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Molecular Characterization of Irish *Salmonella enterica* Serotype Typhimurium: Detection of Class I Integrons and Assessment of Genetic Relationships by DNA Amplification Fingerprinting

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Salmonella enterica **is among the principal etiological agents of food-borne illness in humans. Increasing antimicrobial resistance in *S. enterica* is a cause for worldwide concern. There is concern at present in relation to the increasing incidence of human infection with antimicrobial agent-resistant strains of *S. enterica* serotype Typhimurium, in particular of phage type DT104. Integrons appear to play an important role in the dissemination of antimicrobial resistance genes in many *Enterobacteriaceae* including *S. enterica*. In this study the antimicrobial susceptibilities and phage types of 74 randomly collected strains of *S. enterica* serotype Typhimurium from the Cork region of southern Ireland, obtained from human, animal (clinical), and food sources, were determined. Each strain was examined for integrons and typed by DNA amplification fingerprinting (DAF). Phage type DT104 predominated ($n = 48$). Phage types DT104b ($n = 3$), -193 ($n = 9$), -195 ($n = 6$), -208 ($n = 3$), -204a ($n = 2$), PT U302 ($n = 1$), and two nontypeable strains accounted for the remainder. All *S. enterica* serotype Typhimurium DT104 strains were resistant to ampicillin, chloramphenicol, streptomycin, Sulfonamide Duplex, and tetracycline, and one strain was additionally resistant to trimethoprim. All DT104 strains but one were of a uniform DAF type (designated DAF-I) and showed a uniform pattern of integrons (designated IP-I). The DT104b and PT U302 strains also exhibited the same resistance phenotype, and both had the DAF-I and IP-I patterns. The DAF-I pattern was also observed in a single DT193 strain in which no integrons were detectable. Greater diversity of antibiograms and DAF and IP patterns among non-DT104 phage types was observed. These data indicate a remarkable degree of homogeneity at a molecular level among contemporary isolates of *S. enterica* serotype Typhimurium DT104 from animal, human, and food sources in this region.**

Two serotypes of *Salmonella* predominate worldwide, the poultry-associated *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium, which has a wider animal reservoir. Both of these serotypes can be subdivided by phage typing; some of the *S. enterica* serotype Typhimurium phage types are referred to as definitive types (DT). *S. enterica* serotype Typhimurium phage type DT104 is causing particular concern because of its increasing prevalence and acquisition of multiple antibiotic resistance (11, 25, 26, 29). The selective pressure created by widespread use of antimicrobial agents in animal and poultry husbandry may have contributed to the dissemination of these multi-drug-resistant (MDR) bacterial strains. Detailed characterization of *S. enterica* serotype Typhimurium strains from animal, human, and clinical sources may improve our understanding of the epidemiology of this zoonotic infection.

S. enterica is one of the most common food-borne pathogens identified in Great Britain and Ireland. In the United States 800,000 to 4 million *Salmonella* infections occur annually, of which approximately 1 to 5% are confirmed as due to *S. enterica* serotype Typhimurium by standard laboratory methods, on the basis of data reported to the Centers for Disease Control and Prevention (11). This organism has the potential to infect a variety of animal species, and therefore a diverse range of foods can become contaminated making control difficult. Recent data signaled the emergence of MDR *S. enterica* serotype Typhimurium (8, 11, 25, 26, 29; M. Cormican, C. O'Hare, D. Morris, G. Corbett-Feeney, and J. Flynn, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. A-103, p. 22, 1999), most isolates of which were found to be resistant to at least five common antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). Furthermore the increasing incidence of trimethoprim and nalidixic acid resistance (8, 14, 20) together with reduced susceptibility to ciprofloxacin (a fluoroquinone) is of particular concern. It is well established that the dissemination of antimicrobial resistance is often plasmid and/or transposon mediated. Integrons, a novel group of mobile DNA elements originally identified in gram-negative bacteria, have the potential to incorporate several antibiotic resistance genes by site-specific recombination (7, 13, 19, 24). Recent reports demonstrated that antimicrobial resistance genes are clustered in the genome of *S. enterica* serotype Typhimurium DT104 (6, 20) and that these genes can be efficiently transduced by P22-like phages (22).

In the United Kingdom, infections with MDR strains are reported to be associated with an increased morbidity and mortality compared to other *Salmonella* infections (29). The

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increasing incidence in animals and humans of infection with a single MDR phage type requires investigation. It is essential that relevant epidemiological data be available to monitor organism spread and patterns of infection through animals, food, and humans. In this study 74 randomly selected *S. enterica* serotype Typhimurium isolates from animal, food, and human sources in the Cork region of Ireland submitted to the Molecular Diagnostics Unit between 1997 and 1998 were studied. Our objectives were to determine the predominant serogroups of *S. enterica* serotype Typhimurium in this region using phenotypic and molecular methods.

MATERIALS AND METHODS

Bacterial strains. A total of 74 *S. enterica* serotype Typhimurium isolates were investigated in this study, all of which are listed in Table 1. These isolates were collected between the end of 1997 and the end of 1998 from animal (clinical), human, and food sources. Cork University Hospital, the Cork Regional Veterinary Laboratory, and the Cork County Council Food Laboratory, respectively, submitted the isolates to the Molecular Diagnostics Unit for study. The majority of the strain collection reported here are bovine isolates taken from different farms and regions in the greater Cork area. All isolates were identified as *S. enterica* serotype Typhimurium based on colony morphology, API 20NE (bioMérieux, Marcy l'Etoile, France) biotyping, and serotyping. Isolates were stored on nutrient agar slopes at 4°C and when required were initially grown on XLD agar (Oxoid, Hampshire, United Kingdom) to assess culture purity and then in tryptone soy broth (Oxoid). The isolates were also maintained on cryostat beads (Mast Diagnostics, Merseyside, United Kingdom) at −20°C for long-term storage.

Antimicrobial susceptibility testing. Susceptibility to antimicrobial agents was determined by the disk diffusion assay on Mueller-Hinton agar (Becton Dickinson, Cockeysville, Md.) with commercial antimicrobial susceptibility disks (Oxoid) according to the recommendations of the National Committee for Clinical Laboratory Standards (30). The antibiotics tested and corresponding concentrations were as follows: ampicillin, 10 μg/disk; chloramphenicol, 30 μg/disk; ciprofloxacin, 5 μg/disk; kanamycin, 30 μg/disk; nalidixic acid, 30 μg/disk; streptomycin, 10 μg/disk; spectinomycin, 5 μg/disk; sulfonamide, 300 μg/disk; tetracycline, 10 μg/disk; trimethoprim, 5 μg/disk.

Phage typing. Phage typing was performed in accordance with the methods of the Public Health Laboratory Service, Collindale, London, United Kingdom (2). Briefly, 4 ml of double-strength nutrient broth (Difco, Dublin, Ireland) was inoculated with a single colony of *S. enterica* serotype Typhimurium and incubated at 37°C for 1 h 15 min. By means of a sterile Pasteur pipette 2 ml of the broth culture was then used to flood a dried double-strength nutrient agar plate (30-ml volume of agar, dried for 1 h 30 min), and the excess broth was removed. After surface drying for 15 min, a series of typing phages were applied to the plate surface according to a defined template using a multipoint inoculator. Each plate was incubated overnight at 37°C, and the pattern of lysis produced by the phages was recorded and interpreted by comparison to standard charts.

Genomic DNA isolation. Bacterial cells were grown in 5 ml of tryptone soy broth overnight at 37°C, and the DNA was extracted as previously described by Daly et al. (9).

5′ CS- and 3′ CS-targeted gene cassette amplification. The *S. enterica* serotype Typhimurium isolates were also investigated for the presence of integrons. A primer set previously designed to anneal to the 5′ conserved sequence (CS) and 3′ CS flanking regions containing the integrated gene cassette were used (5, 17). All PCRs were performed in 50-μl final volumes containing 100 ng of genomic DNA, 25 pmol of both forward (Int 1F, 5′-GGC ATC CAA GCA GCA AG-3′) and reverse (Int 1B, 5′-AAG CAG ACT TGA CCT GA-3′) (5, 17) primers, 5 μl of 10× PCR buffer (100 mM Tris-HCl [pH 9.0], 500 mM KCl, 1% Triton X-100), 8 μl of deoxynucleoside triphosphate mixture (consisting of 1.25 mM dATP, dCTP, dGTP, and dTTP), 2.5 mM $MgCl_2$, and 2.5 U of *Taq* DNA polymerase (Sigma, St. Louis, Mo.). Integron primers were synthesized by Eurogentec (Abingdon, United Kingdom) and subsequently purified by high-performance liquid chromatography. All amplification reactions were performed in a MiniCycler (MJ Research, Watertown, Mass.) using the following temperature profile: predenaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 5 min and a final extension step at 72°C for 5 min. Each amplification reaction included a negative control, which contained all reagents except target DNA. Amplified DNA products were resolved by conventional electrophoresis through horizontal 2% agarose gels at 100 V, and the results were visualized and photographed over a UV transilluminator.

DNA amplification fingerprinting (DAF). Genome fingerprint amplification reaction conditions were similar to those described for the analysis of gene cassettes above. Briefly, 200 ng of genomic DNA and 100 pmol of the 10-mer arbitrary primer P1254 (5′-CCG CAG CCA A-3′) (15), were included in each reaction mixture. The amplification thermal profile consisted of a predenaturation step at 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 40°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 5 min. All reactions were performed in duplicate, and results were found to be reproducible.

Nucleotide sequence accession number. The sequence of the partial open reading frame of the *purG* gene has been assigned GenBank accession no. AF151984.

RESULTS

Phage type and antimicrobial susceptibility. Seventy-four *S. enterica* serotype Typhimurium isolates were studied. All were phage typed, with 70% belonging to the DT104 family, of which 65% were found to be DT104 ($n = 48$), 4% were found to be DT104b ($n = 3$), and 1% were found to be PT U302 ($n = 1$). Other phage types included DT193 ($n = 9$ [12%]), the next most common phage type in this collection, DT195 ($n = 6$, [8%]), DT204a ($n = 2$, [3%]), and DT208 ($n = 3$, [4%]). Phage types for two isolates (CIT-F40 and -F105; Table 1) could not be determined.

All of the DT104 isolates were resistant to ACSSuT, and one isolate was resistant to ACSSuT and trimethoprim (ACSSuTTp) CIT-V36; Table 1). Three DT104b isolates (CIT-F107, -F108, and -F109; Table 1) were resistant to ACSSuT and kanamycin. Similarly CIT-V37, which was phage typed as U302, was resistant to ACSSuTTp and CIT-F34 (cultured from a milk sample) was the only isolate in this collection resistant to nalidixic acid. Twenty-one of the remaining 22 isolates in the study population demonstrated differing resistance types, the most notable feature of which was the consistent sensitivity to chloramphenicol. No resistance to ciprofloxacin was detected, and only one isolate tested (CIT-F40) was sensitive to all antimicrobials.

Amplification of integrated gene cassettes by PCR. As 90% (67 of 74) of the strains were resistant to sulfonamide, a feature commonly associated with the presence of class I integrons, all isolates were analyzed for these novel (mobile) genetic elements. Use of the previously described Int 1F and Int 1B primers (5, 17) showed that strains of phage types DT104 ($n = 48$), DT104b ($n = 3$), DT193 ($n = 5$), DT195 ($n = 2$), DT208 ($n = 2$), and PT U302 ($n = 1$) and two nontypeable isolates, representing 85% (63 of 74) of the collection, contained class I integrons. The predominant amplicon pattern (denoted integron pattern IP-I; Fig. 1) detected in all DT104, DT104b, and PT U302 isolates contained three integrons. This IP-I amplicon pattern consisted of two intense DNA fragments of approximately 1.0 and 1.2 kbp as previously described (20, 21) and a weaker 210-bp fragment (Fig. 1, lane 1). *Escherichia coli* strains carrying plasmids R100.1 and R751 (21) were included as control strains (data not shown; Table 1). A 1.0-kbp DNA fragment, similar to that amplified in DT104 isolates, containing the *ant*(3")-*Ia* gene was detected in the control strain carrying R100.1; this fragment corresponded to the 1.0-kb fragment of IP-I. A smaller DNA amplicon (approximately 850 bp) was detected in the control strain carrying R751 (data not shown). For the purposes of comparison these profiles were designated IP groups A and B, respectively (Table 1). However the smaller 210-bp amplicon of IP-I was absent. All remaining isolates produced five different patterns, denoted IP-II through -VI (Fig. 1, lanes 2 to 6, and Table 1). With the exception of IP-VI, all IP groups contained the small integron structure previously outlined for IP-I above. Eleven isolates did not show any evidence of a detectable gene cassette after PCR. The latter isolates displayed phage types DT193 ($n = 4$), DT195 ($n = 4$), DT204a ($n = 2$), and DT208 ($n = 1$).

Careful inspection of Fig. 1 shows that the two large DNA fragments amplified in IP-I were also present, but with reduced intensity, in IP-II, -V, and -VI (Fig. 1, lanes 3, 6, and 5, respectively). These gene cassettes were previously reported in

TABLE 1. Data for *S. enterica* serotype Typhimurium isolates

Isolate	Yr	Source[a]	Phage type[b]	R type[d]	DAF pattern	IP group
CIT-H 8	1997	Human	104	ACSSuT	I	I
CIT-H23	1997	Human	104	ACSSuT	I	I
CIT-F30	1997	Swine	104	ACSSuT	I	I
CIT-V31	1997	Bovine feces	104	ACSSuT	I	I
CIT-F32	1997	Premince beef	104	ACSSuT	I	I
CIT-F33	1997	Poultry carcass	104	ACSSuT	I	I
CIT-H35	1997	Human	104	ACSSuT	I	I
CIT-V39	1998	Swine	104	ACSSuT	I	I
CIT-F45	1998	Swine	104	ACSSuT	I	I
CIT-V57	1998	Bovine feces	104	ACSSuT	I	I
CIT-V59	1998	Bovine feces	104	ACSSuT	I	I
CIT-V61	1998	Bovine fetus	104	ACSSuT	I	I
CIT-V62	1997	Bovine feces	104	ACSSuT	I	I
CIT-V63	1997	Bovine feces	104	ACSSuT	I	I
CIT-V64	1998	Swab pens	104	ACSSuT	I	I
CIT-V65	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V66	1998	Bovine feces	104	ACSSuT	I	I
CIT-V67	1998	Bovine feces	104	ACSSuT	I	I
CIT-V68	1998	Bovine feces	104	ACSSuT	I	I
CIT-V69	1998	Ovine tissue	104	ACSSuT	I	I
CIT-V70	1998	Bovine feces	104	ACSSuT	I	I
CIT-V71	1998	Bovine feces	104	ACSSuT	I	I
CIT-V72	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V73	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V74	1998	Bovine feces	104	ACSSuT	I	I
CIT-V78	1998	Bovine feces	104	ACSSuT	I	I
CIT-V80	1998	Bovine feces	104	ACSSuT	I	I
CIT-V81	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V82	1998	Bovine feces	104	ACSSuT	I	I
CIT-V83	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V84	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V85	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V86	1998	Ovine tissue	104	ACSSuT	I	I
CIT-V87	1998	Bovine feces	104	ACSSuT	I	I
CIT-V89	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V90	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V91	1998	Bovine feces	104	ACSSuT	I	I
CIT-V92	1998	Bovine feces	104	ACSSuT	I	I
CIT-V93	1998	Bovine feces	104	ACSSuT	I	I
CIT-V94	1998	Bovine tissue	104	ACSSuT	I	I
CIT-V95	1998	Bovine feces	104	ACSSuT	I	I
CIT-V96	1998	Bovine feces	104	ACSSuT	I	I
CIT-F100	1998	Cooked poultry	104	ACSSuT	I	I
CIT-F101	1998	Black pudding	104	ACSSuT	I	I
CIT-F103	1998	Cooked poultry	104	ACSSuT	I	I
CIT-F104	1998	Cooked poultry	104	ACSSuT	I	I
CIT-F107	1998	Cooked swine	104b	ACSSuTK	I	I
CIT-F108	1998	Cooked swine	104b	ACSSuTK	I	I
CIT-F109	1998	Cooked swine	104b	ACSSuTK	I	I
CIT-V36	1998	Unknown	104	ACSSuTTp	I	I
CIT-V38	1998	Swine	104	ACSSuT	XI	I
CIT-F44	1998	Swine	193	ASu	I	III
CIT-F41	1998	Swine	193	ASSuTTp	II	II
CIT-F34	1997	Bovine milk	193	ASSuTN	III	None[c]
CIT-F43	1998	Swine	193	ASSuT	III	None
CIT-F47	1998	Swine	193	ASSuT	III	None
CIT-V56	1998	Porcine tissue	193	ASSuT	III	None
CIT-V77	1998	Porcine feces	193	SuTTp	IV	IV
CIT-V79	1998	Canine feces	193	ASuTTp	IV	IV
CIT-V88	1998	Porcine tissue	193	ASSuT	IV	IV
CIT-H12	1997	Human	195	T	II	None
CIT-H16	1997	Human	195	T	II	None
CIT-V60	1998	Bovine tissue	195	SuTTp	III	None
CIT-F102	1998	White pudding	195	STp	V	IV
CIT-F106	1998	Raw poultry	195	SuTp	VI	IV
CIT-H11	1997	Human	195	ASSuT	VII	None
CIT-F42	1998	Swine	204a	ASSuT	XI	None
CIT-F46	1998	Swine	204a	ASSuT	VIII	None

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TABLE 1—*Continued*

Isolate	Yr	Source[a]	Phage type[b]	R type[d]	DAF pattern	IP group
CIT-V58	1998	Porcine feces	208	T	III	None
CIT-V75	1998	Porcine feces	208	T	III	IV
CIT-V76	1998	Porcine feces	208	T	III	IV
CIT-V37	1998	Unknown	PT U302	ACSSuTTp	I	I
CIT-F105	1998	Raw poultry	NT	SuTp	IX	VI
CIT-F40	1998	Swine	NT	Sensitive	X	V
E. coli K12/R100.1[e]		Unknown	nd	nd	nd	A
E. coli K12/R751[e]		Unknown	nd	nd	nd	B

[a] Isolates of human origin were from Cork University Hospital; isolates of veterinary origin were from the Regional Veterinary Laboratory; isolates of food origin were from the Cork County Council Food Laboratory.
[b] NT, not phage typeable; nd, not determined or the relevant information was not available.
[c] None, no gene cassette amplicon was detected.
[d] R type, drugs to which isolates were resistant. A, ampicillin; C, chloramphenicol; K, kanamycin; N, nalidixic acid; S, streptomycin; Su, Sulfonomide Duplex; T, tetracycline; Tp, trimethoprim.
[e] Control strain, kindly provided by D. Sandvang (21).

DT104 isolates (20, 21) and are known to contain the aminoglycoside resistance *ant*(3")-*Ia* gene on the 1.0-kb amplicon together with *pse-1* encoding β-lactamase on the 1.2-kb amplicon. The primers outlined previously by Sandvang et al. (21) were used to gel purify these amplicons from several isolates in this collection and map them by PCR. The structural arrangements of the antimicrobial resistance-encoding genes were found to be conserved in these amplicons as outlined previously (data not shown). In contrast, however, none of the previous reports (20, 21) identified the smaller 210-bp gene cassette amplicon shown in Fig. 1. This fragment appears to be unique to *S. enterica* serotype Typhimurium and was cloned and sequenced. Analysis of the nucleotide sequence (data not shown) identified a 178-bp open reading frame containing part of the *purG* gene (encoding phosphoribosylformylglycinamidine synthetase).

In considering the other IP groups, four additional amplicons were detected, at approximately 1.5 kbp (IP-II), 700 bp and 2.4 kbp (both IP-V), and 300 bp (IP-VI). These bands are not present as part of the DT104 pattern. As the average size of a bacterial gene is approximately 800 bp, it is possible that these larger amplicons (i.e., 1.5 and 2.4 kbp) contain two and three antimicrobial resistance determinants, respectively, within these cassettes, arranged in a "head-to-tail" configuration (6; M. Daly and S. Fanning, unpublished data). Similar structures (6) have recently been identified in *S. enterica* serotype Typhimurium isolates cultured from Albanian refugees entering Italy (27).

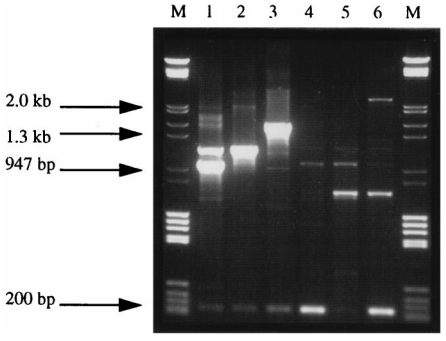

FIG. 1. Amplified gene cassettes and corresponding IP groups of representative strains of *S. enterica* serotype Typhimurium. After PCR 10 μl of the amplified reaction mixture was loaded onto a 1% agarose gel in 1× Tris-EDTA-acetate buffer containing 0.1 μg of ethidium bromide per ml. Samples were horizontally electrophoresed at 100 V for 90 min. Lanes M, molecular weight markers, grade III (Boehringer GmbH, Mannheim, Germany), ranging in size from 0.56 to 21.2 kb; lane 1, CIT-F45 (DT104, IP-I); lane 2, CIT-F44 (DT193, IP-III); lane 3, CIT-F41 (DT193, IP-II); lane 4, CIT-V77 (DT193, IP-IV); lane 5, CIT-V105 (not typeable [NT] IP-VI); lane 6, CIT-F40 (NT, IP-V).

DAF analysis. DNA fingerprint analysis using the previously characterized random 10-mer (15) was performed on purified genomic DNA from all 74 isolates. Typically, between 5 and 11 amplicons were observed after agarose gel electrophoresis and ethidium bromide staining. DNA fragments ranged in size from 300 bp to 2.0 kbp (Fig. 2). The DNA fingerprints of all isolates were grouped into 11 pattern types (DAF groups I through XI), based on a comparison of all profiles shown in the agarose gels (Fig. 2). DAF group I accounted for 70% of all isolates investigated, and this group had a typical DNA fragment pattern consisting of 10 bands (Fig. 2, lanes 1 through 52).

These data suggest (Table 1) an association between DAF pattern I and IP group I for all DT104 strains. Only CIT-V38, which was phage typed as DT104, belonging to IP group I, was distinguished by a DAF pattern (DAF group XI) different from those of all other DT104 and DT104b isolates. In comparison CIT-F44, phage type DT193, had a fingerprint consistent with DAF group I and a gene cassette amplicon profile associated with IP group III. A second fingerprint group (DAF group II) consisted of three isolates (CIT-H12, -H16, and -F41; Fig. 2, lanes 53 through 55). The banding patterns observed in the last were similar to those in DAF group I, with differences represented by a single band at approximately 600 bp. The isolates in this group were typed as DT193 and DT195 (Table 1). DAF group III contained 11% (8 of 74) of the collected strains (Fig. 2, lanes 56 through 63), with all remaining isolates being grouped into eight additional categories (DAF groups IV to XI), wherein 1 to 3 amplicons were detected (Fig. 2, lanes 64 through 74).

DISCUSSION

During the 1980s *S. enterica* serotype Enteritidis PT4 was often reported to be the causative agent in outbreaks of salmomellosis linked to food poisoning. However, in the 1990s *S. enterica* serotype Typhimurium DT104 has emerged as a new epidemic strain (20, 25). Reservoirs for the latter exist among bovine, poultry, and other animal populations from which this pathogen is transmitted to humans via the food chain. *S. enterica* serotype Typhimurium DT104 is characterized by its simultaneous resistance to many antimicrobial agents, with resistance to ACSSuT common among isolates (Table 1). Ac-

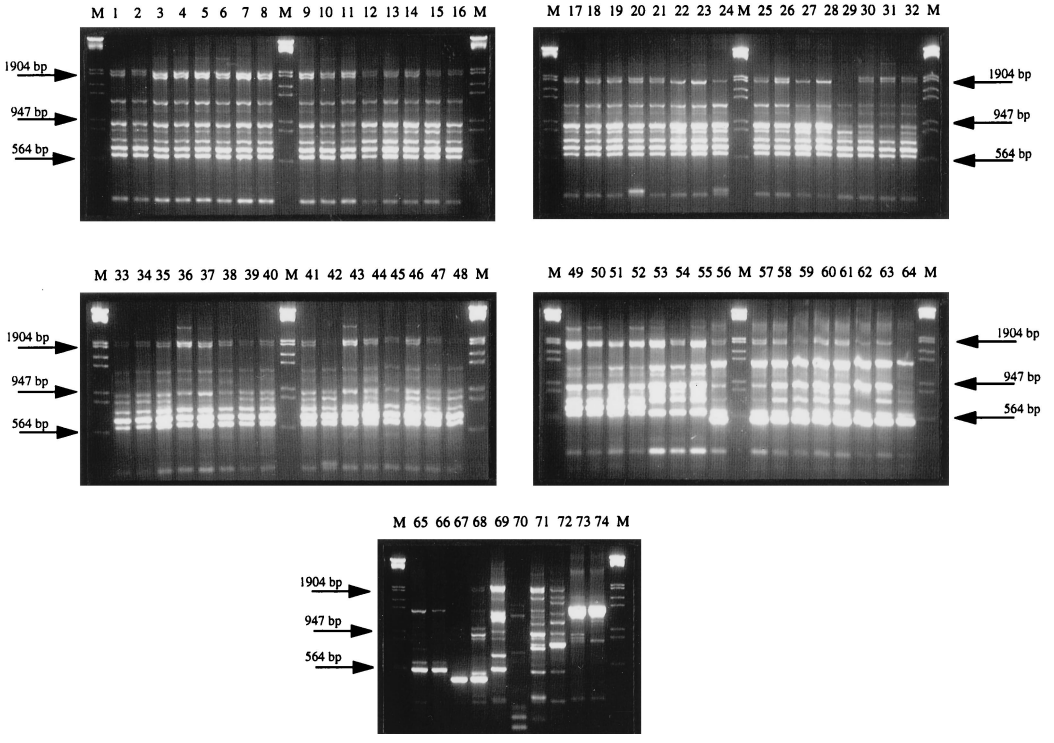

FIG. 2. DAF patterns of all *S. enterica* serotype Typhimurium strains typed using the 10-mer primer P1254 (15). After PCR 10 μl of each reaction mixture was loaded onto a 2% agarose gel in 1× Tris-EDTA-acetate buffer containing 0.1 μg of ethidium bromide per ml. Samples were electrophoresed as outlined for Fig. 1. Lanes (designated DAF groups are in parentheses [Table 1]): M (all gels), molecular weight markers, grade III (Boehringer), ranging in size from 0.56 to 21.2 kb; 1, CIT-H8 (I); 2, CIT-H23 (I); 3, CIT-F30 (I); 4, CIT-F31 (I); 5, CIT-F32 (I); 6, CIT-F33 (I); 7, CIT-F35 (I); 8, CIT-F36 (I); 9, CIT-F37 (I); 10, CIT-F39 (I); 11, CIT-F44 (I); 12, CIT-F45 (I); 13, CIT-V57 (I); 14, CIT-V59 (I); 15, CIT-V61 (I); 16, CIT-V62 (I); 17, CIT-V63 (I); 18, CIT-V64 (I); 19, CIT-V65 (I); 20, CIT-V66 (I); 21, CIT-V67 (I); 22, CIT-V68 (I); 23, CIT-V69 (I); 24, CIT-V70 (I); 25, CIT-V71 (I); 26, CIT-V72 (I); 27, CIT-V73 (I); 28, CIT-V74 (I); 29, CIT-V78 (I); 30, CIT-V80 (I); 31, CIT-V81 (I); 32, CIT-V82 (I); 33, CIT-V83 (I); 34, CIT-V84 (I); 35, CIT-V85 (I); 36, CIT-V86 (I); 37, CIT-V87 (I); 38, CIT-V89 (I); 39, CIT-V90 (I); 40, CIT-V91 (I); 41, CIT-V92 (I); 42, CIT-V93 (I); 43, CIT-V94 (I); 44, CIT-V95 (I); 45, CIT-V96 (I); 46, CIT-V100 (I); 47, CIT-F101 (I); 48, CIT-F103 (I); 49, CIT-F104 (I); 50, CIT-F107 (I); 51, CIT-F108 (I); 52, CIT-F109 (I); 53, CIT-H12 (II); 54, CIT-H16 (II); 55, CIT-F41 (I); 56, CIT-F34 (III); 57, CIT-F43 (III); 58, CIT-F47 (III); 59, CIT-V56 (III); 60, CIT-V58 (III); 61, CIT-V60 (III); 62, CIT-V75 (III); 63, CIT-V76 (III); 64, CIT-V77 (IV); 65, CIT-V79 (IV); 66, CIT-V88 (IV); 67, CIT-F102 (V); 68, CIT-F106 (V); 69, CIT-H11 (VI); 70, CIT-F46 (VII); 71, CIT-F105 (VIII); 72, CIT-F40 (IX); 73, CIT-F38 (X); 74, CIT-F42 (X).

quisition of these resistance genes results from numerous independent events (20, 22).

This study investigated 74 randomly collected *S. enterica* serotype Typhimurium isolates from the Cork region of southern Ireland. The majority of these isolates (Table 1) were derived from bovines, accounting for 56% of all strains analyzed, represented by DAF group I and phage type DT104, (with the exception of two isolates, CIT-F34 [DT193] and -V60 [DT195], belonging to DAF group III]. These data suggest that *S. enterica* serotype Typhimurium DT104 has been remarkably successful, relative to other phage types, in colonizing bovines in this region. The detection of indistinguishable isolates from cattle, food, and humans suggests that cattle are a significant reservoir for human infection with *S. enterica* serotype Typhimurium in the Cork region. In contrast, swine-derived *S. enterica* serotype Typhimurium isolates were more heterogenous. Swine-derived isolates accounted for 29% of the total collection and, while being a smaller proportion of the study population than the bovine group, were more diverse based on their phage types, antibiograms, and DNA fingerprint patterns. Swine-derived isolates were distributed among 4 of the phage types, 8 of the 11 DAF groups, and 5 of the 6 integron groups. It is well recognized that DT193 isolates are genetically heterogenous (4). In this study, DT193 isolates cultured from porcine ($n = 7$), bovine ($n = 1$), and canine ($n = 1$) sources could be divided into four DNA fingerprint groups (DAF groups I to IV). A similar result was also noted recently by Baggesen and Aarestrup (3) when pulsed-field gel electrophoresis patterns of Danish porcine isolates were compared. All remaining isolates cultured from various animal, food, and human sources were distributed among 6 of the 11 DAF groups.

Each isolate was investigated for drug resistance and the presence of class I integrons (based on the large number of isolates resistant to sulfonamide). A conserved integron structure (IP-I; Table 1) similar to that recently reported (20, 21) was identified among all DT104 isolates, three DT104b isolates, and one PT U302 isolate. Eleven of the non-DT104 isolates in the study group did not contain an amplified gene cassette. Molecular analysis of the Penta resistance in *S. enterica* serotype Typhimurium DT104 strains was previously shown to be chromosomally localized. Of particular concern in the United Kingdom is the increasing additional resistance of these isolates to nalidixic acid (14) and trimethoprim (11, 20) and their reduced susceptability to ciprofloxacin (a fluoroquinone). There is evidence for a similar trend, although less marked, in Ireland (8; Cormican et al., Abstr. 99th Gen. Meet. Am. Soc. Microbiol.), where a significant incidence of resistance to nalidixic acid is observed. Resistance to nalidixic acid in one of our strains is a marker for reduced susceptibility to ciprofloxacin (8). While resistance to ACSSuT may be useful as a marker for DT104, further identification by molecular sub-

typing can provide useful information (1, 3, 10, 12, 15, 16, 18, 23, 28).

In conclusion, based on our data, we have identified a probable reservoir for *S. enterica* serotype Typhimurium DT104 among the bovine population in the Cork region of southern Ireland. Many of these isolates were resistant to five or more antimicrobials. This observation highlights two important issues for agriculture, environmental, food, and public health authorities in Ireland. Use of antimicrobial agents in animals and humans contributes to a selective pressure favoring acquisition of multivalent antimicrobial resistance. More-limited use of antimicrobials, in particular in relation to their use for growth promotion and routine flock- or herd-wide prophylaxis in animal husbandry may be expected to reduce the opportunities for the emergence and spread of new antimicrobial agent-resistant pathogens among animals intended for human food. Furthermore, the transmission of DT104 and other strains from the livestock reservoir to humans via the food chain, must be carefully monitored. It is essential that the epidemiology of this organism be fully elucidated. Phenotypic analysis as a "front-line" measure supported by genotyping can facilitate the directed implementation of an effective surveillance and control strategy.

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ERRATUM

Molecular Characterization of Irish *Salmonella enterica* Serotype Typhimurium: Detection of Class I Integrons and Assessment of Genetic Relationships by DNA Amplification Fingerprinting

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Volume 66, no. 2, p. 614–619, 2000. Page 617, legend to Fig. 1, line 9: "CIT-V105" should read "CIT-F105.".

Page 618, legend to Fig. 2, line 4: "4, CIT-F31 (I)," "7, CIT-F35 (I)," "8, CIT-F36 (I)," "9, CIT-F37 (I)," and "10, CIT-F39 (I)" should read "4, CIT-V31 (I)," "7, CIT-H35 (I)," "8, CIT-V36 (I)," "9, CIT-V37 (I), and "10, CIT-V39 (I)," respectively.

Line 8: "46, CIT-V100 (I)" should read "46, CIT-F100 (I)."

Line 9: "55, CIT-F41 (I)" should read "55, CIT-F41 (II)."

Line 11: "68, CIT-F106 (V)," "69, CIT-H11 (VI)," "70, CIT-F46 (VII)," "71, CIT-F105 (VIII)," "72, CIT-F40 (IX)," "73, CIT-F38 (X)," and "74, CIT-F42 (X)" should read "68, CIT-F106 (VI)," "69, CIT-H11 (VII)," "70, CIT-F46 (VIII)," "71, CIT-F105 (IX)," "72, CIT-F40 (X)," "73, CIT-V38 (XI)," and "74, CIT-F42 (XI)," respectively.