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# Evaluation of a New Bacteriophage Set for Typing of *Staphylococcus epidermidis* Strains

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A new set of typing phages was evaluated for typing 821 *Staphylococcus epidermidis* strains isolated from normal human skin and from acne lesions. This method was compared with two different systems for biochemical differentiation of *S. epidermidis*. Distinct subgroups of cocci, which differed in phage susceptibility as well as in biochemical properties, were found. A tentative subdivision of *S. epidermidis* strains by use of 16 phages arranged into four groups is proposed, together with additional biochemical differentiation of nontypable strains.

*Staphylococcus epidermidis* are typical bacteria indigenous to normal skin but, under certain conditions, for example, in the presence of a foreign body in the bloodstream, after instrumentation of the urinary tract, or after immunosuppression, the organism can invade the human body and cause apparent infection. Many authors have suggested the need for an efficient method for the identification of *S. epidermidis* strains, as this would permit more efficient studies on epidemiology and the mechanisms of infection (6, 8, 11, 13, 17). A system for type identification would also be of importance for ecological studies on the carriage of these bacteria from various skin sites. For this purpose, several groups of workers in various countries isolated species-specific phages and evaluated them for type identification (8, 20-22).

In a series of papers, we have described the isolation and characterization of 90 different phages obtained from *S. epidermidis* strains. These contrasted with phages formerly examined in that they possessed a broader generic specificity (15, 16). These phages appear to be useful for type identification, as a considerable proportion of clinical isolates of *S. epidermidis* are susceptible to at least one phage of the group.

The aim of this study was to evaluate our selected *S. epidermidis* phages and compare these with two different biotyping schemes, based on the examination of several hundred *S. epidermidis* strains isolated from human skin. This might enable us to select procedures for further epidemiological and ecological studies on the species.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 821 strains of *S. epidermidis* were tested. The bacteria were isolated from healthy skin from forehead, forearm, and interscapular regions and also from acne lesions of young individuals of both sexes. Specimens were obtained by using either the cotton swab method (9) or aseptic puncture of closed comedones (12). The isolates were selected on the basis of colonial morphology on blood agar plates and Gram-staining characteristics. Selection was also based on the specific biochemical characteristics of the isolates, i.e., production of catalase and anaerobic fermentation of glucose on a recommended medium (18) for 10 days. Absence of coagulase and anaerobic fermentation of mannitol were also noted (2, 18).

**Phages.** Sixteen *S. epidermidis*-typing phages, together with their propagating strains, were selected from the group of 90 bacteriophages previously described (15, 16). This collection consisted of the following phages: Ph5, Ph6, Ph9, Ph10, Ph12, Ph13, Ph14, Ph15, Ph16, U4, U14, U15, U16, U20, U33, and U46. All phages of Ph series were propagated on a host strain, *S. epidermidis* Q 239. The phages of the U series were propagated on a host staphylococcus coded V 505, except for phages U4 and U20, which were propagated on staphylococcal strains 569D and 87, respectively. A standard agar technique for propagation was used throughout (5).

**Phage typing.** Staphylococcal strains were cultured on tryptone-yeast extract broth overnight at 37°C. Diluted broth cultures were flooded onto the surfaces of well-dried tryptone-yeast extract agar plates. Phage suspensions with titers of $10^8$ to $10^9$ plaque-forming units/ml were used for routine test dilutions only. These suspensions were applied to the surfaces of the inoculated and dried plates. The plates were examined after overnight incubation at 37°C. Results were recorded as positive when more than 50 plaques per drop were visible (5).

**Biochemical typing.** Strains were classified according to Baird-Parker's scheme (1), with further modifications (2), and also according to our biochemical-typing system (10). Fermentation of the carbohydrates glucose, mannitol, lactose, and maltose was tested in tubes of medium (18) under aerobic and anaerobic conditions. Phosphatase production was determined by the plate method of Barber and Kuper (3), as modified by Baird-Parker (1). Acetoin was detected on Barrit medium (4), and urease activity was detected on Christensen medium (7). Hydrolysis of Tween 20 was tested on nutrient agar containing 1% Tween 20 and 0.1% $CaCl_2$. Catalase was detected by application of 3% hydrogen peroxide to nutrient agar cultures, and production of coagulase was determined by using diluted rabbit plasma according to published recommendations (18).

## RESULTS

The results of phage typing of the 821 cultures of *S. epidermidis* with sixteen selected phage types are shown in Table 1. Ninety-six different patterns were observed. Twenty patterns occurred more than five times, whereas the remaining 76 patterns were represented mostly by single strains. More than 15% of the bacteria showed a uniform pattern of phage susceptibility: Ph10/Ph12/U14/U16, and about 10% were sensitive to all of the phages used. A considerable number of the strains exhibited exclusive resistance only to phage U20. The remaining patterns were related to the pattern Ph10/Ph12/U14/U16 mentioned above, or else they showed susceptibility to various different phages. In general, 72.5% of all strains were typable with at least one of the phages used at the routine test dilutions, leaving only about one-fourth untypable.

Since a high proportion of the strains were

TABLE 1. *Tentative grouping and frequency of phage patterns among 821 Staphylococcus epidermidis strains*

| Group | Phage pattern | No. of strains with each phage pattern | Strains in each phage group (%) |
|---|---|---|---|
| I | (1) Sensitive to all phages | 86 | 17.4 |
| | (2) Sensitive to all but phage U20 | 56 | |
| II | (3) Ph10/Ph12/U14/U16 | 131 | 25.1 |
| | (4) Ph10/U14/U16 | 46 | |
| | (5) U14/U16 | 12 | |
| | (6) Ph10/Ph12 | 11 | |
| | (7) Ph10/U14 | 6 | |
| III | (8) 30 combinations of phages: Ph5, Ph6, Ph9, Ph13, Ph14, Ph15, Ph16, U4, U15, U20, U33, and U46, less than 5 each | 37 | 4.5 |
| Mixed | (9) Ph10/Ph12/U4/U14/U16 | 25 | 25.5 |
| | (10) Ph10/Ph12/U14/U15/U16 | 22 | |
| | (11) Ph5/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/U4/U14/U16/U20 | 13 | |
| | (12) Ph5/Ph9/Ph12/Ph13/Ph14/Ph15/Ph16/U4/U15/U33/U46 | 12 | |
| | (13) Ph5/Ph6/Ph9/Ph12/Ph13/Ph14/Ph15/Ph16/U4/U15/U33/U46 | 11 | |
| | (14) Ph10/Ph12/U14/U16/U20 | 10 | |
| | (15) Ph10/Ph12/U14/U16/U33/U46 | 10 | |
| | (16) Ph5/Ph6/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/Ph16/U4/U15/U20/U33/U46 | 9 | |
| | (17) Ph5/Ph6/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/Ph16/U15/U33/U46 | 9 | |
| | (18) Ph10/Ph12/Ph13/Ph15/U4 | 9 | |
| | (19) Ph5/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/U4/U14/U15/U16/U20 | 8 | |
| | (20) Ph10/U14/U15/U16 | 8 | |
| | (21) Ph10/Ph12/Ph15/U14/U16 | 7 | |
| | (22) 46 other combinations of all phages, less than 5 each | 57 | |
| Non-typable | | 226 | 27.5 |

exclusively lysed by phages Ph10, Ph12, U14, and U16 (in various combinations), or were susceptible to all phages, we decided to divide our cocci into five groups. In this tentative division, the first group was represented by strains that were generally sensitive or those that only lacked sensitivity to lysis by phage U20. The second group consisted of the bacteria lysed by phages Ph10, Ph12, U14, and U16. The isolates sensitive to the remaining phages were included in group 3. There was also a mixed group, which consisted of the *S. epidermidis* strains typed according to various phage sensitivities, but this group did not include those classified into phage group I. The frequency of the strains contained in these four groups varied from 37 to 210.

These four groups were compared with groupings of the same strains based on their biochemical characteristics. Classification according to Baird-Parker's biotyping system (Table 2) revealed that over 90% of our staphylococci belonged to biotype 1 (groups SII and SV in his previous system of classification [1]) and to biotype 4 (group SV1). The staphylococci were rarely classified into other biotypes. We were unable to type 22 organisms because of their atypical biochemical patterns. It was also noted that the strains included in our phage group I fell almost exclusively into Baird-Parker's biotype 1, and that the cocci typable by sensitivity to phages of the second group were classified in biotype 4 more frequently than others. The strains for the mixed group and those that were nontypable by phages were distributed randomly into Baird-Parker's biotypes.

The second biochemical method of classification applied was our biotyping scheme, which was based on aerobic fermentation of mannitol and production of phosphatase, urease, and lipase. This scheme differs from Baird-Parker's in only two aspects, i.e., the inclusion of tests for urease and lipase activities. But this method is designed mainly for epidemiological purposes and permits the differentiation of coagulase-negative staphylococci into 16 independent groups. As can be seen in Table 3, the majority of the strains were classified into groups 5 and 10 and were also fairly frequently found in the four other groups (6, 11, 12, and 13). Some differences in the distribution of strains sensitive to phage groups I and II were noted. Staphylococci lysed by all phages (eventually with the exception of phage U20) mainly belonged to groups 5 and 10 of our biotyping system, whereas those sensitive to phages Ph10, Ph12, U14, and U16 were more frequently found in groups 6 and 12 than were strains of other phage groups.

## DISCUSSION

An evaluation of our phage collection by the typing of 821 *S. epidermidis* strains of human origin showed that it may be useful both for epidemiological and ecological studies. The high percentage of typability by our phages was comparable to that obtained during routine testing of *S. aureus* strains with the International Phage Collection (14). Very similar data are given by Dean et al. (8). This result was achieved in spite of the fact that we used stricter criteria for typing than we used in our previous study (16).

In contrast to other workers who used different phage sets (6, 8, 20–22), we were able to demonstrate some segregation of the different phage patterns into particular biotypes. The possible existence of natural subgroups within a group of already typable strains was also postulated by Dean et al. (8).

Various authors have reported difficulty in the separation of *S. epidermidis* strains by phage typing because of long, complex lytic patterns of some of the staphylococci (8, 20). Since these strains also differ in their biochemical properties, as was shown by subsequent biotyping, we consider them to be a biologically

TABLE 2. *Correlation of phage typing to Baird-Parker's biotyping of S. epidermidis strains*

| Phage group | No. of strains in Baird-Parker's biotype | | | | No. of strains nontypable |
|---|---|---|---|---|---|
| | 1 (SII + SV)[a] | 2 (SIII) | 3 (SIV) | 4 (SVII) | |
| I | 117 (111 + 6) | 1 | 1 | 2 | 2 |
| II | 134 (117 + 17) | 1 | 11 | 49 | 8 |
| III | 33 (31 + 2) | 0 | 2 | 2 | 0 |
| Mixed | 174 (152 + 22) | 1 | 6 | 26 | 3 |
| Nontypable | 181 (153 + 28) | 1 | 3 | 32 | 9 |
| Total | 639 (564 + 75) | 4 | 23 | 111 | 22 |
| Percent | 79.9 (70.5 + 9.4) | 0.5 | 2.9 | 14.0 | 2.7 |

[a] Subgroups (SII-SIII) comprise Baird-Parker's previous system of biotyping (reference 1); biotypes (1-4) comprise his current system of biotyping (reference 2).

TABLE 3. *Correlation of phage susceptibility to biotyping of S. epidermidis strains according to Heczko et al. (10)*

| Phage group | Biochemical group | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| I | 0 | 0 | 0 | 0 | 87 | 1 | 0 | 0 | 0 | 14 | 2 | 1 | 5 | 0 | 0 | 0 |
| II | 3 | 3 | 2 | 3 | 57 | 16 | 3 | 1 | 0 | 31 | 7 | 16 | 10 | 1 | 1 | 6 |
| III | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 0 |
| Mixed | 1 | 0 | 0 | 1 | 94 | 13 | 0 | 0 | 2 | 27 | 11 | 9 | 9 | 0 | 0 | 6 |
| Nontypable | 2 | 3 | 1 | 6 | 90 | 7 | 5 | 1 | 2 | 32 | 10 | 6 | 12 | 2 | 2 | 0 |
| Total | 6 | 6 | 3 | 10 | 351 | 37 | 8 | 2 | 4 | 105 | 32 | 32 | 37 | 3 | 3 | 12 |
| Percent | 0.9 | 0.9 | 0.4 | 1.5 | 53.9 | 5.7 | 1.2 | 0.3 | 0.6 | 16.1 | 4.9 | 5.3 | 5.7 | 0.4 | 0.4 | 1.8 |

distinct group, and we have segregated them from the other strains in our tentative divisions. This group of *S. epidermidis* should probably be classified as so-called "restriction-modification-less staphylococci" described by Verhoef et al. (23). Possibly, a lack of the enzymes restricting or modifying the phage deoxyribonucleic acid may provide an explanation for the unusual susceptibility of these bacteria to all of the phages used.

It is of interest that particular phage patterns and their frequent appearance in the *S. epidermidis* strains found on human skin resemble those reported by us from clinical isolates of the species (15). This may indicate that a distinct group of *S. epidermidis* with special pathogenic properties, an idea postulated by some authors (6), does not, in fact, exist and that it is more likely that all of these organisms can become pathogenic under favorable conditions as determined by the host.

In this study, about 80% of tested *S. epidermidis* strains were classified into Baird-Parker's biotype 1, and 70% of them were placed in groups 5 and 10 of our scheme, but these strains were distributed in all four phage groups. This fact indicates that phage typing seems be much more useful as an epidemiological tool. Also, Blouse et al. (6) stated that Baird-Parker's biotyping scheme offers very little information with regard to strain characterization, since almost all *S. epidermidis* strains isolated from human sources belong to biotype 1 or, more rarely, to biotype 4. Obviously, with the use of a biotyping scheme, it is possible to biochemically differentiate strains that are nontypable by the use of phages, and this seems to be of particular importance for epidemiological studies.

In this study the frequency of 16 biochemical groups into which 651 staphylococci were classified were similar to groups described previously for clinical isolates of coagulase-negative staphylococci (10), with the exception that only a few skin strains were classified into group 7. This group has been described in a former study as being isolated almost exclusively from urine, and it is likely that these strains correspond to a newly recognized species, *S. saprophyticus* (19).

It is obvious that there is a need for a common system upon which comparisons of *S. epidermidis* phage-typing results from different laboratories can be based. Perhaps this may be achieved only by the selection of a small group taken from the vast number of lytic phages already isolated in different countries.

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