APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2005, p. 2587–2591 Vol. 71, No. 5
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.5.2587–2591.2005

Clonal Expansion May Account for High Levels of Quinolone Resistance in *Salmonella enterica* Serovar Enteritidis

Donna Kilmartin, D. Morris,* C. O'Hare, G. Corbett-Feeney, and M. Cormican

Department of Bacteriology, National University of Ireland, Galway, Ireland

Received 16 June 2004/Accepted 11 October 2004

We have observed a high incidence of isolated nalidixic acid resistance in *Salmonella enterica* serovar Enteritidis isolates in Ireland, particularly isolates of phage type 1 (PT1). A group of nalidixic acid-resistant ($n = 22$) and nalidixic acid-susceptible ($n = 28$) isolates of serovar Enteritidis from multiple sites in Ireland were selected. Isolates were typed by pulsed-field gel electrophoresis (PFGE) with XbaI, and the MICs for nalidixic acid and ciprofloxacin were determined. Mutations associated with nalidixic acid resistance in clinical isolates and laboratory mutants of serovar Enteritidis and 32 nalidixic acid-resistant isolates of 15 other salmonella serovars were identified. PFGE had limited discriminatory power. A specific point mutation (G246T) associated with amino acid substitution Asp87Tyr in the quinolone resistance determining region of the *gyrA* gene accounted for 95% of all mutations in serovar Enteritidis and for all mutations in PT1 isolates. Greater diversity of mutations was observed among all non-Enteritidis salmonella serovars studied. Rates of nalidixic acid resistance in serovar Enteritidis may predominantly reflect clonal expansion after infrequent mutation or selection events.

Salmonella enterica subsp. *enterica* serovar Enteritidis is the most common etiological agent of food-borne salmonellosis worldwide (7). Infection usually results in a self-limiting gastroenteritis (16). Fluoroquinolones are widely used for therapy of salmonellosis since they have potent activity against *Salmonella enterica*, with ciprofloxacin MICs in the range 0.016 to 0.12 mg/liter for wild-type strains (13). In recent years there have been frequent reports of reduced susceptibility to fluoroquinolones (ciprofloxacin MICs of 0.25 to 1.0 mg/liter) among *S. enterica* strains (3, 4, 13), although ciprofloxacin resistance (MIC $\geq$ 4 mg/liter) is rare (4). Resistance to the quinolone nalidixic acid is a marker for reduced fluoroquinolone susceptibility. The clinical significance of reduced susceptibility to fluoroquinolones is the subject of debate at present with a recently published proposal that the current National Committee for Clinical Laboratory Standards (NCCLS) interpretive criteria for ciprofloxacin (susceptible at an MIC of $\leq$1 μg/ml) be reduced significantly (1).

In gram-negative bacteria the principal target of quinolone/fluoroquinolone activity is the type II topoisomerase, DNA gyrase (11). DNA gyrase is a tetramer composed of two GyrA subunits (encoded by *gyrA* gene) and two GyrB subunits (encoded by *gyrB* gene). Raised MICs to quinolones and fluoroquinolones are associated with mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene. Less frequently, quinolone resistance is associated with point mutations in the type IV topoisomerase. This, too, has a tetramer structure and is encoded by the genes *parC* and *parE*. The QRDR of DNA gyrase A in *S. enterica* is between amino acids 67 and 122 encoded by nucleotides 185 to 361 in *gyrA* gene (3). The most common amino acid substitutions in the GyrA subunit associated with quinolone resistance occur at codons Ser83 and Asp87 (14).

We observed an association in Ireland between isolated nalidixic acid resistance and serovar Enteritidis (9). In 2000, 13.7% of serovar Enteritidis strains ($n = 255$) from Ireland were nalidixic acid resistant compared to 2.3% of serovar Typhimurium strains. Quinolone resistance was particularly associated with phage type 1 (PT1); of 26, 23 (88%) were nalidixic acid resistant compared to 6% of serovar Enteritidis of all other phage types. Isolated nalidixic acid resistance is also common in serovars Hadar, Kentucky, and Virchow.

A high incidence of nalidixic acid resistance could reflect frequent mutation/selection events. Alternatively high incidence of nalidixic acid resistance may arise from rare mutation/selection events with subsequent clonal expansion and dissemination. One would predict that clonal expansion would result in the nalidixic acid-resistant organisms being relatively homogeneous, whereas the frequent mutation/selection hypothesis should result in greater heterogeneity. We have studied a collection of nalidixic acid-susceptible and -resistant isolates of serovar Enteritidis and other salmonella serovars to determine whether there is evidence to support either hypothesis.

MATERIALS AND METHODS

Bacterial Strains. Fifty serovar Enteritidis isolates (22 nalidixic acid resistant and 28 nalidixic acid susceptible) submitted to the National Salmonella Reference Laboratory in 2000 were selected. Isolates were selected to represent a wide geographic distribution within Ireland, and 11 isolates were associated with travel outside of Ireland. PT1 ($n = 20$) and PT4 ($n = 17$) accounted for most isolates (Table 1). Thirty-two nalidixic acid-resistant isolates of serovars other than Enteritidis were selected for sequencing of the QRDR of the *gyrA* gene to provide a basis for comparison (Table 2).

The control organisms used were *Escherichia coli* ATCC 25922, *E. coli* J53-2, *S. enterica* serovar Typhimurium DT36 Type Strain (LEP Colindale), and *S. enterica* serovar Braenderup H9812 (Centers for Disease Control and Prevention, Atlanta, Ga.).

Antimicrobial susceptibility testing. MICs were determined by agar dilution method as described by the NCCLS (8). The antimicrobial agents tested were

* Corresponding author. Mailing address: Department of Bacteriology, National University of Ireland, Galway, Galway, Ireland. Phone: (353) 91-544628. Fax: (353) 91-512514. E-mail: Dearbhaile.Morris@nuigalway.ie.

TABLE 1. Phage types, nalidixic acid and ciprofloxacin MICs, pulsed-field profiles, *gyrA* amino acid substitutions, and mutations of 50 serovar Enteritidis isolates

Bank no.	Phage type	MIC (μg/ml)		PFP	*gyrA* amino acid substitution (mutation)
		Nalidixic acid	Ciprofloxacin		
3436/00	1	4	0.016	A	Not tested
3574/00	1	4	0.016	A	Not tested
3332/00	4	8	0.016	A	None detected[a]
3424/00	4	8	0.016	A	Not tested
3446/00	4	4	0.016	A	Not tested
3449/00	4	8	0.016	A	Not tested
3486/00	4	8	0.016	A	Not tested
3494/00	4	8	0.016	A	Not tested
3495/00	4	8	0.016	A	Not tested
3530/00	4	8	0.016	A	Not tested
3545/00	4	8	0.016	A	Not tested
3546/00	4	8	0.016	A	Not tested
3550/00	4	8	0.016	A	Not tested
3566/00	4	8	0.016	A	Not tested
3584/00	4	8	0.016	A	Not tested
3614/00	4	8	0.016	A	Not tested
3679/00	4	8	0.016	A	Not tested
3694/00	4	8	0.016	A	Not tested
3716/00	4	8	0.016	A	Not tested
3490/00	6a	4	0.016	A_2	Not tested
3547/00	6a	8	0.063	A_3	Not tested
3576/00	6a	8	0.016	A	Not tested
3582/00	6a	8	0.063	A_3	Not tested
3595/00	6a	8	0.016	A_1	Not tested
3536/00	21	8	0.016	A	Not tested
3558/00	21	8	0.016	A_6	None detected
3415/00	44	8	0.016	A_5	Not tested
3658/00	44	4	0.016	A_5	Not tested
3307/00	1	>128	0.25	A	Asp87Tyr (G259T)[b]
3324/00	1	>128	0.25	A	Asp87Tyr (G259T)
3371/00	1	>128	0.25	A	Asp87Tyr (G259T)
3416/00	1	>128	0.25	A	Asp87Tyr (G259T)
3438/00	1	>128	0.25	A	Asp87Tyr (G259T)
3452/00	1	>128	0.25	A_4	Asp87Tyr (G259T)
3477/00	1	>128	0.25	A	Asp87Tyr (G259T)
3478/00	1	>128	0.25	A	Asp87Tyr (G259T)
3499/00	1	>128	0.25	A	Asp87Tyr (G259T)
3500/00	1	>128	0.25	A	Asp87Tyr (G259T)
3501/00	1	>128	0.25	A	Asp87Tyr (G259T)
3510/00	1	>128	0.25	A	Asp87Tyr (G259T)
3511/00	1	>128	0.25	A	Asp87Tyr (G259T)
3581/00	1	>128	0.25	A	Asp87Tyr (G259T)
3620/00	1	>128	0.5	A	Asp87Tyr (G259T)
3622/00	1	>128	0.25	A_5	Asp87Tyr (G259T)
3678/00	1	>128	0.25	A	Asp87Tyr (G259T)
3712/00	1	>128	0.25	A	Asp87Tyr (G259T)
3419/00	6a	>128	0.25	A_2	Asp87Asn (G259A)[c]
3361/00	21	>128	0.25	A_1	Asp87Tyr (G259T)
3487/00	21	>128	0.25	A_1	Asp87Tyr (G259T)
3493/00	21	>128	0.25	A	Asp87Tyr (G259T)

[a] Registered under GenBank accession number AY082383.
[b] Registered under GenBank accession number AF487420.
[c] Registered under GenBank accession number AF487421.

nalidixic acid, cinoxacin, flumequine, oxolinic acid, ofloxacin, and lomefloxacin (all from Sigma-Aldrich, Steinheim, Germany) and enrofloxacin (Baytril, Shawnee Mission, Kans.). It was not possible to access ciprofloxacin and marbofloxacin powders of defined potency; therefore, it was necessary to use suspensions normally used for parenteral administration (Ciproxin I.V. Flexibag [Bayer, Berkshire, United Kingdom] and marbofloxacin [Vetoquinol Ireland, Oranmore, Ireland]). *E. coli* ATCC 25922 and *E. coli* J53-2 were included as controls.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed in accordance with the PulseNet Protocol (Centers for Disease Control and Prevention, Atlanta, Ga.) with the restriction endonuclease XbaI (Fig. 1). The isolate *Salmonella* serovar Braenderup H9812 was the control organism used (2).

DNA extraction and PCR. Bacteria were grown overnight, and DNA was extracted with a Wizard genomic DNA purification kit (Promega, Madison, Wis.). PCR was performed by using the ICycler (Bio-Rad, Hercules, Calif.). A 313-bp fragment of the *gyrA* gene of 22 nalidixic acid-resistant serovar Enteritidis isolates, 2 nalidixic acid-susceptible serovar Enteritidis isolates, and 32 non-Enteritidis *S. enterica* isolates was amplified. A 261-bp fragment of the *parC* gene of all quinolone-resistant serovar Enteritidis and individual *Salmonella* Stanley

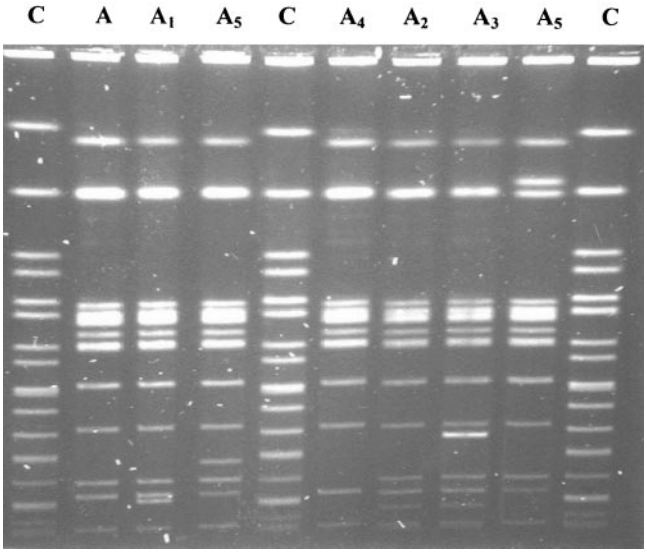

FIG. 1. Gel illustrating the seven different pulsed-field profiles obtained from 50 serovar Enteritidis isolates by using PFGE-XbaI. The label "C" indicates the PFP of the control strain serovar Braenderup H9814. A, the most commonly observed PFP; A_1 to A_6, the variant PFP observed.

and *Salmonella* Reading isolates was amplified. PCRs were performed essentially as described by Wiuff et al. (15). Oligonucleotide primers were P1 (5′-GTCCG AACTGTACCTGGTGG) and P2 (5′-AACAGCAGCGTACGAATGTG) for *gyrA* gene and STPARC1 (5′-CTATGCGATGTCAGAGCTGG-3′) and STPARC2 (5′-TAACAGCAGCTCGGCGTATT-3′) for the *parC* gene (15). PCR products were visualized after electrophoresis on 1% agarose gels (1.65 mg of ethidium bromide/liter). PCR products were purified by using the PCR product presequencing kit (Amersham Biosciences Corp.). Sequencing was performed in both directions by using the BigDye method on ABI 3700 sequencers by MWG Biotech, Milton Keynes, United Kingdom.

Nalidixic acid-resistant laboratory mutants. Nalidixic acid-resistant mutants were derived from serovar Enteritidis strains (3332/00, 3486/00, and 3558/00) by plating a heavy inoculum on Mueller-Hinton broth (MHB) containing a quinolone or fluoroquinolone agent at a concentration three doubling dilution steps above the organism's MIC. Suspect mutant colonies were confirmed as salmonella by colonial morphology on xylose lysine deoxycholate agar (Becton Dickinson, Oxford, United Kingdom). Nalidixic acid resistance was confirmed by NCCLS disk diffusion. Thirty-eight isolates with growth up to the nalidixic acid disk were included in further studies. The QRDR mutations present in these laboratory mutants were defined by PCR amplification, followed by restriction endonuclease digestion (5) or DNA sequencing.

Growth rate studies. Growth rate studies were performed in triplicate with serovar Enteritidis 3332/00 and two different nalidixic acid-resistant derivative mutants: M3332/1 (Asp87Tyr) and M3332/14 (Asp87Asn). Growth studies were performed in 20 ml of MHB with an initial inoculum of 2×10^5 CFU/ml. For M3332/1 and M3332/14 growth rates were repeated in triplicate in the presence of 32 μg of nalidixic acid/ml. Viable counts were performed at times: 0, 180, 240, 300, 330, 360, 390, 420, 450, 480, 510, and 540 min. A growth curve was constructed, and the specific growth rate of each strain was determined. The nalidixic acid MICs for both mutants were assessed before and after each experiment to confirm that the phenotype of nalidixic acid resistance was stable.

RESULTS

Antimicrobial susceptibility testing. The phenotype of nalidixic acid-resistant or -susceptible determined by disk diffusion was confirmed by agar dilution (Table 1). The antibiotic MICs for the nalidixic acid-resistant isolates were >128 μg/ml for nalidixic acid and 0.25 to 0.5 μg/ml for ciprofloxacin (Table 1). Susceptible isolates had nalidixic acid MICs of 4 to 8 μg/ml and ciprofloxacin MICs of 0.016 to 0.064 μg/ml. For all isolates, there was consistent relationship between MICs for all quinolones and fluoroquinolones tested.

PFGE. On PFGE typing, 38 isolates were indistinguishable, and there were six minor variants (one to two band differences) among the remaining 12 isolates. For the predominant phage types studied, PT1 (90% nalidixic acid resistant) and PT4 (100% nalidixic acid susceptible), all but two isolates were indistinguishable (pulsed field profile [PFP] A). PFGE with XbaI was not sufficiently discriminatory to provide support for either clonal expansion or frequent mutation/selection as the basis for the high frequency of nalidixic acid resistance observed.

Sequence data. The *gyrA* QRDR sequences obtained for quinolone-susceptible serovar Enteritidis was identical to that reported for serovar Typhimurium (6). All of the nalidixic acid-resistant PT1 ($n = 18$) and PT21 ($n = 3$) isolates showed the transversion thymine for guanine at position 259 (G259T) in the *gyrA* gene. The single nalidixic acid-resistant PT6a isolate had the transition G259A. The derived amino acid sequences from these mutations indicate a substitution of tyrosine (G-T) or asparagine (G-A) for aspartic acid in the amino acid sequence at position 87 in the *gyrA* gene (see Table 1). No mutations were detected in the *parC* QRDR of the 22 quinolone-resistant isolates of serovar Enteritidis.

Five distinct *gyrA* mutations were found among 32 isolates of serovars other than Enteritidis, as detailed in Table 2. It is noteworthy that a G259A mutation was detected in four of five serovar Hadar isolates and that C248T was detected in all seven serovar Kentucky isolates and in four of six serovar Virchow isolates. The QRDR of the *parC* gene was also sequenced for a nalidixic acid-resistant serovar Reading isolate and a serovar Stanley isolate that had no *gyrA* mutation. No mutations were identified in the *parC* gene of the serovar Reading isolate, whereas the serovar Stanley had a significant mutation (G170C) in the second nucleotide of codon 57, (predicted substitution Thr57Ser) and six additional silent mutations.

Thirty-eight nalidixic acid-resistant mutants were obtained from three nalidixic acid-susceptible parent serovar Enteritidis isolates. The G259T mutation present in all of the clinical nalidixic acid-resistant PT1 isolates was detected in only nine (24%) of the laboratory mutants. A total of 14 (37%) laboratory mutants had a mutation at nucleotide 248 (codon Ser83), 4 (11%) had A260G, 1 (3%) had G259A, 2 (5%) had C241T, and 2 (5%) had C356A. No mutation was identified in the QRDR of the *gyrA* or *parC* genes in 6 (16%) of laboratory-generated mutants.

Growth studies. Growth studies indicated that both parent (3332/00) and mutants had similar specific growth rates in MHB. In the presence of nalidixic acid 32 mg/liter the mutants M3332/1 (G259T) and M3332/14 (G259A) had similar growth rates.

DISCUSSION

We sought to determine whether the high rate of nalidixic acid resistance observed in serovar Enteritidis is related to clonal expansion or frequent selection/mutation events. Although a change in phage type may occur as a result of hori-

TABLE 2. Mutations in QRDR of the *gyrA* gene in 32 representative quinolone-resistant isolates of 17 *S. enterica* serovars

Bank no.	Serovar	*gyrA* amino acid substitution (mutation)	Accession no.
932/01	Albany	Asp87Asn (G259A)	AF487421
827/01	Brandenburg	Asp87Asn (G259A)	AY220512
3919/00	Braenderup	Ser83Phe (C248T)	AF500124
112/01	Cholerae-Suis	Ser83Tyr (C248A)	AF498911
651/01	Derby	Asp87Gly (A260G)	AF498910
3664/00	Hadar	Ser83Phe (C248T)	AY561172
415/01	Hadar	Asp87Asn (G259A)	AY220518
509/01	Hadar	Asp87Asn (G259A)	
861/02	Hadar	Asp87Asn (G259A)	
127/03	Hadar	Asp87Asn (G259A)	
7/01	Haifa	Ser83Tyr (C248A)	AF500126
288/01	Heidelberg	Asp87Gly (A260G)	AF498909
103/02	Infantis	Ser83Tyr (C248A)	AF498913
731/01	Java	Asp87Tyr (G259T)	AF498909
3296/00	Kentucky	Ser83Phe (C248T)	AF500125
3901/00	Kentucky	Ser83Phe (C248T)	
671/03	Kentucky	Ser83Phe (C248T)	
672/03	Kentucky	Ser83Phe (C248T)	
673/03	Kentucky	Ser83Phe (C248T)	
676/03	Kentucky	Ser83Phe (C248T)	
898/03[a]	Kentucky	Ser83Phe (C248T) Asp87Gly (A260G)	AY563421
3721/00	Kottbus	Asp87Gly (A260G)	AY220517
3252/00	Mbandaka	Asp87Gly (A260G)	AF500123
409/01	Oranienburg	Ser83Phe (C248T)	AY220513
97/01[b]	Reading	None detected	
41/01[c]	Reading	None detected	
3508/00	Virchow	Ser83Phe (C248T)	AY498999
18/01	Virchow	Asp87Tyr (G259T)	AF498912
31/01	Virchow	Ser83Phe (C248T)	
824/01	Virchow	Asp87Tyr (G259T)	
1002/02	Virchow	Ser83Phe (C248T)	
54/03	Virchow	Ser83Phe (C248T)	

[a] Ciprofloxacin-resistant isolate.
[b] Isolate with no *parC* mutation; GenBank accession no. AY561171.
[c] Isolate with *parC* mutation G170C, corresponding to the amino acid substitution Thr57Ser; GenBank accession no. AY343990.

zontal genetic change (phage or plasmid), phage types of serovar Enteritidis are generally considered to represent specific lineages. Molecular typing was intended to identify different groups within a given phage type; however, the application of PFGE with XbaI to the isolates provided little additional discrimination. Many isolates of distinct phage type shared an indistinguishable XbaI PFP. Among isolates of PT1 differences were infrequent and minor and were not considered as representing a reliable basis for subcategorization. A small subset of PT1 and PT4 isolates was also typed by PFGE with BlnI (data not shown); however, these were also indistinguishable by this method.

Therefore, we determined the specific point mutations associated with nalidixic acid resistance and compared the diversity of mutations observed with that of mutations observed in nalidixic acid-resistant isolates of other serovars and of laboratory mutants derived from susceptible *Salmonella* Enteritidis parent strains. The isolates studied included five phage types/lineages (1, 4, 6a, 21, and 44) with representative nalidixic acid-resistant isolates included for three (1, 6a, and 21) of the five phage types. All 18 representatives of PT1 and three of PT21 shared the same G259T mutation (Table 1), and the single PT6a isolate had a different (G259A) mutation.

To further investigate this observation, we determined the mutations associated with nalidixic acid resistance in 32 isolates representing 16 other serovars of *S. enterica* (Table 2). Distinct serovars represent distinct lineages in which mutations conferring resistance have clearly arisen in nature independently of mutations in serovar Enteritidis. Five distinct *gyrA* mutations associated with nalidixic acid resistance were observed among the 14 serovars, and a *parC* mutation was observed in a serovar Stanley isolate. A tendency for an association between specific mutations and particular serovars was observed when multiple isolates of a given serovar were studied, as noted with serovars Hadar, Kentucky, and Virchow. Of necessity, the isolates studied are limited in number, are subject to selection bias, and cannot be truly representative of the biology of salmonella in the environment. In order to address in some way the possibility of our results representing a chance occurrence, we performed a limited statistical assessment. If all of the mutations associated with nalidixic acid resistance described in all salmonella serovars in the present study are equally likely to occur and persist, the probability that the pattern of associations of specific mutations with specific serovars actually observed arising by repeated independent mutation alone are as follows: 1.68×10^{-16} for serovar Enteritidis; 9.0×10^{-3} for serovar Hadar, 3.0×10^{-5} serovar Kentucky, and 1.0×10^{-3} serovar Virchow.

In laboratory-generated nalidixic acid-resistant mutants that have clearly arisen independently of each other from a well-characterized parent strain, the basis of acquired nalidixic acid resistance was more diverse. In this group, G259T was detected in only 24% of serovar Enteritidis nalidixic acid-resistant laboratory mutants, with other *gyrA* mutations detected in 60% of isolates and no *gyrA* or *parC* mutation detected in 16%. This finding indicates that a bias toward the repeated independent occurrence of the G259T mutation as distinct from other mutations in serovar Enteritidis is not likely to adequately explain the predominance of this specific mutation in a given lineage.

It is also conceivable that the specific G259T mutation could come to predominate through convergent evolution if the associated Asp87Tyr substitution confers an advantage over strains with other possible nalidixic acid resistance-associated substitutions. It is difficult to construct an experiment to test this hypothesis fully; however, within the limits of the approaches available to us we have demonstrated that there is no difference in growth rate in the absence or in the presence of nalidixic acid between mutants with G259T (M3332/1) and G259A (M3332/14) derived from the same parent strain. Therefore, we found no evidence of a selective advantage for a strain possessing the predominant G259T (Asp87Tyr) mutation.

We have identified no previous reports of *parC* mutations in naturally occurring *S. enterica*. Previously described *parC* mutations in *E. coli* have been at codons 80 and 84 (10). The codon 57 has not previously been associated with quinolone resistance in *E. coli*; however, the association observed does not confirm that the mutation is the cause of this resistance.

The predominance of specific point mutations in nalidixic acid-resistant serovar Enteritidis has previously been described. Wiuff et al. identified the amino acid substitution Ser83Phe as predominant among 15 quinolone-resistant serovar Enteritidis PT8 isolates in the Denmark (15) and suggested

that this is indicative of the spread of a resistant clone of serovar Enteritidis. Another recent study from Spain demonstrated that mutations in codon 87 was predominant (91%) in the nalidixic acid-resistant *S. enterica* serovar Enteritidis found. The three isolates sequenced in that study possessed the Asp87Tyr (G259T) amino acid substitution, identified as predominant in our study (12). The present study expands the evidence supporting clonal expansion as a major contributor to a high incidence of nalidixic acid resistance in serovar Enteritidis. We also present some preliminary evidence of a similar phenomenon of clonal expansion of nalidixic acid resistance in serovars Hadar, Kentucky, and Virchow.

REFERENCES

1. **Aarestrup, F. M., C. Wiuff, K. Molbak, and E. J. Threlfall.** 2003. Is it time to change fluoroquinolone breakpoints for *Salmonella* spp.? Antimicrob. Agents Chemother. **47:**827–829.
2. **Centers for Disease Control and Prevention.** 2001. Standardized molecular subtyping of foodborne bacterial pathogens by pulse-field gel electrophoresis. Centers for Disease Control and Prevention, Atlanta, Ga.
3. **Hakanen, A., P. Kotilainen, J. Jalava, A. Siitonen, and P. Huovinen.** 1999. Detection of decreased fluoroquinolone susceptibility in salmonellas and validation of nalidixic acid screening test. J. Clin. Microbiol. **37:**3572–3577.
4. **Herikstad, H., P. Hayes, M. Mokhtar, M. L. Fracaro, E. J. Threlfall, and F. J. Angulo.** 1997. Emerging quinolone-resistant salmonella in the United States. Emerg. Infect. Dis. **3:**371–372.
5. **Kilmartin, D. M., D. Morris, M. Glennon, G. Corbett-Feeney, and M. G. Cormican.** 2003. Rapid detection of common mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene of *Salmonella enterica*. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-100. American Society for Microbiology, Washington, D.C.
6. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852–856.
7. **Molbak, K., P. Gerner-Smidt, and H. C. Wegener.** 2002. Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerg. Infect. Dis. **8:**514–515.
8. **NCCLS.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria the grow aerobically. Approved standard M7-A5, 5th ed., vol. 20. NCCLS, Wayne, Pa.
9. **O'Hare, C., G. Doran, N. Delappe, D. Morris, V. Buckley, P. McKeown, W. Anderson, and M. G. Cormican.** 2004. Antimicrobial resistance and phage types of human and non-human *Salmonella enterica* isolates from 1998–2003. Commun. Dis. Public Health **7:**193–199.
10. **Qiang, Y. Z., T. Qin, W. Fu, W. P. Cheng, Y. S. Li, and G. Yi.** 2002. Use of a rapid mismatch PCR method to detect *gyrA* and *parC* mutations in ciprofloxacin-resistant clinical isolates of *Escherichia coli*. J. Antimicrob. Chemother. **49:**549–552.
11. **Snyder, L., and W. Champness.** 1997. Molecular genetics of bacteria, vol. 1. ASM Press, Washington, D.C.
12. **Soto, S. M., M. A. Gonzalez-Hevia, and M. C. Mendoza.** 2003. Antimicrobial resistance in clinical isolates of *Salmonella enterica* serotype Enteritidis: relationships between mutations conferring quinolone resistance, integrons, plasmids, and genetic types. J. Antimicrob. Chemother. **51:**1287–1291.
13. **Stock, I., and B. Wiedemann.** 2000. Natural antibiotic susceptibility of *Salmonella enterica* strains. Int. J. Antimicrob. Agents **16:**211–217.
14. **Walker, R. A., N. Saunders, A. J. Lawson, E. A. Lindsay, M. Dassama, L. R. Ward, M. J. Woodward, R. H. Davies, E. Liebana, and E. J. Threlfall.** 2001. Use of a LightCycler *gyrA* mutation assay for rapid identification of mutations conferring decreased susceptibility to ciprofloxacin in multiresistant *Salmonella enterica* serotype Typhimurium DT104 isolates. J. Clin. Microbiol. **39:**1443–1448.
15. **Wiuff, C., M. Madsen, D. L. Baggesen, and F. M. Aarestrup.** 2000. Quinolone resistance among *Salmonella enterica* from cattle, broilers, and swine in Denmark. Microb. Drug Resist. **6:**11–17.
16. **Younmans, G. P., P. Y. Paterson, and H. M. Sommers.** 1985. The biological and clinical basis of infectious disease. The W. B. Saunders Co., Philadelphia, Pa.