Epidemiological markers of *Salmonella typhimurium* isolates in Rome

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Summary. The phage type, antimicrobial resistance pattern, colicinogenic activity and DNA plasmid content of 172 strains of *Salmonella typhimurium* isolated in Rome from 1984 to 1986 were determined; 142 isolates were from patients with enteritis, 30 were from asymptomatic subjects. Most of the phage types identified were isolated during 2 or 3 of the study years; others, e.g., PT141, PT 204c and PT 194 were isolated during 1 year only, and only the last of these was isolated in large numbers. Phage typing proved most valuable in identifying epidemiologically related strains; DNA plasmid analysis was most useful in characterising further cultures of the same phage type, especially those isolated during suspected epidemics.

Introduction

Salmonella typhimurium is currently the commonest salmonella serotype isolated in Italy from both sick and healthy people and animals;¹ it has also often caused food-poisoning epidemics in Italy² and many other countries.^{3,4} To determine the differences or similarities between isolates, the phage type and antimicrobial resistance pattern of *S. typhimurium* isolates received by the National Enterobacteria Centre at Istituto Superiore di Sanità, Rome, were determined and, because many strains are not sensitive to standard phages, DNA plasmid profiles were also determined. This has been shown to be particularly useful in identifying epidemiologically related isolates.^{5–7} Colicinogenic activity, considered to be important in the establishment of intestinal infections,^{8,9} was also measured and its epidemiological usefulness was examined.

This study compared the characteristics of strains isolated from people in the Rome area to determine whether analysis of plasmid DNA is as valuable an epidemiological marker as phage type or antibiotic resistance pattern.

Materials and methods

Bacterial strains

During the 3 years 1984–1986, 172 *S. typhimurium* strains isolated from people in nine districts of Rome were sent to the National Enterobacteria Centre at the Istituto Superiore di Sanità, Rome, for serotyping. All

had been isolated from faeces; 142 from patients with enteritis and 30 from asymptomatic subjects. Forty-eight of the former were isolated from patients in the Palestrina Hospital, district number 28, during May 1985; 45 strains were isolated at the Military Hospital in Rome, district number 1, during June and July 1985, 44 of them from patients suffering from enteritis and one from a healthy man; four strains were isolated at the Anzio Hospital, district number 35, between 22 and 27 July 1985, from children aged from 5 months to 8 years suffering from diarrhoea; the isolation of the remaining 75 strains was scattered in time and place.

Antimicrobial susceptibility was determined by a disk-diffusion method¹⁰ with disks (Baltimore Biological Laboratories, PO Box 243 Cockeysville, MD 21030, USA) containing ampicillin (AM) 10 µg, cephalothin (CF) 30 µg, chloramphenicol (C) 30 µg, colistin (CL) 10 µg, gentamicin (GM) 10 µg, kanamycin (K) 30 µg, streptomycin (S) 10 µg, sulphisoxazole (ST) 1 mg, tetracycline (TE) 30 µg, tobramycin (TM) 10 µg, and trimethoprim sulphamethoxazole (SXT) 25 µg.

Colicinogenic activity. Cultures were examined for colicin production by an overlay method.¹¹

Phage typing. The strains were typed by Anderson's method¹² with 31 type phages; untypable strains were sent for typing to the International Reference Laboratory for Enteric Phage Typing, Central Public Health Laboratory, Colindale Avenue, London.

Plasmid profile analysis. Plasmid DNA was extracted by the method of Kado and Liu¹³ modified by adding two volumes of *n*-amyl alcohol:chloroform (1:24) solution after adding two volumes of phenol:chloroform (1:1) solution. Crude preparations were analysed by agarose gel electrophoresis¹⁴ with the following plasmid mol. wt standards: PBr 322, V 517, RP4, R 144, R27, RA 1.

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Transfer of resistance plasmids was attempted by Datta's method.¹⁵

Plasmid compatibility groups were determined by the method of Grindley *et al.*¹⁶

Results

Of the 172 *S. typhimurium* strains isolated in the Rome area (20 in 1984, 112 in 1985 and 40 in 1986), 89.5% belonged to 20 different phage types (PT). Seven cultures did not conform to a recognised pattern (RDNC) and 12 were not typable (U) with standard typing phages (table I). Of the 20 strains isolated during 1984 (11 from sick and nine from healthy people), 16 belonged to seven different phage types; four were untypable. Of the 112 strains isolated during 1985 (108 from sick and four from healthy people), 107 belonged to 11 different phage types; of these, 45 were PT141, 46 PT194, two were untypable and three were RDNC. Of the 40 strains isolated in 1986 (23 from sick and 17 from healthy people), 30 belonged to 17 different phage types, six were untypable and four were RDNC.

Eighty-seven isolates were sensitive to all the antimicrobial agents tested; 59 isolates were resistant to three or more agents.

Fourteen isolates had no plasmids; 17 had one large plasmid each, ranging in size from 47 to 90 mDa; two isolates had two plasmids, of 25 and 100 mDa; 124 isolates had both small and large plasmids of 1.34 and 150 mDa and 15 isolates had small plasmids of 1.0–4.55 mDa. The phage types, antibiotic resistance and plasmid patterns of the isolates studied are listed in table II. Generally, strains of each phage type were heterogeneous with respect to antibiotic sensitivity and plasmid pattern. In two instances only were strains of the same phage type homogeneous in these respects; 40 of the 46 strains of PT 141 and 43 of the 47 strains of PT 194 showed the same resistance and plasmid patterns.

All 45 strains isolated between 22 June and 2 July 1985 at the Laboratory of the Military Hospital in Rome, district 1, (44 of them from patients with enteritis and one from a healthy subject, all of whom had eaten chicken of the same batch), were of PT 141 and were colicinogenic. All but five isolates were sensitive to all the antimicrobial agents tested; three were resistant to ST and SXT, one to S, ST and SXT, and one to S, ST and TE. These strains were of two different plasmid profiles: all but one had a plasmid of 55 mDa and two small plasmids of 3.5 and 2 mDa, respectively; four had a fourth plasmid of 65 mDa. A further strain of PT 141 carrying plasmids of 50 and 3.31 mDa was isolated in 1986 in district 35. Of the strains of PT 141 lacking the 65 mDa plasmid, 40 were sensitive to all the antimicrobial agents tested and one was resistant to S and SXT. Of four strains harbouring the 65 mDa plasmid, two were resistant to S and SXT, one to S, ST and SXT, and one to S, ST and TE.

Table I. The most frequent *S. typhimurium* phage types isolated in the Rome area by year

Phage type	Number of isolates in			Total number of isolates
	1984	1985	1986	
10	3	—	3	6
12	7	—	4	11
141	—	45	1	46
170	1	1	2	4
194	—	46	1	47
195	2	2	3	7
U285	1	4	3	8
204c	—	4	—	4
RDNC	—	3	4	7
U	4	2	6	12
Others (12)	2	5	13	20
Total	20	112	40	172

RDNC, cultures did not conform to a recognised pattern.

U, untypable.

Forty-five strains of PT 194 were isolated during May 1985 at the laboratory of the Palestrina Hospital, district 28, from patients with enteritis, all of whom shortly before admission had eaten pork at a wedding dinner at the same restaurant. Unfortunately, no isolates from the pork were available for characterisation, but all 45 strains showed the same plasmid profile: two large plasmids of 65 and 55 mDa and five small plasmids of 3.9, 3.1, 2.4, 2 and 1.7 mDa. The 65-mDa plasmid was autotransferable. Forty-three of these isolates were resistant to S, ST, SXT and TE, one was resistant to CF, CL, S, ST, SXT and TE, and one to S, ST and SXT; only this last was colicinogenic. From further subjects attending the same wedding dinner and who had also eaten pork, a PT 193 culture resistant to TE and two untypable cultures of *S. typhimurium* resistant to CF, ST and TE were isolated; they carried plasmids differing in number and weight from those of PT 194 strains. Two further cultures of PT 194 were isolated in two different districts of Rome. One, isolated in 1985, was resistant to AM, S and ST and carried plasmids of 52, 1.8 and 1.6 mDa, and one isolated in 1986 was resistant to Te and carried plasmids of 75 and 52 mDa.

Four strains of PT 204c were isolated between 22 and 27 July 1985 in the laboratory of Anzio Hospital, district 35, from children aged 5 months to 8 years suffering from diarrhoea. All produced colicins and all showed the same antibiotic resistance pattern. These strains carried three plasmids of 150, 60 and 30 mDa respectively; three of them carried a small plasmid of 3.5 mDa; the fourth had a small plasmid of 2.5 mDa. The three larger plasmids were autotransferable: the largest, of 150 mDa and of compatibility group H, coded for resistance to C, S, ST, SXT and TE; the plasmid of 60 mDa, of compatibility group I α coded for resistance to Gm, S and TM and for colicin production.

Table II. *S. typhimurium* strains isolated in the Rome area characterised by phage type, antibiotic-resistance pattern and DNA plasmid profile

Phage type (number of isolates)	Resistance pattern (number of isolates)	DNA Plasmid size in mDa (number of isolates)
PT 8 (3)	sens (3)	50 (2) 1-94 (1)
PT 10 (6)	AM S ST TE (1) AM S ST TE (1) sens (4)	80, 24, 4-5, 3-3 1-73 — (2) 1-62 (1) 2-04 (1)
PT 12 (11)	sens (8)	50, 5 (2) 50 (2) 50, 3-95, 2-57, 1-9 (1) 60 (1) 60, 40 (1) 4-55, 2-57 (1) 50, 5 (2) 50, 3-95, 2-57, 1-9 (1)
PT 15a (1)	sens	50, 2-69
PT 29 (2)	TE (1) TE (1)	50, 5 45, 3-01
PT 36 (1)	sens	50, 3-31
PT 49b (1)	sens	—
PT 109 (1)	sens	47
PT 121 (1)	sens	45, 2-8, 1-6
PT 135 (3)	sens (1) S ST TE (1) C S ST SXT TE (1)	50, 25 50 72, 57, 33, 22-4, 1-34
PT 141 (46)	sens (41) ST SXT (3) S ST SXT (1) S ST TE (1)	55, 3-5, 2 (40) 50, 3-31 (1) 55, 3-5, 2 (1) 65, 55, 3-5, 2 (2) 65, 55, 3-5, 2 65, 55, 3-5, 2
PT 160 (2)	sens	52
PT 170 (4)	sens (1) SXT (1) S SXT (1) ST SXT (1)	— 24, 3-0 — —
PT 171 (1)	sens	1-77, 1-44
PT 178 (1)	sens	50
PT 193 (3)	TE (1) TE (1) S (1)	55, 4-7, 3-7, 3, 2-2, 1-8 60 52
PT 194 (47)	TE (1) Am S ST (1) S ST SXT (1) CF CL S ST SXT TE (1) S ST SXT TE (43)	75, 52 52, 1-8, 1-6 65, 55, 3-9, 3-1, 2-4, 2, 1-7 65, 55, 3-9, 3-1, 2-4, 2, 1-7 65, 55, 3-9, 3-1, 2-4, 2, 1-7
PT 195 (7)	TE (7)	36, 4-36, 4-07, 3-31, 2-95, 2-57, 2-04 (1) 50 (1) 60 (1) 50, 3-95, 2-57, 1-9 (1) 45, 5-49, 2-8, 1-6 (1) 90 (1) 54, 5 (1) — (3)
U285 (8)	sens (8)	1-54, 1-2 (2) 1-54 (2) 4-36, 3-0, 1-54 (1) 150, 60, 30, 2-5 (1) 150, 60, 30, 3-5 (3)
204c (4)	AM C GM K NA S ST SXT TE TM (4)	— (4) 1-0 (1) 3-95, 2-57 (1) 1-34
RDNC (7)	sens (6)	— (1) 50 (1) 52 (1) 45, 2-08 (1) 60, 3-95, 2-57 (1) 3-71, 2-23, 1-90 (1)
U (12)	K S ST TE (1) sens (6) TE (2) ST (1) S ST (1) CF ST TE (2)	50, 5 (1) 20, 4-07, 3-09, 2-04 (1) 60, 3-95, 2-57 60 100, 25

RDNC, cultures did not conform to recognised pattern.

U, untypable.

Discussion

Twenty different phage types of *S. typhimurium* were represented among 172 strains isolated in Rome during the 3 years 1984–1986. Three phage types were prominent. Firstly, four multi-resistant strains of PT 204c were isolated from children with diarrhoea in district 35 during July 1985. Although PT 204c strains have been isolated in the UK with increasing frequency from both man and animals¹⁷ they have not until now been recognised in Italy as a cause of human gastroenteritis. All four isolates were resistant to gentamicin, as described by Threlfall *et al.*^{18,19} for strains isolated from cattle and man and the plasmid profile also resembles that described by Threlfall *et al.*²⁰ for strains isolated in the UK. A plasmid of compatibility group H, conferring resistance to C, S, ST, SXT and TE, and a plasmid of compatibility group X, conferring resistance to AM and K, were present. The plasmid responsible for resistance to GM, S and TM belonged to compatibility group I α and, unlike plasmids of strains isolated from people studied by Threlfall *et al.*,²⁰ it also specified colicinogeny. Except for the small plasmids, the four strains had similar characteristics; unfortunately, although all were isolated in the same month the circumstances in which they were found were not investigated epidemiologically. Multi-resistant strains of *S. typhimurium* PT 204c circulate among cattle in Europe:¹⁹ in Italy, multi-resistant strains of PT 204 and 204a have been isolated from cattle²¹ and, recently, gentamicin-resistant isolates from cattle in Italy showed a phage lysis pattern resembling that of PT 204c and harboured plasmids with the same electrophoretic motility as those found in strains from man. Only three of the PT 204c strains produced colicins.

Secondly, of 46 PT 141 isolates, 45 were isolated during 2 consecutive months from patients in the Military Hospital, Rome, and probably represent a point-source outbreak of food-poisoning from chicken

although none was available for bacteriological examination. Salmonellae colonise poultry easily and this forms a major worldwide source of salmonellosis.¹⁷ Strains of PT 141 are often isolated from poultry,^{22,23} two have been isolated from chicken in Northern Italy. Such strains are usually fully sensitive to antibiotics and often cause food poisoning.^{23–25} Of the 45 epidemiologically related strains of PT 141, all were colicinogenic; 40 were sensitive to all the antimicrobial agents tested and all carried the same plasmids. These strains were isolated early in the outbreak; the remaining PT 141 strain was isolated later, had a different plasmid profile and was antibiotic resistant. A 55-mDa plasmid also present in the antibiotic-sensitive strains has been described as specifying virulence characteristics.^{26,27}

Forty-five strains of PT 194 were isolated from patients in district 28 during May, 1985. This phage type, while not frequent in the UK nor in Spain, Portugal or Greece, was so often isolated from pigs in Belgium and from people in France and Germany as to constitute a widespread clonal outbreak.²⁸ Forty-three of the 45 PT 194 strains did not produce colicins were resistant to S, ST, SXT and TE, and had the same plasmid profile. The remaining two strains had the same plasmid profile, produced colicins, but differed from the former strains and from each other in their antibiotic-resistance patterns. Such variations among strains of the same phage type have been noted;⁶ the possibility that the strains belong to the same clone cannot be ruled out.

These results confirm that not all salmonella strains isolated from epidemiologically related patients belong to the same phage type and show that, among those that do, secondary tests for antibiotic sensitivity, colicin production and plasmid content are of limited value in determining epidemiologically related subsets.

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