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Staphylococcus epidermidis Bacteriophages from the Anterior Nares of Humans[▽]

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The role of virulent bacteriophages in staphylococcal colonization of the human anterior nares is not known. This report of lytic bacteriophages against *Staphylococcus epidermidis* in the anterior nares of 5.5% of human subjects ($n = 202$) suggests their potential role in modulating staphylococcal colonization in this ecological niche.

Microbial colonization of the anterior nares of humans is expected to be a dynamic process modulated by a variety of competing bacterial species, viruses, and health conditions (19). Limited reports (8, 25) have suggested that the anterior nares represent a complex ecological niche in which the microbial species interaction may well determine the colonization and prevalence of staphylococci, one of the most common flora in the human anterior nares. *Staphylococcus epidermidis* represents 8% to 43% of staphylococcal abundance in the nares (14), while *Staphylococcus aureus* represents about 25 to 30% (15). The presence of *S. epidermidis* in the anterior nares has been shown to reduce *S. aureus* colonization (14, 18). *S. aureus* colonization of the anterior nares is additionally influenced by the host, bacterial diversity, and abiotic factors (20, 22). The role of lytic phages in this setting, if any, has not been described.

Virulent phages, which lyse their host bacterium as a normal part of their life cycle, have been isolated from several human microenvironments where the host bacterium is likely to be present, including the oral cavity (23, 3), the vagina (17), stool (12), and the gut (5, 6). As far as *S. epidermidis* phages are concerned, nearly all reports in the literature are linked to temperate phages, which integrate their genome into that of the bacterial chromosome (4, 16, 18, 21, 24, 11). A recent study reported the failure to isolate *S. epidermidis* phages from skin, mucous surface exudates, and breast milk (16). To our knowledge, virulent bacteriophages have never been reported from the anterior nares of humans. This study attempted to determine if virulent bacteriophages against *S. aureus* and *S. epidermidis* are found in the anterior nares and if they play a role in modulating staphylococcal colonization.

We screened for the presence of lytic phages against *S. epidermidis* and *S. aureus* in the anterior nares of ambulatory patients and health care environment workers of a Midwestern rural multispecialty clinic. The study design and methodological details are described elsewhere (2). Briefly, the anterior nares of 202 human subjects were swabbed with dry swabs. Each swab was suspended in 3 ml of Trypticase soy broth and incubated at room temperature for 20 min. Phages against *S. epidermidis* and *S. aureus* were detected by a modified plate assay technique (2) using *S. epidermidis* ATCC 35983 and *S. aureus* ATCC 29213, respectively, as host strains. A 30-μl aliquot of each of the nasal swab filtrate sample was spotted onto each strain (2). The 202 samples were also pooled into 8 groups, numbered I to VIII (Table 1), of 25 samples each, and 50-μl aliquots of each pool were rechecked for the presence of phages on Trypticase soy agar (TSA) plates incubated at 37°C for 48 h.

Lysis zones were observed only on TSA plates containing *S. epidermidis* and not on those containing *S. aureus*. Putative *S. epidermidis* lytic phages were recovered from these lysis zones with a sterile truncated tip and placed into 500 μl of ammonium acetate (100 mM, pH 7.0). Plates of pooled samples exhibited large lysis zones rather than individual plaques, most likely because of the larger aliquot volume loaded. Phages were allowed to diffuse (60 min) at room temperature. Each sample was then centrifuged (13,000 × *g*, 2 min), and the supernatants were transferred to a fresh microcentrifuge tube and centrifuged again at 23,500 × *g* for 1 h at 4°C. The phage pellet was washed and observed with an electron microscope as described elsewhere (13). The specimens were observed at 80 kV using a JEOL 1230 transmission electron microscope located at the Pavillon Marchand of the Université Laval.

Overall, 11 individual samples out of 202 (5.4%) nasal swabs were positive for the presence of virulent bacteriophages against *S. epidermidis* ATCC 35983. The presence of phages was confirmed by the observation of clear phage plaques on TSA plates, as well as virion particles, with the electron microscope (Fig. 1). Figure 1 shows representative transmission electron micrographs of the seven bacteriophages: four *Podoviridae* phages (Fig. 1A) and three *Siphoviridae* phages (Fig. 1B). In two cases, pooled samples showed the presence of phages

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TABLE 1. *S. epidermidis* virulent bacteriophages detected by plate plaque assay

Samples	Individual sample(s) containing phages[a]	Sample pool	Pooled samples with phages[b]	Mean capsid (tail) length [width] ± SEM (nm)	
				Podoviridae	*Siphoviridae*
1–25	None	I	Negative		
26–50	None	II	Positive	37 ± 2 (28 ± 2)	
51–75	26, 60	III	Positive	36 ± 1 (24 ± 1)	
76–100	None	IV	Positive	36 ± 1 (28 ± 1)	
101–125	111	V	Positive		73 ± 2 (366 ± 4 [10 ± 1])
126–150	129, 131, 146	VI	Positive	41 ± 1 (38 ± 1)	69 ± 1 (362 ± 1 [12 ± 1])
151–175	154, 162	VII	Positive	37 ± 5 (30 ± 4)	74 ± 1 (338 ± 1 [10 ± 1])
176–202	177, 192, 195	VIII	Positive	40 ± 1 (31 ± 2)	

[a] Individual samples tested for the presence of virulent phages by plaque assay.
[b] Pooled samples tested for the presence of virulent phages.

(pools II and IV, Table 1), while no phages were detected in the individual samples of those pools. This was probably due to the use of a larger aliquot of the pooled samples on the TSA plates for phage detection. Hurdles to the isolation of phages from individual samples from the anterior nares included low sample volumes (each was obtained from a single swab taken from the anterior nares of an individual patient) and low phage numbers. Since a lysis zone from pooled extracts was observed under an electron microscope, it was not surprising to see a mixture of different phages in some of the samples. Seven of the positive samples were from males, and four were from females. Coagulase-negative staphylococci were ubiquitous flora in these subjects (data not shown), while only 24% of the samples grew *S. aureus* (2). None of the samples yielded phages against *S. aureus*. The presence of virulent phages against *S. epidermidis* but not *S. aureus* was surprising but could be linked to the predominance of *S. epidermidis* host bacteria in the anterior nares of the subjects at the time of sampling.

All of the *S. epidermidis* phages found in the anterior nares possessed a tail and thus belong to the *Caudovirales* order (1). Phages belonging to the *Podoviridae* family (short tail, Fig. 1A) were more predominant, as they were found in all but one of the positive pools (II, III, IV, V, VI, VII, and VIII). Our PubMed searches failed to identify any reports of *Podoviridae* phages infecting *S. epidermidis*, and thus, this study may represent the first description of podophages for this staphylococcal species. The sizes of the phages observed are presented in Table 1.

Phages belonging to the *Siphoviridae* family (long noncontractile tail) (Fig. 1B) were also found but only in three positive pools (V, VI, and VII). Based on morphological criteria, pool VI contained the most diversified phages (Table 1). Of note, the *S. epidermidis* phages belonging to the *Siphoviridae* family observed here had a very long tail (>300 nm), twice the length of those of the five most recently characterized temperate *S. epidermidis* siphophages (10, 16), as well as that of the sole *S.*

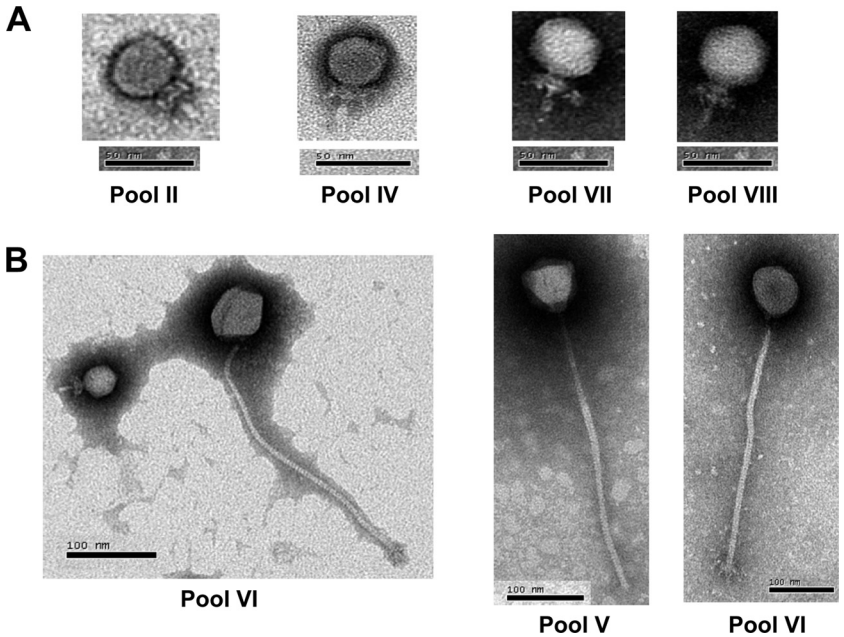

FIG. 1. Transmission electron micrographs of virulent bacteriophages against *S. epidermidis* ATCC 35983 isolated from the anterior nares of humans. Panels: A, *Podoviridae*; B, *Siphoviridae*. Roman numerals represent pooled samples (see Table 1 and text). Two pictures from pool VI are shown in panel B, one of a *Siphoviridae* phage alone and the other of a *Siphoviridae* and a *Podoviridae* phage.

epidermidis phage available at the Félix d'Hérelle Reference Center for Bacterial Viruses (http://www.phage.ulaval.ca).

The lysis activity of diverse phages against *S. epidermidis* ATCC 35983, a known antibiotic-resistant, moderate slime-producing pathogen (7), suggests that they may be useful as antimicrobial agents (9) and that the human anterior nares may represent a reservoir of such novel natural antimicrobials. In conclusion, in this report, we describe the isolation of lytic phages from the anterior nares of humans against a very common human commensal and potential pathogen. We also document the lack of bacteriophages against *S. aureus* in our study. This study lays the foundation for future experiments to determine whether these phages play a modulating role in the competition between *S. epidermidis* and *S. aureus* in the microbial ecology of the anterior nares.

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