Experiences and Observations with the Typing of Staphylococcus aureus Phage 94

R. V. MARRARO1* AND J. L. MITCHELL2

United States Air Force School of Aerospace Medicine, Aerospace Medical Division, Brooks Air Force Base, Texas 78235

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During a 28-month period, 5,078 cultures from a variety of anatomical sites were received for staphylococcal phage typing. Of these, 503 (10%) were not suitable for the procedure requested. Of the 4,575 viable cultures, 1,030 (23%) of the microorganisms were nontypable at both the routine test dilution (RTD) and at 100 × RTD. Of the 3,545 typable organisms, 3,061 (86%) were lysed at RTD, whereas 484 (14%) were typed only at 100 × RTD. Observations pertaining to the typing efficacy of staphylococcal phage 94 indicate that 651 (18%) of the typable microorganisms were lysed only by phage 94 at RTD or at 100 × RTD. Without the addition of this new phage to the international basic set, the number of nontypable strains (1,030 or 23%) would have been 1,681 (39%). Data regarding the geographic distribution of Staphylococcus aureus phage 94 point to the occurrence of the host strain in 13 (68%) of 19 states and 18 (62%) of the 29 hospitals submitting specimens to this laboratory. The assumed origin and speculated mode of dissemination of this microorganism are discussed.

MATERIALS AND METHODS

Isolates. Specimens (5,078 cultures) recovered from a variety of anatomical sites and identified as S. aureus by the submitting laboratories were received from military field medical facilities throughout the United States. These microorganisms were confirmed as S. aureus at this facility by means of morphological, tinctorial, and enzymatic parameters utilizing standard techniques. Selected single colonies from pure cultures were identified according to phage type.

Phage typing. S. aureus isolates were phage typed by standard methods (6) at a routine test dilution (RTD) or, when applicable, at concentrations of 100 × RTD. Phages used for typing staphylococcal strains were those of the currently accepted international basic set: 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 42D, 81, and 187. Also included was the new staphylococcus phage 94 (4).

RESULTS

During the period encompassed by these observations (16 April 1971 to 15 August 1973), it was established that 503 (10%) of the 5,078 total specimens submitted were not suitable for phage typing. Forty-five (<1%) of the cultures were nonviable and 458 (9%) of the remainder were not S. aureus coagulase positive (Table 1).

The 4,575 cultures confirmed as S. aureus were subjected to standard phage-typing procedures. A total of 1,030 (23%) of these microorganisms were nontypable either at the RTD or at
TABLE 1. Cultures of S. aureus received for phage typing from 16 April 1971 to 15 August 1973 (5,078 strains)

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Total no.</th>
<th>No. nonviable</th>
<th>No. not S. aureus</th>
<th>No. typable at RTD*</th>
<th>No. typable only at 100 x RTD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonviable/not S. aureus</td>
<td>503 (10%)</td>
<td>45 (&lt;1%)</td>
<td>458 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontypable</td>
<td>1,030 (23%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typable including phage 94</td>
<td>3,545 (77%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typable only with phage 94</td>
<td>651 (18%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* No lytic reaction.
* Nontypable at the RTD.

100 x RTD (Table 1). The remaining 3,545 (77%) cultures were typable by the system employed. At the RTD, 3,061 (86%) specimens were susceptible to one or more of the phages utilized, whereas 484 (14%) cultures were typable only at 100 x RTD.

Staphylococcus phage 94 has been employed by this facility with the international basic set of phages since 16 April 1971, and it has been a useful addition; 651 (18%) of the typable staphylococci were susceptible only to this new phage either at the RTD or at 100 x RTD (Table 1).

Cultures were received from 29 different field medical facilities located in a number of geographic areas (Fig. 1). However, S. aureus phage 94 was recovered from only 18 of these submitting facilities and in the locations noted in Fig. 1. Five of the major hospitals, each located in a different geographic area and/or state, were among the most constant contributors of specimens for phage typing. Phage 94 was first isolated on 16 April 1971 from the Wilford Hall USAF Medical Center and was recovered routinely thereafter. Specimens were received on a daily to weekly basis from the other major hospitals, but this organism was not recovered from another hospital until 25 June 1971 at the USAF Medical Center Keesler. It was identified at the USAF Regional Hospital Carswell on 30 August 1971, at the USAF Hospital Elmendorf on 18 November 1971, and at the USAF Regional Hospital Sheppard on 21 April 1972. The isolation rates of S. aureus phage 94 from these five installations ranged from 13% to 43% (Table 2). The remaining 13 hospitals were sporadic contributors and, although S. aureus phage 94 was recovered from each, data were not sufficient to form valid conclusions.

DISCUSSION

Since the discovery of bacteriophages, made independently by Twort in England and D’Herelle in France in 1916-1917, a number of bacterial viruses have been adopted for use in current diagnostic methodology. Bacteriophage typing has been a useful epidemiological tool and has been utilized in typing Salmonella, Shigella sonnei, Pseudomonas aeruginosa, Streptococcus pyogenes, and Staphylococcus aureus. The organisms most frequently phage typed are the staphylococci, and the literature contains ample material of historical significance (1, 10, 11). Since bacteriophage typing is not a routine procedure, its use should be restricted to the analyses of those specimens collected during overt epidemiological outbreaks. The use of this technique in tracing the source and routes of spread of hospital-acquired infections has been most effective. The observations resulting from the 28-month period of the study discussed here have been of significant value in the epidemiological surveillance of nosocomial disease in military medical facilities.

The 4,575 cultures confirmed as S. aureus represented isolates from a variety of clinical sources. It was established that 1,030 (23%) of these strains were nontypable. Consideration was given to three concepts that might be employed singly or in combination to reduce the percentage of nontypable strains. First, an expansion of the international basic set of phages might be considered, i.e., incorporation of additional available phages isolated from human sources (2). Second, it is conceivable that some of the nontypable strains might be susceptible to phages of domestic animal origin, especially those recovered from canine sources (3). Third, the determination of antimicrobial resistance patterns of refractile strains might be of additional use. This latter technique has been employed with increasing frequency, and investigators have noted a variety of patterns displayed by staphylococci (8). These suggested methodologies, used in conjunction with the currently accepted basic set of typing phages, may be the additional tools necessary in the
epidemiological investigation of presently refractile staphylococcus strains.

For the most part, the data derived from the phage typing of the 3,545 staphylococcal strains were considered to be of value in the epidemiological surveillance of nosocomial infections. However, with 86% (3,061) of the specimens typable at the RTD and 14% (484) typable only at 100 × RTD, the question arises as to the desirability of initially typing strains at both the RTD and 100 × RTD. Considering the cost factors, however, it seems more reasonable to restrict the use of the higher concentration for phage typing to those staphylococcal strains refractile to bacteriophage at the RTD.

Our isolation of a new and useful typing phage has been reported elsewhere (4). This phage, originally designated as WH-1, was assigned by the Subcommittee on Phage Typing of Staphylococci of the International Committee on Nomenclature of Bacteria in 1972 (10). The phage was tested against the 4,575 specimens of viable staphylococci received and of the 77% (3,545) cultures found typable, 18% (651) were lysed only by phage 94 at the RTD or at 100 × RTD. Thus, without the addition of typing phage 94 to the international basic set, the number of nontypable strains (Table 1) would have risen from 1,030 (23%) to 1,681 (39%). These findings attest to the efficacy of phage 94 in typing some heretofore nontypable staphylococcal strains.

From the geographic distribution (Fig. 1) of the 29 medical facilities submitting S. aureus for phage typing, it can be noted that 19 different states were involved from the East to the West coasts and Alaska. Furthermore, S. aureus phage 94 was present in 13 of the 19 states, but in only 18 of the 29 hospitals submitting specimens to this laboratory (Fig. 1). These data raise the question as to the possible origin and distribution of S. aureus phage 94.

The initial lysogenic host strain 94, from which phage 94 was isolated, was received from Wilford Hall USAF Medical Center, Lackland Air Force Base, Tex., on 16 April 1971. This strain was isolated from a routine bacteriological culture of the cord of a newborn, and neither the infant nor the mother experienced staphylococcal infection during hospitalization. Our assumption is, in light of this isolation and recovery, that the origin of phage 94 was the Wilford Hall USAF Medical Center. Such a hypothesis would appear reasonable since, in our experiences (Fig. 1), S. aureus phage 94 was not recovered from all hospital environments nor was it identified within all of the states serviced.

The speculated mode of dissemination of this microorganism from its possible origin at the Wilford Hall USAF Medical Center aroused a degree of curiosity. This facility is the largest medical treatment facility (1,000 operating beds) within the Air Force and serves as the worldwide reference center for active duty per-

Fig. 1. Geographic distribution of medical facilities submitting S. aureus for phage typing, and the localities of recovery of S. aureus phage 94 (16 April 1971 through 15 August 1973).
### Table 2—Continued

<table>
<thead>
<tr>
<th>Hospital name and location</th>
<th>Total no. of S. aureus submitted</th>
<th>No. of Phage 94 isolates</th>
<th>Rate of Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USAF Hospital George, George AFB, Victorville, Calif.</td>
<td>6</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>USAF Hospital Griffiss, Griffiss AFB, Rome, N.Y.</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>USAF Hospital F. E. Warren, F. E. Warren AFB, Cheyenne, Wyo.</td>
<td>3</td>
<td>2</td>
<td>67</td>
</tr>
</tbody>
</table>

The original report (4) indicated that staphylococcus phage 94 typed approximately 13% of the clinical isolates that were untypable with the basic series of typing phages. In our experience, this rate has increased to approximately 39% or 1,681 otherwise untypable cultures. The data in Table 2 indicate that four of the major hospitals experienced an average rate of recovery of approximately 17% for staphylococcus host strain 94 within their environments. However, the fifth major hospital experienced a 43% rate and, at this point, it may be speculated that an unusually high number of personnel transfers occurred from Lackland AFB or from other medical facilities harboring this strain to Carswell AFB during the period of these observations. Carswell AFB was not considered as the origin of this new strain because the recovery of the host organism there did not occur until 4 months after the first isolation at Lackland AFB and at several other medical installations outside of the state of Texas. Further observations are being conducted in our laboratories, and perhaps as additional data are compiled the origin and dissemination of *S. aureus* phage 94 will become more apparent.

**LITERATURE CITED**


