

## Characterization of *Salmonella enterica* Serotype Typhimurium Isolates from Human, Food, and Animal Sources in the Republic of Ireland

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**A potential epidemic clone of *Salmonella enterica* serotype Typhimurium DT104, and the possible emergence of *S. enterica* serotype Typhimurium DT104b, has been identified from the characterization of 67 *S. enterica* serotype Typhimurium strains from three sources, human gastroenteritis isolates, isolates from food samples, and veterinary isolates, by antimicrobial resistance profiling, phage typing, and pulsed-field gel electrophoresis. Resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline was found in 77.6% of these strains.**

Worldwide, *Salmonella* has been recognized as an important food-borne pathogen. It can be isolated from raw meats, poultry and poultry products, and milk and milk products. *Salmonella* outbreaks have also been associated with poor cooking, reheating of foods, and improper handling of food by food preparers (12).

Representing 30.4% of all *Salmonella* strains isolated from humans, *Salmonella enterica* serotype Typhimurium was the second most commonly isolated *Salmonella* serotype in the Republic of Ireland in 2001, exceeded only by *S. enterica* serotype Enteritidis (18). Phage type DT104 is the phage type most frequently isolated from both cattle (8) and human patients (9).

In 1984, the first strain of *S. enterica* serotype Typhimurium DT104 resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (resistance profile ACSSuT) was isolated in the United Kingdom (31). However, it was in the early 1990s that a dramatic increase of this pentaresistance profile was observed not just in the United Kingdom (30) but worldwide (2, 4, 6, 10, 21).

Despite the importance of *Salmonella* serotype Typhimurium in causing salmonellosis in Ireland, there have been a limited number of epidemiological studies of this organism (5, 6, 9, 15). This study has applied a combination of phenotypic and genotypic typing methods, including pulsed-field gel electrophoresis (PFGE), which has been recognized as a powerful tool and the “gold standard” of molecular typing methods (19, 24), in the analysis of *S. enterica* serotype Typhimurium DT104 and DT104b from human, food, and animal sources over a 2-year period in the midwest region of Ireland.

A total of 67 *S. enterica* serotype Typhimurium isolates were obtained during a 2-year study period from June 2000 to May 2002. These isolates included 28 strains from human gastroenteritis stool samples, 3 strains from food samples, and 36 veterinary strains, for a total of 67 strains. The strains were obtained from the Regional Hospital and Regional Veterinary Laboratory, Limerick, Ireland.

Antimicrobial disk susceptibility testing according to the method of the National Committee for Clinical Laboratory Standards (NCCLS) (16, 17) was carried out using 15 antimicrobial agents: ampicillin, coamoxiclavulanic acid, cefotaxime, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, norfloxacin, ofloxacin, pefloxacin, streptomycin, sulfonamides, tetracycline, and trimethoprim-sulfamethoxazole. *Escherichia coli* ATCC 25922 was used as a control on each test occasion. An isolate was defined as being drug resistant if it was resistant to at least one of the tested antimicrobial agents and multidrug resistant if it was resistant to four or more of the antimicrobial agents tested (25). Phage typing based on the Colindale method (1) was carried out in the *Salmonella* Reference Laboratory, Galway, Ireland.

PFGE was performed with the 54 *S. enterica* serotype Typhimurium DT104 and DT104b strains as well as two non-phage-typeable strains. The preparation of *Salmonella* plugs for PFGE was carried out according to the manufacturer's instructions (Bio-Rad). The restriction enzymes XbaI, SpeI, and BlnI were used to develop the PFGE profiles. Chromosomal DNA restriction patterns produced by PFGE were interpreted by the criteria of Tenover et al. (24) for bacterial strain typing. Statistical analysis of PFGE profiles was carried out by using the Dice coefficient (7).

Antimicrobial susceptibility test results showed a total of 16 resistance profiles for *S. enterica* serotype Typhimurium as summarized in Table 1. All but one strain of *S. enterica* serotype Typhimurium (66 of 67 strains [98.5%]) collected in this study expressed drug resistance, and 88% (59 of 67 strains) expressed multidrug resistance. The pentaresistance profile ACSSuT was observed in 77.6% (52 of 67) of the *S. enterica* serotype Typhimurium strains. In addition, 19.4% (13 of 67) of the isolates expressed trimethoprim-sulfamethoxazole resistance, 3% (2 of 67) expressed nalidixic acid resistance, and 7.5% (5 of 67) expressed intermediate resistance to coamoxiclavulanic acid (Table 1).

Phage type DT104 accounted for 63% (42 of 67) of the isolates and was observed in all three sources, followed by DT104b at 17.9% (12 of 67 isolates) (Table 2). DT104b occurred 50% more frequently in human clinical strains (8 of the 12 strains of phage type DT104b) than in veterinary isolates (4 of the 12 strains of phage type DT104b) and was absent in

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TABLE 1. *S. enterica* serotype Typhimurium resistance profiles

Resistance profile <sup>a</sup>	No. of isolates with resistance profile			
	Clinical	Veterinary	Food	Total
ACSSuT	14	25	1	40
ACSSuTX	4	4	1	9
ACSSuT; Amc-I	0	1		1
ACSSuTNa; Amc-I	0	1	0	1
ACSSuTX; Amc-I	1			1
ASSuT	3	0	0	3
ACSSu; TAmc-I	0	2	0	2
T	1	1		2
ASSuTX	0	0	1	1
ASuTX	1	0	0	1
STNa		1		1
SSuT		1		1
SSuX	1			1
ASu	1			1
ASu; S-I	1			1
S-I	1			1
Total	28	36	3	67

<sup>a</sup> A, ampicillin; Amc, coamoxiclavulanic acid; C, chloramphenicol; Na, nalidixic acid; S, streptomycin; Su, sulfonamides; T, tetracycline; X, trimethoprim-sulfamethoxazole; I, intermediate resistance. The 52 strains with the ACSSuT pentaresistance profile are shown in the first five rows.

strains obtained from food sources. Five other phage types were observed (Table 2).

Upon visualization of the PFGE results for the 56 strains of *S. enterica* serotype Typhimurium DT104 and DT104b digested with XbaI, BlnI, and SpeI, PFGE gels identified 15 individual PFGE banding patterns. The most predominant banding pattern observed was designated X1aB1aS1a and was observed in 21 isolates of both phage type DT104 and phage type DT104b from all three sources (Table 3). The remaining PFGE banding patterns are summarized in Table 3.

This study has utilized a combination of phenotypic and genotypic typing methods to observe a relationship among strains of *S. enterica* serotype Typhimurium isolated from human, food, and animal sources in the midwest region of Ireland. The data reported here demonstrate a high level of drug resistance (98.5% [66 of 67 strains]) associated with *S. enterica* serotype Typhimurium, a result which correlates with those of studies performed in the European Union (6, 14, 30, 22). Multidrug resistance was observed in 88% (59 of 67) of *S. enterica* serotype Typhimurium strains, and the resistance profile for 77.6% (52 of 67) of these strains was ACSSuT. In the

TABLE 2. Phage typing of *S. enterica* serotype Typhimurium isolates by source

Type of isolate	No. of isolates	No. of isolates with <i>S. enterica</i> serotype Typhimurium phage type						
		DT104	DT104b	DT15a	DT120	DT193	DT10	UT <sup>a</sup>
Clinical	28	12	8		1	6	1	
Veterinary	36	29	4	2	1			
Food	3	1						2
Total	67	42	12	2	2	6	1	2

<sup>a</sup> UT, untypeable.

TABLE 3. Summary of *S. enterica* serotype Typhimurium DT104 and DT104b PFGE pattern profiles

PFGE type <sup>a</sup>	No. of isolates of PFGE type					Total
	Human		Veterinary		Food (DT104)	
	DT104	DT104b	DT104	DT104b		
X1aB1aS1a	2	5	11	3		21
X1dB1aS1a	2	2	8			12
X1aB1aS1b			4		1 <sup>b</sup>	5
X1cB1aS1a	4					4
X1aB1aS1c			1	1	0	2
X1aB1bS1b			1			1
X1aB1cS1a	1					1
X1bB1aS1a			2			2
X1bB1fS1a					1	1
X1bB1gS1a	1					1
X1dB1aS1b		1	1			2
X1eB1dS1d					1 <sup>b</sup>	1
X1cB1eS1a	1					1
X1cB1gS1a	1					1
X1aB3S2			1			1

<sup>a</sup> X, XbaI; B, BlnI; S, SpeI.

<sup>b</sup> Non-phage-typeable strain.

1990s, a new *S. enterica* serotype Typhimurium phage type DT104 expressing this pentaresistance profile emerged in many countries and has been reported in Ireland (15), the United Kingdom (30, 31), the United States (10, 23), Canada (21), and Denmark (2, 3). The ACSSuT resistance profile has been shown to be chromosomally integrated (26), suggesting that the withdrawal of antimicrobial agents would not have any effect on the current epidemic of DT104. Portentously, strains of *S. enterica* serotype Typhimurium expressing resistance to trimethoprim-sulfamethoxazole in addition to this pentaresistance profile are highlighted in this study. Furthermore, all trimethoprim-sulfamethoxazole resistance was associated with additional resistance to one or more of the five antimicrobials with the pentaresistance profile ACSSuT. Resistance such as this may be associated with the use of trimethoprim to combat bovine infections, as was observed by Threlfall et al. in the United Kingdom during the late 1970s (28) and more recently in the early 1990s (29).

All strains of *S. enterica* serotype Typhimurium were susceptible to the second-generation fluoroquinolones; however, two bovine isolates expressed resistance to the first-generation quinolone nalidixic acid. The emergence of nalidixic acid-resistant *Salmonella* is a cause for concern, as fluoroquinolone and reduced fluoroquinolone resistance have been associated with nalidixic acid resistance (11, 20).

During this study, six different *S. enterica* serotype Typhimurium phage types were observed. Accounting for 75% of all *S. enterica* serotype Typhimurium strains, DT104 was the most prevalent phage type observed in both cattle and human patients. Similar observations have been reported in other studies in the United Kingdom (27, 31), the United States (10, 23), and Canada (21). DT104 was first observed in cattle in Ireland in 1995, and incidences of this phage type have continued to escalate from 2 in 1995 to 85 in 1998. In comparison, the first case of DT104b was observed in cattle in 1996 and incidences have since remained relatively low (8).

Although PFGE has been used in international studies of *S.*

*enterica* serotype Typhimurium, it is difficult to compare published PFGE profiles for DT104 due to differences in pulse times and electrophoresis run conditions. However, with this limitation in mind, the size and number of fragments in the XbaI PFGE type X1a observed in our study appeared similar to the PFGE profile of the multiresistant DT104 isolates in previous Irish studies (5, 15), in a multinational study which included Denmark, Germany, Italy, Spain, and the United States (3), and in a study in France (13), thus confirming the spread and persistence of this multidrug-resistant, epidemic strain of *S. enterica* serotype Typhimurium. However, molecular epidemiological analysis by PFGE was unable to differentiate between *S. enterica* serotype Typhimurium phage types DT104 and DT104b. One suggested explanation for this result is that chromosomally integrated pentaresistant *S. enterica* serotype Typhimurium phage types DT104 and DT104b differ only in their numbers of lysis reactions to phage, which are not associated with the genetic pattern of chromosomal DNA.

This study suggests that at present Ireland is suffering from an epidemic of *S. enterica* serotype Typhimurium DT104. It is therefore imperative that food handlers, veterinarians, and people working with animals recognize the possibility of cross-contamination of this microorganism. Furthermore, this study identified the need for future surveillance and monitoring of the potential epidemic strains of *S. enterica* serotype Typhimurium DT104b expressing multiple resistances.

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