Phage Typing of Staphylococcus intermedius

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Staphylococcus intermedius, a coagulase-positive staphylococcal species, is a common canine pathogen and a rare human wound pathogen. A total of 145 strains of S. intermedius (ATCC 29663, 4 reference strains, 4 human isolates, 44 canine infection isolates, and 92 isolates from canine gingiva) were screened for lysogenic phage by a modified Fisk method. Nineteen phage preparations were prepared for preliminary typing experiments. Lytic activity was observed on 93 of 145 (64.1%) isolates, yielding 44 lytic patterns with individual strains susceptible to one or more phages. Five phages lysed only a single strain, but lytic patterns varied from 1 to 11 lytic phages per isolate. A distinct lytic pattern did not separate canine or human wound isolates from canine gingival isolates. All human wound isolates fell into the two most common canine gingival or wound patterns; the single human nasopharyngeal isolate was not lysed by any phage. Twenty-two of 44 (55%) canine wound isolates and 65 of 92 (71%) gingival isolates yielded lytic patterns. Lysogenic phages are common in S. intermedius. This preliminary study suggests that phage typing may be a useful tool in distinguishing epidemiologically related strains.

Staphylococcus intermedius, a coagulase-positive staphylococcus, is a common component of the skin and oral or nasal flora of normal dogs, horses, and other lower animals including some birds (4). It is distinguishable from Staphylococcus aureus by its slow fermentation of mannitol, a negative acetoin reaction, and a positive β-galactosidase reaction in the API Staph Ident system (7). S. intermedius is also a common skin or wound pathogen in dogs (4) and an occasional pathogen in humans bitten by dogs (7).

Limited data in humans suggest that S. intermedius is only rarely cultured from the normal nasopharynx of humans (9). The ecology and epidemiology of this organism has not been defined, in part because of the lack of an adequate typing system. Phage typing systems for canine staphylococci similar to S. intermedius have been described previously by American (1) and French (3) investigators. Early phage sets consisted of four to five phages and were capable of typing 67 to 74% of canine isolates (1, 3), whereas traditional bovine and human phage sets were capable of typing less than 10% of canine staphylococci (11). Chinese and Japanese investigators have also described phage sets derived from presumptive S. intermedius isolates (i.e., S. aureus biotypes E and F) utilizing eight to nine phages (6, 10, 11). Although Shimizu and Kato (6) were able to classify 72.4% of their isolates in 13 lytic patterns, Wang (10, 11) was able to type only 10% of isolates in 12 lytic patterns. We describe our preliminary studies of phage typing utilizing 19 recently isolated phages against 145 S. intermedius strains.

MATERIALS AND METHODS

Bacterial strains. All but four isolates of S. intermedius were isolated from normal canine gingiva, wounds, or other clinical infections; four isolates were from human sources including three dog bite wounds and one nasopharyngeal culture (Table 1). All strains were identified by previously defined methods (8). S. aureus strains (n = 49) used for comparison were obtained from normal canines or from human clinical infections. Reference strains of S. intermedius were obtained from the American Type Culture Collection (ATCC) and those provided by Wesley Kloos, University of North Carolina. Additional ATCC reference strains for Staphylococcus sciuri, Staphylococcus aureus, Staphylococcus simulans, and Staphylococcus epidermidis were included for comparison.

Media. The liquid medium utilized for cultures to be typed was unsupplemented tryptic soy broth (BBL Microbiology Systems, Cockeysville, Md.). Tryptic soy broth supplemented with 400 μg of CaCl₂ per ml (TSBC) was utilized for cultivation of phage pools, phage propagation, and all phage titrations. Solid media for phage propagation and typing consisted of semisolid overlays of 0.7% Noble agar in tryptic soy broth which were dispensed in 4-ml amounts in capped tubes (1, 2). Basal layers of tryptic soy agar (BBL) were supplemented with 400 μg of CaCl₂ per ml (TSAC), sterilized, cooled to 50°C, and poured in 12-ml amounts in sterile petri dishes.

Isolation and purification of phages. A modified version of the classic Fisk method was utilized for phage isolations (2). Strains to be screened for lysogenicity were grown on a shaker (100 rpm) for 4 h at 37°C. One-milliliter samples of six to nine strains were pooled, incubated for 4 h at 37°C, and then centrifuged at 2,500 × g for 20 min. The supernatant fluids were spotted on seeded semisolid agar lawns of each of the single strains contained in the mixture. The lawns were prepared by inoculating 0.2 ml (4 drops) of a 4-h culture into a tube of melted and cooled (50°C) semisolid agar which was poured over a plate containing the hard basal layer.

Each phage was purified by three transfers of single plaques. Crude high-titer phage stocks were prepared by flooding an appropriately seeded plate with 4 to 10 drops of TSBC containing sufficient phage from the last purification transfer, incubated at 37°C for 18 h. The lysed plate was then flooded with 5 ml of calcium-supplemented tryptic soy broth and refrigerated for 24 h. The broth was removed, mixed

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gently, and centrifuged at 2,500 × g for 30 min and filtered through a Millex-GS filter (pore size, 0.22 μm; Millipore, Bedford, Mass.).

Preparation of stock phage. Tenfold serial dilutions (10⁻¹ to 10⁻⁵) of the crude phage suspension were prepared in TSAC, and each dilution was spotted on the homologous host strain. A 0.1-ml sample of the dilution that produced confluent lysis (and 0.1 ml of each dilution above and below this level) was inoculated into semisolid agar and overlaid to determine the concentration of phage which just produced confluent lysis of the entire plate. Five plates were overlaid with this dilution, incubated, and harvested as noted previously (crude phage stock preparation). Stock phage was serially diluted (10⁻¹ to 10⁻⁸) and spotted on homologous hosts. That dilution just producing confluent lysis constituted the routine test dose (RTD). Phage typing was done in duplicate with the RTD and 10× RTD for all phages (except K-4 and K-13) that failed to yield concentrations allowing the 10× RTD concentration to be employed. A lytic pattern with ≥50 plaques was considered a positive result. Approximately three-fourths of phage preparations were maintained at high titer for up to 6 months (4°C), while five phages stored conventionally exhibited a rapid decrease in titer. For these latter preparations, typing was accomplished by preparations of new phage for each experiment.

RESULTS

Twenty phages were isolated with preliminary lytic activity and used as possible typing phages (Table 2). Of 145 S. intermedius strains, 93 (64.6%) were lysed by at least one isolated phage, while 51 of 144 strains (31.5%) were phage resistant and showed no detectable lysis by any of the isolated phages. None of the 19 phages tested produced a lytic pattern on human S. aureus, canine or human coagulase-negative staphylococci, or reference isolates of S. aureus, S. sciuri, S. simulans, or S. epidermidis. Three strains (6.1%) of canine S. aureus isolates were lysed by a single phage (K-3) with ≥50 plaques in a single spot; all three isolates were from normal gingival or wound cultures. These three strains exhibited the only cross-species lysis observed.

Three of the four human wound isolates were found to be lysed by phages similar to those lysing canine gingival isolates (Table 3). The single human nasopharyngeal isolate was not lysed by any phage. Twenty-two of the 44 (55%) canine wound isolates displayed nine lytic patterns, while 65 of the 92 (71%) canine gingival isolates displayed 37 lytic patterns. S. intermedius ATCC 29633 had a unique lytic pattern (K-2 and K-8) not common to any other strain. Similarly, the lytic patterns against two Klos reference strains were unique (K-4 and K-8 and K-2, -11, and -20).

No distinct lytic pattern separated canine or human wound isolates from the lytic patterns of canine gingival isolates. However, 20 of the 44 (45.5%) canine wound isolates were susceptible to lysis by phage K-2, while only 27 of 92 (29.3%) of gingival isolates were lysed by phage K-2. Three phages, K-2, K-3, and K-4, lysed all human wound isolates; these three phages lysed from 25 to 45.5% and 18.7 to 29.7% of canine wound and gingival canine isolates, respectively.

### TABLE 2. Bacteriophage and adapted (mutant) bacteriophages from S. intermedius typing set (n = 19)

<table>
<thead>
<tr>
<th>Phage no./source strain</th>
<th>Original host source</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-1/87-302 (87-287)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-2/87-290 (87-287)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-3/87-347 (87-287)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-4/87-349 (87-287)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-7/87-362 (87-387)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-8/88-362 (87-346)</td>
<td>Canine infection</td>
</tr>
<tr>
<td>K-9/88-45 (87-364)</td>
<td>Canine infection</td>
</tr>
<tr>
<td>K-11/88-49 (87-367)</td>
<td>Canine infection</td>
</tr>
<tr>
<td>K-13/89-29 (89-58)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-15/88-64 (89-34)</td>
<td>Reference (Kloos)</td>
</tr>
<tr>
<td>K-16/89-70 (89-40)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-17/89-77 (89-79)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-18/89-83 (89-82)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>aK-18/89-83 (89-82)*</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-19/89-8 (89-15)</td>
<td>Human NF (Davis)</td>
</tr>
<tr>
<td>K-20/89-12 (89-19)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>aK-20/89-12 (89-19)*</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-22/89-67 (89-40)</td>
<td>Canine gingiva</td>
</tr>
<tr>
<td>K-23/89-89 (89-34)</td>
<td>Canine gingiva</td>
</tr>
</tbody>
</table>

* Adapted phages.

### TABLE 3. Lytic activity of 11 phages by isolate source of S. intermedius

<table>
<thead>
<tr>
<th>Phage*</th>
<th>Canine gingiva</th>
<th>Canine wound</th>
<th>Reference</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-2</td>
<td>27 (29.7)</td>
<td>20 (45.5)</td>
<td>1 (25)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>K-3</td>
<td>24 (26.4)</td>
<td>13 (29.5)</td>
<td></td>
<td>2 (50)</td>
</tr>
<tr>
<td>K-4</td>
<td>17 (18.7)</td>
<td>11 (25.0)</td>
<td>1 (25)</td>
<td></td>
</tr>
<tr>
<td>K-5</td>
<td>18 (19.8)</td>
<td>13 (29.5)</td>
<td>1 (25)</td>
<td></td>
</tr>
<tr>
<td>K-8</td>
<td>3 (3.3)</td>
<td>5 (11.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-9</td>
<td>6 (6.6)</td>
<td>11 (25.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-11</td>
<td>3 (3.3)</td>
<td>2 (4.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-16</td>
<td>1 (1.0)</td>
<td>6 (13.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-19</td>
<td>2 (2.2)</td>
<td>6 (13.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aK-20</td>
<td>2 (2.2)</td>
<td>5 (11.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-23</td>
<td>2 (2.2)</td>
<td>6 (13.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although overall canine wound isolates were less frequently lysed by any phage compared with gingival isolates, 6 phages (K-20, K-11, K-19, aK-20, K-20, and K-23) more frequently lysed canine wound isolates than canine gingival isolates (13 versus 52% for gingival versus wound isolates, respectively).

DISCUSSION

In this preliminary evaluation of S. intermedius-derived lysogenic bacteriophages, approximately two-thirds of S. intermedius isolates from various sources displayed lytic patterns. However, there appeared to be a more limited number of lytic patterns associated with canine and human wounds. Also, certain phage patterns were almost exclusively associated with isolates from dogs without disease (i.e., canine gingiva), whereas lysis by certain phages was observed more frequently among isolates from clinically infected canine tissues. These preliminary results indicate that canine wound isolates may be a unique subset of normal canine gingival isolates, thereby suggesting an associated pathogenicity with certain phage types. Although the numbers of isolates were very small, all three isolates from dog bite wound infections in humans were contained within the patterns observed among the canine gingival isolates. Phages isolated in these studies appeared to be highly specific for S. intermedius, since few related species were found to be susceptible to lysis.

In this preliminary study, we have not examined in detail the reproducibility of the lytic patterns or the stability of S. intermedius bacteriophage preparations. These are crucial issues to be defined before phage typing could be recommended as a practical or useful tool for defining S. intermedius epidemiology. Alternative methods of epidemiologic typing, such as plasmid typing, do not appear to be practical, since these staphylococci infrequently have definable plasmids. We have submitted 73 strains to plasmid typing and found extrachromosomal DNA bands in only six isolates (5). The stability of approximately two-thirds of the phage preparations examined here was satisfactory at 4°C for up to 6 months, maintaining high titers of phage activity. In approximately one-third of strains, the bacteriophages could be maintained only in the cryopreserved host.

In the future, more specific and stable phages for S. intermedius must be identified and the optimal conditions for storage and maintenance of phage preparations should be defined. In addition, the interexperimental variation and stability of individual lytic patterns must be determined. The specificity of the phages isolated in this study appeared to be highly specific for lytic activity against S. intermedius. Prospective phage typing of S. intermedius isolated from various canine and human anatomic sites may identify specific pathogenic subtypes. Prospective epidemiologic studies of canine outbreaks of infections due to S. intermedius are needed to determine the feasibility of using phage typing methods as tools in studying epidemiology.

REFERENCES