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Phage type and DNA plasmid profile of *Salmonella typhimurium* isolates in the area of Isernia, Italy

M. FANTASIA[1], N. RICCI[2], A. MANUPPELLA[2], A. MARTINI[1], E. FILETICI[1] AND T. LAURELLI[3]

[1] *Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanità, Rome, Italy*

[2] *Presidio Multizonale di Igiene e Prevenzione, Isernia, Italy*

[3] *Laboratorio di Analisi, Ospedale di Isernia, Italy*

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SUMMARY

Thirty-eight *Salmonella typhimurium* strains isolated from December 1987 to March 1988 in Isernia, Central Italy, were characterized on the basis of their phage type, resistance to antimicrobials and plasmid profiles. According to their phage types, the isolates could be assigned to one of six groups, the prevalent one being PT 195 which accounted for 73·6% of isolates.

On the basis of their plasmid content, the isolates could be assigned to one of ten groups. The prevalent plasmid profile (60·0; 6·0; 4·3; 4·0; 3·2 megadaltons) was found in 60·4% of isolates.

All the isolates from a particular food (salsicce), and as most of isolates from humans who had consumed this food belonged to phage type 195 and were of the same plasmid profile.

The combined use of phage typing and DNA plasmid analysis proved to be a useful tool in identifying epidemiologically related isolates in this investigation.

INTRODUCTION

Antimicrobial susceptibility determination, biotyping and phage typing are well-established methods used as epidemiological markers [1–3]. Quite recently plasmid profile analysis was introduced to determine the molecular relatedness of isolates of *Salmonella* spp. and as a further method of strain differentiation. However, opinions on the usefulness of this method are conflicting [4, 5]. In the present study we applied various methods to the characterization of strains of *Salmonella typhimurium* isolated in Italy during a suspected epidemic.

In February 1988 health officers noted an unusual high number of cases of salmonellosis requiring admission to the paediatric department of a hospital in Isernia, Central Italy. All isolates were identified as *S. typhimurium*. An epidemiological investigation was instigated on these cases and on all other cases of salmonellosis caused by *S. typhimurium* which had occurred since December 1987 using a standardized questionnaire. The household contacts of cases were also investigated microbiologically. As processed pork (salsicce) derived from the

same commercial source, had been consumed by most of patients in the 2 days before isolation of *S. typhimurium*, samples of this food were examined for the presence of such a microorganism.

All strains of *S. typhimurium* isolated in the area of Isernia from late December 1987 to the end of March 1988 were studied in order to determine their phage type [6], resistance to antimicrobial drugs [7] and plasmid profile [8]. The purpose of these investigations was to determine whether plasmid analysis used either as single test, or combined with other tests such as resistance pattern determination and phage typing is a useful epidemiological tool in determining relatedness between isolates in a limited geographical area.

MATERIALS AND METHODS

Bacterial strains

Thirty strains of *S. typhimurium* were obtained from faeces of humans, 22 suffering from enteritis and 8 asymptomatic; 19 were isolated from children and 11 from adults; 6 strains were from food (a kind of sausage hereafter referred to as salsicce), 1 from a rectal swab of a pig and 1 from butcher's work table.

Isolation

Isolation of *Salmonella* sp. from human source was achieved by standard procedures [9]; for isolates from food and other sources an enrichment procedure was employed, based on methods of Edel and Kampelmacher [10, 11].

Biochemical and serological characterization were performed by standard procedures [9].

Phage typing

S. typhimurium isolates were typed by the method of Callow [12]; the phage type designations were those of Anderson and colleagues [1]; the strains which did not react with the standard typing phages were sent of Dr B. Rowe, WHO Collaborating Centre, London, for further phage typing.

Resistance typing

Bacterial susceptibility to antimicrobial agents was determined by the method of agar disk diffusion [7] using Isosensitest agar (Oxoid) and the following antimicrobials (BBL): ampicillin (AM) 10 μg; cefalotin (CF) 30 μg; chloramphenicol (C) 30 μg: gentamicin (GM) 10 μg; kanamycin (K) 30 μg; nalidixic acid (NA) 30 μg; streptomycin (S) 10 μg; sulfathiazole (ST) 1 mg; tetracycline (TE) 30 μg; tobramicin (TM) 10 μg; sulfamethoxazole-trimethoprim (SXT) 25 μg.

Plasmid DNA extraction and analysis

Plasmid DNA was extracted according to the method of Kado and Liu [8]. Plasmids were separated by electrophoresis in a 0·7 % agarose gel with tris-borate-EDTA buffer for 2·15 h at room temperature at 90 volts; the gel was stained with ethidium bromide for 1 h. Photographs of the DNA bands were taken under UV light exposure. Molecular weights were calculated according to the method of Aay

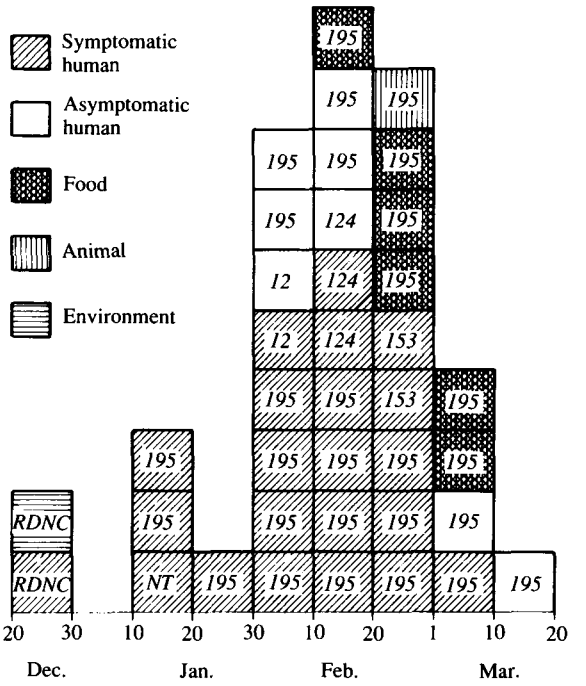

Fig. 1. Number of *S. typhimurium* phage type isolates by day and source.

and colleagues [13]. *E. coli* K12 strains containing the plasmids p IP40a, R702, R16 and the strain V517, were used as standards in each run.

RESULTS

Outbreak

In the 5-month period December 1987 to March 1988, *S. typhimurium* was isolated in the area of Isernia from 38 specimens; 30 isolates were from humans and 8 from non-human sources. Of the 30 human isolates, 12 were from hospitalized patients, 10 from outpatients and 8 from asymptomatic subjects. The distribution of cases of enteritis caused by *S. typhimurium* as established by the onset of symptoms revealed the highest incidence in February (Fig. 1). 77·3% of cases were children aged 1–3 years, 4·5% were 4–7 years and 18·2% were adults with ages ranging from 19–82 years. The most frequent symptoms were fever, vomiting, diarrhoea, with blood and mucus in a few cases. The clinical picture was complicated by convulsion in one patient and by a severe dehydration in another. Symptoms disappeared in 4–10 days.

Phage typing

Of the 38 *S. typhimurium* isolates, 35 belonged to 4 different phage types (PT's), 2 did not conform to recognized patterns and the remaining isolate was untypable with the standard phages.

Twenty-eight isolated belonged to PT 195, 21 of which had been isolated from

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Table 1. *Phage type, antimicrobial resistance pattern and molecular weight of plasmid DNA in* S. typhimurium *isolates (number of strains)*

PT	Source	Resistance pattern	Plasmic molecular weight in MDa
195 (28)	A [1]	sens	60; 6; 4·3; 4; 3·2
	F [6]	sens	60; 6; 4·3; 4; 3·2
	H [16]*	sens	60; 6; 4·3; 4; 3·2
	H [1]*	sens	100; 60; 6; 4·3; 4; 3·2
	H [1]	sens	60; 6; 5·6; 4·3; 4
	H [2]	TE	60; 6; 5·6; 4·3; 4
	H [1]	AM C S SXT TE	100; 6; 4; 2·7
124 (3)	H [1]	sens	80; 7; 4·5
	H [2]†	AM CF S	80; 7; 4·5
12 (2)	H [1]	sens	—
	H [1]	AM C S TE	—
153 (2)	H	sens	5; 4·6; 3·2; 2·6
RDNC (2)	E [1]	sens	80; 6·8; 4·2
	H [1]	sens	80; 6·8; 4·2
NT (1)	H	sens	60; 6; 5·6; 5·1; 4·4; 3·8; 3·4; 3; 2·6

*, the patients consumed 'salsiccia', †, the patients consumed 'ricotta', PT, = Phage type, MDA, megadalton, A, animal (pig), E, environment, F, food (salsiccia), H, human, AM, ampicillin 10 μg, C, chloramphenicol 30 μg, CF, cefalothin 30 μg, S, streptomycin 10 μg, SXT, sulfamethoxazole-trimethoprim 25 μg, TE, tetracycline 30 μg, RDNC, cultures did not conform to recognized pattern, NT, non typable.

humans (8 from hospitalized patients, 7 from outpatients and 6 from healthy carriers). All isolates from food and the isolate from the rectal swab of a pig were PT 195. Twelve out of 15 sick patients positive for PT 195 were children, 11 under 3 years and one 7 years old, respectively. Of the remaining cultures, 3 belonged to PT 124, 2 to PT 12 and 2 to PT 153.

All isolates from people who had consumed raw salsicce belonged to PT 195. Of the isolates from people who denied consuming this food, 4 belonged to PT 195, 3 to PT 124 and 2 to PT 153. Two of 3 patients with PT 124 had consumed a light cheese called 'ricotta' a few days before onset of symptoms.

Plasmid studies

The phage types, molecular weight of plasmid DNA and antimicrobial resistance patterns of the *S. typhimurium* isolates are summarized in Table 1 and the plasmid profiles of representative *S. typhimurium* isolates of different phage types are shown in Fig. 2.

According to their plasmid content, the 28 PT 195 cultures could be assigned to one of four groups. The predominant plasmid pattern (60·0; 6·0; 4·3; 4·0; 3·2 Mda) was found in 23 cultures; the other 5 PT 195 cultures showed 3 different patterns. Twenty-five of the 28 PT 195 isolates were sensitive to all antimicrobials tested, 2 were TE-resistant and 1 isolate, from stool of a 3-year-old outpatient suffering from enteritis, was multi-resistant with the pattern AM, C, S, SXT, TE. With the exception of the multi-resistant isolate, all PT 195 cultures harboured a plasmid

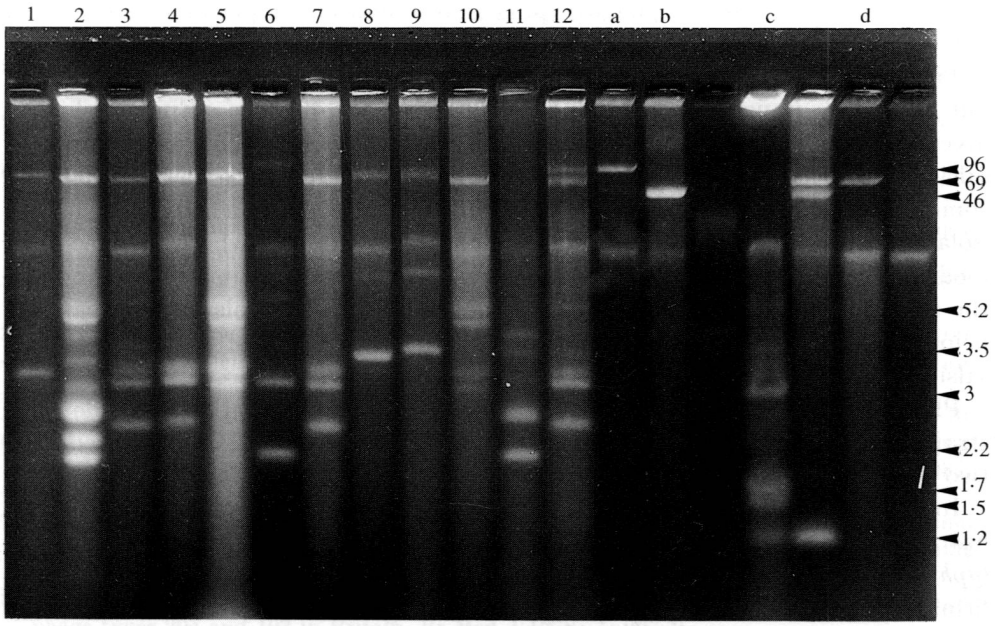

Fig. 2. Agarose gel electrophoresis (0·7% agarose) of DNA plasmid from *S. typhimurium* strains. Molecular weight (Mda). Lanes: 1 = RDNC (80·0; 6·8; 4·2); 2 = NT (60·0; 6·0; 5·6; 5·1; 4·4; 3·8; 3·4; 3·0; 2·6); 3, 4, 7 = PT 195 (60·0; 6·0; 4·3; 4·0; 3·2); 5, 10 = PT 195 (60·0; 6·0; 5·6; 4·3; 4·0); 6 = PT 195 (100; 6·0; 4·0; 2·7); 8, 9 = PT 124 (80·0; 7·0; 4·5); 11 = PT 153 (5·0; 4·6; 3·2; 2·6); 12 = PT 195 (100; 60·0; 6·0; 4·3; 4·0; 3·2); a = pI P 40 (96·0); b = R 702 (46·0); c = V 517 (32; 5·2; 3·5; 3·0; 2·2; 1·7; 1·5; 1·2); d = R 16 (69·0).

of 60·0 Mda; the multi-resistant isolate did not possess this plasmid but carried an additional plasmid of 100 Mda. All PT 195 cultures carried at least three small plasmids with molecular weight ranging from 3·2 to 6·0 Mda. The three PT 124 isolates had identical plasmid profiles but differed in antimicrobial resistance; one of the two PT 12 isolates was multi-resistant (AM, C, S, TE) and one was drug-sensitive: no plasmid DNA was detected in either strain. The two PT 153 strains, although isolated in different places, were both drug-sensitive and had identical plasmid profiles.

DISCUSSION

In view of an usually high number of strains of *Salmonella typhimurium* isolated from patients in a pediatric department of a hospital in Isernia (region Molise, Central Italy) in a short period of time, an epidemiological investigation was instigated. Most of the cases and their household contacts were found to have consumed raw processed pork (salsicce) a few days before isolation of organisms. As *S. typhimurium* is the serotype most frequently isolated in Italy from both man and food [14, 15] and as processed pork is one of the foods most frequently contaminated by this serotype, serological identification was not sufficient to establish an epidemiological link between the isolates. Although epidemiological investigations had suggested that salsicce was the source of infection in this

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outbreak, other tools i.e. phage typing determination of plasmid profiles and antimicrobial resistance patterns were necessary to confirm this hypothesis.

The present investigation demonstrated that PT 195 was endemic in Isernia, but with an unusual spread in a short period of time. Epidemiological investigations had already demonstrated correlation between consumption of salsicce and isolation of *S. typhimurium* PT 195. This hypothesis was confirmed by combined tests, which demonstrated that although PT 195 was heterogeneous, all isolates of this phage type from salsicce and from subjects who consumed such food belonged to the same phage type, were all sensitive to antimicrobiols and had the same plasmid profile. These results confirmed that the isolates from patients belonged to the epidemic clone and proved that the origin of the outbreak was salsicce.

Phage typing and plasmid profile analysis also revealed that two identical strains belonging to a different phage type, phage type 124 had been isolated from two patients who had consumed a kind of fresh cheese (ricotta).

In conclusion, the combined use of phage typing and plasmid profile analysis has considerably enhanced the differentiation of a ubiquitous serotype, *S. typhimurium*, which is frequently isolated in Italy, and have provided confirmatory evidence of epidemiological investigations [16]. In contrast, the resistance pattern was not of epidemiological value. This was because resistance in *S. typhimurium* was rarely encountered in this study (only four strains were multi-resistant), and when present, there was no correspondence with other parameters i.e. phage type and plasmid content.

Our data also show that in the outbreak isolates there was close correlation between phage type and plasmid profile. However, previous studies have shown that plasmids can be lost or gained, although rarely, in a short period of time [17]. Finally, in our study plasmids do not appear to have interfered with the lysotype of their host in contrast to what has been demonstrated in other types in the past [18, 19].

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