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 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 Infoscot 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%
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; i,v:7) according to the Kauffmann-White 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 (30 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 restested 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 gels. Plasmid DNA was adsorbed onto a silica gel membrane in the presence of a proprietary neutralization buffer (composition not specified), and plasmid DNA was assayed by ethidium bromide staining using ethidium bromide and photographed under UV light. DNA ladder (Promega, Madison, Wis.) was run as size standards. The gel was destained, and visualized 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%), A2 (n = 26, 23%), and A3 (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 bof-
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. 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 \textit{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 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. 1. UPGMA-generated dendrogram (Bionumerics software) and PFGE patterns generated with XbaI of representative isolates of \textit{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); lane 5, S149 (type A); lane 6, S614 (type A\textsuperscript{M}); lane 7, 95/B58 (type H); lane 8, 95/B72 (type H); lane 9, S335 (type B); lane 10, 95/B71 (type E); lane 11, 95/B53 (type D).](image)

![FIG. 2. DAF patterns of representative \textit{S. enterica} strains. The DAF type for each strain is in parentheses. lane 1, \textit{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.](image)
approximately 2, 3.5, and 7 kb were associated with the 28 PFGE isolates of type A or subtype A² that were examined for plasmids. The 7-kb plasmid was present in all 21 SuTm-resistant PFGE type A/A² 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 SS44 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 (PFGE 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 (*sulI*), 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.

**REFERENCES**