee scheme. Both the present study and other recent reports (2, 3, 6) suggest that staphylococci of the species disregarded by the Subcommittee scheme may have clinical relevance. No misidentification was obtained using this method. Non-identifications, even of isolates of the three species regarded, were rather frequent (4% of clinical isolates of *S. aureus*, 12% of *S. epidermidis*, and 18% of *S. saprophyticus*). As for the API STAPH micro-

method, its potentially high resolving power was lowered by the fact that some species turned out to be only occasionally identifiable: e.g., not even 1 strain of *S. simulans*, out of 17 tested, could be identified. No misidentification was obtained using the micromethod; non-identifications, however, were rather frequent. An acceptable rate of successful identification was obtained only with strains of *S. aureus* and *S. epidermidis* (98 and 95%, respectively, of clinical isolates). Apart from these two species, about 60% of the clinical isolates yielded numerical profiles that did not appear in the index. In these cases, the alternative identification with the aid of the differential chart was seldom successful. This may also be due to the fact that in this chart some reactions are listed in direct contradiction to what is commonly accepted: e.g., phosphatase production is shown as positive in *S. cohnii* and negative in *S. xylosus*, and acetoin production is given as positive in *S. simulans*, whereas usually opposite reactions would be expected on the basis of both our experience (7, 14) and the original descriptions of the species (4, 10). Moreover, aside from the manufacturer's directions, no information is available about the actual variation within the different species of some characteristics tested for by the micromethod (e.g., acid production from α-methylglucoside or from *N*-acetylglucosamine); these reactions were neither included in the original species descriptions (4, 10) nor accounted for in the study (1) on which the API STAPH micromethod is based.

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