

Plasmid profile typing can be used to subdivide phage-type 49 of *Salmonella typhimurium* in outbreak investigations

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SUMMARY

Plasmid profile typing has been used to subdivide phage-type 49 of *Salmonella typhimurium*, the most common phage type in humans in England and Wales since 1985. Twenty profile patterns have been identified in 350 strains examined.

Four profile patterns have been identified in 143 isolates from patients infected in 33 epidemiologically unrelated incidents and two patterns have predominated, ST49:62 and ST49:62,1. These patterns were also common amongst *S. typhimurium* phage-type 49 isolated from cattle and poultry; however ST49:62 was more common in bovines whereas ST49:62,1 predominated in poultry. *S. typhimurium* phage-type 49 with a different profile pattern, ST49:62,3, was responsible for a large outbreak in London in 1988 which was traced to mayonnaise made from eggs supplied by one producer. Plasmid profile typing can now be regarded as a method of supplementing phage typing in investigating outbreaks caused by this organism.

INTRODUCTION

In the 8 years 1981–8, 51 806 strains of *Salmonella typhimurium* isolated from humans in England and Wales were identified in the Division of Enteric Pathogens (B. Rowe, unpublished observations). Subdivision of this serotype is essential for epidemiological investigations and phage typing has proved a rapid and reliable method. Two hundred and eleven phage types have been designated [1].

From 1981–4 the most common phage type in humans was phage-type 12 [2]. In the last 4 years phage-type 49 (PT49) has predominated and from 1985 to 1988 comprised 14.5% of *S. typhimurium* from humans (B. Rowe, unpublished observations). *S. typhimurium* PT49 is also common in cattle and poultry [3] and these food animals probably constitute important reservoirs of this phage type.

Subdivision of *S. typhimurium* PT49 is often required to assist in epidemiological investigations and plasmid profile typing is a method which has been used to subdivide other phage types of *S. typhimurium* [4–6]. The technique has also been used to differentiate phage types of *S. enteritidis* [7]. The present study describes how subdivision of strains of *S. typhimurium* PT49 isolated in England and Wales from 1981–8 has been attempted by using plasmid profile typing. The application

of the results to a number of outbreaks is described and the epidemiological implications of the findings are discussed.

MATERIALS AND METHODS

Bacterial strains

Three hundred and fifty strains of *S. typhimurium* PT49 isolated from humans, food animals, their environment and their products, and from human food in England and Wales during the 8-year period 1981–8 were studied. These had been submitted by laboratories of the Public Health Laboratory Service, by hospital laboratories and by laboratories of the Veterinary Investigation Service.

Phage typing and testing for resistance to antimicrobial drugs

Strains were phage-typed [8] and tested for resistance to antimicrobial drugs [9, 10]. To ensure that results were not influenced by the carriage of resistance plasmids, only drug-sensitive representatives were chosen for plasmid profile analysis.

Extraction of plasmid DNA and agarose gel electrophoresis

Partially purified plasmid DNA was extracted using a modification of the method of Kado and Liu [11] as described by Threlfall and colleagues [7]. Plasmid DNA was analysed by electrophoresis at 140 V for 2.5 h on vertical slab agarose gels containing 0.8% agarose (w/v, Sigma, Type II). Molecular weights (MWs) were determined in relation to the MW of four reference plasmids with MWs ranging from 98.0 to 4.6 MDa, carried in *Escherichia coli* K12, strain 39R861 [12]. Plasmids of less than 5.0 MDa were sized in relation to plasmids carried in *E. coli*, strain V517 [13].

RESULTS

Plasmid profile types in S. typhimurium phage-type 49

Plasmids were found in all strains of *S. typhimurium* PT49 and twenty profile patterns were identified. The MWs of the plasmids and the corresponding profile pattern designations are shown in Table 1.

All strains carried a plasmid of approximately 62 MDa. The profile pattern of strains which carried only this plasmid was designated ST49:62. Nineteen other profile patterns were identified on the basis of a varying number of additional plasmids of different MWs (Table 1). These were designated ST49:61, 1 through to ST49:62, 19.

Distribution of plasmid profile types in S. typhimurium phage-type 49

Human isolations

One hundred and forty-three strains of *S. typhimurium* PT49 isolated from patients infected in 33 epidemiologically-unrelated incidents were plasmid typed according to the scheme shown above. Four profile patterns were identified: ST49:62, ST49:62, 1, ST49:62, 3 and ST49:62, 4 (Table 2). ST49:62 was found in 16 patients infected in seven outbreaks, ST49:62, 1 in 102 patients from twenty outbreaks, ST49:62, 3 in 4 patients from one outbreak and ST49:62, 4 in 21 patients from five outbreaks.

Table 1. Plasmid profile patterns in 350 strains of *S. typhimurium* phage-type 49

62	MW of plasmid DNA (MDa)										Profile pattern (PP)	
	—	—	—	—	—	—	—	—	—	—		
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62
62	—	—	—	—	—	—	—	—	—	—	1·2	ST49:62, 1
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 2
62	—	—	—	—	—	—	—	—	—	—	1·2	ST49:62, 3
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 4
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 5
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 6
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 7
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 8
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 9
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 10
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 11
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 12
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 13
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 14
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 15
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 16
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 17
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 18
62	—	—	—	—	—	—	—	—	—	—	—	ST49:62, 19

Table 2. *Profile patterns in S. typhimurium phage-type 49 from humans*

PP	No. of strains	No. of outbreaks/incidents
ST49:62	16	7 (21.2)
ST49:62, 1	102	20 (60.6)
ST49:62, 3	4	1 (3.0)
ST49:62, 4	21	5 (15.1)
Totals	143	33

Figures in parentheses indicate percent of outbreaks with each plasmid profile pattern.

Isolations from food animals and their products

In order to identify the food animal reservoirs of *S. typhimurium* PT49, isolates from cattle, milk, poultry, egg products and poultry houses, and from pigs, sheep and human food were plasmid typed. The results are shown in Table 3.

Cattle

Ten profile patterns were identified in 98 bovine isolates. The most common was ST49:62 (48% of isolates) followed by ST49:62, 1 (39%). The four patterns found in *S. typhimurium* PT49 from humans were all found in cattle. Two of these, ST49:62 and ST49:62, 1, were also identified in *S. typhimurium* PT49 from milk. A further six patterns, ST49:62, 5, ST49:62, 6, ST49:62, 7, ST49:62, 10, ST49:62, 12 and ST49:62, 14 were identified, none of which have been found in strains from humans.

Poultry

Six profile patterns were found in 54 poultry isolates. The most common was ST49:62, 1 (72%) followed by ST49:62 (10%). Of the four patterns in humans, three were identified in poultry, ST49:62, ST49:62, 1 and ST49:62, 4. Three other patterns, ST49:62, 10, ST49:62, 11 and ST49:62, 16 were found exclusively in poultry.

Egg products

Twenty-seven strains from egg products were examined and 10 profile patterns were found, including the 4 identified in humans and 3 of the 6 in poultry. The most common was ST49:62, 1 (56%). Patterns found exclusively in *S. typhimurium* PT49 from egg products were ST49:62, 8, ST49:62, 17, ST49:62, 18 and ST49:62, 19.

Poultry houses

The patterns ST49:62, 1, ST49:62, 2, ST49:62, 3 and ST49:62, 9 were identified in *S. typhimurium* PT49 isolated from poultry houses. Of these ST49:62, 1 had previously been found in poultry and egg products. In contrast ST49:62, 2 and ST49:62, 3 had been identified in egg products but not in poultry. As yet ST49:62, 9 has not been found in *S. typhimurium* PT49 from humans or food animals and their products.

Table 3. Distribution of profile patterns in strains of *S. typhimurium* phage-type 49 from food animals, food animal products and human food

PP	No. of strains											Total	%
	Cattle	Milk	Poultry	Eggs*	Pigs	Sheep	Food	Environment†					
ST49:62	47 (48)	1	10 (19)	1 (4)	0	2	0	0	0	0	0	61	29.5
ST49:62, 1	38 (39)	1	39 (72)	15 (56)	5	3	2	2	2	2	2	105	50.2
ST49:62, 2	0	0	0	1 (4)	0	0	0	0	5	0	0	6	2.9
ST49:62, 3	2 (2)	0	0	1 (4)	0	0	1	3	3	0	0	7	3.4
ST49:62, 4	3 (3)	0	2 (4)	3 (11)	0	0	0	0	0	0	0	8	3.9
ST49:62, 5	1 (1)	0	0	1 (4)	0	0	0	0	0	0	0	2	1.0
ST49:62, 6	3 (3)	0	0	0	0	0	0	0	0	0	0	3	1.4
ST49:62, 7	1 (1)	0	0	0	0	0	0	0	0	0	0	1	0.5
ST49:62, 8	0	0	0	2 (7)	0	0	0	0	0	0	0	2	1.0
ST49:62, 9	0	0	0	0	0	0	0	1	1	0	0	1	0.5
ST49:62, 10	1 (1)	0	1 (2)	0	0	0	0	0	0	0	0	2	1.0
ST49:62, 11	0	0	1 (2)	0	0	0	0	0	0	0	0	1	0.5
ST49:62, 12	1 (1)	0	0	0	0	0	0	0	0	0	0	1	0.5
ST49:62, 13	0	0	0	0	0	0	1	0	0	0	0	1	0.5
ST49:62, 14	1 (4)	0	0	0	0	0	0	0	0	0	0	1	0.5
ST49:62, 15	0	0	0	0	1	0	0	0	0	0	0	1	0.5
ST49:62, 16	0	0	1 (2)	0	0	0	0	0	0	0	0	1	0.5
ST49:62, 17	0	0	0	1 (4)	0	0	0	0	0	0	0	1	0.5
ST49:62, 18	0	0	0	1 (4)	0	0	0	0	0	0	0	1	0.5
ST49:62, 19	0	0	0	1 (4)	0	0	0	0	0	0	0	1	0.5
Totals	98	2	54	27	6	5	4	4	11	11	11	207	

* Egg products.

† Environment, poultry associated.

Figures in parentheses indicate percent of profile patterns from respective sources.

Table 4. *Incidence of ST49:62 and ST49:62,1 in S. typhimurium PT49 from cattle and poultry*

Animal host	Year	Plasmid profile pattern			Total
		ST49:62	ST49:62,1	Others	
Cattle	1981	6	1	0	7
	1984	16	0	2	18
	1988	13	19	4	36
Poultry	1981	0	3	0	3
	1984	0	15	4	19
	1988	1	7	1	9

Sheep and pigs

Profile pattern ST49:62 was identified in 2 of 5 isolates from sheep and ST49:62,1 in the remaining 3. Five of six pig isolates had the ST49:62,1 pattern and one the ST49:62,15 pattern. This was the only isolate of PT49 with this pattern.

Human food

Two isolates from ham, one from a mayonnaise-based food and one from a carrot were examined. The two isolates from ham had the ST49:62,1 pattern, that from the mayonnaise-based food, ST49:62,3 and that from a carrot, the unique pattern ST49:62,13.

Incidence of plasmid profile patterns in S. typhimurium PT49 from cattle and poultry

The incidence of the two most common profile patterns, ST49:62 and ST49:62,1, in *S. typhimurium* PT49 isolated from cattle and poultry in 1981, 1984 and 1988 is shown in Table 4.

In *S. typhimurium* PT49 from cattle, 6 of 7 1981 isolates and 16 of 18 1984 isolates were of ST49:62 and only one strain, which was isolated in 1981, had the ST49:62,1 pattern. In contrast, only 13 of 36 1988 isolates had the ST49:62 pattern whereas 19 were of ST49:62,1.

In *S. typhimurium* PT49 from poultry, all of 3 strains isolated in 1981, 15 of 19 from 1984 and 7 of 9 from 1988 were of ST49:62,1; only one strain, which had been isolated in 1988, displayed the ST49:62 pattern.

Outbreak investigations

Three *S. typhimurium* PT49 outbreaks have been investigated in which the source of infection was identified.

Outbreak 1

In 1984, 357 patients and staff were affected in an outbreak in a hospital in the North of England. The vehicle of infection was a roast beef meal but the source was probably raw chickens prepared in the hospital kitchens [14]. Nine isolates from patients, 10 from staff and 4 from the hospital environment were plasmid typed and all were found to have the ST49:62,1 pattern.

Outbreak 2

In 1986 at least 20 people were infected with *S. typhimurium* PT49 after consuming a roast beef meal at a function in the Midlands. Strains from two patients were examined and both had the ST49:62 profile pattern.

Outbreak 3

In 1988, at least 120 people were affected in an outbreak in a large metropolitan building in London [15]. The vehicle of infection was a mayonnaise sauce used in several food preparations. *S. typhimurium* PT49 with the profile pattern ST49:62,3 was isolated from all of four patients examined. Strains with an identical pattern were subsequently isolated from the mayonnaise sauce and from the farm which supplied the eggs used in this sauce.

DISCUSSION

One of the purposes of this investigation was to determine whether plasmid profile typing could be used to subdivide phage-type 49 of *S. typhimurium*, the most common phage type in humans in England and Wales since 1985. The results have shown that *S. typhimurium* PT49 can be divided into 20 sub-types on the basis of variation in plasmid profile.

Four of these profile patterns have been found in strains of *S. typhimurium* PT49 which have caused outbreaks in humans. ST49:62,1 was the most common pattern and 20 of 33 incidents investigated had been caused by strains with this pattern. In one such outbreak, in a hospital in the North of England in 1984, the source of infection was thought to have been raw chickens [14]. This epidemiological finding was supported by the plasmid profile results which demonstrated that the causative organism had a profile pattern which, up to 1984, had been confined almost exclusively to *S. typhimurium* PT49 from poultry. The second most common pattern, ST49:62, has been implicated in seven outbreaks, including an outbreak in the Midlands in 1986 in which a beef joint was the source of infection. Profile pattern ST49:62,3 has been identified in only one outbreak, that which occurred in London in 1988. This outbreak was traced to mayonnaise made from eggs supplied by one producer and this finding was confirmed by plasmid profile typing [15]. Profile pattern ST49:62,4 has been identified in *S. typhimurium* PT49 from five outbreaks which have occurred in widely separated areas of the country. The sources of these outbreaks have not been established.

Cattle and poultry are known to be important reservoirs of *S. typhimurium* PT49 [3]. Examination of isolates from these food animals has shown that the two most common profile patterns in humans, PT49:62 and PT49:62,1, also predominated in cattle and poultry. However, ST49:62 was more common in strains from cattle and milk, whereas ST49:62,1 predominated in poultry and egg products. Up to 1984, ST49:62 was almost exclusive to bovine isolates of *S. typhimurium* PT49 and ST49:62,1 to poultry. However, by 1988 *S. typhimurium* PT49 with the ST49:62,1 pattern had become established in cattle and now predominates in this host. ST49:62 was not identified in *S. typhimurium* PT49 isolated from poultry isolated before 1984. Although this pattern has subsequently been found in poultry, it does not seem to have become established to the extent

that ST49:62, 1 has become in cattle. ST49:62, 1 has also found in over 50 % of *S. typhimurium* PT49 from egg products whereas ST49:62 has been identified in only one egg product.

In 1988, *S. typhimurium* PT49 with the pattern ST49:62, 3 was isolated from humans and from mayonnaise prepared from eggs. Although this pattern has not been found in any *S. typhimurium* PT49 from poultry, it has been identified in environmental isolates from an egg-producing farm. This would suggest a poultry reservoir for this profile type. However ST49:62, 3 has subsequently been identified in two strains from cattle, and may now have become established in bovines. Profile pattern ST49:62, 4, which has caused several outbreaks in humans, has been found in *S. typhimurium* PT49 from cattle, poultry and egg products, and does not appear to have a specific food animal host. As yet, 16 profile pattern types in *S. typhimurium* PT49 from food animals or their products have not been identified in human isolates of this phage type.

This investigation has demonstrated that plasmid profile typing can be used to divide *S. typhimurium* phage-type 49 into 20 subtypes of which the two most common are associated predominantly with cattle (ST49:62) or with poultry (ST49:62, 1). However it is noteworthy that differences in the distribution of these two types in their respective food animal hosts has become less marked over the 8-year period. The results have also shown that plasmid profile typing enables strains of *S. typhimurium* PT49 with less common profile types to be recognized and their food animal source identified, particularly in some outbreak situations. This technique can now be regarded as an important method of supplementing phage typing in investigating the epidemiology of *S. typhimurium* phage-type 49 in humans and food animals.

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