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Use of plasmid profile typing for surveillance of *Salmonella enteritidis* phage type 4 from humans, poultry and eggs

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SUMMARY

Plasmids were found in 1022 of 1089 (94%) of drug-sensitive strains of *Salmonella enteritidis* phage type 4 from humans (sporadic and outbreak cases), poultry (chickens) and eggs in England and Wales in the 5-year period 1988–92 and 25 plasmid profile patterns were identified. Strains characterized by a single plasmid of 38 MDa predominated (= plasmid profile type SE 38), comprising over 90% of isolates from humans, 70% from poultry and 92% from eggs. Eleven profile types were identified in strains from humans, 21 in strains from poultry and 3 in strains from eggs. Eight of the 11 patterns identified in human isolates were found in strains from poultry and 2 in strains from eggs. In contrast 15 patterns seen in poultry were not found in strains from humans. Four percent of strains from humans and 13% from poultry did not carry the 38 MDa plasmid but all strains from eggs were found to carry this plasmid. The second most common profile type in strains isolated between 1981 and 1988 was not identified in strains isolated from 1988–92. It is concluded that plasmid profile typing is a useful method for rapid differentiation within phage type 4 of *S. enteritidis* but that methods which can discriminate within the predominant profile type, SE 38, are now required.

INTRODUCTION

Since 1988 *Salmonella enteritidis* has been the most common serotype isolated from food animals in England and Wales. In 1981 the Laboratory of Enteric Pathogens (LEP) identified 1087 strains of this serotype from humans [1] whereas in 1992, 20381 strains were identified, an increase of approximately 19-fold over the 12-year period. The primary method for the differentiation of *S. enteritidis* is phage typing and using a scheme developed in LEP [2], it has been demonstrated that in 1992, 84% of *S. enteritidis* from humans belonged to phage type (PT) 4 [3] and provisional figures for the first quarter of 1993 [4] indicate that the epidemic is still continuing.

The reservoir of infection is poultry (chickens) and outbreaks associated with the consumption of poultry meat [5] and shell eggs have been reported [1, 6]. Underlying the human epidemic has been the spread of *S. enteritidis* PT 4 in poultry flocks. Since 1989, strains of *S. enteritidis* PT 4 have also been isolated from cattle and pigs as well as human food not of poultry origin.

To assist epidemiological investigations a rapid method for differentiating

within PT 4 is obviously required and in a previous communication we compared the differentiation of *S. enteritidis* by phage typing to differentiation by plasmid profile typing [7]. Eleven plasmid profile types were identified within the 27 *S. enteritidis* phage types described by Ward and colleagues [2] and within 247 strains of *S. enteritidis* PT 4 isolated between 1981 and 1987, 9 profile types were identified. Strains characterized by a single plasmid of 38 MDa predominated, followed by strains carrying this plasmid and two additional plasmids of 2·8 and 2·4 MDa. A small number of strains were plasmid-free. We now describe the distribution of plasmid profile types among strains of *S. enteritidis* PT 4 isolated from humans, poultry (chickens) and eggs in England and Wales over the 5-year period 1988–92.

MATERIALS AND METHODS

Bacterial strains

Strains of *S. enteritidis* examined in this study had been isolated from humans, poultry (chickens) and eggs in England and Wales over the 5-year period 1988–92, and had been referred to LEP by Public Health and hospital laboratories, and by laboratories of the Veterinary Investigation Service. Strains were phage-typed by the method of Ward and colleagues [2] and were tested for resistance to antimicrobial drugs by the methods of Anderson and Threlfall [8] and Ward and colleagues [9]. To ensure that plasmid profile types were not affected by the presence of drug resistance plasmids, only strains which were sensitive to antimicrobial drugs were included. Both family and general outbreaks were investigated, the latter occurring in food service establishments such as hotels and restaurants as well as in the community and in hospitals. Although at least two, and in some cases up to 50 isolates were examined from each outbreak, for the purposes of analysis only the results of a single representative isolate from each outbreak have been included. Strains from sporadic cases were also studied and were randomly chosen without regard to the sex or age of the patient and as far is known, were from symptomatic patients. Strains from poultry were randomly selected from isolates submitted by Veterinary Investigation Centres. For 1991 and 1992, random selection of isolates was done by computer.

Isolation of partially-purified plasmid DNA and agarose gel electrophoresis

Partially-purified plasmid DNA was extracted by a modification of the method of Kado and Liu [10], as described previously [7]. Samples were analysed by electrophoresis at 100 V for 2–2·5 h on 0·6% horizontal agarose gels (w/v, Sigma, Type II medium EEO), using a model H5 horizontal gel apparatus (Gibco/BRL), and were sized in relation to plasmids carried in the *Escherichia coli* strains 39R861 [11] and V517 [12], as previously described [7].

RESULTS

Plasmid profile patterns

Plasmids were found in 1022 of 1089 strains of *S. enteritidis* PT 4 studied (94%) and 25 plasmid profile patterns were identified (Table 1). The plasmid profile types were designated in accordance with the scheme of Threlfall and colleagues [7] and in relation to the possession of the *S. enteritidis* 'serotype-specific' 38 MDa

Table 1. *Plasmid profile patterns in strains of* S. enteritidis *PT 4 from humans and poultry, 1988–92*

MW of plasmid DNA (MDa)																Plasmid profile pattern
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	SE 0
—	—	—	—	—	—	—	—	6·0	—	—	—	—	—	—	—	SE 0a*
—	—	—	—	—	—	—	—	—	—	4·0	3·0	—	—	—	—	SE 0b*
—	—	—	—	—	—	—	—	6·0	—	4·0	3·0	—	—	—	—	SE 0c*
—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·0	1·0	SE 0d*
—	—	60	—	—	—	—	—	—	—	—	—	—	—	—	—	SE 0e*
—	—	—	—	—	38	—	—	—	—	—	—	—	—	—	—	SE 38
—	70	—	—	—	38	—	—	—	—	—	—	—	—	—	—	SE 38a
—	—	—	—	—	38	—	—	—	—	—	3·0	2·8	—	—	—	SE 38b
—	—	—	—	—	38	34	—	—	—	—	—	—	—	—	—	SE 38c
—	—	—	—	—	38	—	—	—	—	—	3·0	—	—	2·0	—	SE 38d
—	—	—	—	—	38	—	—	—	—	—	3·0	—	—	—	—	SE 38g
—	—	—	—	—	38	—	—	—	—	—	—	—	—	2·0	—	SE 38m
—	—	—	50	—	38	—	—	—	—	—	—	—	—	2·0	—	SE 38p*
—	—	—	—	—	38	—	—	—	5·0	—	3·0	—	—	—	—	SE 38q*
—	—	—	—	—	38	—	—	—	—	—	—	—	2·6	—	—	SE 38r*
—	—	—	—	—	38	—	—	—	5·0	—	—	—	—	—	—	SE 38s*
—	—	60	—	—	38	—	—	—	—	—	3·0	—	—	—	—	SE 38t*
—	—	60	—	—	38	—	—	—	—	—	3·0	2·8	—	—	—	SE 38u*
—	—	60	—	—	38	34	—	—	—	—	—	—	—	—	—	SE 38v*
—	—	60	—	—	38	—	—	—	—	—	—	—	—	—	—	SE 38w*
—	—	—	—	—	38	—	8·0	—	5·0	—	—	—	—	—	1·0	SE 38x*
—	—	—	—	—	38	—	—	—	5·0	—	—	—	—	—	1·0	SE 38y*
80	—	—	—	45	38	—	—	—	—	—	—	—	—	—	—	SE 38z*
—	—	—	—	—	38	—	—	—	—	—	—	—	—	2·0	1·0	SE 38ma*

* indicates newly identified plasmid profile types.

plasmid [13]. Thus, strains which were plasmid-free were designated plasmid profile type (PP) SE 0. Those which carried only the 38 MDa plasmid were designated SE 38, and those with the 38 MDa plasmid and additional plasmids with MWs ranging from 80 MDa to 1·0 MDa, SE 38a through to SE 38z and SE 38 ma. Strains which did not carry the 38 MDa plasmid but possessed other plasmids with MWs ranging from 60 MDa to 1·0 MDa were designated SE 0a through to SE 0e.

Of the 25 plasmid profile patterns identified in the strains of *S. enteritidis* PT 4, eight had previously been identified in *S. enteritidis* strains of various phage types and four (PPs SE 0, SE 38, SE 38a, SE 38c) in *S. enteritidis* PT 4 [7]. Thus in all, a total of 17 new plasmid profile patterns have been identified and designated in the strains of *S. enteritidis* PT 4 examined in this study.

Distribution of plasmid profile types in S. enteritidis *PT 4 from humans, poultry and eggs*

A total of 11 PPs were observed in strains of *S. enteritidis* PT 4 from humans, 21 in strains from poultry and 3 in strains from eggs (Table 2). Of the 21 patterns identified in poultry isolates, 8 appeared in humans. All of the 3 patterns identified in isolates from eggs (PPs SE 38, SE 38d, SE 38g) were also found in strains from poultry but only 2 of these (SE 38 and SE 38g) were found in strains from humans.

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Table 2. *Distribution of plasmid profile types in* S. enteritidis *PT 4 from humans, poultry (chicken) and eggs, 1988–92*

Year		Source*	Plasmid profile type (PP)																								
			0	0a	0b	0c	0d	0e	38	38a	38b	38c	38d	38g	38m	38p	38q	38r	38s	38t	38u	38v	38w	38x	38y	38z	38ma
1988	H:	S ($n = 106$)	2	—	—	—	—	—	102	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
		O ($n = 45$)	2	—	—	—	—	—	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	P:	($n = 10$)	1	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	E:	($n = 14$)	—	—	—	—	—	—	13	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
1989	H:	S ($n = 64$)	1	—	—	—	—	—	61	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
		O ($n = 84$)	3	1	—	—	—	—	77	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
	P:	($n = 66$)	9	—	—	—	—	—	47	1	1	—	—	5	—	—	2	1	—	—	—	—	—	—	—	—	—
	E:	($n = 10$)	—	—	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1990	H:	S ($n = 112$)	4	—	—	—	—	—	102	—	—	2	—	1	1	1	1	—	—	—	—	—	—	—	—	—	—
		O ($n = 77$)	2	—	—	—	—	—	69	—	—	1	—	—	1	2	2	—	—	—	—	—	—	—	—	—	—
	P:	($n = 164$)	26	—	1	1	—	1	101	2	1	—	1	4	5	2	—	—	9	2	1	1	1	3	2	—	—
	E:	($n = 13$)	—	—	—	—	—	—	10	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—
1991	H:	S ($n = 96$)	9	—	—	—	1	—	81	—	—	1	—	—	2	1	—	—	—	—	—	—	—	—	—	1	—
		O ($n = 19$)	—	—	—	—	—	—	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	P:	($n = 30$)	2	—	—	—	—	—	25	—	—	—	—	—	—	1	—	—	2	—	—	—	—	—	—	—	—
	E:	($n = 4$)	—		—	—			4	—		—	—	—		—	—	—			—	—	—		—	—	—
1992	H:	S ($n = 96$)	5	—	—	—	—	—	86	1	—	1	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—
		O ($n = 39$)	1	—	—	—	—	—	36	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
	P:	($n = 31$)	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	E:	($n = 2$)	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals:	H:	S ($n = 474$)	21	—	—	—	1	—	432	1	—	4	—	1	7	5	1	—	—	—	—	—	—	—	—	1	—
		O ($n = 264$)	8	1	—	—	—	—	244	1	—	1	—	—	1	6	2	—	—	—	—	—	—	—	—	—	—
	P:	($n = 301$)	38	—	1	1	—	1	212	3	2	—	1	9	5	3	2	1	11	2	1	1	1	3	2	—	1
	E:	($n = 52$)	—	—	—	—	—	—	48	—	—	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—

* H, human; S, sporadic; O, outbreak; P, poultry (chickens); E, egg.

Three patterns identified in human isolates (PPs SE 0a, SE 0d, SE 38c) were not found in strains from poultry; similarly, 15 patterns identified in poultry were not found in human isolates of PT 4.

The most common pattern identified in isolates from humans (both sporadic and outbreak), poultry and eggs was SE 38 and 91% of sporadic and 92% of outbreak isolates from humans and 70% of poultry isolates belonged to this profile type. Ninety-two percent of isolates of PT 4 from eggs also belonged to this profile type but the number of isolates examined was small in contrast to the numbers of strains from humans or poultry. The second most common type in humans and poultry was SE 0 (plasmid-free). However, whereas only 4% of human isolates belonged to this profile type, 13% of strains from poultry were plasmid-free. In contrast, all of the strains from eggs carried the 38 MDa plasmid.

The greatest variation in PPs was seen in isolates made in 1990. In that year, 7 PPs were identified in 112 sporadic human isolates, 6 in 77 strains from outbreaks, 18 in 164 isolates from poultry and 3 in 13 isolates from eggs. Prior to 1990 there was little variation in profile pattern and although some variation was observed in subsequent years, since 1990 only 8 different patterns have been found in strains from humans and 5 in strains from poultry, with the SE 38 pattern comprising 89% of human and 90% of poultry isolates since 1991. All of the egg isolates studied since 1990 have been of the SE 38 profile type.

DISCUSSION

For epidemiological purposes, there is a clear need for rapid and reproducible methods which can differentiate within PT 4 of *S. enteritidis*, which has been the predominate phage type in human salmonellosis in England and Wales since 1988. Previous studies based on the distribution of insertion sequence IS*200* elements [14] in the chromosome of strains of *S. enteritidis* PT 4 have demonstrated the clonality of this phage type [15, 16], which extends to strains isolated in England and Wales before the present epidemic [16] and to strains of PT 4 isolated in Switzerland [17]. Because of the clonal nature of PT 4, it is unlikely that a method of differentiation based on the distribution of conserved gene sequences in the chromosome will provide sufficient discrimination for epidemiological studies. In lieu of such a genotypic method, differentiation based on the numbers and MWs of extrachromosomal elements (plasmid profiles) and their restriction enzyme fingerprints may be more appropriate for PT 4. However recent studies have demonstrated that for *S. enteritidis*, restriction enzyme fingerprinting is unlikely to extend the degree of discrimination that can be achieved by plasmid profile typing [18]. For this investigation, plasmid profile typing was therefore chosen as the method most likely to demonstrate variation within strains of *S. enteritidis* PT 4 isolated in England and Wales since 1988.

In the present study, plasmid profile typing demonstrated the existence of 25 plasmid profile types in strains of *S. enteritidis* PT 4 isolated since 1988. However strains belonging to the profile type SE 38, characterized by a single plasmid of 38 MDa predominated, comprising over 90% of human and 70% of poultry isolates. Obviously, subdivision within the SE 38 profile type is desirable. The second most common profile type was that designated PP 0 and strains of this

profile type did not carry any plasmids. Strains of PP SE 0 have been isolated from both humans and poultry but it is noteworthy that whereas only 4% of human isolates belonged to this profile type, 13% of strains from poultry were plasmid-free. The 38 MDa plasmid has been designated the *S. enteritidis* 'serotype-specific' plasmid (SSP) [13] and has been demonstrated to contribute to the virulence of *S. enteritidis* for BALB/c mice [13, 19]. SSP-like plasmids have been shown to be necessary for the intracellular survival of their host organisms in mouse phagocytes [20] and it has been postulated that such plasmids may be involved in the virulence of their host organisms for humans [13, 21], although this has not been proven. The present results show that for *S. enteritidis*, the SSP is not necessary for the induction of enteritis in humans as 3% of the outbreak strains and 5% of the sporadic isolates did not carry this plasmid. Furthermore, in 1990 an outbreak in a hospital in Wales in which over 20 patients were infected was caused by a plasmid-free strain of *S. enteritidis* PT 4. It may, however, be significant that all of the egg isolates of PT 4 studied here carried the 38 MDa plasmid. Infection of the egg within the oviduct is thought to be an important factor in the internal contamination of shell eggs with *S. enteritidis* PT 4 [22] and it is possible that the 38 MDa plasmid may be necessary for the intracellular survival of strains of PT 4 within the reproductive organs of poultry.

In our previous study of strains isolated from 1981–7, the second most common pattern in *S. enteritidis* PT 4 was that designated SE 38f, which was characterized by carriage of the 38 MDa plasmid and two additional plasmids of 2·8 and 2·4 MDa. This profile type was common from 1981–5 and was prevalent in travellers with a history of recent return from Spain, the Balearic Isles and the Canaries. This plasmid profile was not found in any of the strains studied which had been isolated between 1988 and 1992 from patients in England and Wales. During these years the majority of patients infected with *S. enteritidis* PT 4 and with a history of recent return from Spain were found to be infected with strains with the SE 38 plasmid profile. This suggests that in Spain, *S. enteritidis* PT 4 of the SE 38f profile type has now been replaced by strains with the SE 38 profile.

A further observation of interest is the finding that 15 profile patterns found in poultry isolates of PT 4 have as yet not been observed in strains from humans. However the presence of a greater number of profile types in strains of PT 4 from poultry is not unexpected, since poultry flocks provide the reservoir of this phage type and plasmid acquisition within a primary host such as poultry, in which the organism is widely disseminated, may be more likely to occur than within a secondary host.

These findings demonstrate that plasmid profile typing is a useful method for the rapid differentiation within PT 4 of *S. enteritidis*. However, since the majority of infections in humans are caused by strains of a single profile type, SE 38, methods which can discriminate within this profile type are now required.

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