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Comparison of Antibiotic Susceptibility Profiles and Molecular Typing Patterns of Clinical and Environmental *Salmonella enterica* Serotype Newport

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ABSTRACT

The genus *Salmonella* is composed of more than 2,400 serotypes, many of which cause enteric diseases in humans and animals. Several *Salmonella* serotypes are multidrug resistant, and there is evidence of the clonal spread of these strains from animals to humans. *Salmonella enterica* serotype Newport is one of the serotypes that increasingly present a multidrug-resistant phenotype. Source tracking and antibiotic resistance testing are important considerations for identifying the outbreak strain. The first goal of this study was to examine the antibiotic susceptibility patterns of clinical and environmental *Salmonella* Newport isolates from various geographic locations and to compare the discriminatory ability of two DNA fingerprinting techniques. The second goal was to determine whether the antibiotic resistance profiles and typing patterns correlated. Thirty *Salmonella* Newport isolates, including environmental and human clinical strains, were subjected to pulsed-field gel electrophoresis (PFGE), ribotyping, and antibiotic susceptibility testing. Eighty percent of the isolates showed total or intermediate resistance to one or more drugs; 75% of the isolates were multidrug resistant. Ribotyping with the *Eco*RI enzyme and PFGE with the *Xba*I enzyme each divided the isolates into 14 groups. Cluster analysis based on antibiotic susceptibility patterns generated 23 profiles. The susceptible and resistant isolates were not differentiated on the basis of either of the molecular typing techniques. Hence, no correlation was observed between the antibiotic resistance profiles and the DNA subtyping patterns. In conclusion, ribotyping is as discriminatory as PFGE and, when used in combination with antibiotic resistance profiles, provides a powerful tool for the source tracking of *Salmonella* Newport.

Nontyphoidal *Salmonella* is a common foodborne pathogen that causes infectious diseases in various animals and humans. Some of the widespread serotypes infecting humans, poultry, and cattle include *Salmonella enterica* serotype Typhimurium, *S. enterica* serotype Enteritidis, and *S. enterica* serotype Newport (http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2002/*Salmonella*Annual Summary2002.pdf). According to a survey by the Centers for Disease Control and Prevention (CDC), there was a 12% increase in the incidence of infections caused by *Salmonella* Newport from 1996 to 2003 (http://www.cdc.gov/foodnet/annual/2003/2003_report.pdf). The emergence of multidrug-resistant *Salmonella* Newport is of serious concern. According to the National Antimicrobial Resistance Monitoring System survey of enteric bacteria, *Salmonella* Newport was one of the most common nontyphoidal *Salmonella* serotypes to show multidrug resistance in 2002 in the United States (http://www.cdc.gov/narms/annual/2002/2002ANNUALREPORTFINAL.pdf). Multidrug-resistant *Salmonella* Newport associated with undercooked ground beef affected 47 persons in five different states in the United States in 2002 *(2).* Rankin et al. *(14)* observed that all the multidrug-resistant *Salmonella* Newport isolates included in their study had the bla_{CMY} gene that encodes resistance to extended-spectrum cephalosporins. Giles et al. *(7)* established that the bla_{CMY} gene is transmitted through plasmid transfer. Resistance to multiple drugs is frequently transmitted through integrons in *Salmonella,* of which class 1 integrons are the most common. A number of studies have shown that the multidrug resistance in *Salmonella* Newport isolates can partly be mediated by class 1 integrons, allowing resistance to several antimicrobials *(14, 16).* An alarming situation is the possible integration of the resistance determinants into the chromosome of *Salmonella* Newport, as observed in *Salmonella* Typhimurium DT104, *S. enterica* serotype Albany, and *S. enterica* serotype Paratyphi *(1, 4, 12).* Indeed, this was observed in a study by Doublet et al. in which they characterized two human clinical *Salmonella* Newport isolates and found that the *aac*(3)-*Id* gene (3′-*N*-aminoglycoside acetyltransferase) was integrated into the chromosome. The *aac*(3)-*Id* gene confers resistance to gentamicin and sisomicin, and the region in the chromosome where the gene was found was named *Salmonella* Genomic Island (SGI)-H *(5).* Within the strains of *Salmonella* Newport studied, Zhao et al. *(16)* showed that the antibiotic susceptibility profiles of *Salmonella* Newport correlated with the pulsed-field gel electrophoresis (PFGE) clusters. Similarly, Fontana et al. *(6)* observed a clear discrimination of resistant and susceptible *Salmonella* New-

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port isolates by PFGE as well as ribotyping. One goal of this study was to determine whether the apparent correlation between PFGE and the antibiotic resistance profiles among *Salmonella* Newport isolates, as observed by Zhao et al. and Fontana et al., could be found in another study group. In this study, the isolates were tested against a wider range of antimicrobials than in the study by Zhao et al. In addition to subtyping by PFGE, typing based on ribosomal DNA restriction fragment patterns was included to test for a correlation between the antibiotic resistance profiling and ribotyping. The second goal was to determine which of the three techniques—ribotyping, PFGE, or the antibiotic susceptibility patterns—was more discriminatory for typing epidemiologically unrelated *Salmonella* Newport. The method that generates the greatest number of discrete profiles will be considered the most discriminatory.

MATERIALS AND METHODS

Bacterial isolates. A total of 30 *Salmonella* Newport isolates, including 17 environmental isolates, 11 clinical isolates, and 2 control strains, i.e., ATCC 6962 and ATCC 27869 (American Type Culture Collection, Manassas, Va.), were used in this study. The clinical isolates were epidemiologically unrelated. *Salmonella* isolated from human stool samples by the Florida Department of Health (FLDOH), $n = 7$, and the Washington Department of Health (WADOH), $n = 4$, were kindly provided for this study. Seventeen *Salmonella* Newport isolates from a midwestern turkey processing plant (collected for 4 days) were generously donated by Dr. Catherine Logue of North Dakota State University *(11).* *Salmonella* Braenderup (CDC H9812, which was used as a molecular-weight standard for the PFGE) was obtained from the FLDOH. All of the isolates were characterized biochemically by an API 20E panel, analyzed with the APILAB Plus Identification Program version 3.3.3/4.0 (bioMérieux, Inc., Hazelwood, Mo.), and supplemented for subspecies differentiation with mucate medium and malonate broth (REMEL, Inc., Lenexa, Kans.). Assimilation profiles were generated with the 95 substrates in Biolog GN-2 Microplates and analyzed by Omnilog ID 1.1 software against the Biolog GP612. KI D database (Biolog, Inc., Hayward, Calif.). The FLDOH clinical isolates were screened for the somatic (O) serogroup with BD *Salmonella* O Antisera (Becton Dickinson, Sparks, Md.), and serotyping was performed by the *Salmonella* reference laboratory of the FLDOH, Bureau of Laboratories (Jacksonville, Fla.). Strains obtained from the WADOH and Dr. Logue were serotyped prior to inclusion in this study.

Antibiotic susceptibility testing. Antibiotic resistance profiles were determined by the Sensititre system (Trek Diagnostics, Cleveland, Ohio), which is based on the classic macrobroth dilution, according to the manufacturers' instructions. Resistance to a total of 31 different antibiotics or antimicrobial combinations was tested with two panels consisting of 23 and 16 antibiotics each. The isolates were tested against various antibiotics, including (i) β-lactams—ampicillin (Amp), piperacillin (Pip), and ticarcillin (Tic); (ii) β-lactam–β-lactamase inhibitor combinations—amoxicillin–clavulanic acid (Aug), ampicillin-sublactum (A/S), piperacillin-tazobactum (P/T), and ticarcillin–clavulanic acid (Tim); (iii) aminoglycosides—amikacin (Ami), gentamicin (G), kanamycin (K), streptomycin (Str), and tobramycin (Tob); (iv) cephams—ceftriaxone (Axo), cephalothin (Cep), cefoxitin (Fox), ceftiofur (Tio), aztreonam (Azt), cefepime (Fep), cefoperazone (Fop), cefotaxime (Fot), and ceftazidime (Taz); (v) quinolones—nalidixic acid (Nal); and (vi) fluoroquinolones—ciprofloxacin (Cip), levofloxacin (Levo), and lomefloxacin (Lome). Other antibiotics tested included chloramphenicol (Ch), tetracycline (Tet), trimethoprim-sulfamethaxazole (Cot), sulfamethoxazole (Smx), sulfizoxazole (Fis), and imipenem (Imi). Results were interpreted according to guidelines published by the NCCLS (currently the Clinical and Laboratory Standards Institute) *(13).* An isolate was considered resistant, intermediately resistant, or susceptible on the basis of the NCCLS breakpoints, where available. An isolate was considered multidrug resistant when it was resistant or intermediately resistant to two or more classes of antibiotics. *Escherichia coli* (ATCC 25922) was used for quality control.

Automated ribotyping. Isolated colonies from freshly subcultured (18 to 20 h) Trypticase soy agar (TSA) plates were used for ribotyping. The automated RiboPrinter (Dupont Qualicon, Wilmington, Del.) performed ribotyping with the restriction enzyme *Eco*RI according to the manufacturers' instructions.

PFGE. All reagents were obtained from Sigma (St. Louis, Mo.) unless otherwise specified. Macrorestriction digestion of genomic DNA was performed by the CDC standardized laboratory protocol for the molecular subtyping of *E. coli* O157:H7. Briefly, *Salmonella* colonies from freshly inoculated TSA plates (incubated for 18 to 20 h) were suspended in 1 ml of cell suspension buffer (100 mM Tris–100 mM EDTA, pH 8.0) to obtain an A_{610} with 1 to 1.4 absorbance on a DU 640 Spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). Two hundred microliters of the above cell suspension, 0.2 mg of proteinase K, and 200 μl of 1% molten agarose (Seakem Gold, Cambrex Bio Science, Rockland, Maine) were dispensed into disposable plug molds (Bio-Rad Laboratories, Hercules, Calif.) and allowed to solidify. The plugs were lysed in cell lysis buffer (50 mM Tris-50 mM EDTA [pH 8.0], 1% sarcosyl) with 0.5 mg of proteinase K for 2.5 h at 54°C in a shaking water bath. They were then washed twice in preheated water (50°C) and four times in Tris-EDTA buffer (50°C) at 10-min intervals. Plug slices of 2-mm width were digested with 50 U each of *Spe*I and *Xba*I (Promega, Madison, Wis.) separately for 2 h. *Xba*I–digested *Salmonella* Braenderup H9812 plug slices were used as molecular-weight standards. The electrophoresis was performed on the CHEF Mapper (Bio-Rad), with an initial switch time of 2.16 s and a final switch time of 63.8 s for 18 h. The gel was stained with ethidium bromide and visualized by the GelDoc (Bio-Rad).

Dendrogram construction. Tagged Image File Format (TIFF) images of PFGE and ribotyping, text files of data from the RiboPrinter, and MICs of antibiotic resistance were analyzed by BioNumerics software version 3.0 (Applied Math, Sint-Martens-Latem, Belgium) with the Dice coefficient. For PFGE, four molecular-weight standards were run on each gel for normalization, and bands below 54 kb were not considered for analysis in order to exclude plasmid DNA. For ribotyping, the data were analyzed in two different ways. In the first method, data normalized by the RiboPrinter (text file) were exported into the BioNumerics database. In the second method, TIFF images obtained from the RiboPrinter were manually analyzed by BioNumerics software. The phylogenetic relationship between isolates was studied by dendrograms constructed with UPGMA (Unweighted Pair Group Method with Arithmetic averages), with 1% position tolerance. Strains that showed 93% or more similarity were considered identical for PFGE; this threshold was established from a cluster analysis of PFGE profiles for molecular-weight standards run on 10 gels with four standards per run. For the RiboPrinter, a comparison of molecular-weight standards between gels showed 99.99% similarity; therefore, strains showing less than 99.99% similarity were

TABLE 1. *Isolates, sources, and antibiotic resistance profiles*[a]

Salmonella isolate no.	Source	Mo/yr of isolation[b]	Antibiotic resistance profile[c]
CBD 30 Newport	ATCC 6962	Unknown	S
CBD 1058 Newport	ATCC 27869	Unknown	S
CBD 67 Newport	FLDOH	10/2001	S
CBD 213 Newport	FLDOH	11/2002	Ch
CBD 425 Newport	WADOH	08/2001	AugAmpFoxTioAxoCepChStrSmxTetA/SAztFopFotTazPipTicTim
CBD 426 Newport	WADOH	02/2001	AugAmpFoxTioAxoCepChStrSmxTetA/SAztFopFotTazPipP/TTic-Tim
CBD 427 Newport	WADOH	03/2002	AugAmpFoxTioAxoCepChStrSmxTetA/SAztFopFotTazPipTimTob
CBD 428 Newport	WADOH	Unknown	AugAmpFoxTioAxoCepChStrSmxTetA/SFopFotTazPipTicTim
CBD 571 Newport	Turkey carcasses	2000-1	GTetTob
CBD 572 Newport	Turkey carcasses	2000-1	GTetTob
CBD 584 Newport	Turkey carcasses	2000-1	A/SGPipTetTicTob
CBD 585 Newport	Turkey carcasses	2000-1	GImi
CBD 586 Newport	Turkey carcasses	2000-1	GSmxTet
CBD 587 Newport	Turkey carcasses	2000-1	GTet
CBD 588 Newport	Turkey carcasses	2000-1	GTet
CBD 589 Newport	Turkey carcasses	2000-1	Tet
CBD 590 Newport	Turkey carcasses	2000-1	StrSmxTet
CBD 591 Newport	Turkey carcasses	2000-1	AugAmpStrSmxA/SFopPipP/TTicTim
CBD 592 Newport	Turkey carcasses	2000-1	StrSmxTetA/SPipTicTim
CBD 593 Newport	Turkey carcasses	2000-1	StrGTetTob
CBD 594 Newport	Turkey carcasses	2000-2	Tet
CBD 595 Newport	Turkey carcasses	2000-3	Ch
CBD 596 Newport	Turkey carcasses	2000-3	Ch
CBD 597 Newport	Turkey carcasses	2000-4	AmpCepGKStrSmxA/SFopPipTicTim*
CBD 598 Newport	Turkey carcasses	2000-4	AmpCepGKNalStrSmxA/SFopPipTicTim*
CBD 604 Newport	FLDOH	11/2003	AmpGKSmx
CBD 759 Newport	FLDOH	04/2004	S
CBD 815 Newport	FLDOH	07/2004	FoxCep
CBD 827 Newport	FLDOH	09/2004	S
CBD 829 Newport	FLDOH	09/2004	S

[a] The turkey carcass isolates were collected for 4 days.

[b] The number following the year refers to the day of isolation.

[c] An asterisk indicates the presence of integrons in that isolate. S, susceptible to all antibiotics tested; Ch, chloramphenicol; Aug, amoxicillin–clavulanic acid; Amp, ampicillin; Fox, cefoxitin; Tio, ceftiofur; Cep, cephalothin; Str, streptomycin; Smx, sulfamethoxazole; Tet, tetracycline; A/S, ampicillin-sublactum; Azt, aztreonam; Fop, cefoperazone; Fot, cefotaxime; Taz, ceftazidime; Pip, piperacillin; Tic, ticarcillin; Tim, ticarcillin–clavulanic acid; P/T, piperacillin-tazobactum; Tob, tobramycin; Imi, imipenem; K, kanamycin; G, gentamicin; Nal, nalidixic acid.

considered different for the automated normalization as well as the TIFF-based manual analysis.

PCR and sequencing. The isolates were screened for the presence of integrons by PCR. The DNA was extracted by means of the Epicenter Masterpure kit (Epicenter Technologies, Madison, Wis.) or the MagNA Pure LC DNA isolation kit with the MagNA Pure LC instrument (Roche Applied Sciences, Indianapolis, Ind.) according to the manufacturers' instructions. The integrons were amplified with primers and conditions as described elsewhere *(8)*. The amplified product was purified by a gel extraction kit (Qiagen, Valencia, Calif.) for sequencing. Sequencing was performed with the CEQ 8000 (Beckman) according to the manufacturers' protocol and reagents. The results were analyzed by Lasergene software version 5.6 (DNAStar Inc., Madison, Wis.) and compared to the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Thirty *Salmonella* Newport isolates from three different geographic locations were characterized by their antibiotic susceptibility profiles, PFGE patterns, and ribotyping patterns.

Antimicrobial susceptibilities. The isolates were tested against 31 antibiotics or a combination of antibiotics. Table 1 shows the information for isolates and their antibiotic susceptibility patterns. Six (20%) of the 30 isolates were susceptible to all of the antibiotics tested. Three were resistant or intermediately resistant to Ch only, and two isolates were resistant or intermediately resistant to Tet only. The four clinical isolates from the WADOH were resistant or intermediately resistant to 17 or more drugs. Most of the environmental isolates showed resistance or intermediate resistance to Tet (11 of 17) and G (10 of 17). Forty-five percent of the clinical isolates were resistant or intermediately resistant to Ch or Fox. Overall, 45% of the clinical isolates and 76% of the environmental isolates showed a multidrug-resistant phenotype, with resistance or intermediate resistance demonstrated toward two or more clas-

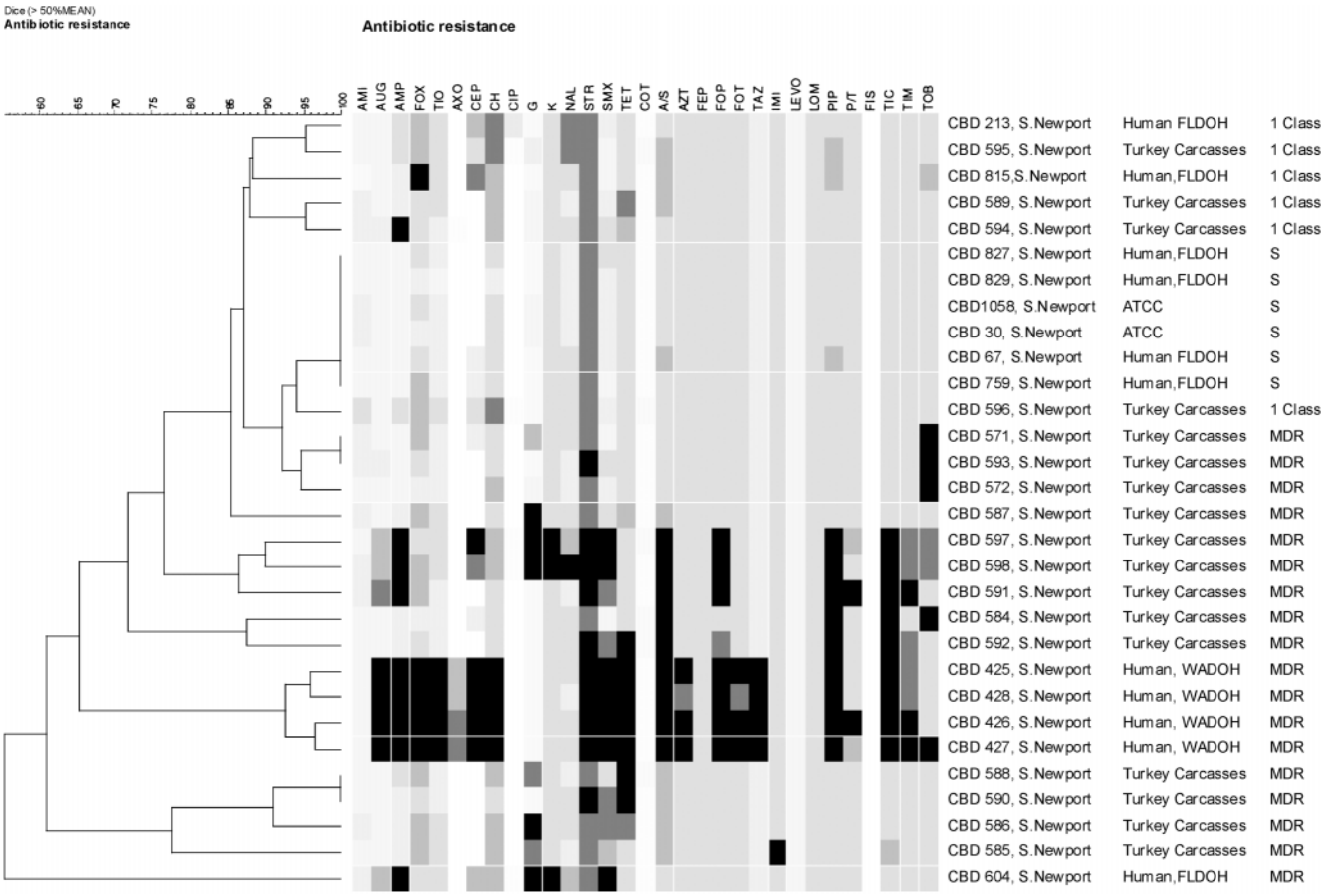

FIGURE 1. *Dendrogram showing the percent similarity between isolates on the basis of their susceptibilities to 31 different antibiotics or antibiotic combinations. Each cell indicates the MIC of the corresponding antimicrobial. The darker the cell, the greater the MIC value, and therefore, the greater the resistance to that particular drug. S, susceptible to all classes of antibiotics; 1 class, resistant to one class of antibiotics; MDR, multidrug resistant to two or more classes of antibiotics.*

ses of antimicrobials. Cluster analysis of the antimicrobial susceptibility results showed 23 distinct profiles. Isolates that were resistant or intermediately resistant to six or more antibiotics formed a separate cluster that was clearly distinguishable from the susceptible or less-resistant isolates (Fig. 1). The clinical isolates from the WADOH formed a cluster at 94% or more similarity. The environmental isolates were distributed among various clusters.

Ribotyping. Thirty *Salmonella* Newport isolates were ribotyped with the enzyme *Eco*RI. When the results were analyzed manually, three major clusters at 74% or more similarity were observed (Fig. 2). The three clusters were further divided into 14 unique ribotypes. On the basis of 99.9% similarity, with the exception of CBD 584, the environmental isolates grouped in the largest cluster, composing 9 of the 14 ribotypes, whereas the clinical isolates were dispersed among the three major clusters. Four clinical isolates, CBD 604, CBD 213, CBD 829, and CBD 759, showed 100% identity with the environmental isolates. When the fragment patterns were normalized by RiboPrinter software, 10 ribotypes at 98% or less similarity were observed by BioNumerics software (data not shown). These 10 ribotypes are essentially similar to the manually analyzed data, with some exceptions. The major difference between the automated and manual analyses was observed among the environmental isolates. Thirteen isolates that showed 100% similarity by the automated analysis were divided into eight ribotypes by the manual analysis (data not shown).

PFGE. The isolates were subtyped by PFGE with two enzymes, *Xba*I and *Spe*I, separately. Two isolates were considered identical only when they showed the same macrorestriction pattern with both the enzymes. With 93% similarity as the threshold, 14 pulsotypes were observed with the enzyme *Xba*I (Fig. 3). Similarly, 14 subtypes were detected with the enzyme *Spe*I (data not shown). The environmental isolates formed one large cluster at 84% that could be further divided into three subgroups and four pulsotypes with the enzyme *Xba*I. The 11 clinical isolates showed greater diversity, forming eight pulsotypes with all of the isolates from the WADOH forming one cluster. The two ATCC control strains were of two distinct pulsotypes. The PFGE results between the two enzymes correlated well (data not shown).

PCR and sequencing. All of the isolates that showed resistance or intermediate resistance to at least one drug were tested for the presence of class 1 integrons. Of the 18

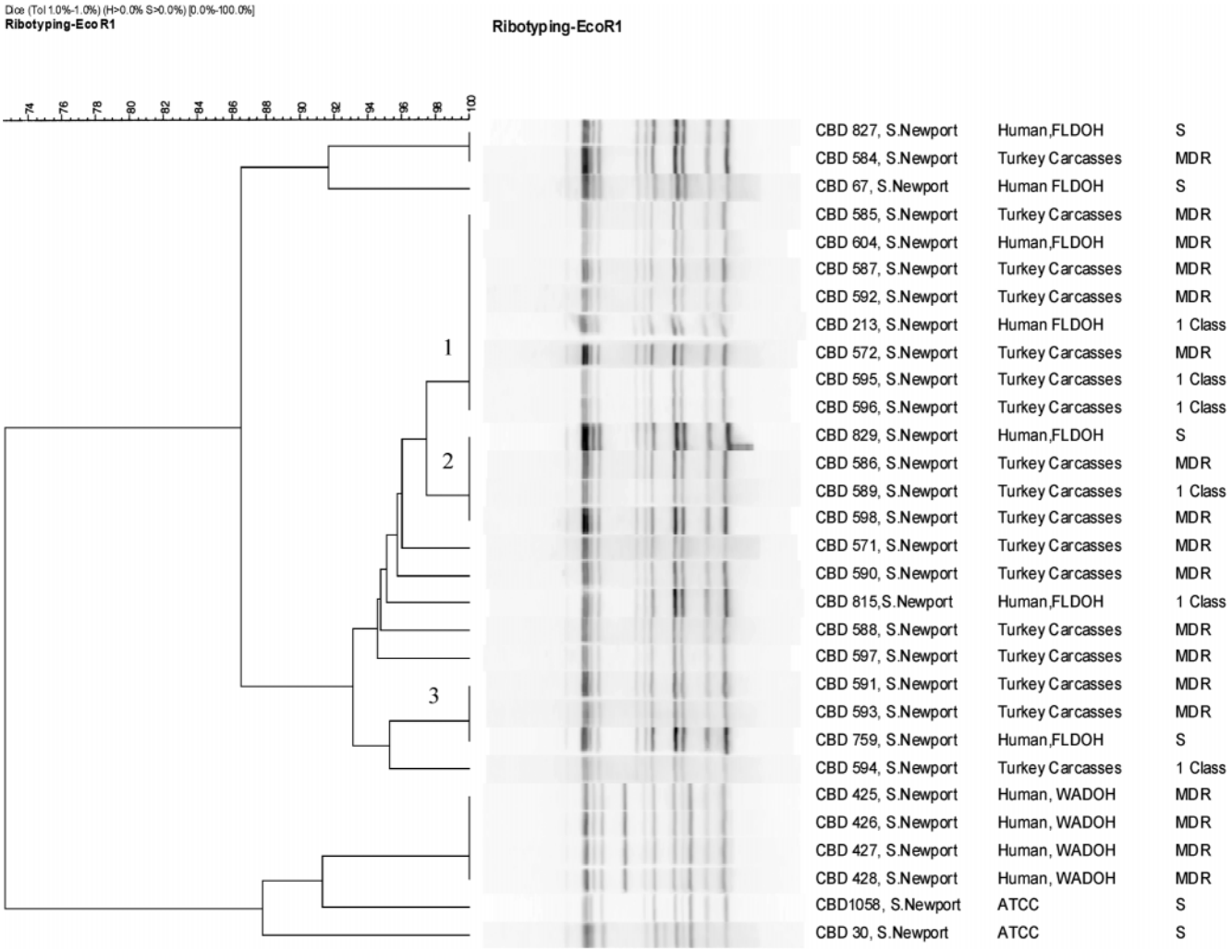

FIGURE 2. *Dendrogram representing the ribotypes of the* Salmonella *Newport isolates with the enzyme* Eco*RI. The isolate number, corresponding source, and resistance pattern are shown. The relatedness among the isolates is depicted by the percent similarity. Cluster 2 depicts the group that was further divided by PFGE; clusters 1 and 3 refer to PFGE group A, which was subdivided by the ribotyping. S, susceptible to all classes of antibiotics; 1 class, resistant to one class of antibiotics; MDR, multidrug resistant to two or more classes of antibiotics.*

isolates that were multidrug resistant, only two showed the presence of class 1 integrons of 1 kb. Sequence analysis of the amplified integrons showed a 98% match to the aminoglycoside adenyl transferase (*aadA1*) of *Salmonella* Typhimurium and *S. enterica* serotype Infantis class 1 integrons encoding resistance to aminoglycosides (Table 2).

DISCUSSION

Thirty *Salmonella* Newport isolates were subjected to molecular typing and antibiotic susceptibility testing to compare the discriminatory power of the two typing techniques and to determine whether the DNA fragment patterns correlated with their respective antibiotic resistance profiles. PFGE with *Xba*I, as well as *Spe*I, generated 14 pulsotypes, illustrating the consensus between the two enzymes. Likewise, ribotyping divided the isolates into 14 types, clearly demonstrating that ribotyping by the RiboPrinter can be as discriminatory as PFGE for typing *Salmonella* Newport. Moreover, ribotyping by the automated RiboPrinter was more discriminatory when the fragment profiles were analyzed manually from TIFF files than when the patterns were normalized by the RiboPrinter. Fourteen profiles were observed by manual analysis compared to the 10 profiles obtained by the automated normalization. A greater discrimination was observed in the environmental isolates by manual analysis than by automated analysis. Previous studies have shown that PFGE further resolved the ribogroups *(3, 15).* However, we observed that PFGE and ribotyping together further resolved groupings that were not differentiated by these methods individually. For example, ribotype cluster 2 (Fig. 2) was further discriminated by PFGE into two groups, B and C (Fig. 3). Likewise, PFGE cluster A (Fig. 3) was further divided into clusters 1 and 3 by ribotyping (Fig. 2). Ribotyping with a combination of enzymes has previously been shown to be more discriminatory than ribotyping with one enzyme. For example, ribotyping with *Pst*I and *Sph*I has proven to be very discriminatory for certain *Salmonella* serotypes, including Enteritidis and Typhimurium *(9, 10).* This is the first report, to our knowledge, that shows that ribotyping by the auto-

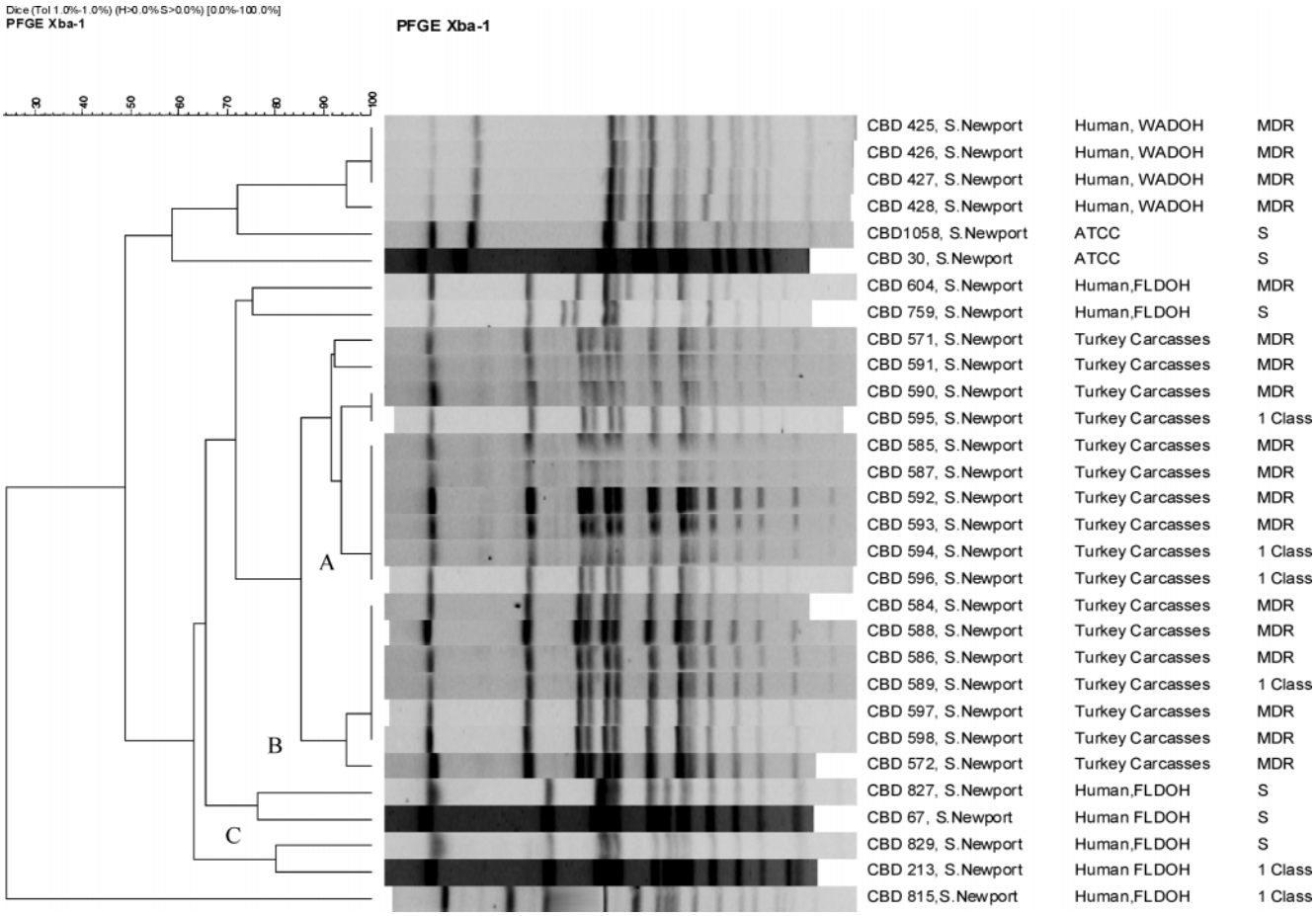

FIGURE 3. *Dendrogram representing the macrorestriction profiling with the enzyme* Xba*I. The percent similarity between various pulsotypes is shown. The isolate number and source are specified. Cluster A was further divided by ribotyping; clusters B and C correlate with ribotyping group 2 that was subdivided by PFGE. S, susceptible to all classes of antibiotics; 1 class, resistant to one class of antibiotics; MDR, multidrug resistant to two or more classes of antibiotics.*

mated RiboPrinter with the enzyme *Eco*R1 is as discriminatory as PFGE for *Salmonella* Newport when analyzed manually from TIFF files.

All of the environmental isolates from turkey carcasses formed a single major cluster by both the ribotyping and PFGE methods. With ribotyping, the environmental isolates clustered at 92% or more similarity, along with some clinical isolates (Fig. 2). With *Xba*I PFGE, all the environmental isolates formed a unique cluster at 88% or more relatedness (Fig. 3). Similarly, PFGE performed on the environmental isolates with *Spe*I generated a distinct group at 90% or more similarity (data not shown). This demonstrates that all the environmental isolates are very closely related and that PFGE clearly distinguished the environmental isolates from the clinical isolates, whereas ribotyping did not. The PFGE and ribotyping profiles of the four clinical isolates from the WADOH were identical. Because these four isolates have the same molecular typing patterns, the possibility of an outbreak strain is indicated, even though they were obtained from different sources (Table 1).

Two isolates, CBD 571 and CBD 572, which were previously serotyped as *Salmonella* Bardo by conventional

TABLE 2. *Integron sequencing*[a]

Salmonella isolate and source	Antibiotic resistance	Integron size (kb)	% match to NCBI BLAST
597 Newport, turkey carcasses	AmpCepGKStrSmxA/SFopPipTicTim	1	98% to *Salmonella* Infantis and *Salmonella* Typhimurium class 1 integron *aadA1*
598 Newport, turkey carcasses	AmpCepGKNalStrSmxA/SFopPipTicTim	1	98% to *Salmonella* Infantis and *Salmonella* Typhimurium class 1 integron *aadA1*

[a] Amp, ampicillin; Cep, cephalothin; G, gentamicin; K, kanamycin; Str, streptomycin; Smx, sulfamethoxazole; A/S, ampicillin-sublactum; Fop, cefoperazone; Pip, piperacillin; Tic, ticarcillin; Tim, ticarcillin–clavulanic acid; *aadA1*, aminoglycoside adenyl transferase; Nal, nalidixic acid.

methods, showed a very high similarity to *Salmonella* Newport by molecular subtyping methods. *Salmonella* Newport and *Salmonella* Bardo have very similar antigenic formulas and differ by the presence or absence of the O:6 antigen. Ribotyping showed that CBD 571 was 96% similar to *Salmonella* Newport (Fig. 2). PFGE with *Xba*I showed a 92% similarity (Fig. 3) to *Salmonella* Newport, and PFGE with *Spe*I showed a 95% relatedness (data not shown) to *Salmonella* Newport. Similarly, the ribotyping of CBD 572 showed 100% identity with *Salmonella* Newport cluster (Fig. 2), and PFGE analysis with *Xba*I and *Spe*I showed 93% (Fig. 3) and 100% identity, respectively (data not shown), with *Salmonella* Newport. This prompted us to reserotype the two putative *Salmonella* Bardo isolates. Both strains typed as *Salmonella* Newport, demonstrating that molecular techniques are not only rapid but are also very powerful tools for identifying *Salmonella* serotypes, provided that the profile from the serotype is present in the database. Discrimination of these *Salmonella* Newport strains also confirms that molecular typing methods are very useful for outbreak investigations.

Eighty percent of the isolates were resistant or intermediately resistant to at least one antibiotic. Overall, 60% of the isolates were multidrug resistant, showing resistance or intermediate resistance to two or more classes of antibiotics. Only two isolates harbored class 1 integrons. Sequencing of the integrons confirmed the presence of the *aadA1* gene, consistent with the antibiotic resistance pattern of the isolates. The typing of the strains on the basis of their antibiotic susceptibilities, which gave rise to 23 profiles, was more discriminatory than either ribotyping or PFGE. When the cluster analyses of the macrorestriction profiles and ribotypes were compared to the antibiotic resistance dendrograms, no correlation was observed. The susceptible isolates, the multidrug-resistant strains, and the isolates that were resistant or intermediately resistant to only one class of antibiotics were distributed among various clusters, as shown in the both the ribotyping and PFGE dendrograms (Figs. 2 and 3). Therefore, there was no clear distinction of the susceptible isolates from the resistant isolates with *Xba*I PFGE, contrary to the results observed by Zhao et al. *(16).* Unlike our study, the study by Zhao et al. focused on the multidrug-resistant–AmpC phenotype. In contrast, four of the eight isolates (Table 1) that showed the multidrug-resistant–AmpC phenotype in our study, CBD 591, CBD 597, CBD 598, and CBD 604, did not show correlation with genotyping, whereas the four WADOH isolates—CBD 425, CBD 426, CBD 427, and CBD 428—did. As mentioned, it is possible that the four WADOH *Salmonella* Newport isolates are clonal and hence have highly similar antibiotypes as well as molecular types, which would thus not support the conclusions of the Zhao et al. study. Likewise, no correlation was observed between the antibiotic resistance patterns and the ribotyping patterns, as reported in a study by Fontana et al. *(6).*

In conclusion, PFGE and manually analyzed ribotyping were shown to be equally discriminatory for the typing of *Salmonella* Newport in this study. The PFGE patterns and ribotypes correlated well in clustering the isolates. Because ribotyping by the automated RiboPrinter gives results in 8 h, it is an excellent tool for the initial identification as well as the source tracking of *Salmonella* Newport. However, a larger study group should be tested to confirm that ribotyping is as discriminatory as PFGE for epidemiological investigations. Cluster analysis of antibiotic susceptibilities was more discriminatory than either of the molecular typing techniques. Because both resistant and susceptible isolates were distributed among different ribogroups as well as PFGE clusters, no correlation was observed between the antibiograms and the molecular typing profiles. This differs from the findings by Zhao et al. *(16).* A greater selection of antimicrobials was used in this study and could be a factor that contributed to the lack of correlation between PFGE and resistance profiles. A combination of macrorestriction patterns from PFGE, ribotyping, and antibiotic resistance profiles will be very useful for the rapid source tracking of *Salmonella* Newport strains in an outbreak situation.

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