Epidem. Inf. (1987), **99**, 291–294
Printed in Great Britain

A phage-typing scheme for *Salmonella enteritidis*

By L. R. WARD, J. D. H. de SA and B. ROWE

WHO Collaborating Centre for Phage Typing and Resistance of Enterobacteria, Division of Enteric Pathogens, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

(*Accepted 3 April 1987*)

SUMMARY

For many years phage typing has proved invaluable in epidemiological studies on *Salmonella typhi*, *S. paratyphi A* and *B*, *S. typhimurium* and a few other serotypes. A phage-typing scheme for *S. enteritidis* is described. This scheme to date differentiates 27 types using 10 typing phages.

INTRODUCTION

Salmonella enteritidis is an important cause of human infection in the United Kingdom. Since 1975 it has always been either the second or third most prevalent serotype. The number of isolates from humans has risen dramatically from 995 in 1980 to 5529 in 1986. This increase has made serotyping alone inadequate for epidemiological purposes. A phage-typing scheme would provide enhanced strain discrimination as has been demonstrated for *S. typhimurium* (Anderson *et al.* 1977) and *S. virchow* (Chambers *et al.* 1987). This paper describes a phage-typing scheme for *S. enteritidis*.

MATERIALS AND METHODS

Isolation of the typing phages

The typing phages were from three sources. Four of the final phages were obtained by direct isolation from lysogenic strains of *S. enteritidis*. Five were phage adaptations and one phage was isolated from sewage.

Lysogenic strains of S. enteritidis

One hundred strains of *S. enteritidis* isolated from humans, animals and sewage were examined for the presence of temperate phages.

Individual strains were tested for lysogenicity by growing them in 20 ml double strength Bacto Nutrient Broth (nutrient broth) at 37 °C for 5 h with agitation. The cultures were then centrifuged at 3000 rev/min for 15 min. The supernatants were carefully removed and either treated with 0·14% toluene (Anderson & Felix, 1953) or incubated at 57 °C for 40 min to kill any residual bacteria.

The supernatants were tested on *S. enteritidis* strains that had been flooded onto nutrient agar plates (double strength Bacto Nutrient Broth with 1·3% Bacto

Nutrient Agar added). Approximately 200 epidemiologically unrelated *S. enteritidis* strains were used. After overnight incubation at 38·5 °C single plaques together with a small amount of surrounding culture were cut out according to the method of Callow (1959).

The isolated phages were inoculated into 20 ml of nutrient broth and incubated with shaking at 37 °C for 4–5 h. The cultures were centrifuged and the supernatants removed and sterilized as described above. The phages obtained were titrated in serial dilutions in broth against their propagating strains to determine the Routine Test Dilution (RTD) (Anderson & Williams, 1956).

Fourteen phages were obtained by this method and were screened against a panel of 206 epidemiologically unrelated strains of *S. enteritidis*. Ten of these phages were discarded because they gave similar or unsatisfactory reactions. Four phages were finally selected and in the final scheme these are designated 1, 2, 3 and 6.

Adapted phages

It is well known that phages of a range of salmonella serotypes can undergo host-induced modification (Callow, 1959). Attempts were made to adapt the temperate phages obtained above, on strains of *S. enteritidis*.

The starting phage was titrated in serial dilution and spotted on strains of *S. enteritidis* that had been flooded onto nutrient agar plates. The plates were incubated at 38·5 °C overnight and discrete plaques of the new phage lines were cut as described previously, inoculated into 3 ml broth and incubated for 3–5 h while lysis occurred. The preparations were centrifuged and sterilized as previously described. Phage 5 and phage 8 in the final scheme were obtained by adaptation of the lysogenic phages 3 and 6 respectively, and phage 4 was obtained by adaptation of a phage of unknown origin. Typing phages 9 and 10 were produced by adaptation of the typing phages 4 and 8 respectively.

Phage from sewage

Sewage samples were centrifuged at 3500 rev/min and the supernatants were separated and sterilized with toluene. The treated samples were suspended in equal parts of double-strength nutrient broth, inoculated with broth cultures of *S. enteritidis* strains and incubated with shaking for 3–4 h. The cultures were then centrifuged at 3500 rev/min and the supernatants removed and sterilized with toluene before being spotted on a selection of *S. enteritidis* strains on nutrient agar plates. The procedure for selection and purification of the phages was the same as previously described. One phage, namely 7, was obtained by this means for the final scheme.

Final typing phages

The selected typing phages were grown in bulk by the standard agar layer technique (Adams, 1950, 1959) on their specific propagating strains.

Table 1. *Reactions of the* S. enteritidis *type strains with the typing phages at Routine Test Dilution*

Phage type	Phages 1	2	3	4	5	6	7	8	9	10	No. of strains with pattern 1981–86
1	OL	SCL	CL	OL	OL	SCL	CL	SCL	OL	OL	354
2	OL	—	CL	OL	CL	SCL	SCL	OL	OL	OL	149
3	OL	—	—	—	—	—	—	OL	—	OL	2
4	—	SCL	CL	OL	CL	SCL	CL	SCL	OL	OL	8771
4a	—	SCL	CL	OL	CL	SCL	CL	—	OL	+++	1
5	—	SCL	+	OL	CL	SCL	+	++	OL	+++	25
6	—	OL	—	OL	—	OL	—	OL	OL	OL	987
6a	—	SCL	—	SCL	—	SCL	—	—	OL	—	38
7	—	SCL	—	OL	—	—	—	—	OL	—	27
8	—	—	SCL	OL	CL	SCL	SCL	SCL	OL	OL	4929
9	—	—	OL	—	OL	—	OL	—	—	—	19
10	—	—	—	OL	++	SCL	—	—	OL	—	5
11	—	—	++	—	CL	—	—	SCL	—	CL	456
12	—	OL	+	OL	+++	—	SCL	—	OL	—	7
13	—	—	—	OL	—	SCL	—	—	OL	—	191
14	—	—	—	—	—	SCL	+	SCL	OL	OL	16
15	—	—	SCL	—	SCL	SCL	—	SCL	—	+++	8
16	—	SCL	—	—	—	SCL	—	SCL	—	OL	6
17	+++	OL	+	OL	+++	—	SCL	+	OL	+++	2
18	—	SCL	SCL	—	CL	SCL	CL	SCL	—	OL	6
19	—	—	++	++	++	SCL	+	OL	+	OL	—
20	OL	—	SCL	—	CL	—	+	CL	—	OL	8
21	OL	SCL	—	OL	—	OL	—	SCL	OL	OL	7
22	OL	—	—	SCL	—	OL	—	OL	OL	OL	3
23	—	—	—	OL	—	—	—	—	OL	—	7
24	—	—	—	—	—	—	—	—	OL	—	57
25	—	—	—	—	—	—	—	+	—	OL	11
Total											16092

—, No reaction; +, 1–20 plaques; ++, 21–80 plaques; +++, 81–100 plaques; SCL, semi-confluent lysis; CL, confluent clear lysis; OL, confluent opaque lysis.

RESULTS

The final *S. enteritidis* phage-typing scheme contains 10 typing phages, defining 27 distinct patterns (Table 1). This scheme was used to phage type 16568 strains of *S. enteritidis* isolated from humans in the United Kingdom during the years 1981–86.

A total of 190 (1·1%) strains were resistant to all the typing phages. In addition there were 286 (1·7%) strains that reacted with the typing phages in patterns which did not comply to any of those described in the paper. At present these will be referred to as RDNC because they react with the typing phages but do not conform to any of the current recognized patterns; in the future if any of these become epidemiologically significant a definitive type will be assigned to them.

DISCUSSION

During the last 6 years the *S. enteritidis* phage-typing scheme has proved valuable for epidemiological purposes. Strains from many common source outbreaks have been typed. The scheme demonstrates a high degree of strain discrimination, 97% of the strains tested are assigned to designated types. Two phage types, type 4 and type 8, predominate and in future it may be desirable to further sub-divide these.

In a reference laboratory phage typing is a rapid and economical method of strain discrimination.

We wish to thank Professor E. S. Anderson and the staff of the former Enteric Reference Laboratory who contributed to the early development of this scheme.

REFERENCES

ADAMS, M. H. (1950). *Bacterial Viruses*. Methods in Medical Research **2**, Chicago Year Book Publishers.

ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers Inc.

ANDERSON, E. S. & FELIX, A. (1953). The Vi-type determining phages carried by *Salmonella typhi*. *Journal of General Microbiology* **9**, 65.

ANDERSON, E. S. & WILLIAMS, R. E. O. (1956). Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *Journal of Clinical Pathology* **9**, 127.

ANDERSON, E. S., WARD, L. R., DE SAXE, M. J. & DE SA, J. D. H. (1977). Bacteriophage-typing designations of *Salmonella typhimurium Journal of Hygiene* **78**, 297–300.

CALLOW, B. R. (1959). A new phage-typing scheme for *Salmonella typhimurium*. *Journal of Hygiene* **57**, 346–359.

CHAMBERS, R. M., MCADAM, P., DE SA, J. D. H., WARD, L. R. & ROWE, B. (1987). A phage-typing scheme for *Salmonella virchow*. *FEMS Microbiology Letters* **40**, 155–157.