APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2002, p. 181–186 Vol. 68, No. 1
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.1.181–186.2002

Salmonella enterica Serotype Bredeney: Antimicrobial Susceptibility and Molecular Diversity of Isolates from Ireland and Northern Ireland

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Received 3 May 2001/Accepted 15 October 2001

***Salmonella enterica* serotype Bredeney has emerged as the third most commonly identified serotype among human clinical isolates referred to the Irish National Salmonella Reference Laboratory in the years 1998 to 2000. A collection of 112 isolates of *S. enterica* serotype Bredeney collected during the period 1995 to 1999 from animal, food, and human sources from both Ireland and Northern Ireland were studied. Antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), and DNA amplification fingerprinting (DAF) were performed on all isolates. Plasmid profiles were examined on a subset of 33 isolates. A high proportion (74%) of isolates were susceptible to all antimicrobial agents tested. Resistance to both sulfonamide and trimethoprim was observed in 21% of isolates, and resistance to multiple (five) antimicrobial agents was observed in a single isolate (0.9%). Eight different PFGE patterns were obtained, with 87% of isolates grouping as PFGE type A. PFGE type A was predominant in animals, food, and humans. There was good overall concordance between the groups identified by PFGE and DAF. Overall results indicate that most *S. enterica* serotype Bredeney isolates in Ireland and Northern Ireland from animal and human sources are clonally related.**

Salmonella enterica-associated gastroenteritis is an important food-borne human disease throughout the world. More than 2,000 serotypes of *Salmonella enterica* are recognized, and most serotypes are capable of infecting a variety of animal species, including humans. There is considerable variation with time and with geographical location in the serotypes most commonly associated with human infection. In many European countries, *S. enterica* serotype Enteritidis and serotype Typhimurium are among the serotypes most commonly associated with human salmonellosis (2, 12, 24, 27). Likewise in Ireland, *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteritidis account for most human isolates referred to the National Salmonella Reference Laboratory, and a similar pattern is observed in Northern Ireland.

In most reference laboratories *S. enterica* serotype Bredeney is an uncommon human pathogen. *S. enterica* serotype Bredeney represented 0.4% (12 of 2,830) of isolates recorded in the Public Health Laboratory Service salmonella data for England and Wales in the second quarter of 2000 (2) and less than 0.5% of human isolates received by the U.S. Centers for Disease Control and Prevention from 1984 to 1986 (15). *S. enterica* serotype Bredeney has a wide geographical distribution (1, 2, 5, 14, 19, 21) and has been isolated from many animal species, including poultry, pigs, cats, and dogs (5, 9, 11, 17, 19), and from the environment (1, 7, 13, 18).

Although *S. enterica* serotype Bredeney accounts for a very small proportion of overall human infections, there are indications that it may achieve local importance in particular regions at specific times. Szilagyi noted *S. enterica* serotype Bredeney as among the three most common serotypes in a region of Romania between 1967 and 1973 (25). Outbreaks of *S. enterica* serotype Bredeney have occurred, including an outbreak in a surgical ward reported in 1977 (8) and a community outbreak in New York in the mid-1980s associated with eating roast beef (14). Large community outbreaks have occurred in Alabama in 1998 involving 170 people (16) and in Australia in 1977 and 1997 (6), and a smaller outbreak was reported in England and Wales (3).

In 1998 we observed the emergence of *S. enterica* serotype Bredeney as the third most frequent serotype identified among human clinical isolates of *Salmonella enterica* from Ireland submitted to the National Salmonella Reference Laboratory. Similarly, the Infectious Disease Bulletin from the eastern region of Ireland (population 1.3 million) for the period 1995 to 1999 reported *S. enterica* serotype Bredeney as the third commonest *Salmonella* serotype (9% of 1,239 isolates) isolated from human cases by clinical laboratories. In 1999 *S. enterica* serotype Bredeney was reported as accounting for 5.5% (12 of 216) of human cases of salmonellosis in the Infoscan bulletin covering the southern region of Ireland (4).

S. enterica serotype Bredeney is not commonly associated with sporadic human infection in Northern Ireland. Only 0.2%

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of 10,500 fecal specimens examined by the Northern Ireland Public Health Laboratory in 2000 yielded *S. enterica* serotype Bredeney. Isolates of *S. enterica* serotype Bredeney from human infections received at the National Salmonella Reference Laboratory in Ireland included in this study were from sources that were widely distributed in time and geographical location, with no evident clustering. No outbreaks of *S. enterica* serotype Bredeney were reported to the Food Safety Authority of Ireland until 1999, after most of the isolates in the current study had been collected.

There has not been any previous comprehensive study of the phenotypic and molecular diversity of *S. enterica* serotype Bredeney strains circulating in a defined geographic area. This paper describes the antimicrobial susceptibility and molecular diversity of a collection of 112 isolates of *S. enterica* serotype Bredeney from human, animal, and food sources throughout Ireland and Northern Ireland. The collection of strains includes 10 clinical isolates and 8 food isolates obtained during investigation of an outbreak of *S. enterica* serotype Bredeney infection in Northern Ireland in 1997.

MATERIALS AND METHODS

Bacterial strains. The collection included 42 clinical isolates from the Department of Medical Microbiology of University College Hospital, Galway ($n = 20$), or other clinical Microbiology laboratories in Ireland ($n = 22$). Thirty-eight isolates were from food or animals from a specific region of Ireland (Cork). Thirty-two isolates were clinical, food, and environmental isolates from Northern Ireland (see Table 1). Isolates were stored at −70°C. The bacterial strains were confirmed as *S. enterica* by API 20E (Biomerieux, Marcy l'Etoile, France) and confirmed as *S. enterica* serotype Bredeney (4,12; l,v:7) according to the Kauffmann-Whyte typing scheme using slide agglutination with standard antisera (Murex Biotech Ltd., Dartford, England, and Dade-Behring Gmbh, Marburg, Germany).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed according to the disk diffusion method of the National Committee for Clinical Laboratory Standards (20). The following antimicrobial agents (disk content indicated in parentheses) were tested: ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), kanamycin (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), sulfonamide (300 μg), streptomycin (10 μg), tetracycline (30 μg), and trimethoprim (5 μg). *Escherichia coli* ATCC 25922 was used as the control. Stability of the expressed resistance of the isolates was determined by subculturing a subset of each of the resistance types each day for 4 weeks. At the end of each week, the isolates were retested against the same panel of antimicrobial agents.

PFGE. For pulsed-field gel electrophoresis (PFGE), a heavy inoculum of an overnight growth on diagnostic sensitivity test agar (DST) was suspended in saline, washed three times, pelleted, and weighed. An equal weight of saline was added to make a stock solution, and working suspensions were prepared by adding 5 μl of stock to 10 μl of saline. The working suspension was made up to 240 μl total volume with TEN buffer (100 mM Tris-HCl [pH 7.5], 100 mM EDTA). Then 230 μl of 2% molten SeaKem GTG agarose was added to the suspensions and mixed, and this was added to Bio-Rad gel molds to make the plugs. Plugs were washed by gentle shaking in EC buffer (6 mM Tris, 1 M NaCl, 100 mM EDTA, 0.5% Brij 58, 0.2% [wt/vol] deoxycholate and 0.5% Sarkosyl) for 5 h and then washed in CHEF buffer (100 mM Tris-HCl [pH 7.5], 100 mM EDTA, 150 mM NaCl). Plugs were digested with proteinase K (20 mg/ml) in ES buffer (400 mM EDTA [pH 9.3], 1% Sarkosyl) overnight at 50°C. Plugs were then washed several times in CHEF buffer and stored in a refrigerator. Total genomic DNA was digested by placing thin slices from the respective plug in 300 μl of DNS buffer (100 mM Tris-HCl [pH 8.0], 5 mM $MgCl_2$), washing four times, and adding 10 U of *Xba*I in H buffer (Boehringer, Mannheim, Germany) and 100 μl of RNase (Sigma, Dorset, England) to the wells and incubating at 37°C overnight.

PFGE was performed with the Pharmacia LKB Gene Navigator system in 0.5× Tris-borate-EDTA. DNA macrorestriction fragments were resolved on 1.2% (wt/vol) agarose gels. Pulse Marker 50 to 1000 kb (Sigma) consisting of concatemers of lambda DNA were used as size standards. Gels were run for 20 h at 5 V/cm with pulse times ramped at 5 to 50 s followed by 4.7 V/cm with a pulse time of 5 to 20 s. Gels were stained by immersion in ethidium bromide (5 μg/ml), destained, and visualized under UV light.

Macrorestriction fragment patterns were initially analyzed by the criteria of Tenover et al. (26). Computer-assisted analysis of the PFGE banding patterns was performed using Bionumerics software (Applied Maths, Kortrijik, Belgium). TIFF images of the gel were normalized by aligning the size standards located in the outer lanes of the gel with the reference standard for the database. Analysis of banding patterns was performed with the Dice coefficient using a 1.0% tolerance for the band migration distance. Clustering of patterns was performed by the unweighted pair group method with arithmetic averaging (UPGMA).

Strains for which PFGE patterns could not be obtained due to degradation of DNA were reprocessed, and electrophoresis was performed with 0.38% (wt/vol) thiourea in the TBE running buffer (22).

DAF. DNA amplification fingerprinting (DAF) was carried out following the DAF protocol described previously (10). Reactions were performed in 50-μl final volumes containing 200 ng of genomic DNA, 100 pmol of a 10-mer arbitrary primer, P1254 (5′-CCGCAGCCAA-3′) (15), 5 μl of 10× PCR buffer (100 mmol of Tris-HCl [pH 9.0], 500 mmol of KCl, and 1% Triton X-100 per liter), 8 μl of deoxynucleoside triphosphate mix (containing 1.25 mmol/liter each of dATP, dCTP, dGTP, and dTTP), 2.5 mmol of $MgCl_2$, and 2.5 U of *Taq* DNA polymerase (Sigma, St. Louis, Mo.) per liter.

Amplification conditions were denaturation 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. Reactions were performed in duplicate. PCR products were analyzed by electrophoresis of 10-μl aliquots of the PCR in ethidium bromide-stained agarose gels. Gels were interpreted by visual comparison of banding patterns. Isolates differing by two or more bands were considered to represent distinct DAF types.

Plasmid profiles. Plasmids were prepared with the Qiaprep Spin miniprep kit (Qiagen, Sussex, United Kingdom). Briefly isolates were grown in 4 ml of Luria-Bertani broth overnight. Cells were harvested by centrifugation and resuspended in 250 μl of resuspension buffer (50 mM Tris HCl [pH 8.0], 10 mM EDTA) and 100 μg of RNase A per ml. Cells were lysed by addition of 250 μl of lysis buffer (200 mM NaOH, 1% [wt/vol] sodium dodecyl sulfate). Lysate was then neutralized with the proprietary neutralization buffer (composition not specified), and plasmid DNA was adsorbed onto a silica gel membrane in the presence of a high-salt buffer. After washing, plasmid DNA was eluted from the gel with 10 mM Tris-Cl (pH 8.5). Electrophoresis was performed in a 0.7% agarose gel with 1× Tris-acetate-EDTA (TAE) buffer for 3 h at 70 V. A 2- to 10-kb supercoiled DNA ladder (Promega, Madison, Wis.) was run as size standards. The gel was stained using ethidium bromide and photographed under UV light.

RESULTS

Results of PFGE typing, DAF typing, and antimicrobial susceptibility testing for each isolate are summarized in Table 1. Five distinctive antimicrobial resistance profiles were identified among the *S. enterica* serotype Bredeney isolates in this collection. The majority (74%) of isolates were susceptible to all the antimicrobial agents tested. Resistance to sulfonamides and trimethoprim (SuTm) was only detected in 23 (21%) isolates. Two (1.8%) *S. enterica* serotype Bredeney isolates (95/B61 and 95/B53, Table 1) were resistant only to ampicillin (A), while a single (0.9%) isolate, 96/B20, was resistant only to tetracycline (T). One isolate (0.9%) (97/23F) was resistant to five antimicrobial agents: ampicillin, streptomycin, sulfonamides, tetracycline, and trimethoprim (ASSuTTm).

Eight distinct PFGE patterns designated A through H were observed (Fig. 1). PFGE pattern A accounted for 97 isolates (87%). PFGE type A predominated in isolates from clinical, food, and environmental sources from both Ireland and Northern Ireland. Within PFGE type A there were three subtypes, A ($n = 68$, 60.7%), A^2 ($n = 26$, 23%), and A^{2b} ($n = 3$, 2.7%).

The remaining 15 isolates (13%) in the collection were grouped as PFGE types B through H. Types B, C, and E through G were each represented by a single isolate (0.9%). PFGE type D was represented by six isolates (5%) from bo-

TABLE 1. *S. enterica* serotype Bredeney isolates from Ireland and Northern Ireland: PFGE type, DAF type, and antimicrobial resistance phenotype

Strain	Source	Origin	Year	PFGE type	DAF type	Antibiogram[a]	Strain	Source	Origin	Year	PPGE type	DAF type	Antibiogram[a]
S147	Human	Dublin	1997	A	I	All	95/B51	Poultry	Cork	1995	A	I	SuTm
S117	Human	Dublin	1998	A	I	All	95/B63	Poultry	Cork	1995	A	I	SuTm
S11	Human	Dublin	1999	A	I	All	95/B64	Poultry	Cork	1995	A	I	All
S41	Human	Dublin	1999	A	I	All	95/B73	Poultry	Cork	1995	A	I	All
S146	Human	Galway	1997	A	I	All	96/B80	Poultry	Cork	1996	A	I	T
S141	Human	Galway	1997	A	I	All	95/B49	Poultry	Cork	1995	A	II	All
S150	Human	Galway	1997	A	I	All	95/B50	Poultry	Cork	1995	A	II	All
S603	Human	Galway	1998	A	I	All	95/B59	Poultry	Cork	1995	A	II	SuTm
S34	Human	Galway	1999	A	I	All	95/B60	Poultry	Cork	1995	A	II	SuTm
S37	Human	Galway	1999	A	I	All	95/B65	Poultry	Cork	1995	A	II	SuTm
S38	Human	Galway	1999	A	I	All	95/B66	Poultry	Cork	1995	A	II	SuTm
S50	Human	Galway	1999	A	I	All	97/86F	Poultry	N. Ireland	1997	A	II	All
S55	Human	Galway	1999	A	I	All	98/34F	Poultry	N. Ireland	1998	A	II	SuTm
S59	Human	Galway	1999	A	I	All	98/64F	Poultry	N. Ireland	1998	A	II	All
S148	Human	Limerick	1997	A	I	All	98/71F	Poultry	N. Ireland	1998	A	II	All
S525	Human	Mayo	1998	A	I	All	99/18F	Poultry	N. Ireland	1999	A	II	All
S31	Human	Mayo	1999	A	I	All	99/25F	Poultry	N. Ireland	1999	A	II	All
S453	Human	Waterford	1998	A	I	All	95/B62	Poultry	Cork	1995	A^2	I	SuTm
S142	Human	Dublin	1997	A	II	All	95/B68	Poultry	Cork	1995	A^2	I	SuTm
S143	Human	Galway	1997	A	II	All	95/B69	Poultry	Cork	1995	A^2	I	All
S43	Human	Galway	1999	A	II	All	95/B74	Poultry	Cork	1995	A^2	I	All
S47	Human	Galway	1999	A	II	All	95/B76	Poultry	Cork	1995	A^2	I	SuTm
S60	Human	Galway	1999	A	II	All	96/B79	Poultry	Cork	1996	A^2	I	SuTm
S149	Human	Mayo	1997	A	II	All	95/B70	Poultry	Cork	1995	A^2	II	SuTm
97/121	Human	N. Ireland	1997	A	II	All	95/B75	Poultry	Cork	1995	A^2	II	SuTm
97/122	Human	N. Ireland	1997	A	II	All	97/10F	Poultry	N. Ireland	1997	A^2	II	SuTm
97/126	Human	N. Ireland	1997	A	II	All	97/29F	Poultry	N. Ireland	1997	A^2	II	All
97/128	Human	N. Ireland	1997	A	II	All	98/21F	Poultry	N. Ireland	1998	A^2	II	All
97/130	Human	N. Ireland	1997	A	II	All	98/35F	Poultry	N. Ireland	1998	A^2	II	All
97/131	Human	N. Ireland	1997	A	II	All	97/19F	Poultry	N. Ireland	1997	A^{2b}	II	All
97/133	Human	N. Ireland	1997	A	II	All	95/B56	Poultry	Cork	1995	D	III	All
97/134	Human	N. Ireland	1997	A	II	All	95/B57	Poultry	Cork	1995	D	III	All
97/135	Human	N. Ireland	1997	A	II	All	97/23F	Poultry	N. Ireland	1997	F	II	ASSuTTm
97/136	Human	N. Ireland	1997	A	II	All	95/B58	Poultry	Cork	1995	H	V	All
99/51	Human	N. Ireland	1999	A	II	All	95/B61	Poultry	Cork	1995	H	V	A
99/52	Human	N. Ireland	1999	A	II	All	95/B44	Bovine	Cork	1995	A	I	SuTm
S3	Human	Roscommon	1998	A	II	All	95/B45	Bovine	Cork	1995	A	I	SuTm
S456	Human	Dublin	1998	A^2	I	All	95/B47	Bovine	Cork	1995	A	I	All
S139	Human	Galway	1997	A^2	I	All	95/B78	Bovine	Cork	1995	A	I	All
S41	Human	Galway	1998	A^2	I	All	95/B53	Bovine	Cork	1995	D	III	A
S507	Human	Galway	1998	A^2	I	All	95/B67	Bovine	Cork	1995	D	III	All
S116	Human	Limerick	1998	A^2	I	All	95/B54	Bovine	Cork	1995	D	IV	All
S601	Human	Offaly	1998	A^2	I	All	95/B55	Bovine	Cork	1995	D	IV	All
S144	Human	Waterford	1997	A^2	I	All	95/B52	Bovine	Cork	1995	H	V	All
S138	Human	Dublin	1997	A^2	II	All	95/B77	Deer	Cork	1995	A^2	I	SuTm
S145	Human	Dublin	1997	A^2	II	All	95/B71	Deer	Cork	1995	E	IX	SuTm
S372	Human	Offaly	1998	A^2	II	All	95/B72	Deer	Cork	1995	H	V	All
S21	Human	Waterford	1998	A^2	II	All	98/47F	Marine Sed	N. Ireland	1998	A	II	All
S614	Human	Dublin	1998	A^{2b}	I	All	98/42F	Marine Sed	N. Ireland	1998	A^2	II	All
S93	Human	Dublin	1999	A^{2b}	I	All	97/65F	Mussels	N. Ireland	1997	A	II	All
S535	Human	Galway	1998	B	VI	All	97/69F	Mussels	N. Ireland	1997	A	II	All
S544	Human	Galway	1998	C	VII	SuTm	97/71F	Mussels	N. Ireland	1997	A	II	All
95/B41	Poultry	Cork	1995	A	I	SuTm	98/6F	Mussels	N. Ireland	1998	A^2	II	SuTm
95/B42	Poultry	Cork	1995	A	I	SuTm	95/B43	Porcine	Cork	1995	A	II	SuTm
95/B46	Poultry	Cork	1995	A	I	All	99/26F	Rice	N. Ireland	1999	A	II	All
95/B48	Poultry	Cork	1995	A	I	All	98/9F	Winkles	N. Ireland	1998	G	VIII	All

[a] Isolates were tested by the disk diffusion susceptibility method of the National Committee for Clinical Laboratory Standards for susceptibility to ampicillin (A), chloramphenicol (C), ciprofloxacin (Cip), kanamycin (K), nalidixic acid (Na), nitrofurantoin (Ni), sulfonamide (Su), streptomycin (S), tetracycline (T), and trimethoprim (Tm). The resistance phenotype is represented by the abbreviations for the antimicrobial agents to which the isolate tested resistant. All indicates susceptibility to all antimicrobial agents tested.

vines ($n = 4$) and poultry ($n = 2$). Type H was represented by four isolates (3.6%). PFGE type H isolates were groupable by PFGE only after incorporation of thiourea into the running buffer.

PFGE banding patterns were also analyzed using Bionumerics software. The similarity percentage shown in the dendrogram generated was 100% for strain numbers 95/B58 and 95/B72 (PFGE type H) (Fig. 1). Similarly, representatives of subtypes A, A^2, and A^{2b} (S149, S139, and S614) were assigned to one cluster with a similarity percentage of >77%. Other PFGE types were identified as significantly different from one another with percentage similarities of <65%.

DAF complemented the PFGE typing scheme and was more discriminatory in some cases, giving nine individual patterns

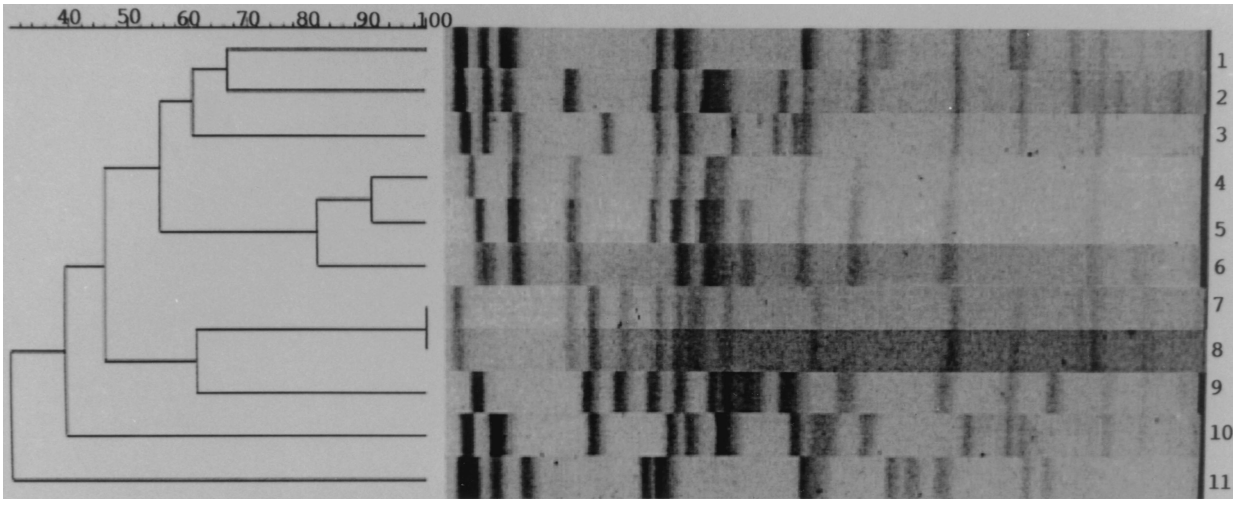

FIG. 1. UPGMA-generated dendrogram (Bionumerics software) and PFGE patterns generated with *Xba*I of representative isolates of *S. enterica* serotype Bredeney from Ireland and Northern Ireland. lane 1, isolate 97/23F (PFGE type F); lane 2, 98/9F (type G); lane 3, S544 (type C); lane 4, S139 (type A^2); lane 5, S149 (type A); lane 6, S614 (type A^{2b}); lane 7, 95/B58 (type H); lane 8, 95/B72 (type H); lane 9, S535 (type B); lane 10, 95/B71 (type E); lane 11, 95/B53 (type D).

(Fig. 2). The DAF protocol subdivided the PFGE type A strains into two separate DAF clusters, DAF type 1 (n = 47, 42.4%) and type 2 (n = 50, 44.6%), and also separated the six isolates of PFGE type D into two groups, DAF type 3 (n = 4, 3.6%) and type 4 (n = 2, 1.8%). The four isolates (3.6%) that comprised PFGE group H were grouped as DAF type 5.

The PFGE patterns which contained a single isolate also fitted into unique DAF types as outlined in Table 1. The pentaresistant strain 97/23F (Table 1) that was of a unique PFGE type F clustered as DAF type 2 with 49 other isolates. All isolates from the clinical cases associated with the 10-person outbreak of *S. enterica* serotype Bredeney in Northern Ireland and some of the isolates from food sampled in association with the outbreak investigation were of PFGE type A and DAF type 2. This suggests that the differentiation between of DAF types 1 and 2 may be of epidemiological significance.

A subset of 33 isolates (29.4%) were examined for the presence of plasmids. These isolates were selected to include a representative of each PFGE type and each antimicrobial resistance phenotype observed. One or more of three plasmids of

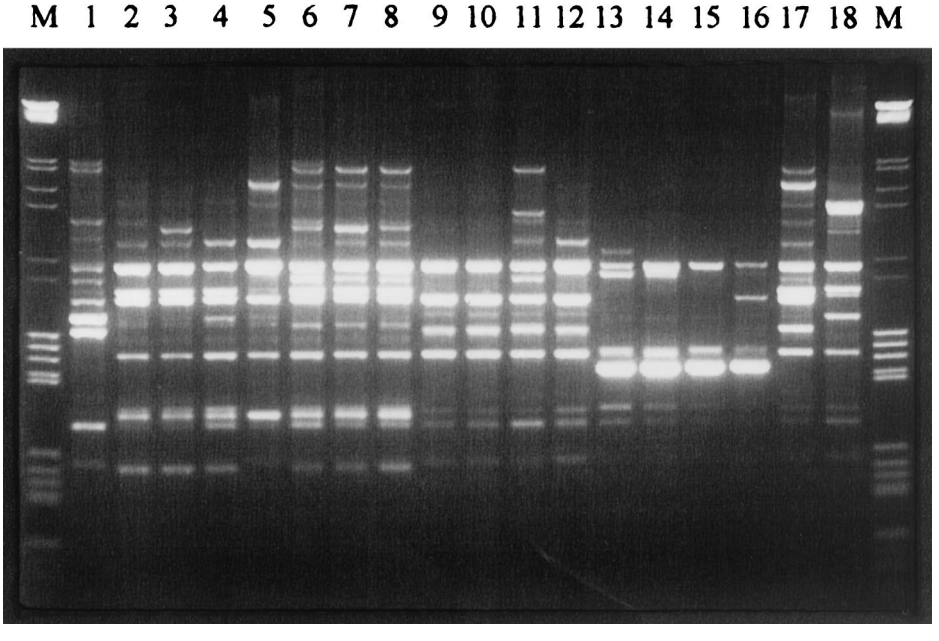

FIG. 2. DAF patterns of representative *S. enterica* strains. The DAF type for each strain is in parentheses. lane 1, *S. enterica* serotype Typhimurium DT104 control; lane 2, S147 (type 1); lane 3, S55 (type 1); lane 4, 2075 (type 1); lane 5, S544 (type 7); lane 6, S149 (type 2); lane 7, S60 (type 2); lane 8, 2140 (type 2); lane 9, 1992 (type 3); lane 10, 2019 (type 3); lane 11, 2012 (type 4); lane 12, 2139 (type 3); lane 13, 2073 (type 5); lane 14, 2135 (type 5); lane 15, 2038 (type 5); lane 16, 1972 (type 5); lane 17, S535 (type 6); lane 18, 2256 (type 9). Lane M, size markers.

approximately 2, 3.5, and 7 kb were associated with the 28 PFGE isolates of type A or subtype A^2 that were examined for plasmids. The 7-kb plasmid was present in all 21 SuTm-resistant PFGE type A/A^2 isolates examined. A 7-kb plasmid was not detected in six PFGE type A isolates that were susceptible to all antimicrobial agents tested and similarly was not detected in a PFGE type A isolate that exhibited resistance to tetracycline only. The 2- and 3.5-kb plasmids were present in the majority of PFGE type A isolates and also in isolates of unrelated PFGE types. The 7-kb plasmid was not detected in strains S544 or 95/B71 (SuTm-resistant isolates of PFGE types C and E, respectively) or in 95/B53 or 95/B61 (ampicillin-resistant PFGE types D and H, respectively) or in 97/23F (multiantibiotic-resistant PFGE type F).

Four strains, 95/B59 (PFGE type A and SuTm resistance), 97/23F (PFGE type F and ASSuTTm resistance), 95/B53 (PFGE type D and A resistance), and 95/B60 (PFEG type A and T resistance) were subcultured daily on an antibiotic-free nonselective medium for 4 weeks and repeatedly tested for susceptibility to antimicrobial agents. Strain 95/B59 but not the other strains reverted to susceptibility within 2 weeks of repeated subculture. The susceptible derivative of 95/B595 had lost the 7-kb plasmid but retained the 2- and 3.5-kb plasmids.

DISCUSSION

S. enterica serotype Bredeney is well recognized as a serotype isolated from poultry, other animals, and the environment and as an uncommon human pathogen associated with occasional outbreaks (1, 2, 5, 7, 9, 11, 13, 14, 17, 19, 21). In recent years *S. enterica* serotype Bredeney has become the third most common *S. enterica* serotype among isolates from human infections submitted for identification to the National Salmonella Reference Laboratory in Ireland. Most human isolates submitted to the reference laboratory are from cases that are dispersed in time and location.

In Ireland at present, the vast majority (97 of 112 [87%]) of *S. enterica* serotype Bredeney isolates from apparently unrelated human illness and from nonhuman sources form a closely related group, as determined by two independent DNA-typing approaches (PFGE and DAF). All but one of the distinct PFGE types observed was also identified as distinctive strains or clusters by DAF typing.

To our knowledge this is the first comprehensive study of the genotypic diversity of *S. enterica* serotype Bredeney circulating in a specific geographic region. Our results are consistent with dissemination of a particular clone of *S. enterica* serotype Bredeney throughout the animate and inanimate environment of the island of Ireland. This observation is significant in relation to the application of molecular techniques to the investigation of suspected links between human cases of *S. enterica* serotype Bredeney infection on this island. Clearly there is a high probability that by chance alone, human infection from unrelated sources may yield isolates that are indistinguishable by molecular typing.

We have not as yet had an opportunity to study strains from other regions; however, it would be interesting to determine how widely disseminated this predominant genotype of *S. enterica* serotype Bredeney is within Europe and globally. One might speculate that this strain of *S. enterica* serotype Bredeney has enhanced virulence for humans and that this may be related to the relative importance of *S. enterica* serotype Bredeney as a human pathogen in Ireland compared with other parts of Europe. It may be that molecular diversity within *Salmonella* serotypes is limited in Ireland because opportunities for introduction of new strains are limited by geographic factors and regulatory controls on the importation of livestock. Comparison of the results of this study with genotyping data from other geographic regions may help to clarify these issues.

A limitation of molecular typing methods for international comparison remains the absence of standardization of techniques, equipment, and conditions, which leads to problems comparing results obtained in different laboratories. A standardized protocol for PFGE typing of *S. enterica* and a reporting network exists (PulseNet from Centers for Disease Control and Prevention). As standardized PFGE typing becomes more widely available, it will become more practical to determine the degree of regional and global diversity that exists in microbial populations important for human health.

Antimicrobial resistance is much less common in *S. enterica* serotype Bredeney than in *S. enterica* serotype Typhimurium, of which the multiresistant (ACSSuT) DT104 clone has become endemic (10, 23). In contrast, in our strains of *S. enterica* serotype Bredeney, only resistance to sulfonamide and trimethoprim is common (observed in 21 of 112 [21%] isolates) and is primarily associated with poultry isolates (16 of 23, [70%]). Among the genetically related PFGE group A strains, the SuTm resistance phenotype was observed almost exclusively in poultry isolates and was associated with the presence of a 7-kb plasmid. This led us to speculate that the SuTm resistance phenotype may be unstable in *S. enterica* serotype Bredeney, persisting only in the presence of antimicrobial selective pressure. The instability of the SuTm resistance phenotype was confirmed in vitro by relatively rapid loss of the resistance phenotype and of the associated 7-kb plasmid on repeated subculture on antimicrobial agent-free media. Sulfonamides and trimethoprim are used in animal husbandry and may provide the selective pressure to retain the SuTm resistance phenotype in *S. enterica* serotype Bredeney in animals.

Sulfonamide resistance is a marker for the presence of class 1 integrons, a novel group of mobile genetic elements that may be important in the dissemination of antimicrobial resistance. Integrons contain interchangeable gene cassettes linked to other structural features, including the sulfonamide resistance gene (*sul1*), and may be present on plasmids. All isolates in the collection were examined for the presence of integron-associated gene cassettes by PCR as previously described (10). Only the multidrug-resistant isolate 97/23F contained an amplifiable gene cassette. It is unlikely, therefore, that antimicrobial resistance is mediated by integrons in most isolates of *S. enterica* serotype Bredeney. This contrasts with the importance of integrons in relation to antimicrobial resistance in *S. enterica* serotype Typhimurium.

Our study indicates that most *S. enterica* serotype Bredeney isolates from the animate and inanimate environment in Ireland and Northern Ireland are closely related. This has practical implications for the use of molecular typing to provide supportive evidence for a common source of infection in a suspected outbreak of *S. enterica* serotype Bredeney. Our observation raises the possibility that this strain may be particu-

larly pathogenic for humans. Further studies of *S. enterica* serotype Bredeney from elsewhere in the world would be valuable, and we would welcome the opportunity to explore this issue with collaborators from other countries.

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