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Phage Typing of *Staphylococcus epidermidis*

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Thirteen phages were isolated from lysogenic cultures of *Staphylococcus epidermidis* from a clinical laboratory and used to type 223 clinical isolates of this organism. The 18 phages isolated in The Netherlands were used to type these same cultures. No correlation was observed between phage type, biotype, or clinical source of isolation. At phage concentrations of 100 times the routine test dilution, 35.0% of the cultures were typable with our phages and 21.5% were typable with the phages from The Netherlands. When only cultures in biotype 1 were considered, 43.3 and 24.1% of 141 cultures were typable with our phages and those from The Netherlands, respectively. The lytic reactions obtained with our phages were generally stronger and easier to read and the lytic patterns were, almost invariably, shorter. The typability of untypable cultures was increased 12.0% by incubation at 45 C prior to phage typing and 20% by heat shock (55 C for 5 min) prior to typing. Phage typing 5 subcultures of 20 typable cultures on 5 successive days showed that the lytic patterns were reproducible. The present status of phage typing *S. epidermidis* and the work needed to obtain a set of typing phages for epidemiological studies of infections by this organism are discussed.

The role of *Staphylococcus epidermidis* as a pathogen in certain clinical conditions is now well established. Colonization of Spitz-Holter valves (5), endocarditis (12), and urinary tract infections (6) are among the more severe infections in which *S. epidermidis* has been implicated. The ubiquitous nature of this organism and our inability to distinguish potentially pathogenic strains make its etiological role difficult to assess. Its resistance to antibiotics (3, 6) can present therapeutic problems in the treatment of infections produced by this organism (12). The biotyping scheme of Baird-Parker (1) is of limited use in epidemiological studies in view of our recent findings of the occurrence of all four biotypes from all categories of clinical sources (6). The 18 bacteriophages specific for *S. epidermidis* isolated in The Netherlands by Verhoef and co-workers (14, 15) and used by investigators outside of the United States in epidemiological studies (4) have not been effective for the identification of strains isolated by us (unpublished data) and by others in this country (7). The recent use of these phages in an epidemiological study in Texas is interesting in view of the high percentage of typability obtained; however, the overwhelming majority of strains isolated subsequently by these investigators have been untypable (3). The purpose of this study was to isolate phages from well-characterized clinical isolates of *S. epidermidis* and to assess their potential use as typing phages. We also compared typability of our cultures with our phages and with those of Verhoef and co-workers (14, 15).

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MATERIALS AND METHODS

Cultures. Except for 5 cultures that were lost, all 223 cultures of *S. epidermidis* used in this study were from the same collection of 228 cultures we characterized recently as to biotype, clinical source of isolation, and the production of enzymes associated with virulence in *Staphylococcus aureus* (6).

Phages. Thirteen phages (Table 1) were isolated from lysogenic cultures of *S. epidermidis* in our collection by use of the improved rapid plate method developed recently in our laboratory (8). Discrete plaques were picked and washed into 1 ml of Trypticase soy broth (BBL) supplemented with 0.3% yeast extract and 400 μg of $CaCl_2$ per ml (YETS-CC broth). This suspension of phages was then used for propagating in YETS-CC broth containing 0.5% agar by the agar layer method (13). This process of phage purification and propagation was repeated three times. Phages were filtered through a 0.45-μm membrane filter (Millipore Corp.) and stored at 4 C. Eighteen typing phages of *S. epidermidis* and their bacterial hosts from the collection of Verhoef et al. (14, 15) also were used. This phage set included the following phage/host combinations: 48/81, 51/41, 71/240, 71A/382, 82/87, 82A/368, 108/407, 130/87, 130A/141, 157/382, 275/98, 275A/380, 275B/48, 448/407, 456/76, 459/407, 471/330, and 489/407.

TABLE 1. *Biotype and clinical source of lysogenic and indicator (propagating) strains of S. epidermidis and bacteriophage designation*

Lysogenic strain			Indicator strain			
No.	Source	Biotype	No.	Source	Biotype	Phage no.
5	Abscess	1	68	Urine	1	68
21	Wound	1	29	Nasopharyngeal	1	29
27	Wound	1	113	Abscess	1	113
34	Urine	1	48	Urogenital	3	48
141	Urine	4	245	Urine	4	245
163	Urine	1	171A	Urine	1	171A
172	Wound	3	127	Blood	3	127
174	Blood	1	188	Urine	1	188
176	Wound	1	207	Urogenital	1	207
182	Wound	1	108	Blood	1	108
233	Wound	1	173	Urogenital	1	173
238	Urine	1	112	Nasopharyngeal	1	112
246	Urine	1	124	Nasopharyngeal	1	124

Phage typing. The routine test dilution (RTD) of each phage suspension was determined prior to use and used in the typing procedure. Cultures to be typed were grown in YETS broth overnight at 37 C and then swabbed onto a YETS-CC agar (1.5%) plate. Either the RTD or 100 times the RTD of each phage was placed in a phage applicator, and phages were applied to the surfaces of the inoculated plates. After overnight incubation at 30 C, plates were examined for lysis. Complete lysis within a drop was recorded as 4+; a 3+ reaction consisted of a few colonies within the drop, and a 2+ reaction consisted of an area that was approximately half lysed or more than 50 plaque-forming units. A 1+ reaction contained 20 to 50 plaque-forming units, whereas a ± reaction was one with less than 20 plaque-forming units within the drop. Reactions weaker than 2+ were not recorded.

Enhancement and reproducibility of typability with phages from Missouri. Twenty-five weakly (reactions less than 2+) typable and 25 untypable (complete unreactivity to phages) cultures were incubated overnight at 45 C prior to typing. In another experiment 25 untypable cultures, after incubation overnight at 45 C, also were subjected to heat shock (55 C for 5 min) prior to typing. Studies of the reproducibility of the lytic patterns obtained were performed with 20 cultures exhibiting all degrees of phage reactivity. Cultures were subcultured for 5 consecutive days and phage typed each day.

RESULTS

The results of phage typing 223 cultures of *S. epidermidis* with 13 phages isolated in this investigation and 18 phages isolated by Verhoef et al. (14, 15) are shown in Table 2. At 100 times the RTD, 39 different phage types were obtained with the 13 phages isolated by us in Missouri, and 32 different phage types were obtained with the 18 phages of Verhoef et al. (14, 15). Five phage types (29, 127, 245, 29/188, and 29/171A/188) accounted for 38.5% of the typable cultures with our phages, and five

TABLE 2. *Phage typing of 223 cultures of S. epidermidis (141 biotype 1 cultures) with 13 phages isolated in Missouri and with 18 phages isolated by Verhoef et al.*

Determination	No. and % of cultures typable with phages from:	
	Missouri	Verhoef et al.
Phage-typable cultures at RTD	43 (19.3)	19 (8.5)
Phage-typable biotype 1 cultures at RTD	31 (22.0)	14 (9.9)
Phage-typable cultures at 100 times RTD	78 (35.0)	48 (21.5)
Phage-typable biotype 1 cultures at 100 times RTD	61 (43.3)	34 (24.1)

phage types (130A, 71A/157, 82/130, 130/130A/456, and 82/130/275A/275B) accounted for 39.6% of the typable cultures with the phages of Verhoef et al. (14, 15). As shown in Table 2, at 100 times the RTD, 61 (43.3%) and 34 (24.1%) of 141 biotype 1 cultures were typable with our phages and those of Verhoef et al., respectively. With our phages, 2 (50%) of 4 biotype 2 cultures, 13 (43.3%) of 30 biotype 3 cultures, and 3 (6.6%) of 45 biotype 4 cultures were typable. No correlation was observed between phage type, biotype, or clinical source of isolation.

Thirty-six of 223 cultures were typable at 100 times the RTD with both sets of phages (Table 3). Compared with the reactions obtained with the phages of Verhoef et al. (14, 15), those obtained with our phages generally were stronger (3+ and 4+) and the lytic patterns were, almost invariably, shorter. When Verhoef et al. (14, 15) phages did produce shorter lytic patterns, usually those produced by our

TABLE 3. *Comparison of the patterns of lysis obtained at 100 times the RTD with 13 phages isolated in Missouri and with 18 phages isolated by Verhoef et al.*

Culture	Pattern of lysis obtained with phages from:	
	Missouri	Verhoef et al.
1	29/108/113/171A	51/71/82/82A/108/130/130A/275/275A/456
5	127	82/130
11	29/68/108/171A/188	48/51/71/71A/82A/108/130A/157/275/448/456/459/471/489
16	29/188	130A
17	29/68/108/113/127/171A/188	51/71/82/82A/108/130/130A/275/275A/448/456/459/471/489
23	29/108/113/188/207	48/51/71/71A/82A/130A/157/275/275A/448/459/471
29	29/108/113/127/171A/188	108/130A/459
30	29/171A/188	130A
38	48/207	82/82A/130/130A/275A/448/471
42	48/127	82/130/130A/275A/275B
48	29/48/68/108/124/127/171A/188/207	82/130/275A/275B
68	29/68/108/113/127/171A/188/207	51/71/71A/82/82A/130/130A/157/275/275A/459
72	29/68/108/113/171A/188	51/82/82A/108/130/130A/275/448/456/459/471/489
81	29/108/127/171A/188	71A/157
95	68/108/124/127/207	82/130
100	173	51
102	29	130A/275
103	29	130A/275
108	29/68/108/113/171A/188	82A/108/130A/275/448
113	29/68/108/112/113/171A	48/51/71/71A/82/82A/108/130/130A/157/275/275A/275B/448/456/459/471/489
124	29/48/108/113/124/127/171A/188/207	82/130/275A/275B
127	29/48/108/113/124/127/171A/188/207	82/130/130A/275A/275B
131	29/108/112/113/127	51/82A/108/130/130A/275/275A/448/456/471/489
138	29/112/127/171A/188	82A/130/130A
142	48/188	82/275A
158	29/108/113/188	48/51/71/71A/130A/157/275A/275B
167A	29/108/113/171A/188	130/130A
168	29/108/173	130/130A/456
170	29/108/173	130A/456
173	108/173	130/130A/456
182	127	82/130
188	29/113/171A/188	130A
197	29/108/113/171A/188	51/82A/108/130A/275/275A/448/459/489
207	48/124/127/207	82/130/275A
209	188	48/71/71A/157/275A
246	48/127	82/130

phages were not much longer.

Elevated (45 C) temperature of incubation before phage typing (100 times the RTD) resulted in 3 (12.0%) of 25 untypable cultures becoming typable and 7 (28.0%) of 25 weakly (reactions less than 2+) typable cultures showing stronger reactions with some phages (data not shown). However, among the weakly typable cultures, some phage reactions were not as strong as those obtained with incubation at 37 C, whereas others were unchanged. Of the 25 untypable cultures subjected to heat shock (55 C for 5 min) prior to typing, 5 (20.0%) became typable. However, the reactions (mostly 2+) resulting after heat shock were not sharp and were difficult to read.

Variations in lytic patterns upon subculturing and phage typing on 5 successive days were minor. Generally, strong (3+ and 4+) reactions remained as such throughout the test period. Few reactions were lost; the loss of a reaction was restricted almost entirely to weak (± and 1+) reactions, which were not considered significant when typability was determined.

DISCUSSION

The results show that strains of *S. epidermidis* can be distinguished by phage typing. One problem appears to be the isolation of phages that will lyse a majority of the strains of *S. epidermidis*, regardless of their geographic origin. Verhoef et al. (15) found 75.5% of 240 strains of *S. epidermidis*, the majority being in biotype 1, typable at either the RTD or 100 times the RTD. In England, Dean et al. (4), using 9 of the phages of Verhoef et al. (14, 15) and 10 they isolated, reported that 39% of 1,517 strains of coagulase-negative staphylococci were lysed at 100 times the RTD; 72% of the biotype 1 strains were typable. One difficulty they encountered was the long complex lytic patterns of their typable strains. In Germany and Czechoslovakia, Pulverer et al. (11), using 13 phages they isolated, found that 58.2% of 170 strains of *S. epidermidis* were lysed at the RTD. As shown in Table 2, strains of *S. epidermidis* obtained from our clinical laboratory were relatively resistant to lysis by the 18 phages of Verhoef et al. (14, 15) at either the RTD or 100 times the RTD. When only biotype 1 cultures were considered, the increase in typability was negligible. On the other hand, with our 13 phages at either the RTD or 100 times the RTD, the percentage of typable cultures increased markedly. Our typability percentage was highest for biotype 1 cultures, which are the ones most often implicated in disease (5, 6, 12). The lytic reactions with our phages were generally stronger (3+ and 4+), easier to read, and, almost invariably, shorter (Table 3). The percentage of typability obtained with our phages, however, is not high enough at this time to suggest their use by others. A combination of our phages with those of Verhoef et al. (14, 15) would only result in a 5.8% increase in typability. It is difficult to explain the high percentage (59.3) of typability with the phages (at 100 times the RTD) of Verhoef et al. (14, 15) obtained by Blouse et al. recently in the outbreak in an Air Force hospital in Texas and the resistance (76.5%) to the same phages of cultures isolated subsequently from the hospital (3). Apparently, the strains implicated in the outbreak came from a source that was no longer active in the dissemination of these typable strains. The recent isolation of 49 new phages by Pulverer et al. in addition to the ones they reported in 1973 indicates the interest in *S. epidermidis* phages (10). These investigators, using a set of 16 phages, including one from the set of Verhoef et al. (14, 15), found that 71.6% of 183 strains of *S. epidermidis* from different parts of the world (United States, Canada, Denmark, Czechoslovakia, The Netherlands, and Germany) were lysed at the RTD; nearly one-half of the resistant strains were lysed subsequently by these phages at 100 times the RTD. Although their percentage of typability was high, it must be mentioned that their criterion of typability (1+ or greater than 20 plaque-forming units) was not that recommended and used conventionally for *S. aureus* (2, 9) and used for *S. epidermidis* by us and by others (4).

The necessity of using concentrated (100 times the RTD) suspensions of phages by us and others (4, 15) to obtain increased percentages of typability shows the greater resistance to phages of *S. epidermidis* than *S. aureus*. Although concentrated suspensions of phages are recommended for strains of *S. aureus* untypable initially at the RTD (2, 9), such phage suspensions appear to be used almost routinely for the initial phage typing of *S. epidermidis*. Although some phages isolated from lysogenic cultures of *S. epidermidis* exhibit a lower percentage of typability for *S. aureus* (10, 14), none of our 13 phages lysed any of 47 different strains of *S. aureus* in our collection. The reproducibility of lytic patterns observed by us with our phages and by others (4, 10, 15) suggests that the slight variations obtained by phage typing *S. epidermidis* are similar to those observed with *S. aureus*.

The results show that phage typing *S. epidermidis* is feasible and could be of immense value in epidemiological studies since biotyping has severe limitations. From our work and that of others (10), there appears to be considerable variation in the typability of cultures from one geographical area when typing phages isolated from cultures from a different area are used. To our knowledge, our phages are the only ones that have been isolated from clinical isolates of *S. epidermidis* in the United States. Whether our phages will be of value for the identification of strains of *S. epidermidis* from other parts of the United States has not yet been determined. Obviously, before a set of typing phages that will distinguish strain differences between the majority of strains of *S. epidermidis* is obtained, more work is needed to isolate such phages from cultures from different parts of the world. This is needed particularly for strains of *S. epidermidis* in biotype 1, which are the ones most often isolated from disease states.

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