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The Typing of *Salmonella dublin* by Means of Bacteriophage

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SUMMARY: By the use of six phages, 294 (94 %) of 306 strains of *Salmonella dublin* were classified into eleven types. The remaining twelve strains were insusceptible to all phages. Unfortunately, for the purposes of distinguishing strains of epidemiological interest, 66.9 % of the strains belonged to the same type. Acquired phage resistance was responsible for many of the strains being regarded as different phage types.

The phages were also active on *S. rostock*, *S. gallinarum* and on a small proportion of *S. pullorum* strains tested. They were not active on *S. enteritidis*. Three phages possessed a different range of lytic activity despite the fact that Bail's cross-resistance tests suggested that they were identical.

The ability to type a species of *Salmonella* satisfactorily by means of anti-O phages appears to depend mainly on a large proportion of the strains having acquired resistance to different phages.

In view of the importance of *Salmonella dublin* infection in cattle in this country, brought to light mainly by the work of Field (1948, 1949*a, b*), and of the fact that *S. dublin* is occasionally responsible for outbreaks of food poisoning in this country, some of which have been traced to infected cows' milk, it was considered worthwhile to search for a method of typing *S. dublin* by means of bacteriophage.

The main investigations were conducted in the hope of finding phages similar to those used by Craigie & Yen (1938) for typing *S. typhi*. Although no such phages were found, it was possible to classify strains of *S. dublin* to some extent with anti-O phages. When this work was nearing completion, Lilleengen (1950) published a classification of *S. dublin* closely resembling mine. The main purpose of this paper is to indicate some of the reasons why it is possible to divide *S. dublin* into types using anti-O phages.

MATERIAL AND METHODS

The methods were exactly those used in investigations on *S. thompson* (Williams Smith, 1951*a, b*). All the phages, except one (phage E), were isolated from lysogenic strains of *S. dublin*; phage E was isolated from a culture of *S. dublin* that was actively infected with it. The strains of *S. dublin* were identified by numbers and the phages by letters.

Since pure phage preparations can be obtained only by using non-lysogenic strains for propagation (Williams Smith, 1951*b*) great care was taken to ensure that all propagating strains were non-lysogenic. All the phages were propagated on the same strain, a Type 1 strain.

RESULTS

Twenty phages were isolated from lysogenic strains of *S. dublin*. These phages, together with the phage E, were tested on 50 strains of *S. dublin*. Six of the phages, A-F, were of value in differentiating the strains; the lytic activity of the remainder resembled that of one or other of these six phages. Phages A-D, were active only on smooth strains; phages E and F were active on smooth, and on some rough strains.

The six phages were applied in test dilution to 306 strains of *S. dublin*. Of those 306 strains, 294 (96 %) were typable and 12 (4 %) were insusceptible to the phages. The 306 strains were divisible into eleven types according to the pattern of their reactions with the six phages (Table 1). The Type 1 strains were fully susceptible to all the test phages; the other types were susceptible only to some of these phages. All Types 1-8 were smooth strains. Types 9-11 and the untypable strains were both smooth and rough. Unfortunately, from the point of view of analysing epidemics, 205 (66.9 %) of the strains belonged to Type 1. An extensive search was made for phages that would subdivide Type 1; but none was found.

Table 1. *The phage-typing of 306 strains of Salmonella dublin*

Type	Typing phages						Strains of <i>S. dublin</i>	
	A	B	C	D	E	F	No.	%
1	CL	CL	CL	CL	CL	CL	205	66.9
2	CL	CL	CL	CL	CL	—	12	4.0
3	CL	—	CL	CL	CL	CL	17	5.6
4	CL	CL	CL	CL	—	CL	29	9.5
5	CL	—	CL	CL	—	CL	2	0.7
6	—	CL	CL	CL	CL	CL	8	2.6
7	—	CL	—	—	CL	CL	3	1.0
8	—	—	—	CL	CL	CL	8	2.6
9	—	—	—	—	CL	CL	3	1.0
10	—	—	—	—	CL	—	6	3.0
11	—	—	—	—	—	CL	1	0.3
Untypable	—	—	—	—	—	—	12	4.0

CL, confluent lysis; —, no lysis. The phage types have been numbered only for the purpose of this table and are not recognized designations.

Most of the 306 strains were isolated from cows that were either suffering from clinical *S. dublin* infection or were symptomless faecal excretors of this organism. Eight strains were responsible for different outbreaks of food poisoning in human beings; phage-typing did not distinguish them from strains obtained from cows.

Some of the strains were tested several times during a period of 2 years and, apart from the variations inherent in any method of phage-typing depending on pattern reactions, they showed no significant variation in their susceptibility to the test phages. During this period they were subcultured several times.

The significance of lysogenic strains in the phage-typing method

Acquired phage resistance was responsible for many strains being regarded as distinct phage types in the case of staphylococci (Williams Smith, 1948) and *S. thompson* (Williams Smith, 1951*b*). The strains had acquired resistance to different phages in the field—indicated by the fact that they were lysogenic—and were consequently resistant to some of the phages used for typing. A similar state of affairs is possible in the typing of *S. dublin*.

If acquired phage resistance is responsible for the heterogenicity of *S. dublin* strains, lysogenicity would not be a common feature of Type 1 strains, since these strains were fully susceptible to all the typing phages, but it would be in strains belonging to Types 2–11 because these strains were resistant to one or more of the typing phages. Accordingly, 70 Type 1 strains were tested, and none of them were lysogenic; 24 (63 %) of 38 strains belonging to Types 2–11 were lysogenic.

Investigations were carried out in a manner similar to that which has been successful in elucidating the relationship among the staphylococcal and *S. thompson* strains. The principle of the method was to prepare a variant of a Type 1 strain, i.e. a strain showing no evidence of acquired phage resistance, resistant to the phage or phages carried by a lysogenic strain in Types 2–11. The resistant variant and the lysogenic strain were then tested by the typing phages, and, if their reactions were similar, it was concluded that acquired phage resistance was responsible for the originally determined phage type of the lysogenic strain. A young broth culture of the lysogenic strain was heated to 56° for 30 min. and tested for bacteriological sterility. Resistant variants of a Type 1 strain were then made to the phage contained in the heat-treated culture. The phage was not purified because some lysogenic cultures may contain more than one phage. The results of some of these experiments (Table 2) indicated that acquired phage resistance appeared to be responsible for the phage reactions of Types 1–3, 5, 7, 9 and 10. Acquired resistance could also account for the insusceptibility of the untypable strain (i) to all the typing phages and for that of the untypable strain (ii) to some of the phages.

The phages from different strains belonging to one lysogenic phage type all induced the same kind of resistance in Type 1. For example, the five variants of a Type 1 strain resistant to the phages carried by five strains of Type 3 all had the same reactions as Type 3 strains. Again, variants of a Type 4 strain, as well as of a Type 1 strain, were sometimes prepared resistant to the phages carried by lysogenic strains. The resistant variants of a Type 4 strain had the same phage reactions as similar variants of a Type 1 strain except, of course, that they were always insusceptible to phage E, the phage that lysed Type 1 but not Type 4 strains.

No evidence for the origin of Types 4, 6, 8, and 11 was discovered since none were lysogenic. It is known that some artificially-prepared resistant variants may be non-lysogenic, and it may be that these strains had developed phage resistance without becoming lysogenic. Support for this view was found by preparing on four occasions variants of a Type 1 strain resistant to phage E,

Table 2. *The action of the typing phages on lysogenic strains and on variants of a Type 1 strain resistant to the phages carried by these lysogenic strains*

The resistant variants of the Type 1 strain are designated by the word Type 1 followed, in brackets, by the type number of the lysogenic strain from which the 'crude' phage was obtained. All the phages were used in test dilution.

Strain	Typing phages					
	A	B	C	D	E	F
Type 1	CL	CL	CL	CL	CL	CL
Type 2	CL	CL	CL	CL	CL	—
Type 1 (Type 2)	CL	CL	CL	CL	CL	—
Type 3	CL	—	CL	CL	CL	CL
Type 1 (Type 3)	CL	—	CL	CL	CL	CL
Type 5	CL	—	CL	CL	—	CL
Type 1 (Type 5)	CL	—	CL	CL	—	CL
Type 7	—	CL	—	—	CL	CL
Type 1 (Type 7)	—	CL	—	—	CL	CL
Type 9	—	—	—	—	CL	CL
Type 1 (Type 9)	—	—	—	—	CL	CL
Type 10	—	—	—	—	CL	—
Type 1 (Type 10)	—	—	—	—	CL	—
Untypable (i)	—	—	—	—	—	—
Type 1 (untypable (ii))	—	—	—	—	—	—
Untypable (ii)	—	—	—	—	—	—
Type 1 (untypable (ii))	—	—	—	—	CL	CL

Symbols as in Table 1.

the phage which distinguishes Type 4 from Type 1. The variants were sub-cultured twice and then tested for lysogenicity; all were non-lysogenic. However, it is quite possible that factors other than acquired phage resistance accounted for the identity of these four types. One obvious and well-known factor which can account for change in phage susceptibility is smooth → rough variation. In the case of the *S. dublin* strains rough variation was associated with insusceptibility to phages A–D. But not all the strains insusceptible to these phages were rough, however. It should also be remembered that bacteriophage action itself can induce rough variation.

The differentiation of the typing phages by Bail's cross-resistance tests

Cross-resistance tests were carried out according to the method of Bail (1923) in order to determine the relationship of the six typing phages to each other. The resistant variants were prepared from a Type 1 strain. These tests showed that phages B, E, and F differed from each other and from the other phages. Each of the variants resistant to phages A, C and D was completely resistant to all three phages; they were fully susceptible to phages B, E, and F. According to Bail's interpretation, phages A, C and D should therefore be considered identical. The phage-typing results (Table 1), however, do not support this since phages C and D were active, and phage A inactive, on Type 6 strains; whereas phage D was active, and phages A and C inactive, on Type 8 strains.

The action of the Salmonella dublin phages on other species of Salmonella

The six phage preparations in test dilution were examined for activity on species of *Salmonella* other than *S. dublin*. The species and number of strains of each species examined were:

Group A: *S. paratyphi* A (2).

Group B: *S. paratyphi* B (2), *typhi-murium* (4), *stanley* (1), *derby* (3), *california* (1), *abortus-equi* (1), *abortus-ovis* (2), *abortus-bovis* (2), and *brancaster* (1).

Group C: *S. paratyphi* C (2), *cholerae-suis* (7), *cardiff* (1), *daytona* (1), *montevideo* (4), *oranienburg* (2), *potsdam* (1), *concord* (9), *barielly* (2), *tennessee* (2), *muenchen* (1), *manhattan* (1), and *düsseldorf* (1).

Group D: *S. typhi* (2), *enteritidis* (20) *rostock* (2), *moscow* (1), *blegdam* (1), *pensacola* (1), *dar-es-salaam* (2), *gallinarum* (12) and *pullorum* (15).

Group E: *S. london*, (1), *give* (1), *anatum* (2), *meleagridis* (1), and *zanzibar* (1).

Other groups: *S. habana* (1), *worthington* (1), *cubana* (1), *kirkee* (1), *kentucky* (1), *carro* (1), *minnesota* (1) and *inverness* (1).

The phages lysed only three of the species tested: *S. gallinarum*, *pullorum* and *rostock*. The twelve strains of *S. gallinarum*, the two *S. rostock* strains and one of the 15 *S. pullorum* strains were fully susceptible to all six typing phages. Two *S. pullorum* strains were lysed by phage F; the remaining 12 *pullorum* strains were resistant. Despite the close antigenic similarity between *S. enteritidis* and *S. dublin*, none of the 20 strains of *S. enteritidis* was susceptible.

DISCUSSION

Acquired phage resistance can account for many strains of *S. dublin* being considered as different phage types. The position is similar to that in staphylococci (Williams Smith, 1948) and *S. thompson* (Williams Smith, 1951*b*).

The different phage types were relatively stable provided, of course, no changes such as rough variation occurred. Whether change of type occurs frequently in the field is another matter. This possibility was discussed previously with regard to *S. thompson* (Williams Smith, 1951*b*) and depends on the opportunity a bacterial population has of becoming phage-infected, acquiring a resistance to the phage with which it has become infected and, as a result, undergoing a change in type. Though such a change might occur during epidemiological investigations conducted over a long period of time, the possibility of its occurrence during a short-term inquiry would be fairly remote.

An important limitation to the use of phage for typing *S. dublin* is that 66.9 % of the strains examined belonged to one type; membership of this type would be of little significance from the epidemiological point of view. This fact is related to the observation that most of the strains were non-lysogenic, exhibited no evidence of acquired phage resistance, and, consequently, were fully susceptible to all the typing phages. *S. thompson* differed (Williams Smith, 1951*b*), since 84 % of the strains examined were lysogenic and could be classified into eleven types, the largest comprising only 28 % of the

whole. In the case of *S. cholerae-suis* (Williams Smith, 1951*b*) 88 % of the strains were lysogenic, but this did not permit a satisfactory classification because these strains were latently infected with the same phage and, consequently, exhibited the same degree of phage resistance. It is clear, therefore, that to classify a species of *Salmonella* satisfactorily by anti-O phages, not only should a high proportion of strains be lysogenic but many of the phages they carry should be different.

Lilleengen (1950) also found that the majority (62 %) of the 129 strains of *S. dublin* he examined were fully susceptible to all his typing phages. The remaining types were distinguished from each other, as in the present work, by their pattern of phage reactions. Lilleengen's phages were obtained by the filtration of such material as sewage and faeces, whereas the phages in this work were isolated from lysogenic strains of *S. dublin*. The phages from the lysogenic strains appear to be more specific in their action than those from sewage or faeces, since none of them were active on *S. enteritidis* and only one of 15 strains of *S. pullorum* was fully susceptible to them; the phages used by Lilleengen were active on most strains of *S. pullorum* tested and some of them were active on *S. enteritidis*.

The cross-resistance tests substantiate previous doubts about the accuracy of such tests (Burnet, 1929; Williams Smith, 1951*b*). The three phages, A, C and D, were considered identical when a Type 1 strain was used as a 'parent' strain for carrying out cross-resistance tests but were shown to have a different range of lytic activity when tested on the series of strains of *S. dublin*. It is conceivable that the variants of the Type 1 strain-resistant to any one of these three phages also lacked receptors for the other two, but that some strains such as those of Types 6 and 8 possessed receptors which were susceptible to attack by some but not all of the three phages. It is noteworthy that no evidence was obtained to suggest that acquired phage resistance was responsible for the identity of Types 6 and 8. No other reasons for their identity were found.

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