PHAGE TYPING OF *STAPHYLOCOCCUS AUREUS*

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The lysis of *Staphylococcus aureus* by bacteriophage was first reported by Twort (1915). It was not until 1942 that Fisk used bacteriophages for the identification of strains of this organism. He described a technique for the detection of lysogenic staphylococci and propagation of phages isolated from them. For the identification of strains of *Staph. aureus* he used undiluted phage suspensions. The number of different types Fisk identified was limited and strains regarded as of one type were possibly not always identical, but very closely related strains.

Wilson & Atkinson (1945) extended the work of Fisk and prepared eighteen lytic phage filtrates which enabled them to identify a high proportion of staphylococci. They, unlike Fisk, used diluted phage filtrates for phage sensitivity tests. The dilutions, which they termed 'test dilutions', were determined by titration of the phage filtrates on their propagating strains of staphylococci. The highest dilution of each phage necessary to cause confluent lysis of its propagating staphylococcus was regarded as the test dilution.

Wilson & Atkinson's technique, with modifications, is used by those at present engaged in phage typing staphylococci. Some employ phages in addition to the set originally isolated by Wilson & Atkinson. In this Unit thirty-two phages are used.

The object of this investigation was to reduce the manipulations at present necessary to type strains of *Staph. aureus* by phage, by the investigation of the effect of pooled phages and by their use for initial screening tests in routine phage typing.

Before adopting phage pools for routine use, certain investigations were necessary. It had to be shown that the components of all pools of phages when brought into contact with staphyloccoci behave as single phages, that staphylococci which have adsorbed phages to which they are resistant still undergo lysis when mixed with phages to which they are normally susceptible, and that a pool of phages will lyse strains of staphylococci which are lysed by any one of its components.

It has been considered that broth cultures of staphylococci used for phage testing should be previously incubated at 37° C. for 6 hr. or 30° C. for 18 hr. If it could be shown that such cultures remained satisfactory for testing after they had subsequently remained at room temperature for 24 hr., fresh subcultures for each testing would then be unnecessary.

The work accordingly fell into the following eight sections:

(1) A determination of the amount of phage which was adsorbed by 'resistant' staphylococci in 18 hr. at 37° C. The term 'resistant' is used to indicate that a strain of staphylococcus was not lysed by the phage with which it was tested.

(2) An attempt to demonstrate the effect of such inactive phage on staphylococci tested for phage sensitivity. A staphylococcus is said to be 'sensitive' to a phage if lysed by it.

(3) The growth of single and mixed phages of one group on separate propagating strains of staphylococci was investigated.

(4) This was repeated using unrelated phages.

(5) The respective lytic effects of each phage and of several pools of the phages on all the propagating strains were assessed. This showed whether the effect of each phage was the same when pooled as when used singly.

(6) The stability of single and pooled phages was determined; any incompatability between the phages would have been indicated in the tests.

(7) Phage pools were used in parallel with the standard technique for routine typing. This would have shown any discrepancies in the lytic results obtained from pooled phages.

(8) The phage reactions of many phage types of staphylococci were tested using broth cultures which were allowed to stand at room temperature for 24 hr., after previous incubation at 30° C. for 18 hr. These results indicated whether it was necessary to use broth cultures incubated for the same length of time as in the standard phage-typing technique.

MATERIALS AND METHODS

The phages used were as follows:

Wilson & Atkinson's set which consisted of phages 3A, 3B, 3C, 6, 7, 29, 31, 42B, 42C, 44, 44A, 47, 47A, 47B, 47C, 51, 52 and 52A. More recently isolated phages 29A, 31A, 31B, 42D, 42E, 47D, 53, 53A, 54 and 57 have been obtained from the Staphylococcal Reference Laboratory, Colindale, and phages 55, 69, 70 and 71 have been isolated from staphylococci by the author using the cross-culture method of Fisk. Numeration of phages is consistent with the Reference Laboratory practice.

The media used were Wright's nutrient broth, nutrient agar made by adding shred agar to this broth to give a concentration of 1·25% and Yeastrel agar (Heimer, G., personal communication).

Yeastrel agar

Yeastrel	3 g.	Sodium di-hyd. phosp.	3 g.
Eupeptone	5 g.	Shred agar	12·5 g.
Sodium chloride	3 g.	Water	1000 ml.

Heimer's original formula included 1 g. calcium chloride, but this has been omitted because it precipitated the phosphates. The medium as used was found to have a calcium content of 55 mg./l. (Baar, S., personal communication).

The above constituents were mixed and steamed to dissolve, made alkaline (pH 8·2–8·4) with 40% sodium hydroxide, filtered, adjusted to pH 7·4–7·6, bottled and autoclaved for 15 min. at a pressure of 15 lb./sq.in. For use the medium was melted and cooled to 50° C.; a concentration of 5% Fildes's peptic blood digest was added and plates were poured.

Phage filtrates of high titre were maintained by propagation at required intervals using a broth or agar-plate method. Techniques used for propagation of phage on agar plates and in broth and for phage titration and typing were those described by Williams & Rippon (1952).

EXPERIMENTAL

The adsorption of phage particles by staphylococci

Burnet & Lush (1935) reported the adsorption of phage particles by staphylococcal cells irrespective of whether these cells were subsequently lysed by the phage. Rountree (1947) showed also that dead staphylococcal cells could adsorb such particles, and adsorption was complete in 30 min. at 37° C. with an initial concentration of 5000 phage particles per ml.

Attempts were made to assess the adsorption of undiluted phage filtrates by the cells present in undiluted broth cultures of staphylococci.

Technique

One ml. amounts of undiluted phage filtrate were added to 1 ml. of broth and to 1 ml. of a broth culture of a staphylococcus resistant to that particular phage. The broth culture had previously been incubated at 37° C. for 6 hr. The phage particle concentration in each mixture was determined by the titration of aliquots of the supernatant fluids with the appropriate staphylococcus. The mixtures were incubated at 37° C. for 18 hr. and aliquots titrated similarly.

An arbitrary selection of seven phages and seven strains resistant to these phages was made. The phages were 7, 44, 3A, 29, 31, 51 and 6 and the strains were respectively PS 52A, PS7, PS47, PS3C, PS6/53A, PS3B and PS31/44. (PS52A is the strain used for propagating phage 52A; PS31/44 is the propagating strain for phages 31 and 44, etc.). These strains were shown not to be lysogenic for the strains subsequently used as indicator strains. The strains used to indicate the respective phage concentrations were the propagating strains for these phages.

Results

The titrations are shown in Table 1 and indicate the following:

The phage in each filtrate was stable when incubated with an equal volume of broth.

The staphylococci with which the phages 7, 44, 3A, 29 and 31 had been incubated had reduced the phage particle concentration almost completely. For example, the initial concentration of phage particles per ml. of phage 7 was about 5×10^7, while after incubation with strain PS52A it was 10,000 approximately.

Phage 51 was reduced in concentration by approximately 30% when incubated with PS3B. A few plaques were observed, however, when PS3B was tested with 1 : 1 dilution of phage 51. It was therefore probable that the comparatively slow rate of adsorption of phage 51 by PS3B was due to a proportion of PS3B cells being lysed and liberating phage particles while the remainder were adsorbing it. The rate of adsorption was apparently greater than the rate at which new phage particles were being liberated from susceptible cells.

Staphylococcus PS31/44 was completely resistant to phage 6, even when undiluted phage was tested. But only about 30% adsorption of phage 6 could be demonstrated; an explanation of this cannot be offered without further investigation.

Table 1. *The adsorption of phages* 7, 44, 3A, 29, 31, 51 *and* 6 *by staphylococci* PS52A, PS7, PS47, PS3C, PS6/53A, PS3B and PS31/44 *respectively*

Mixture	Hours at 37° C.	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Phage 7 and broth	0	++	++	+	±	6
	18	++	+	±	21	0
Phage 7 and staph. PS52A	0	++	++	+	±	9
	18	12	2	0	0	0
Phage 44 and broth	0	++	++	++	+	27
	18	++	++	++	70	5
Phage 44 and staph. PS7	0	++	++	++	+	24
	18	±	35	4	0	0
Phage 3A and broth	0	++	++	++	+	51
	18	++	++	++	+	71
Phage 3A and staph. PS47	0	++	++	++	+	56
	18	±	104	12	1	0
Phage 29 and broth	0	++	++	+	±	7
	18	++	++	+	±	9
Phage 29 and staph. PS3C	0	++	++	+	±	11
	18	0	0	0	0	0
Phage 31 and broth	0	++	++	++	+	51
	18	++	++	++	+	30
Phage 31 and staph. PS6/53A	0	++	++	++	+	58
	18	+	36	3	0	0
Phage 51 and broth	0	++	++	++	+	82
	18	++	++	++	+	107
Phage 51 and staph. PS3B	0	++	++	++	+	72
	18	++	++	+	±	53
Phage 6 and broth	0	++	++	++	64	8
	18	++	++	++	62	8
Phage 6 and staph. PS31/44	0	++	++	++	51	6
	18	++	++	++	15	2

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques. Figures indicate number of plaques.

The phage sensitivity of staphylococci that have previously adsorbed phages to which they are resistant

That phage particles were adsorbed by resistant staphylococci was shown in the previous section. Attempts were next made to demonstrate the effect of such particles on the phage sensitivity of the staphylococci, for if interference could be demonstrated this might prevent pooled phages from lysing strains normally sensitive to one or more of the phages in the pool. The phage-coated cells obtained in the previous section were treated as follows:

Technique

A few drops of each suspension of staphylococci that had been coated with phage particles to which they were resistant were placed on agar plates and spread evenly over the surface. Normal broth cultures of the same strains of staphylococci were also spread on agar plates in a similar way. Phages appropriate for each strain were then titrated with each inoculated strain and the phage particle concentration per ml. of each phage calculated.

The results are shown in Table 2 and were as follows:

Results

The titrations of each phage on its normal and phage-coated propagating strain were alike.

It was concluded therefore that the activity of a phage on its propagating strain was not altered significantly by phage adsorbed on that strain.

Table 2. *Titrations of phages on staphylococci with and without previously adsorbed phage particles*

Phage	Staph.	Phage previously adsorbed	Dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
52A	PS52A	7	++	++	+	±	27
		0	++	++	+	±	23
7	PS7	44	++	++	+	±	31
		0	++	++	+	±	37
47	PS47	3A	++	++	++	++	+
		0	++	++	++	++	+
3C	PS3C	29	++	++	++	++	+
		0	++	++	++	++	+
6	PS6/53A	31	++	++	++	++	+
		0	++	++	++	++	+
3B	PS3B	51	++	+	±	21	0
		0	++	+	±	38	3
31	PS31/44	6	++	++	++	+	64
		0	++	++	++	+	73

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques. Figures indicate number of plaques.

The effect of pooling phages upon the lytic action of the phages in the pool

Most of the staphylococcal phages fall naturally into three groups, the members of which are commonly associated in forming patterns of lysis on strains of *Staph. aureus*. It would appear to be an advantage to pool such related phages for use in routine typing, because on the initial testing of a strain lysis should usually occur with one pool only. It would then be necessary to test the strain separately only against the components of the pool which had lysed.

One group, the 3A group, consisting of phages 3A, 3B, 3C, 51 and 55, gives very frequent cross-reactions. Sensitivity to almost every combination of these phages has been observed; some common patterns are 3B/55, 3C/51 and 3C/51/55.

Strains sensitive to each phage alone have also been found; strains sensitive to 3C alone were relatively common in material received by this Unit over one period. Cross-reactions between phages of this group and their propagating strains were assessed by titration of each phage on plates seeded with each strain.

Table 3. *The relative lytic effect of phages* 3A, 3B, 3C, 51 *and* 55 *on their propagating strains of staphylococci*

	Staphylococcus														
	PS3A Dilution			PS3B Dilution			PS3C Dilution			PS51 Dilution			PS55 Dilution		
Phage	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
3A	++	++	++	0	0	0	0	0	0	0	0	0	±	14	0
3B	+	0	0	++	++	++	0	0	0	0	0	0	++	++	+
3C	+	±	0	++	++	++	++	++	++	++	++	+	++	++	++
51	±	0	0	0	0	0	+	±	0	++	++	++	0	0	0
55	+	±	0	0	0	0	+	±	6	0	0	0	++	++	+

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques. Figures indicate number of plaques.

The results of the titrations (Table 3) indicated the following. The propagating strains for phages 3A, 3C, 51 and 55 were most susceptible to their appropriate phages. Strain PS3B was equally susceptible to phages 3B and 3C. Each of the five strains was lysed by at least one phage other than its own. The effect of pooled related phages upon the lytic action of the phages in the pool was assessed by incubating the pool in turn with each of the propagating strains for the phages in the pool. The results were compared with those obtained on incubating each phage separately with each of the propagating strains for the phages under investigation.

Technique

Dilutions in broth of the phages 3A, 3B, 3C, 51 and 55 were made such that their plaque counts on their propagating strains were alike. A sixth preparation, of pooled phages, was made such that it contained a similar concentration of each of these phages. One ml. of each phage suspension was mixed with 1 ml. of broth culture of each propagating strain for the five phages. Aliquots of the mixtures were centrifuged and the supernatant fluids titrated on the five strains. The mixtures were incubated at 37° C. for 18 hr., centrifuged and again titrated.

Results

The results of the titrations are shown in Table 4 and discussed below. Whenever the phage pool was incubated with a propagating strain for one of the phages in the pool, there was phage propagated which lysed this strain. This fact alone strongly indicated that the use of pooled related phages for a screening test in routine typing would give clear-cut results. There was also some evidence that the phage that propagated when the pooled phages were incubated with a strain was the same as that which propagated when these phages were incubated singly with that strain. For example, incubation of strain PS3C with the single phages showed

that only phage 3C propagated. Phage 3C has a titre of 10^3, 10^3, 10^2, 10^3 respectively with strains PS3B, PS3C, PS51 and PS55 (Table 3). The phage produced during incubation of PS3C with the pooled phages showed identical titres on these strains. The two phages therefore were probably identical.

There are phages which rarely associate with others to give patterns with strains of staphylococci. It might be an advantage for some purposes to pool such phages for use in typing.

Cross-titrations of many phages therefore were made on all their propagating strains to indicate which were the most specific. Phages 3A, 42E, 47 and 70 were found to be specific in that each lysed its own propagating strain but none of the propagating strains for the other three phages significantly (Table 5). These phages accordingly were investigated as previously described for the investigation of a group of related phages (Table 6).

The phages incubated singly or pooled multiplied with their propagating strains only. The phages to which any strain was resistant were adsorbed.

The stability of pooled phages in broth at 37° C.

The stability of pooled phages in broth at 37° C. was assessed by comparison of the stability of each of the phages in the pool.

Technique

Dilutions in broth of the phages 3A, 3B, 3C, 51 and 55 were made, and also a pool of these phages similar to those described in the previous section. Aliquots of each phage suspension were cross-titrated on the propagating strains for these phages before and after they had been incubated at 37° C. for 18 hr.

Results

The plaque counts obtained before and after incubation were alike (Table 7). The phages in this group therefore were stable in broth for at least 18 hr. at 37° C.

Similar results were obtained when phages 3A, 42E, 47 and 70 were assessed for stability at 37° C. (Table 8).

The lytic action of thirty-two phages at test dilutions, singly and when pooled

Broth cultures of each propagating strain of staphylococcus appropriate for the thirty-two phages used in this Unit, were incubated at 37° C. for 6 hr. Agar plates were seeded with each strain and allowed to dry at room temperature. Each phage was diluted in broth to its predetermined test dilution. The phages were mixed in several pools. The selection for each pool was made in the first instance by a survey of phage patterns which had previously occurred in routine typing results. The second selection was made by careful inspection of the phage reactions on 500 strains of *Staph. aureus* in routine typing.

The most satisfactory phage pools, as indicated in routine work, and the individual phages of those pools were tested on all the agar plates of each

Table 4. *Titrations of phages* 3A, 3B, 3C, 51 *and* 55 *before and after incubation with their propagating strains*

Staph. + Phage	Hours at 37° C.	Staphylococcus PS3A Dilution 10^{-1}	PS3A 10^{-2}	PS3A 10^{-3}	PS3B Dilution 10^{-1}	PS3B 10^{-2}	PS3B 10^{-3}	PS3C Dilution 10^{-1}	PS3C 10^{-2}	PS3C 10^{-3}	PS51 Dilution 10^{-1}	PS51 10^{-2}	PS51 10^{-3}	PS55 Dilution 10^{-1}	PS55 10^{-2}	PS55 10^{-3}
PS3A+3A	0	36	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	++	++	++	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+3B	0	±	0	0	70	0	0	0	0	0	0	0	0	±	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+3C	0	10	0	0	±	6	0	±	0	0	0	0	0	0	0	0
	18	±	31	5	±	0	0	±	0	0	±	±	0	±	0	0
PS3A+51	0	0	0	0	0	0	0	0	0	0	88	6	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+55	0	0	0	0	0	0	0	0	0	0	0	0	0	+	±	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+3A, 3B, 3C, 51 and 55	0	++	+	+	++	+	±	++	+	+	+	+	27	++	++	+
	18	++	++	++	+	0	0	+	±	0	±	5	0	±	0	0
PS3B+3A	0	29	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3B+3B	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0
	18	+	0	0	++	++	++	0	0	0	0	0	0	++	++	+
PS3B+3C	0	+	±	0	++	+	+	++	+	±	+	±	7	+	±	2
	18	++	++	++	++	++	++	++	++	++	++	+	+	++	++	+
PS3B+51	0	0	0	0	0	0	0	0	0	0	±	7	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3B+55	0	0	0	0	0	0	0	0	0	0	0	0	0	+	±	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3B+3A, 3B, 3C, 51 and 55	0	++	+	14	++	+	±	+	+	±	++	±	12	++	+	±
	18	++	±	0	++	++	++	++	++	++	++	+	±	++	++	+

PS3C+3A	0	43	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3C+3B	0	0	0	0	+	±	0	0	0	0	0	0	0	±	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3C+3C	0	0	0	0	+	±	0	+	±	0	±	0	0	+	±	0
	18	+	±	0	++	++	++	++	++	++	++	++	+	++	++	++
PS3C+51	0	0	0	0	0	0	0	0	0	0	74	3	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3C+55	0	0	0	0	0	0	0	0	0	0	0	0	0	±	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS3C+3A, 3B, 3C, 51 and 55	0	±	41	3	+	±	23	+	±	0	++	+	54	++	+	48
	18	+	0	0	++	++	++	++	++	++	++	++	+	++	++	++
PS51+3A	0	47	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS51+3B	0	0	0	0	+	±	3	0	0	0	0	0	0	±	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS51+3C	0	±	68	9	+	±	0	+	±	0	±	14	0	±	0	0
	18	±	0	0	+	±	0	+	+	0	±	41	4	0	0	0
PS51+51	0	0	0	0	0	0	0	0	0	0	42	0	0	0	0	0
	18	±	0	0	0	0	0	0	0	0	++	++	++	0	0	0
PS51+55	0	0	0	0	0	0	0	0	0	0	0	0	0	±	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS51+3A, 3B, 3C, 51 and 55	0	+	±	24	++	+	±	++	+	±	++	+	51	++	+	33
	18	+	±	0	++	38	5	++	+	±	++	++	++	55	6	0
PS55+3A	0	35	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS55+3B	0	0	0	0	±	4	1	0	0	0	0	0	0	±	0	0
	18	±	0	0	++	+	+	0	0	0	+	0	0	++	+	55
PS55+3C	0	±	5	0	±	12	2	+	±	0	+	±	29	±	0	0
	18	+	±	6	++	++	+	++	++	+	++	++	+	++	+	±
PS55+51	0	0	0	0	0	0	0	0	0	0	±	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS55+55	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0
	18	+	±	0	0	0	0	+	±	0	0	0	0	++	++	++
PS55+3A, 3B, 3C, 51 and 55	0	+	±	64	++	+	±	++	++	+	++	+	47	++	+	47
	18	+	+	±	++	++	+	++	++	+	+	±	±	++	++	++

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques. Figures indicate number of plaques.

propagating strain. The phages in the pools were, like the single phages, at their test dilutions.

The lytic actions obtained are shown in Table 9 which shows complete correlation between the phage pool effects and the effects of their single components.

Table 5. *The relative lytic action of phages* 3A, 42E, 47 *and* 70 *on their propagating strains of staphylococci*

	Staphylococcus													
	PS3A Dilution				PS42E Dilution				PS47 Dilution			PS70 Dilution		
Phage	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
3A	++	++	+	±	0	0	0	0	0	0	0	0	0	0
42E	0	0	0	0	++	++	++	++	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	++	++	++	±	0	0
70	0	0	0	0	±	0	0	0	0	0	0	++	++	++

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques.

The stability of single and pooled phages at their test dilutions

The single and pooled phages were kept at 0–5° C. and tested periodically in the following way. The phages were diluted in broth to their predetermined test dilutions and pools were made so that each phage was at its test dilution.

A large loopful of broth culture of each propagating staphylococcus which had been previously incubated at 37° C. for 6 hr., was spread over an agar plate. Two 6 in. diameter agar plates were adequate for the thirty-two inocula required for the single phages. When the cultures had dried at room temperature a loopful of each phage was placed on to its appropriate staphylococcus. Lysis was shown after subsequent incubation of the plates. The phage pools were tested in the same way. A loopful of each pool was placed on previously inoculated staphylococci appropriate for each phage component of the mixture. One agar plate 6 in. in diameter was sufficient for the five phage pools tested.

Semi-confluent or confluent lysis was recorded as ++ and regarded as satisfactory for typing purposes. When dilutions gave less than a ++ reaction they were replaced by fresh dilutions for use in routine typing.

These tests have been made on several batches of phages and phage pools over a period of some months. A typical result is shown in Table 10(*a*) and (*b*) and may be summarized as follows:

The phages that belong to the serological group A (Rountree, 1949) were stable for at least 1 month. The stability of the group B phages was variable but most remained stable only for 2–3 weeks.

Twenty-six of the phages were equally stable when kept singly or in pools. For example, phage 42C gave a ++ reaction with the appropriate strain PS42C for 2 weeks only. The phage pool 'S' which had 42C as one of its components, gave the same result with this strain. Subsequent tests of the phage and the phage pool showed a gradual decline in the activity of both, which ended in complete inactivity by the seventh week.

Table 6. *Titrations of phages* 3A, 42E, 47 *and* 70 *before and after incubation with their propagating strains*

		Staphylococcus											
		PS3A Dilution			PS42E Dilution			PS47 Dilution			PS70 Dilution		
Staph. + Phage	Hours at 37° C.	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
PS70+3A	0	++	+	47	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS70+42E	0	0	0	0	±	11	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS70+47	0	0	0	0	0	0	0	++	+	31	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS70+70	0	0	0	0	0	0	0	0	0	0	+	±	28
	18	0	0	0	0	0	0	0	0	0	++	++	++
PS70+3A, 42E, 47 and 70	0	++	+	0	+	±	31	+	38	0	+	±	113
	18	+	0	0	0	0	0	0	0	0	++	++	++
PS3A+3A	0	61	9	0	0	0	0	0	0	0	0	0	0
	18	++	++	++	0	0	0	0	0	0	0	0	0
PS3A+42E	0	0	0	0	+	±	7	0	0	0	0	0	0
	18	0	0	0	+	0	0	0	0	0	0	0	0
PS3A+47	0	0	0	0	0	0	0	45	2	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+70	0	0	0	0	0	0	0	0	0	0	26	1	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS3A+3A, 42E, 47 and 70	0	45	1	0	+	+	±	+	±	9	+	±	46
	18	++	++	++	+	50	4	+	10	0	0	0	0
PS42E+3A	0	±	14	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS42E+42E	0	0	0	0	+	±	15	0	0	0	0	0	0
	18	0	0	0	++	++	++	0	0	0	0	0	0
PS42E+47	0	0	0	0	0	0	0	+	±	24	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS42E+70	0	0	0	0	0	0	0	0	0	0	+	±	22
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS42E+3A, 42E, 47 and 70	0	±	0	0	++	±	57	+	±	16	+	±	42
	18	0	0	0	++	++	++	0	0	0	0	0	0
PS47+3A	0	++	+	56	0	0	0	0	0	0	0	0	0
	18	12	1	0	0	0	0	0	0	0	0	0	0
PS47+42E	0	0	0	0	±	8	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS47+47	0	0	0	0	0	0	0	++	+	24	0	0	0
	18	0	0	0	0	0	0	++	++	++	0	0	0
PS47+70	0	0	0	0	0	0	0	0	0	0	+	±	18
	18	0	0	0	0	0	0	0	0	0	0	0	0
PS47+3A, 42E, 47 and 70	0	32	2	0	++	±	51	+	±	61	±	±	41
	18	0	0	0	+	±	23	++	++	++	++	+	±

++ = confluent or semi-confluent lysis; + = numerous discrete plaques; ± = > 100 plaques. Figures indicate number of plaques.

The stability of the remaining six phages in the pools was difficult to assess accurately, because their propagating strains were also lysed by some of the other phages present in the pools. Phage 55, in particular, could not be assessed as it was present with phage 3B which also lyses strain PS55 to titre, and furthermore is a more stable phage. This difficulty was less with the other phage pools. The stability of the phage components of the pools could therefore be assessed.

Table 7. *Phage plaque counts of phages* 3A, 3B, 3C, 51 *and* 55 *before and after incubation in broth*

Phage	Hours at 37° C.	Staphylococcus PS3A	PS3B	PS3C	PS51	PS55
3A	0	35	0	0	0	0
	18	28	0	0	0	0
3B	0	0	62	0	0	0
	18	0	59	0	0	0
3C	0	0	0	9	0	0
	18	0	0	12	0	0
51	0	0	0	0	85	0
	18	0	0	0	76	0
55	0	0	0	0	0	31
	18	0	0	0	0	25
3A+3B+3C+51+55	0	7	12	2	10	13
	18	11	15	3	6	14

The standard error of a single plaque count is probably about 4. Experimental estimates of the standard error based on ten replicate observations at means 31·3, 87·2 and 112·4 were 3·8, 3·4 and 5·4 respectively.

Table 8. *Phage plaque counts of phages* 3A, 42E, 47 *and* 70 *before and after incubation in broth*

Phage	Hours at 37° C.	Staphylococcus PS3A	PS42E	PS47	PS70
3A	0	48	0	0	0
	18	35	0	0	0
42E	0	0	14	0	0
	18	0	17	0	0
47	0	0	0	59	0
	18	0	0	42	0
70	0	0	0	0	37
	18	0	0	0	41
3A+42E+47+70	0	14	17	65	8
	18	3	25	54	9

The use of phage pools in parallel with the standard method of phage typing

For 3 months phage pools were used in parallel with individual phages in routine testing of strains of *Staph. aureus*. A total of 511 strains were tested comprising 59 of the 3A group, 68 of the 29/52 group, 163 of the 6/47 group, 19 of the miscellaneous group and 202 of the non-typable group. The strains were equally well distinguished by both methods.

Table 9. *A comparison of the effect of single phages and phage pools on propagating strains of staphylococci*

	Phage pools					Single phages																													
						P					*Q*										*R*									*S*				*T*			
Staph.	*P*	*Q*	*R*	*S*	*T*	3A	3B	3C	51	55	6	7	47	47A	47B	47C	47D	53	53A	54	29	29A	31	31A	31B	52	52A	69	70	42B	42C	42D	42E	44	44A	57	71
PS3A	++	0	0	0	±	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
PS3B	++	0	0	0	±	0	++	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
PS3C	++	0	0	0	±	0	0	++	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±
PS51	++	0	0	0	0	0	±	0	++	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS55	++	0	0	0	++	0	++	±	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++
PS6/53A	0	++	0	0	0	0	0	0	0	0	++	0	++	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS7	0	++	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS47	0	++	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS47A	0	++	+	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
PS47B	0	++	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS42B/47C	0	++	0	++	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0
PS47D	0	++	++	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	++	0	0	0	0	++	±	0	0	0	0	0	0	0	0	0
PS53	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS54	0	++	+	0	0	0	0	0	0	0	0	++	0	0	0	±	+	++	++	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
PS29	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS29A	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PS31/44	0	++	++	0	++	0	0	0	0	0	0	±	±	0	0	0	+	0	++	0	±	0	++	0	0	±	0	0	++	0	0	0	0	++	0	0	0
PS31A	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0
PS31B	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0
PS52	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	±	0	0	0	0	0	0	0	0	0
PS52A	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0	0	0
PS69	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	±	0	0	0	++	0	0	0	0	0	0	0	0
PS70	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0
PS42C	0	++	++	++	++	0	0	0	0	0	0	±	+	0	±	±	+	0	++	±	+	0	++	±	0	±	+	0	++	±	++	0	±	++	0	0	0
PS42D	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	0
PS42E	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0
PS44A	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0
PS57	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0
PS71	0	0	0	0	++	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++

P = 3A, 3B, 3C, 51, 55. *Q* = 6, 7, 47, 47A, 47B, 47C, 47D, 53, 53A, 54. *R* = 29, 29A, 31, 31A, 31B, 52, 52A, 69, 70. *S* = 42B, 42C, 42D, 42E. *T* = 44, 44A, 57, 71.
++ = confluent or semi-confluent lysis; + = numerous numbers of plaques; ± = < 50 plaques.

Table 10(*a*). *The stability of test dilutions of single phages at* 0–5° C. *in nutrient broth*

Time	Phages																																		
(weeks)	3A	3B	3C	51	55	6	7	47	47A	47B	47C	47D	53	53A	54	29	29A	31	31A	31B	52	52A	69	70	42B	42C	42D	42E	44	44A	57	71
1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	+	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++	+
4	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	+	±	++	±	++	+	+	++	++	++	±	+	++	++	++	++	++	++	++	±
5	++	++	+	++	±	++	++	++	++	++	+	++	++	++	++	+	±	++	±	++	+	+	++	++	++	±	+	++	++	+	+	0
6	++	++	±	++	±	++	++	++	++	++	+	+	+	++	++	±	0	++	0	++	±	±	+	++	++	±	+	++	++	+	+	0
7	++	++	±	+	0	+	++	+	++	++	±	±	±	++	+	±	0	++	0	++	±	±	±	±	+	0	±	++	++	±	±	0
8	+	+	0	+	0	+	+	+	++	++	±	0	±	++	+	0	0	++	0	++	±	±	±	0	+	0	0	++	++	±	0	0

Table 10(*b*). *The stability of test dilutions of pooled phages at* 0–5° C. *in nutrient broth*

Time	Phage pool																																		
	P					*Q*										*R*									*S*				*T*							
(weeks)																																			
1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++	+
4	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	+	++	+	+	++	++	++	±	++	++	++	++	++	++	++	++	±
5	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	±	++	+	++	+	+	++	++	++	±	+	++	++	+	+	±
6	++	++	±	++	++	++	++	++	++	++	++	+	++	++	++	++	±	++	±	++	±	±	++	+	++	±	±	++	++	+	+	0
7	++	++	±	+	++	++	++	+	++	++	+	±	++	++	+	+	±	++	±	++	±	±	+	±	+	±	±	++	++	±	±	0
8	+	+	0	+	+	++	+	+	++	++	+	±	+	++	+	±	0	++	0	++	±	±	+	0	+	0	0	++	++	±	±	0

P = 3A, 3B, 3C, 51, 55. *Q* = 6, 7, 47, 47A, 47B, 47C, 47D, 53, 53A, 54. *R* = 29, 29A, 31, 31A, 31B, 52, 52A, 69, 70. *S* = 42B, 42C, 42D, 42E. *T* = 44, 44A, 57, 71.
++ = confluent or semi-confluent lysis; + = numerous numbers of plaques; ± = < 50 plaques.

The use of staphylococcal broth cultures for phage typing

In the standard method of phage typing *Staph. aureus*, broth cultures, previously incubated at 37° C. for 6 hr. or 30° C. for 18 hr., are used. That such broth cultures can be used for a second time, after they have been kept at room temperature for 24 hr., was shown thus:

Forty-two strains of staphylococci, including all the propagating strains, were selected, which gave different phage patterns. Five ml. of broth inoculated from a single colony of each strain were incubated at 30° C. for 18 hr. Agar plates inoculated from each culture were allowed to dry at room temperature. A small drop (0·01 ml.) of each of the thirty-two stock typing phages was placed on each inoculated plate, and allowed to dry at room temperature. The plates were then incubated for 6 hr. at 37° C. and left overnight at room temperature before results were recorded. This procedure was repeated with the staphylococcal broth cultures that had been left at room temperature (15–20° C.) for 24 hr.

The phage patterns of the strains at each testing were identical.

The technique now used for typing strains of Staphylococcus aureus *in this Unit*

Isolated colonies of *Staph. aureus* from blood agar plates are inoculated into 5 ml. amounts of broth and incubated for 6 hr. at 37° C. or 18 hr. at 30° C. Two loopfuls of each culture are spread over the surface of agar plates to cover five squares. Five strains are tested on one plate. When the cultures have dried each square is 'spotted' with a drop of a phage pool. Five phage pools are used and called respectively, *P*, *Q*, *R*, *S* and *T*. They are as follows:

P = 3A, 3B, 3C, 51 and 55.
Q = 6, 7, 47, 47A, 47B, 47C, 47D, 53, 53A and 54.
R = 29, 29A, 31, 31A, 31B, 52, 52A, 69 and 70.
S = 42B, 42C, 42D and 42E
T = 44, 44A, 57 and 71.

When dry the plates are incubated for 18 hr. at 30° C. or 6 hr. at 37° C. and then overnight at room temperature. If lysis is then observed, a fresh agar plate or portion of it is inoculated, using the original broth culture. The components of the phage pool or pools which had caused lysis are spotted separately into squares and the plate incubated in one of the above ways. In this way the phage pattern (or type) of the staphylococcus is obtained.

DISCUSSION

When a phage pool was incubated with a strain of *Staph. aureus* in broth culture, those phages multiplied which would do so when incubated separately with the same strain. In such an experiment, those phages which failed to multiply did not alter the behaviour of the rest of the pool, although reduction in their titres suggested that they were absorbed, or that they were inactivated by some constituent of the lysate (Rountree, 1947), derived from either the membrane or the contents of the cell.

The action of a phage on a staphylococcus is the same whether acting alone or mixed with other phages, whatever their relationship may be. Phages therefore can be used in pools for screening tests, making it unnecessary to test each strain with each individual phage. The phages normally required may be pooled into five mixtures for testing strains, which if lysed by a pool are subsequently tested with the separate phages in that pool. This method gives the same type or phage pattern of a staphylococcus as the standard typing method.

Adsorption of undiluted phage filtrates by resistant staphylococci is not complete in 18 hr. at 37° C. The amount adsorbed varies according to the phage and strain used. It was expected that since boiled suspensions of staphylococcal cells will adsorb phage (Rountree, 1947) most if not all the cells present in broth cultures would adsorb phage particles. The surface of a cell membrane is sufficiently large to allow several thousand phage particles to be attached to it. Since the ratio of cells to particles was approximately equal in the experiments, either it is for some reason impossible for many particles to be adsorbed on one cell or only a small proportion of cells adsorb. The latter explanation would account for the results obtained when phages were titrated on propagating strains that had previously adsorbed phage to which they were resistant.

SUMMARY

Phage adsorption by resistant staphylococci was studied.

Staphylococcal cultures previously allowed to adsorb phage to which they were resistant remained as sensitive to their appropriate phages as normal cultures of the same strains.

When pooled related and unrelated phages were incubated with their propagating strains, the results resembled those obtained when these phages were incubated separately with the same strains.

The lytic effects of thirty-two phages at test dilutions were tested singly and when pooled into five mixtures. All the phage-propagating strains and over 500 strains received for typing were tested. The results were in agreement.

Thirty-two phages at their test dilution in nutrient broth remained stable, whether pooled or not, at 0 to +5° C. for various times. Briefly summarized, serological group A phages were stable for at least 1 month, and group B phages were stable for only 2–3 weeks.

Broth cultures of staphylococci incubated for 18 hr. at 30° C. were as phage-sensitive as the same cultures when left at room temperature for 24 hr.

A new method of phage typing staphylococci by means of phage pools, as satisfactory as the standard method, and less laborious, is described.

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