

## Phage typing and drug resistance of *Shigella sonnei* isolated in England and Wales

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### SUMMARY

Phage typing of *Shigella sonnei* has been used to examine isolates from the 1991–2 *sonnei* dysentery outbreak in England and Wales and compare them with strains isolated during and following a widespread foodborne outbreak in 1994 which was associated with consumption of imported lettuce. The distribution of phage types was different in the three periods studied with PT 3 predominating during 1991–2, PT 2 during the ‘lettuce’ outbreak in the summer months and PT 6 during the subsequent months. PT 6 was frequently associated with travel outside the UK. Variation was also seen in the distribution of drug resistance patterns.

### INTRODUCTION

Dysentery due to *Shigella sonnei* is endemic in England and Wales. The majority of cases are young children and outbreaks of *S. sonnei* are mainly centred around primary schools and nurseries with the usual mode of transmission being person-to-person by the faecal-oral route [1]. Control has focussed on effective hygiene measures and on exclusion of symptomatic excretors.

The systems most widely used for subtyping *S. sonnei* are colicin typing [2] and phage typing [3, 4]. In 1949 Hammarstrom published a phage typing scheme developed in Sweden [3] and a subsequent report showed that, between 1946 and 1966, 80% of isolates belonged to the 12 most common phage types, with types 5 and 3 predominating [4]. There was observable variation in the distribution of phage types over time.

This phage typing scheme is now used in several European countries including Sweden, Germany [5], Spain [6] and Italy [7]. In a survey of *S. sonnei* from Czechoslovakia [8], 83% typability was reported with phage types 2 and 6 accounting for 34% and 19% respectively.

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An association between phage type, colicin type and antibiogram within geographical areas was reported from S. Australia indicating the prevalence of local epidemic strains [9]. Studies in Germany demonstrated that some phage types are plasmid determined [10], although in 1988 Toth and Hajnal [11] reported that phage type, antibiogram, biotype, colicin type and pathogenicity appeared to be independent factors.

Between 1987 and 1990 laboratory reports of *S. sonnei* infection to the Public Health Laboratory Service's Communicable Diseases Surveillance Centre (CDSC), averaged 2850 per year with peak incidence in the winter. In 1991 the total had risen to 9840 and in 1992 to 17237. This countrywide epidemic declined during 1993 with the number of *S. sonnei* reports to CDSC totalling 6024 in 1993, and 5639 in 1994. However, in June 1994 there was an outbreak of *S. sonnei* food poisoning in several countries in North West Europe where imported iceberg lettuce was shown epidemiologically to be the vehicle of infection [12–14]. Initial reports from Sweden indicated that two phage types of *S. sonnei* were involved.

The present paper describes the distribution of phage types and drug resistance patterns among

isolates from England and Wales during the 1991 and 1992 epidemic, during the 'lettuce' outbreak and between September and December 1994.

## MATERIALS AND METHODS

### Strains

#### *S. sonnei* isolated in England and Wales: 1991–2

The *S. sonnei* Reference Unit at Guildford Public Health Laboratory supplied a collection of 342 strains isolated in England and Wales during 1991 and 1992. These were selected on the basis of isolation date, geographical origin and colicin type. The aim was to sample strains from known clusters together with apparently sporadic isolates from the same laboratory during the same time period.

#### *S. sonnei* isolated in England and Wales: June–August 1994

Following initial reports of an outbreak of *S. sonnei* infection associated with consumption of iceberg lettuce, public health and hospital laboratories were asked to refer to LEP isolates of *S. sonnei* from adults particularly where food poisoning was suspected. Five hundred and ninety-three strains were received between June and August.

#### *S. sonnei* isolated in England and Wales: September–December 1994

Two hundred and thirty-nine strains isolated from adults and children were received by LEP between September and December. These included isolates from two primary school outbreaks and a significant number of isolates from patients who had recently returned from abroad.

### Phage typing reagents

Phages and representative type strains were supplied by Dr R. Wollin of the Statens Bakteriologiska Institute Stockholm, Sweden.

### Phage typing method

The strains were phage typed following a protocol based on that supplied by Dr Wollin. *S. sonnei* shows phase variation between smooth, phase I, and rough, phase II, forms. Phase variation is plasmid-determined

and phase I colonies segregate phase II cells at a high frequency in culture. Because of this instability and since both cell types react with the phages, two rough colonies are picked from each isolate and phage typed. Phage XII of the scheme only lyses rough cells and colonies which are resistant to phage XII are discarded. Two rough colonies from MacConkey agar plates were inoculated into duplicate nutrient broths and incubated overnight at 37 °C. Cultures were then flooded onto nutrient agar plates and spotted with phages at Routine Test Dilution (RTD). Plates were incubated for 5 h at 38 °C and then patterns of lysis recorded and interpreted according to the chart supplied from Sweden.

### Testing for resistance to antimicrobial drugs

All isolates were screened for resistance to the following antimicrobial agents using iso-sensitest agar (Oxoid) in an agar incorporation method [15] at the levels quoted: ampicillin (abbreviation A; 8 and 128 mg/l), chloramphenicol (C; 8 mg/l), ciprofloxacin (Cp; 0.125 and 1 mg/l), furazolidine (Fu; 8 and 32 mg/l), gentamicin (G; 4 and 32 mg/l), kanamycin (K; 16 mg/l), nalidixic acid (Nx; 16 mg/l), streptomycin (S; 16 and 128 mg/l), sulphamethoxazole (Su; 64 mg/l), spectinomycin (Sp; 64 mg/l), tetracycline (T; 8 and 128 mg/l), trimethoprim (Tm; 2 mg/l).

## RESULTS

### The phage typing scheme

#### Discrimination

All of the 1174 *S. sonnei* strains were phage typable and a total of 29 phage types (PT) defined in the chart supplied by Dr Wollin was identified. Eight possible new types were defined as reaction patterns which did not conform to any of those previously described but which were represented by more than one isolate in the LEP collection; these types have been given provisional alphabetic designations. The 29 phage types and eight new types identified among UK isolates are shown in Table 1. A further 25 isolates each gave different non-conforming patterns (designated RDNC).

#### Reproducibility

Variation between the two replicate colonies from the

Table 1. *S. sonnei* phage types identified in England and Wales\*

Phage type	I	II	III	IV	VI	VII	VIII	IX	X	XI	XII
2	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
3	—	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
3A	—	CL	CL	CL	CL	CL	CL	CL	CL	CL	+
4†	CL	CL	CL	SCL	CL	CL	CL	CL	CL	CL	CL
5	—	—	CL	CL	CL	CL	CL	CL	CL	CL	CL
6	CL	CL	—	CL	CL	CL	CL	CL	CL	CL	CL
7	—	CL	—	CL	CL	CL	CL	CL	CL	CL	CL
9	—	CL	CL	CL	CL	+	CL	CL	CL	CL	CL
12	CL	CL	—	—	CL	CL	CL	CL	CL	CL	CL
19	—	CL	—	CL	—	CL	CL	CL	CL	CL	CL
23	—	CL	CL	CL	CL	—	CL	—	CL	CL	CL
26	—	—	—	CL	CL	CL	—	CL	CL	CL	CL
28†	—	SCL	CL	CL	CL	SCL	CL	CL	—	CL	++
29	—	—	CL	CL	CL	—	CL	—	CL	CL	CL
32	—	CL	—	CL	CL	—	CL	—	CL	CL	CL
44	i	+++	—	—	CL	CL	CL	CL	—	CL	CL
50	—	CL	—	—	CL	—	—	—	CL	CL	CL
62	—	SCL	—	CL	CL	CL	CL	CL	—	CL	CL
65	—	CL	—	—	i	—	—	+	CL	CL	i
66	—	CL	—	—	—	—	—	—	CL	CL	i
75	CL	CL	—	—	—	CL	—	CL	CL	CL	CL
78	—	CL	—	++	CL	—	CL	—	CL	CL	CL
79	—	CL	—	—	—	CL	—	CL	CL	CL	CL
81	++	—	CL	CL	CL	CL	CL	CL	—	CL	CL
82	+	CL	CL	CL	CL	CL	CL	CL	—	CL	CL
85†	—	CL	—	CL	++	CL	CL	CL	CL	CL	CL
87	—	CL	—	CL	—	CL	—	CL	CL	CL	CL
89	—	CL	—	CL	—	—	CL	—	CL	CL	CL
93	i	SCL	—	—	—	CL	—	CL	CL	CL	CL
A	< CL	SCL	—	SCL	< CL	—	< CL	—	< CL	SCL	< CL
B	—	< CL	—	+++	—	—	—	—	CL	CL	SCL
D	—	+	—	< CL	SCL	CL	< CL	< CL	—	< CL	CL
F	—	—	—	+++	CL	SCL	++	< CL	SCL	+	—
K	< CL	SCL	—	+++	CL	SCL	CL	CL	CL	—	< CL
L	—	< CL	—	—	—	—	—	SCL	CL	< CL	SCL
M	—	< CL	—	< CL	—	—	—	< CL	< CL	< CL	+
N	CL	—	CL	CL	CL	CL	CL	CL	—	CL	CL

\* CL, confluent lysis; SCL, semi-confluent lysis; + + +, 500+ plaques; + +, 100–500 plaques; +, 10–100 plaques; i, isolated plaques with peripheral opaque lysis; —, no reaction.

† Reactions observed with type strains supplied by Sweden.

same isolate was seen in only 93 (7.9%) isolates. Nineteen of the 1174 (1.6%) paired colonies differed in respect of phage XII only. In this case the colony resistant to phage XII, i.e. which was phase I, was discarded and the result for the remaining colony reported.

Seventy-four isolates did not conform to designated types or differed in respect of one or more phages other than phage XII. These were replated and further colonies phage typed to obtain stable colonies which gave consistent reactions on repeated subculture. On

this testing of multiple colonies from the same isolate some problems were encountered particularly with types which differed from each other by degree of sensitivity to a single phage such as types 3 and 9 with respect to phage VII or 65 and L with phage IX (Table 1). On retyping 25 strains of *S. sonnei* chosen at random after 6 months storage on Dorset egg medium, three showed a change in lysis pattern with one phage only. Two of these, previously typed as PT 65, were redesignated PT L and one was redesignated PT 3 having previously been typed as PT 9.

Table 2. *S. sonnei* isolated in England and Wales, 1991 and 1992

Phage type	Total	Sensitive	ASSu	A	ASSuTm	ASSuT	SSuTm	Other R-types
3	150	17	87	31	—	4	—	11
82	54	12	32	9	—	—	—	1
7	36	6	19	8	—	3	—	—
85	29	3	20	3	1	—	—	2
28	13	—	13	—	—	—	—	—
23	11	—	1	—	7	—	—	3
79	7	1	5	—	—	—	—	1
6	4	—	—	—	—	—	4	—
L	4	2	1	—	—	—	—	1
RDNC*	10	1	1	1	—	—	3	4
Other phage types (15)	23	4	7	3	1	1	1	6
Total	342	46	187	55	9	8	8	29

\* Reacts with phages, but does not conform to a designated phage type.

### *S. sonnei* isolated in England and Wales: 1991–2

A total of 342 *S. sonnei* from the Guildford collection, were phage typed, 153 isolated in 1991 and 189 isolated in 1992. As there was little variation in phage type distribution between strains isolated in 1991 and 1992 the 2 years data have been pooled (Table 2). The two most frequent phage types were PT 3 (43.9%) and PT 82 (15.8%). Together they accounted for 59.7% of the strains tested.

Only 46 (13.4%) were sensitive to all drugs tested. A total of 19 resistance patterns (R-types) were identified, the most frequent of which was ASSu (54.4%) which was identified in almost all of the phage types represented. Resistance to ampicillin alone (16.1%) was also widely distributed. In contrast resistance to ASSuTm was associated with PT 23 and SSuTm with PT 6. All four of the isolates belonging to PT 6 were of R-type SSuTm, two of these were from patients who had travelled to Egypt and Tunisia. Of 11 PT 23, 7 (63.6%) were R-type ASSuTm; two isolates were from the same community in Wales and five were from different infant school outbreaks in Bristol.

Strains from a total of 39 local outbreaks within the general epidemic during this period were included in the study. All strains tested from 27 of these outbreaks belonged to a single phage type with PT 3 accounting for 18 outbreaks. Variation in phage type was seen in 12 outbreaks of which 11 included representatives of two phage types. One outbreak in 1992 which was studied in more detail had seven isolates which were PT 3 and sensitive to all antibiotics tested, and two isolates which were PT 23 and R-type AKSSuTm. It

was shown that change from PT 3 to PT 23 was due to acquisition of a transferable plasmid encoding resistance to trimethoprim, and also production of colicin Ib. Resistance to AKSSu was also plasmid determined, but transfer to PT 3 resulted in no phage type change. The remaining five strains tested each had a different phage and R type; although they had been epidemiologically defined as an outbreak the laboratory results indicated that they were in fact not closely related.

### *S. sonnei* isolated in England and Wales: June–August 1994

A total of 593 isolates were referred to LEP between June and August 1994 from 51 different laboratories in England and Wales (Table 3). Two hundred and thirty-four (39.4%) isolates belonged to PT 2 and 94 (15.9%) were PT L. Although most of these isolates were from patients presenting as sporadic infections, there were a number of local clusters in which there was a strong epidemiological association between illness and consumption of iceberg lettuce [13]. Although a small number of isolates of PT L were identified among strains isolated in the UK in 1991 and 1992, no isolates of PT 2 were seen before May 1994.

PT 2 was the predominant phage type found in Sweden among patients associated with the 'lettuce' outbreak [14]. The second phage type reported from Sweden as being associated with lettuce was PT 65. This differs from PT L by the reaction with phage IX (Table 1). Two isolates of PT 65, and four isolates of

Table 3. *S. sonnei* isolated in England and Wales June, July and August 1994

Phage type	Total	Sensitive	SSuTTm	A	K	AKSSuT	ASSu	ASSuTm	Other R types [14]
2	234	205	9	1	—	2	8	3	6
L	94	90	—	—	—	—	—	1	3
62	75	73	—	2	—	—	—	—	—
3	63	24	1	19	1	14	—	1	3
6	57	2	41	—	—	—	3	1	10
23	22	3	1	1	14	—	1	1	1
82	20	10	2	8	—	—	—	—	—
RDNC*	11	1	4	—	—	—	1	—	5
Other phage types [12]	17	3	1	—	1	—	2	3	7
Total	593	411	59	31	16	16	15	10	35

\* Reacts with phages but does not conform to a designated phage type.

PT L were identified in the 1991 and 1992 collection of strains and were clearly distinguishable from each other. A single isolate received from Sweden as belonging to PT 65, when tested in parallel with UK isolates typed as PT L.

All PT 62 isolates during this period were related to an outbreak in North Wales where infection was associated with eating ice cream at a particular establishment. Secondary cases occurred among family members or other children having contact with primary cases. This outbreak peaked during July. Phage types 3 (10.6%) and 6 (9.6%) were the only other phage types identified in large numbers during this period.

A total of 411 isolates (69.3%) received between June and August 1994, were fully sensitive to all antibiotics tested. PT 2 and PT L isolates (associated with contaminated lettuce) were predominantly sensitive, as were PT 62 isolates. As in previous years, the most common R-type among isolates of PT 3 was resistance to A alone while PT 6 was associated with resistance to SSuTTm.

#### ***S. sonnei* isolates in England and Wales: September–December 1994**

The numbers of *S. sonnei* referred to LEP declined during August and averaged 60 isolates per month between September and December. Of the 239 isolates received during this period, PT 6 was the most frequently identified phage type (30.5%) and 44 (60.2%) of these were of R-type SSuTTm (Table 4). Phage type 62 continued to be isolated in North Wales

but by September numbers were falling. Between September and December PT 62 was the fifth most common phage type. Phage types 2 and L also declined in frequency during this period.

Of the 22 isolates of PT 50, 17 were associated with a primary school in Hull, 14 of which were of R-type ASuTm. Between September and December, 7 isolates identified as a new type, PT D were received all of which were associated with an outbreak in a primary school in North London. Six of these were of the R-type ASSuTm and 1 ASSu. PT D is similar to PT 62, but is distinguished by being more resistant to phage II. Two isolates of PT D were identified from the 1991 and 1992 collection of strains.

The isolates from sporadic infections showed a similar distribution of both phage type and drug resistance to that seen in isolates from 1991 and 1992 with ampicillin-resistant PT 3 predominating.

#### ***S. sonnei* isolated from patients recently returned from abroad**

During the second half of 1994, 137 of the 787 (17.4%) *S. sonnei* phage typed were from patients who had recently returned from abroad (Table 5). Strains acquired abroad were more likely to be drug resistant, 69% as opposed to 27% from patients giving no history of recent foreign travel. SSuTTm was the most common resistance pattern overall heavily biased by isolates of PT 6 which was particularly associated with patients who had travelled abroad, predominantly to India, Africa or the Middle East. This resistance pattern was only identified in one isolate of

Table 4. *S. sonnei* isolated in England and Wales September to December 1994

Phage type	Total	Sensitive	SSuTTm	A	ASuTm	ASSuTm	ASSuTTm	Other R-types [12]
6	73	9	44	—	—	3	3	14
3	59	15	—	39	1	—	1	3
50	22	—	1	—	14	4	2	1
2	21	16	2	1	1	—	—	1
62	18	14	—	—	—	—	—	4
L	11	5	1	—	—	—	—	5
D	7	—	—	—	—	6	—	1
RDNC*	4	—	3	—	—	1	—	—
Other phage types [13]	24	7	5	1	—	—	3	8
Total	239	66	56	41	16	14	9	36

\* Reacts with phages but does not conform to designated phage type.

Table 5. Association between resistance and foreign travel in *S. sonnei* from England and Wales: June–December 1994

Phage type	Total	Patient: source of infection	Total	Total (%)	Sensitive (%)	SSuTTm (%)	Other (%)
All phage types	787	Abroad	137	17	31	45	23
		Not abroad	650	83	73	18	9
PT 6	130	Abroad	76	59	11	63	26
		Not abroad	54	41	6	69	25
PT 3	122	Abroad	2	2	50	0	50
		Not abroad	120	98	31	1	68

PT 3, a phage type rarely associated with foreign travel.

## DISCUSSION

Of the 96 phage types defined by the scheme a total of 29 types were identified among isolations from England and Wales included in the present study. A further eight provisional types have been described and 25 isolates with unique patterns have been designated RDNC. Because some phage types differ only in their degree of sensitivity to a single phage, in these instances it proved necessary to type a number of colonies of such strains in parallel with type strains for both of the related phage types.

There was considerable variation in phage type distribution between isolates from the three periods examined. Phage type 3 predominated amongst isolates from the 1991–2 countrywide *S. sonnei* epidemic in the UK. Although epidemiological data identified a number of localized outbreaks during this

period most involved PT 3 as did the 'sporadic' isolates. In some instances, where isolates from a single local outbreak were identified as belonging to more than one phage type, it was shown that plasmid acquisition had resulted in a phage type conversion, such as has been demonstrated in *Salmonella enteritidis* [16] and *Salmonella typhimurium* [17]. The identification of five different phage and R-types among strains isolated in the same locality over a short time period and therefore defined epidemiologically as an outbreak, underlines the value of detailed microbiological typing.

In June 1994 reports from Sweden, Germany, Norway and the UK described food poisoning outbreaks involving adults and associated with the consumption of imported iceberg lettuce. The use of the same phage typing scheme across several European countries has facilitated cross referencing between the British, German and Swedish outbreaks. The same phage types were identified in several countries. Exchange of cultures and type strains is



advisable for direct comparison under identical conditions where minor differences are used to define closely related phage types as is the case with phage type 65 and L. Gericke and colleagues [18] have shown that a PT L isolate from the UK had three additional bands when compared with PT 65 isolates from Germany and Sweden using pulsed field gel electrophoresis indicating that some variation was present. In Sweden eight of 53 patients were infected with a second enteric pathogen: four with enterotoxigenic *Escherichia coli*, two with *Giardia lamblia*, one with *Salmonella* sp. and one with *Campylobacter* spp. [15]. It has been suggested that faecal contamination of the water used to irrigate the lettuce fields was the source of contamination [14, 15] so the presence of a number of strains or the same or different organisms in the outbreak might be expected.

All but one isolate of PT 62 were associated with an outbreak among children in North Wales. Isolates from this and the lettuce outbreak were unusual in that most of the isolates were fully drug sensitive.

Towards the end of the summer PT 3 was becoming re-established as the predominant phage type among isolates from patients infected in the UK. It was evident from studies on the 1991 and 1992 isolates that PT 3 and derivative types such as PT 23 probably represent 'endemic' UK *S. sonnei* types. During the late summer and autumn there were a significant number of isolates of PT 6, the majority of which were associated with foreign travel. Since travel history is known to be under-reported it is probable that most infection with PT 6 originates abroad.

This study demonstrated that phage typing of *S. sonnei* provided data which validated the epidemiological recognition of outbreaks and enabled identification of infections related to overseas travel or the consumption of an imported foodstuff. A national reference facility based on phage typing will increase the discriminatory ability of epidemiological investigations and is particularly suited to dealing with large numbers of bacterial isolates.

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