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Development of a Bacteriophage-Typing Set for *Staphylococcus epidermidis*

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Six phages isolated from lysogenic cultures of *Staphylococcus epidermidis* were used to type cultures in our collection classified previously as "untypable" with our original typing set of 13 phages, new clinical isolates from Missouri, and clinical isolates from Connecticut, Georgia, Pennsylvania, and Virginia. Typability with the new set of 6 phages and with our 13 original phages was compared. For 203 cultures, 81 (39.9%) cultures were typable with the set of 6 phages and 73 (36.0%) cultures were typable with the set of 13 phages. When the two typing sets were combined, the percentage of typability for 425 cultures increased to 49.9%, compared with 35.7% with the original set of 13 phages. For 109 cultures from outstate, the percentage of typability was 56.9%, whereas that for 316 cultures from Missouri was 47.5%. When only cultures in biotype 1 were considered, the percentage of typability for 268 cultures with the combined set increased to 58.2%, compared with 39.5% with the original set of 13 phages. Analysis of individual reactions for all 425 cultures typed in our laboratory indicated that 11 of the 19 phages in the combined set accounted for the majority of the reactions, and a provisional set containing these 11 phages is proposed.

Acceptance of the role of *Staphylococcus epidermidis* as a pathogen in certain clinical conditions has necessitated development of methods for epidemiological studies. The biotyping scheme of Baird-Parker (1) is of limited use in such studies, based on our recent findings (4). A bacteriophage-typing set, if developed for *S. epidermidis* as it has been for *Staphylococcus aureus*, offers one practical method for identifying specific strains of this organism. The 18 bacteriophages specific for *S. epidermidis* isolated in The Netherlands by Verhoef et al. (11) have not been effective in the identification of strains isolated by us (10) and by others in the United States (2, 5) and Germany (7). Recently we reported the isolation of 13 bacteriophages from lysogenic cultures of *S. epidermidis* (10). These were used to type 223 isolates from clinical sources in Missouri. The percentage of typability at 100 times the routine test dilution was 35.0%; for biotype 1 cultures, it was 43.3%. In this study we report the isolation of (i) six new phages and their use in typing cultures in our collection classified previously as "untypable" and (ii) new clinical isolates of *S. epidermidis* from Missouri and four other geographical regions. We also compare typability of these cultures with our new phages and with our original set of 13 phages, provide data for typability when the two sets are combined, and, based on all of our typing data, propose the use of a provisional typing set consisting of phages from both sets.

MATERIALS AND METHODS

Cultures of *S. epidermidis* used in the phage isolation experiments from our collection were characterized recently (4). Cultures of *S. epidermidis* used in typing experiments were obtained as follows: (i) 143 cultures from our collection (4) that were not typable with the 13 phages we isolated recently (10); (ii) 95 new cultures obtained from the University of Missouri Medical Center, Columbia, over a 3-month period; and (iii) 109 cultures from Connecticut, Georgia, Pennsylvania, and Virginia. Identification of each culture as *S. epidermidis* and its classification into biotype were the same as described previously (4). Six phages isolated from lysogenic cultures in biotype 1 and propagated on cultures in biotype 1 were designated 38, 95, 100, 113A, 124A, and 165. Methods for isolation, propagation, purification, and phage typing have been described (6, 10). Our original set of 13 phages has also been described (10). All typing was done with phages at 100 times the routine test dilution. Only reactions in which the degree of lysis was equal to 2+ or greater than the amount of bacterial growth within the drop of phage were recorded.

RESULTS AND DISCUSSION

We reported previously (10) that 78 (35.0%) of 223 cultures were typable with 13 phages isolated from lysogenic cultures of *S. epidermidis*. When 143 cultures in this collection previously

classified as "untypable" by the original 13 phages were typed with the 6 new phages, 24 (16.8%) typed. The results of phage typing new clinical isolates of *S. epidermidis* from Missouri and four other geographical regions with both sets of phages are shown in Table 1. Typability with the set of 6 phages varied from 20.0 to 46.7%; with the set of 13 phages, it varied from 26.3 to 80.0%. In all, 81 (39.9%) and 73 (36.0%) of 203 cultures were typable with the new set of 6 phages and the original set of 13 phages, respectively. When the two sets were combined to form a set of 19 phages, the percentage of typability for 222 cultures in our original collection increased from 35.0 to 46.4%, whereas the typability of our new collection from Missouri was 50.0% (Table 2). Typability of cultures from the four geographical regions varied from 47.4 to 80.0%. Interestingly, for 109 cultures from outstate, the percentage of typability was 56.9%, whereas that for 316 cultures from Missouri was 47.5%. With the combined set of 19 phages, the typability for 425 cultures from all sources was 49.9%. For this same number of cultures typed with the set of 13 original phages, 35.7% were typable (not shown).

One obvious question in the application of a phage-typing system on a national or international basis is its effectiveness in lysing cultures from different geographical regions. As we reported earlier (10), typability of our cultures from Missouri with the 18 phages isolated by Verhoef et al. (11) and specific for *S. epidermidis* from The Netherlands was only 21.5%; a combination of our original 13 phages with the phages of Verhoef et al. would have resulted in an increase in typability of only 5.8%. Blouse et al. (2) and Minshew and Rosenblum (5) in this country have experienced similar difficulties in typability with the phages from The Netherlands. Pulverer et al. (7) in Germany have reported marked differences in typability with phages obtained from cultures from one country in Europe used to type cultures from another country; also, the phages of Verhoef et al.

TABLE 1. *Phage typing S. epidermidis from different geographical regions with 6 new phages and 13 original phages*

Source of culture	No. of cultures typed	No. (%) of cultures typable with the set containing: 6 new phages	13 original phages
Missouri	94	40 (42.6)	26 (27.7)
Connecticut	50	18 (36.0)	19 (38.0)
Georgia	10	2 (20.0)	8 (80.0)
Pennsylvania	19	7 (36.8)	5 (26.3)
Virginia	30	14 (46.7)	15 (50.0)

TABLE 2. *Phage typing S. epidermidis from different geographical regions with the combined set of 19 phages*

Source of culture	No. of cultures typed	No. (%) of cultures typable
Original Missouri collection	222	103 (46.4)
New Missouri collection	94	47 (50.0)
Connecticut	50	26 (52.0)
Georgia	10	9 (80.0)
Pennsylvania	19	9 (47.4)
Virginia	30	19 (63.3)
All Missouri	316	150 (47.5)
Outstate	109	62 (56.9)
All sources	425	212 (49.9)

were less effective for their cultures than phages they isolated. On the other hand, Dean et al. (3) in England have met with greater success in typability with some of the phages from Verhoef et al. (11). According to Schmidt and Jeffries (8), due to peculiarities of either the system or the organism, typing systems are usually not interchangeable, and international standardization of typing systems is the exception rather than the rule. Although the number of cultures from outstate that we typed was not large and was not representative of a cross-section of the United States, our combined typing set was, at least, equally effective in lysing cultures from the same and different geographical regions, and represents a marked improvement in typability over our original set of 13 phages.

With our original set of typing phages, 61 (43.3%) of 141 cultures in biotype 1 were typable (10). When 80 cultures in biotype 1 classified as "untypable" with our typing set were typed subsequently with our new set, 21 (26.3%) of these typed. When the two typing sets were combined, 79 (56.4%) of 140 biotype 1 cultures from our previous collection typed, whereas the typability of our new collection of biotype 1 cultures from Missouri was 63.5% (Table 3). Also shown are the typing results with the combined sets of phages for all biotype 1 cultures obtained from three geographical regions. For biotype 1 cultures from outstate, 37 (56.9%) of 65 cultures typed, whereas for biotype 1 cultures from Missouri, 119 (58.6%) of 203 cultures typed. From all sources, 156 (58.2%) of 268 cultures in biotype 1 were typable with the combined set of 19 phages. For this same number of cultures typed with the set of 13 original phages, 39.6% were typable (not shown). Since it has been shown that cultures in biotype 1 are the ones most often isolated from disease states (4, 9), our combined set offers a substantial improvement

TABLE 3. *Phage typing of biotype 1 cultures of S. epidermidis from different geographical regions with the combined set of 19 phages*

Source of culture	No. of cultures typed	No. (%) of cultures typable
Original Missouri collection	140	79 (56.4)
New Missouri collection	63	40 (63.5)
Connecticut	31	17 (54.8)
Pennsylvania	15	8 (53.3)
Virginia	19	12 (63.2)
All Missouri	203	119 (58.6)
Outstate	65	37 (56.9)
All sources	268	156 (58.2)

over our previous set in identifying organisms in this biotype.

In developing a typing set as a tool for practical use in epidemiological studies, decisions must be made as to which are the most effective lysing phages and which phages can be deleted without affecting markedly the sensitivity of the system. When all of the individual phage reactions for all the cultures typed in our laboratory were analyzed, it was apparent that some phages lysed so few organisms that retaining them in the set would be impractical. In the combined set of 19 phages, 8 (48, 124, 173, 245, 38, 100, 124A, and 165) did not lyse at least 4.7% of the 425 cultures we have typed to date and were deleted from the set. The result was that the percentages of typability changed only for cultures from Missouri, since no culture from outstate was lysed solely by any of the eight deleted phages. The decrease in typability for cultures from Missouri was from 150 (47.5%) to 142 (44.9%) for 316 cultures. When only cultures in biotype 1 were considered, the percentage of typability for cultures from Missouri decreased from 119 (58.6%) to 113 (55.7%) for 203 cultures. Obviously, this slight decrease in typability of cultures from Missouri was reflected by a corresponding decrease in the percentage of typability for cultures from all sources. Our new typing set would contain phages 29, 68, 108, 112, 113, 127, 171A, 188, 207, 95, and 113A, the latter two phages obtained from our set of six. Although the use of this set of 11 phages would decrease slightly the percentage of typability with our system, we feel that the advantages of maintaining a set of 11 phages over a set of 19 phages outweighs the slight loss of sensitivity. New phages will be added to our typing set as more discriminating phages are isolated. Until then, we believe that our typing set of 11 phages offers a feasible and reliable method to conduct epidemiological studies of infections by *S. epidermidis* in the United States. Obviously, more work is needed before an international typing set can be proposed.

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LITERATURE CITED

1. **Baird-Parker, A. C.** 1974. The basis for the present classification of staphylococci and micrococci. Ann. N.Y. Acad. Sci. 236:7-13.
2. **Blouse, L. E., L. N. Kolonel, C. A. Watkins, and J. M. Atherton.** 1975. Efficacy of phage typing epidemiologically related *Staphylococcus epidermidis* strains. J. Clin. Microbiol. 2:318-321.
3. **Dean, B. A., R. E. O. Williams, F. Hall, and J. Corse.** 1973. Phage typing of coagulase-negative staphylococci and micrococci. J. Hyg. 71:261-270.
4. **Males, B. M., W. A. Rogers, Jr., and J. T. Parisi.** 1975. Virulence factors of biotypes of *Staphylococcus epidermidis* from clinical sources. J. Clin. Microbiol. 1:256-261.
5. **Minshew, B. H., and E. D. Rosenblum.** 1972. Transduction of tetracycline resistance in *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. 1:508-511.
6. **Parisi, J. T., and H. W. Talbot, Jr.** 1974. Improved rapid plate method for the isolation of bacteriophages from lysogenic bacteria. Appl. Microbiol. 28:503-504.
7. **Pulverer, G., J. Pillich, and A. Klein.** 1975. New bacteriophages of *Staphylococcus epidermidis*. J. Infect. Dis. 132:524-531.
8. **Schmidt, W. C., and C. D. Jeffries.** 1975. Bacteriophage typing of gram-negative rod-shaped bacteria. Crit. Rev. Clin. Lab. Sci. 6:201-246.
9. **Speller, D. C. E., and R. G. Mitchell.** 1973. Coagulase-negative staphylococci causing endocarditis after cardiac surgery. J. Clin. Pathol. 26:517-522.
10. **Talbot, H. W., Jr., and J. T. Parisi.** 1976. Phage typing of *Staphylococcus epidermidis*. J. Clin. Microbiol. 3:519-523.
11. **Verhoef, J., C. P. A. van Boven, and K. C. Winkler.** 1971. Characters of phages from coagulase-negative staphylococci. J. Med. Microbiol. 4:413-424.