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Bacteriophage Typing of Coagulase-Negative Staphylococci

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Cultures comprising the 10 species of coagulase-negative staphylococci proposed by Kloos and Schleifer (J. Clin. Microbiol. 1:82-88, 1975) were typed with bacteriophages isolated from *Staphylococcus epidermidis*. Although only 10.5% were typable, 50% of those identified as *S. epidermidis* were typed. Cultures from patients with middle ear infections were also classified by this system and phage typed.

Interest in coagulase-negative staphylococci is increasing because of their roles as pathogens in certain clinical conditions and their marked resistance to antibiotics (3, 4). The ubiquity of these organisms does present problems when one is faced with making a decision as to whether their isolation in the clinical laboratory represents true infection or merely contamination. Studies have shown that coagulase-negative staphylococci are a heterogeneous group of microorganisms, and many investigators have identified subgroups with them (1, 2). Recent systematic studies by Kloos and Schleifer (6) of coagulase-negative staphylococci as normal flora from humans have suggested the classification of these organisms into nine species based on morphological, physiological, and biochemical characteristics. One additional species was proposed for coagulase-negative staphylococci from animals (7). Currently, only two species (*Staphylococcus epidermidis* and *Staphylococcus saprophyticus*) are recognized in the 8th edition of *Bergey's Manual* (2). Because of our research in the development of a phage typing set for *S. epidermidis* (9, 10), we thought it of interest to determine the typability of the 10 species proposed by Kloos and Schleifer with our typing set, which has been used for cultures classified as *S. epidermidis* by currently accepted tests, and to correlate the phage-typable cultures with the biotypes of Baird-Parker established for *S. epidermidis* (2). We also report on the use of the Kloos and Schleifer classification scheme in a phage typing study.

Ten isolates each of *Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus capitis, Staphylococcus simulans, S. epidermidis, Staphylococcus hominis, S. saprophyticus, Staphylococcus cohnii* subsp. A, *Staphylococcus xylosus, and Staphylococcus sciuri*, and five isolates of *S. cohnii* subsp. B were obtained from W. E. Kloos of North Carolina State University, Raleigh, N.C. These had been classified according to the parameters established by Kloos and Schleifer (6). Procedures used for biotyping were the same as reported previously (4). The phages used were the 11 (29, 68, 95, 108, 112, 113, 113A, 127, 171A, 188, and 207) phages we have used in previous studies (9, 10). All typing was done with phages at 100 times the routine test dilution. Reactions were recorded only if the degree of lysis was equal to 2+, or greater than the amount of bacterial growth within the drop of phage.

The results of phage typing 105 cultures of coagulase-negative staphylococci classified by Kloos and Schleifer (6) are shown in Table 1. Of the 105 cultures typed, only 11 (10.5%) were typable. When separated into the 10 proposed species, the percentages of typable cultures within each species were as follows: *S. haemolyticus*, 10%; *S. warneri*, 10%; *S. capitis*, 30%; *S. simulans*, 10%; *S. epidermidis*, 50%; *S. hominis*, 0%; *S. saprophyticus*, 0%; *S. cohnii* subsp. A and B, 0%; *S. xylosus*, 0%; and *S. sciuri*, 0%. Interestingly, all three cultures of *S. capitis* were lysed by phage 188, although no information regarding their sources was available. The 50% typability of cultures classified by Kloos and Schleifer as *S. epidermidis* agrees favorably with our previous reported typability of 49.9% for 425 cultures of *S. epidermidis* from five different regions in the United States, although our identification of this species was according to currently accepted criteria (2, 9). If the "two strong differences" rule is used in the interpretation of the lytic patterns of cultures from a common outbreak, two (JRM1 and MK258) of the cultures of *S. epidermidis* can be considered related. However, no information was available regarding the sources of these two cultures. Of the 11 phage-typable cultures, 7 belonged to biotype 1 and 4 belonged to biotype 4 (Table 1). Of the 10 cultures of *S. epidermidis* biotyped, 7

TABLE 1. *Phage types and biotypes of five different species of coagulase-negative staphylococci*

Strain no.[a]	Species	Phage type	Biotype
KL109	*S. haemolyticus*	29, 68, 95, 127, 171A, 207	1
JM25	*S. warneri*	113, 113A, 127, 171A, 188	4
HM11	*S. capitis*	188	4
DBM213	*S. capitis*	188	4
ATCC 27843	*S. capitis*	188	4
HK12	*S. simulans*	29	1
RK13	*S. epidermidis*	68, 171A	1
SM225	*S. epidermidis*	112	1
JRM1	*S. epidermidis*	95, 108, 127, 207	1
MK258	*S. epidermidis*	68, 95, 108, 127, 207	1
KL280	*S. epidermidis*	29, 68, 95, 108, 112, 113, 113A, 171A, 188, 207	1

[a] Courtesy of W. E. Kloos, North Carolina State University.

belonged to biotype 1, the biotype most frequently isolated from hospitalized individuals (1, 4).

We have used the Kloos and Schleifer classification scheme in one study involving the role of *S. epidermidis* in middle ear infections. We received from K. Wicher (Erie County Laboratory, Buffalo, N.Y.) 25 isolates of this organism, which had been obtained from six patients. These represented isolates from ear fluid, ear drum skin, and other surrounding sites. Only 3 (12%) of the 25 cultures were typable with our phages. Interestingly, when the classification scheme of Kloos and Schleifer was applied to these cultures, only 12 were classified as *S. epidermidis*, and these included the three typable cultures. It could not be determined, however, whether the cultures identified as *S. epidermidis* by the Kloos and Schleifer system were more important in the etiology of the middle ear infections than the non-*S. epidermidis* cultures.

Whether the proposed classification scheme of Kloos and Schleifer for coagulase-negative staphylococci can be used to advantage in a clinical setting is, at this time, uncertain. It has been used by several investigators and found to be reliable and of obvious value in ecological studies (5, 8). Our phages were isolated from lysogenic cultures of staphylococci classified as *S. epidermidis* by the currently accepted tests (2). Apparently, the use of this traditional classification system resulted in the isolation of phages which have a greater ability to lyse cultures classified as *S. epidermidis* by Kloos and Schleifer than the non-*S. epidermidis* cultures identified by their system, and it would be advantageous in epidemiological studies with our typing phages. However, whether this classification system is effective in detecting cultures that are more likely to be pathogenic or important clinically has not been determined fully. In one recent study where the Kloos and Schleifer classification system was utilized with coagulase-negative staphylococci from human infections, the most frequently isolated organisms were identified as *S. epidermidis* (8). Obviously, more studies using this classification system are needed with coagulase-negative staphylococci from clinical sources to determine its usefulness in identifying potentially pathogenic species.

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