

THE EMERGENCE IN TAIWAN OF FLUOROQUINOLONE RESISTANCE  
IN *SALMONELLA ENTERICA* SEROTYPE CHOLERAESUISCHENG-HSUN CHIU, M.D., PH.D., TSU-LAN WU, M.S., LIN-HUI SU, M.S., CHISHIH CHU, PH.D., JU-HSIN CHIA, M.S.,  
AN-JING KUO, M.S., MAW-SHENG CHIEN, PH.D., AND TZOU-YIEN LIN, M.D.**ABSTRACT**

**Background** *Salmonella enterica* serotype choleraesuis is a cause of serious systemic infections. Because fluoroquinolones are the drug of choice for the treatment of severe salmonella infections, the emergence and dissemination of fluoroquinolone-resistant *S. enterica* serotype choleraesuis have clinical consequences.

**Methods** In Taiwan, a hospital-based surveillance system has been in place since 1987 to monitor the incidence of *S. enterica* serotype choleraesuis infections and the antimicrobial susceptibility of the isolates. We investigated the rapid emergence of fluoroquinolone resistance in this serotype in 2000 and 2001. Pigs in Taiwan were evaluated as a potential source of the resistant salmonella.

**Results** A total of 501 clinical isolates of *S. enterica* serotype choleraesuis were recovered in our hospital from 1987 through 2000. The proportion of total salmonella isolates made up by *S. enterica* serotype choleraesuis decreased from an average of 8.4 percent before 1995 to 2.7 percent in 1996 through 1998. During 1999 and 2000, this proportion increased significantly, to an average of 5.0 percent. Ciprofloxacin resistance in *S. enterica* serotype choleraesuis has been observed since 2000. In the third quarter of 2001, 60 percent of isolates were resistant to ciprofloxacin. Molecular typing indicated that the primary source of *S. enterica* serotype choleraesuis isolates was herds of swine. All the resistant isolates from humans and swine had mutations that led to the substitution of phenylalanine for serine at position 83 and asparagine for aspartic acid at position 87 in the gene for DNA gyrase A.

**Conclusions** This investigation in Taiwan indicates that fluoroquinolone-resistant *S. enterica* serotype choleraesuis can spread from swine to humans. The use of fluoroquinolones in food animals should be prohibited. (N Engl J Med 2002;346:413-9.)

Copyright © 2002 Massachusetts Medical Society.

**S**ALMONELLOSIS is an important public health problem throughout the world.<sup>1-3</sup> Although most salmonella infections are self-limiting, serious sequelae, including systemic infection and death, can occur.<sup>1-4</sup> Among more than 2000 salmonella serotypes, *Salmonella enterica* serotype choleraesuis has a high predilection for causing systemic infection in humans.<sup>4</sup> *S. enterica* serotype choleraesuis usually causes bacteremia and metastat-

ic focal infections that require parenteral antimicrobial therapy.<sup>4</sup> Appropriate drugs include ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol; however, resistance to these agents is increasing in many areas of the world.<sup>3-5</sup> Fluoroquinolones and the third-generation cephalosporins are recommended for use in areas where there are resistant organisms.<sup>6,7</sup>

The first recognized outbreak of fluoroquinolone-resistant salmonella infection in the United States occurred in 1997.<sup>8</sup> Increasing resistance to fluoroquinolones has been detected in many other countries,<sup>9-12</sup> and it has also emerged in the multidrug-resistant clone of *S. enterica* serotype typhimurium definitive type 104 (DT104).<sup>12,13</sup> Most of the resistant salmonella strains belong to serotypes typhimurium, Hadar, Schwarzengrund, and enteritidis,<sup>8-13</sup> which usually cause gastroenteritis and sometimes cause extraintestinal infections.<sup>4,14</sup> The development of fluoroquinolone resistance among serotypes that often cause systemic infections would have serious implications for public health, as well as implications for the care of individual patients. We report the emergence in Taiwan of fluoroquinolone resistance in *S. enterica* serotype choleraesuis. Molecular epidemiologic investigation indicated that swine served as a reservoir for the resistant bacteria.

**METHODS****Hospital-Based Surveillance and Identification of Isolates**

Since 1987, infections caused by salmonella in Taiwan have been monitored by review of the records of the isolation of salmonella in the Clinical Microbiology Laboratory, Department of Clinical Pathology, Chang Gung Memorial Hospital and Chang Gung Children's Hospital. The data, including dates and sites of isolation and information on antimicrobial susceptibility, have been incorporated into computerized data bases and shared in a timely fashion with physicians. We analyzed data on salmonella isolates and investigated resistance to fluoroquinolones in *S. enterica* serotype choleraesuis isolates from 2000 and 2001. All the isolates described in this report came from different patients or, rarely, from unrelated episodes in the same patient. Any additional isolates from the same patient during a single episode were excluded.

From the Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, Taoyuan (C.-H.C., T.-Y.L.); the Department of Clinical Pathology, Chang Gung Memorial Hospital, Taoyuan (T.-L.W., L.-H.S., J.-H.C., A.-J.K.); the Department of Microbiology and Immunology, Chang Gung University College of Medicine, Taoyuan (C.C.); and the Graduate Institute of Veterinary Pathology, National Chung-Hsing University, Taichung (M.-S.C.) — all in Taiwan. Address reprint requests to Dr. Chiu at the Chang Gung Children's Hospital, 5 Fu-Hsin St., Kweishan 333, Taoyuan, Taiwan, or at chchiu@adm.cgmh.org.tw.

ed. The Chang Gung Memorial Hospital is a 3500-bed, university-affiliated medical center, and the Chang Gung Children's Hospital is a 450-bed hospital for children. Both are located in northern Taiwan, but the patients came from throughout Taiwan, including the scattered islands.

All isolates were cultured and identified according to standard methods,<sup>15</sup> with no major changes over time in the policy for the identification of salmonella. All isolates were serotyped by the slide agglutination test with the use of O antiserum to detect O antigen and by the tube agglutination test with the use of H antiserum to detect H antigen (Difco). The identification of *S. enterica* serotype choleraesuis followed the Kauffman-White scheme.

The antimicrobial susceptibility of all clinical isolates of salmonella was investigated by the standard disk-diffusion method for nonblood isolates and by the microbroth-dilution method for blood isolates.<sup>16,17</sup> The antimicrobial agents examined included ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, cefixime, ceftazidime, ceftizoxime, ceftriaxone, cefepime, and ciprofloxacin. Ciprofloxacin was first used at the hospitals in October 1996. Susceptible and resistant isolates were defined according to the criteria suggested by the National Committee for Clinical Laboratory Standards.<sup>16,17</sup>

### Microbiologic Examination

Forty-eight clinical isolates of *S. enterica* serotype choleraesuis collected between January 2000 and June 2001 were subjected to molecular microbiologic examination. In addition, 17 clinical isolates collected in 1997<sup>18</sup> and another 26 recovered from pigs with scours (dysentery) in 2000 and 2001 were tested. The minimal inhibitory concentration (MIC) of various antibiotics was determined in these isolates by the E test (AB Biodisk) in accordance with guidelines of the National Committee for Clinical Laboratory Standards.<sup>17</sup> The plasmid profiles of the isolates were determined by the method of Kado and Liu.<sup>19</sup> A pair of oligonucleotide primers synthesized according to the published DNA sequence of *spvC*, a conserved gene located on the salmonella virulence plasmid, was used to detect the plasmid in the isolates.<sup>20,21</sup> These isolates were also genotyped by infrequent-restriction-site-polymerase-chain-reaction (IRS-PCR) analysis. This method followed the procedures described previously,<sup>22</sup> except that 15  $\mu$ l of adapter-ligated DNA templates was used in the final amplification. Each isolate was analyzed at least twice to ensure the reproducibility of the result. The criteria proposed by Tenover et al.<sup>23</sup> were used to analyze the DNA fingerprints generated by IRS-PCR.

A 313-bp DNA fragment was amplified with the primers P1 (5'TACCGTCATAGTTATCCACGA) and P2 (5'GTACTTTACGCCATGAACGT),<sup>12,13</sup> which correspond to nucleotides 434 to 454 and 142 to 161 of the gene for DNA gyrase A (*gyrA*), respectively. The amplified fragment contains the quinolone-resistance-determining region of *gyrA*.<sup>12</sup> PCR products obtained after amplification were purified with use of the Wizard PCR Preps kit (Promega) and were sequenced with an automatic sequencer (ABI 373A, Perkin-Elmer, Applied Biosystems). The sequences obtained were analyzed by PCGene software (IntelliGenetics). The search for homologous sequences was performed in the GenBank data base with use of FASTA software.

To examine whether the resistance was mediated by a plasmid, plasmids of the resistant isolates underwent electrophoresis on agarose gels and were then transferred onto Zeta-Probe membranes (Bio-Rad). To prepare a probe, the purified PCR product was labeled with [ $\alpha$ -<sup>32</sup>P]2'-deoxycytidine-5'-triphosphate with use of the Random Primers DNA Labeling System (GIBCO-BRL). DNA-DNA hybridization was performed as described earlier.<sup>18</sup>

### Statistical Analysis

The chi-square test was used to determine the significance of differences. A difference was considered statistically significant if

the P value was less than 0.05. All statistical analyses were performed with the use of Epi Info software (version 6.04).

## RESULTS

### Surveillance of *S. enterica* Serotype Choleraesuis

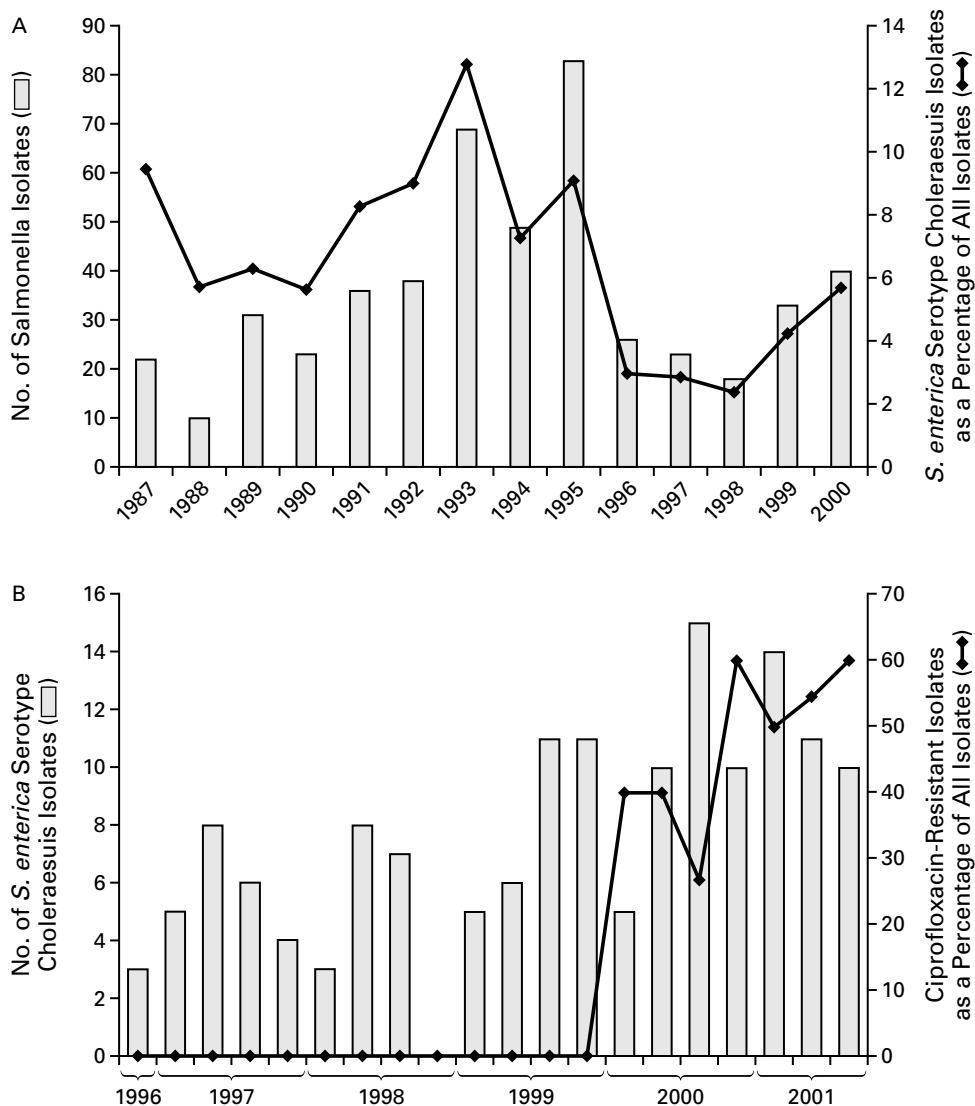
A total of 8196 salmonella isolates were analyzed. The annual number of isolates increased from 232 in 1987 to 700 in 2000 (average, 585; maximum, 910 in 1995). As a proportion of all bacterial isolates tested in this laboratory (26,731 in 1987 and 49,778 in 2000), the proportion of salmonella isolates increased significantly ( $P < 0.001$ ), from 0.9 percent in 1987 to 1.4 percent in 2000 (average, 1.5 percent; maximum, 2.1 percent in 1995). A total of 501 separate clinical isolates of *S. enterica* serotype choleraesuis were recovered in our laboratory from 1987 through 2000. A total of 359 (72 percent) were isolated from blood. Other sources of isolates included urine, wound, bone, tissue from a mycotic aneurysm, and rarely, stool. As shown in Figure 1, the annual number of isolates increased gradually up to 1995, with a substantial decline in 1996 through 1998 and an increase thereafter. The proportion of *S. enterica* serotype choleraesuis isolates among all salmonella isolates also decreased from an average of 8.4 percent before 1995 to 2.7 percent in 1996 through 1998 ( $P < 0.001$ ). During 1999 and 2000, this proportion increased significantly to an average of 5.0 percent ( $P < 0.001$ ).

In our hospitals, serogroup B has been the most prevalent type of salmonella isolate through the years. The proportion of serogroup B isolates among all salmonella isolates has remained steady at about 60 to 70 percent (average, 64.6 percent) during these years. Sixty-six percent of serogroup B isolates belonged to serotype typhimurium.<sup>14,24</sup>

### Emergence of Fluoroquinolone Resistance in *S. enterica* Serotype Choleraesuis

Before 1991, with rates of resistance below 40 percent, most *S. enterica* serotype choleraesuis isolates were susceptible to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Since then, the rates of resistance to these antibiotics have increased substantially (Fig. 2). In 2000, resistance to at least one of the three antibiotics was found in approximately 90 percent of *S. enterica* serotype choleraesuis isolates (Fig. 2). Seventy-eight percent of the isolates were resistant to all three antibiotics in 2000, when ciprofloxacin resistance began to emerge.

*S. enterica* serotype choleraesuis isolates have remained susceptible to the newer-generation cephalosporins. There were no reports of resistance to ciprofloxacin through 1999. However, since March 2000 a dramatic and rapid increase in the incidence of ciprofloxacin resistance in *S. enterica* serotype cholerae-



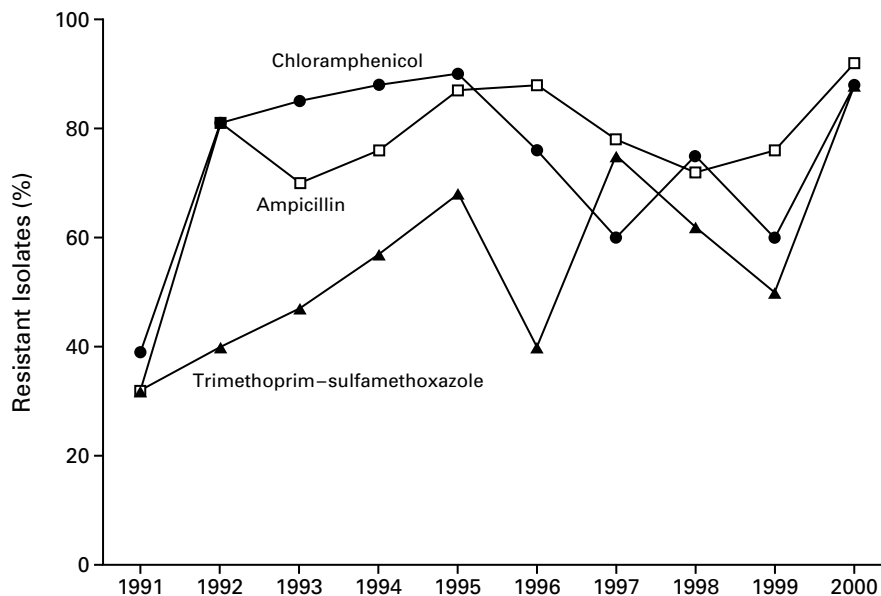
**Figure 1.** Emergence of Fluoroquinolone Resistance among *Salmonella enterica* Serotype Choleraesuis Isolates in Taiwan. Panel A shows the total annual numbers of salmonella isolates from Chang Gung Memorial Hospital and Chang Gung Children's Hospital from 1987 through 2000 (bars) and the percentage of these isolates that were *S. enterica* serotype choleraesuis (curve). Panel B shows the total quarterly numbers of *S. enterica* serotype choleraesuis isolates from these hospitals from the fourth quarter of 1996 through the third quarter of 2001 (bars) and the percentage of these isolates that were resistant to ciprofloxacin (curve). Ciprofloxacin was not available in these hospitals before October 1996.

suis has been observed (Fig. 1). In the third quarter of 2001, the rate of resistance was 60 percent (Fig. 1).

#### Microbiologic Examination

According to the criteria of Tenover et al.,<sup>23</sup> four types (1 to 4) were identified among the *S. enterica* serotype choleraesuis isolates on IRS-PCR analysis.

The four major types were further differentiated into 12 subtypes, with a difference in at least one band used to define a subtype (Fig. 3). Seventy-one of the 91 isolates analyzed (78 percent) were of subtype 1a, indicating that there was an endemic strain of *S. enterica* serotype choleraesuis circulating in Taiwan (Table 1). The isolates recovered from different spe-



**Figure 2.** Percentage of Clinical Isolates of *Salmonella enterica* Serotype Choleraesuis from Chang Gung Memorial Hospital and Chang Gung Children's Hospital That Were Resistant to Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole from 1991 through 2000.

cies (humans and swine) and in different years (1997 and 2000 to 2001) did not differ significantly in the distribution of their IRS-PCR patterns (Table 1). There was also no significant difference in the distribution of the patterns between ciprofloxacin-susceptible and ciprofloxacin-resistant isolates (Table 1).

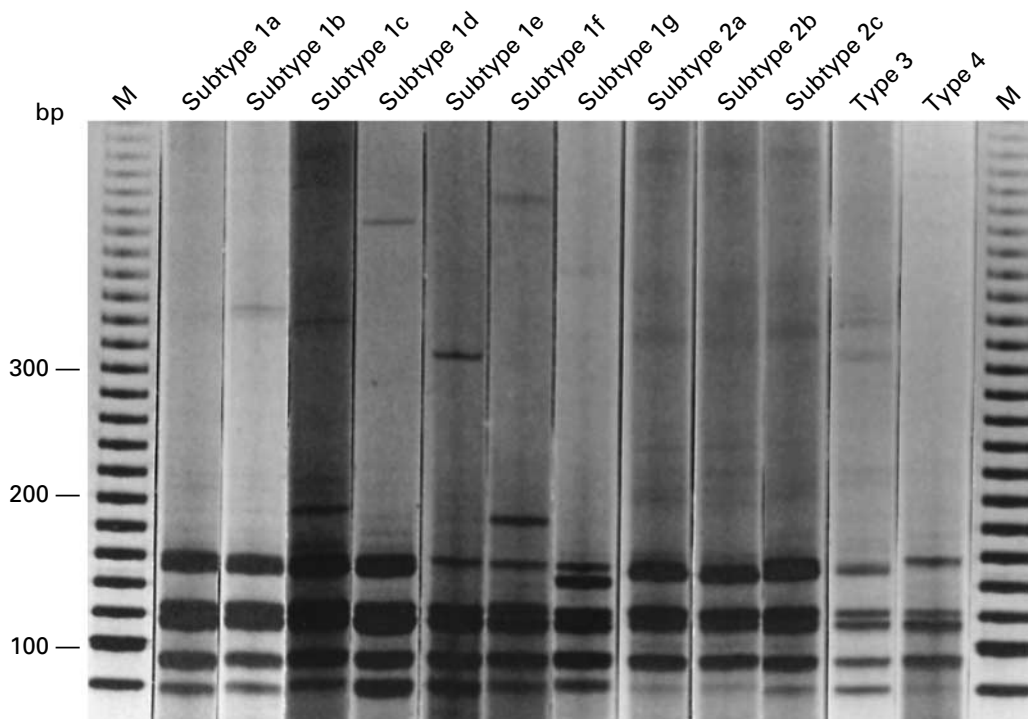
Of the 48 isolates obtained from humans in 2000 and 2001, 22 (46 percent) contained a 50-kb plasmid plus another plasmid of 75, 90, or 125 kb; 13 (27 percent) had only the 50-kb plasmid; and 13 (27 percent) had only one of the larger plasmids of 75, 90, or 125 kb. Among the 26 isolates from swine, 16 (62 percent) contained a 50-kb plasmid plus another plasmid of 75, 90, or 125 kb; 4 (15 percent) had only the 50-kb plasmid; and 6 (23 percent) had only one of the larger plasmids. The plasmid patterns of *S. enterica* serotype choleraesuis isolates, irrespective of the source and antimicrobial-susceptibility profile, were similar. PCR analysis showed that only three isolates from humans and one isolate from swine were negative for *spvC*, indicating that almost all isolates examined possessed a virulence plasmid.

DNA sequencing of the PCR-amplified *gyrA* region of 65 isolates from humans and 26 from swine identified a base substitution at codons 83 and 87 (according to *Escherichia coli* numbering<sup>12</sup>) in all ciprofloxacin-resistant isolates (Table 1). The substitu-

tions, TCC→TTC at codon 83 and GAC→AAC at codon 87, caused amino acid changes of serine to phenylalanine and aspartic acid to asparagine, respectively. All resistant isolates from humans contained the two substitutions, as did resistant isolates from swine. Some ciprofloxacin-susceptible isolates possessed different nucleotide substitutions at the two positions, leading to different amino acid changes (Table 1). Most susceptible strains, including those isolated from humans in 1997, had a single amino acid change — either Ser83Phe or Asp87Asn (Table 1). Thus, the two mutations are equally important in permitting *S. enterica* serotype choleraesuis to attain resistance to ciprofloxacin. The amplified PCR product of *gyrA* did not hybridize to any plasmid of the 35 resistant isolates examined in DNA-DNA hybridization.

## DISCUSSION

Infection with *S. enterica* serotype choleraesuis has become common in Taiwan.<sup>14,24</sup> *S. enterica* serotype choleraesuis is the most frequently isolated serotype of *S. enterica* after typhimurium and Schwarzengrund.<sup>14,24</sup> The number of *S. enterica* serotype choleraesuis infections decreased significantly from 1996 through 1998. In Taiwan in 1996, an epidemic of foot-and-mouth disease in swine resulted in the



**Figure 3.** Patterns of Clinical Isolates of *Salmonella enterica* Serotype Choleraesuis on Infrequent-Restriction-Site–Polymerase-Chain-Reaction Analysis.

Four types (1 to 4) were identified among the *S. enterica* serotype choleraesuis isolates. The four major types were further differentiated into 12 subtypes. M represents the 20-bp DNA ladder. Band sizes are shown at the left.

slaughter of many pigs across the island. Because humans acquire salmonella infections by eating infected animals, the decrease in *S. enterica* serotype choleraesuis infections in humans may have been the result of the extensive reduction in the pig population, which served as the reservoir of this serotype.

The proportion of clinical isolates of *S. enterica* serotype choleraesuis that were fluoroquinolone-resistant increased rapidly and dramatically from none through 1999 to 60 percent in the third quarter of 2001. All of the isolates from humans and swine that we examined had similar IRS-PCR and plasmid patterns, suggesting that human *S. enterica* serotype choleraesuis infections were acquired from pigs. It appears that there was an endemic strain of *S. enterica* serotype choleraesuis circulating in humans and pigs. The sequencing results support this hypothesis, since all resistant isolates, irrespective of source, had the same amino acid changes in *gyrA*. We previously demonstrated that *S. enterica* serotype choleraesuis isolates were resistant to ampicillin and sulfonamides as a re-

sult of the acquisition of a large R (resistance) plasmid, the recombination of the R plasmid with the virulence plasmid, or both.<sup>18</sup> It appears, however, that the gene for resistance to fluoroquinolones in *S. enterica* serotype choleraesuis is located on the chromosome rather than on the plasmid. Our data suggest that the two mutations in *gyrA* gave rise to the resistance and that the rapid emergence of the highly resistant population was due mainly to the clonal spread of an endemic resistant strain.

The two most common mutations associated with fluoroquinolone resistance in *S. enterica* serotype typhimurium DT104 are at codons 83 and 87 and involve amino acid changes Ser83Phe and Asp87Asn, respectively.<sup>13</sup> The same mutations were responsible for fluoroquinolone resistance in *S. enterica* serotype choleraesuis. We could not completely exclude the possibility that mutations in *gyrB*, *parC*, or *parE* might also contribute to the resistance phenotype; however, this is unlikely, since mutations in *gyrB* and *parC* are not normally associated with resistance to

**TABLE 1.** CHARACTERISTICS OF *SALMONELLA ENTERICA* SEROTYPE CHOLERAESUIS ISOLATES FROM HUMANS AND SWINE IN TAIWAN.\*

SOURCE, YEAR OF ISOLATION, AND SUSCEPTIBILITY TO CIPROFLOXACIN	NO. OF ISOLATES (% OF TOTAL)	MIC		AMINO ACIDS AT CODONS 83 AND 87 OF <i>gyrA</i> †						IRS-PCR TYPES													
		RANGE	MIC <sub>90</sub>	Phe and Asn	Phe and Asp	Ser and Asn	Ser and Asp‡	Ser and Gly	Ser and His	1a	1b	1c	1d	1e	1f	1g	2a	2b	2c	3	4		
		μg/ml		number of isolates																			
Humans (1997)																							
Susceptible	17 (100)	0.006 to 0.25	0.125	0	2	6	4	2	3	15	0	0	0	0	1	0	0	0	0	0	1		
Swine (2000–2001)																							
Resistant	12 (46)	8 to 16	16	12	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	1
Susceptible	14 (54)	0.094 to 0.25	0.25	0	2	7	0	5	0	13	0	0	0	0	0	0	0	0	0	0	0	0	1
Humans (2000–2001)																							
Resistant	23 (48)	8 to >32	16	23	0	0	0	0	0	15	2	0	1	0	0	1	3	0	1	0	0	0	0
Susceptible	25 (52)	0.012 to 0.5	0.19	0	0	15	2	8	0	17	3	1	0	1	0	0	1	1	0	1	0	1	0

\*MIC denotes minimal inhibitory concentration, MIC<sub>90</sub> the lowest antibiotic concentration that inhibited the growth of 90 percent of the organisms, and IRS-PCR infrequent-restriction-site polymerase chain reaction.

†The amino acid changes at codons 83 and 87 were found on comparison with the sequence of the gene for DNA gyrase A (*gyrA*) from *Escherichia coli*.

‡The amino acids at codons 83 and 87 were the same as those of *gyrA* from *E. coli*.

fluoroquinolones in salmonella,<sup>25,26</sup> and strains with a single *gyrA* mutation were present among human isolates as early as 1997. This fact implies that if the antecedent strains with a single mutation had been detected earlier, by either phenotypic or molecular methods, the emergence of full resistance might have been predicted. Since resistant isolates probably spread from animals to humans, regular surveillance of salmonella isolates from animals could be helpful.<sup>27</sup>

There is increasing concern in the public health community about the possibility that antibiotics fed to animals that are then consumed by humans may contribute to resistance in human pathogens.<sup>1,3,10,13,27-30</sup> Certain antibiotics are critical to human medicine because they are effective against pathogens that are resistant to other antibiotics. Fluoroquinolones are among these critical antibiotics, so the emergence of fluoroquinolone-resistant *S. enterica* serotype choleraesuis is potentially a serious problem. A recent survey by the National Health Research Institute of Taiwan found that five antibiotics important to human medicine, including a fluoroquinolone (enrofloxacin), have been widely added to animal feed for years.<sup>31</sup> Half of the feed-mill operators surveyed said they added enrofloxacin to pig feed as a growth promoter.<sup>31</sup> The nontherapeutic use of enrofloxacin in domestic pigs, therefore, can reasonably be expected to select for resistance to fluoroquinolones, including ciprofloxacin, in *S. enterica* serotype choleraesuis.

In 2000, the U.S. Food and Drug Administration announced plans for withdrawing two fluoroquino-

lones, sarafloxacin and enrofloxacin, from use to treat respiratory and diarrheal diseases in poultry. However, many experts within the agricultural and pharmaceutical industries opposed the proposed ban, arguing that improper use of such drugs in hospital settings represented the chief source of resistance. To explore this question, we reviewed the medical records of the patients infected by ciprofloxacin-resistant *S. enterica* serotype choleraesuis. None of these patients had received quinolone therapy before the onset of the infection (data not shown). Fluoroquinolone use in animals, rather than in humans, appears to account for most of the problem of resistance.

The emergence of fluoroquinolone resistance would change the policy for the treatment of *S. enterica* serotype choleraesuis infections. Because the majority of *S. enterica* serotype choleraesuis isolates are also resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, the third-generation cephalosporins are now the only antibiotics with reliable activity against this serotype in Taiwan. In view of the severe adverse consequences for human health of the use of fluoroquinolones in food animals, we suggest that such use should be prohibited.

Supported by grants (CMRP1197, to Dr. Chiu, and CMRP798-III, to Ms. Wu) from Chang Gung Memorial Hospital and Chang Gung Children's Hospital.

**REFERENCES**

1. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. *Science* 1986;234:964-9.

2. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis* 1999;5:607-25.
3. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. *Emerg Infect Dis* 1997;3:425-34.
4. Cohen JI, Bartlett JA, Corey GR. Extra-intestinal manifestations of *Salmonella* infections. *Medicine (Baltimore)* 1987;66:349-88.
5. Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *J Infect Dis* 1994;170:128-34.
6. Jacobson MA, Hahn SM, Gerberding JL, Lee B, Sande MA. Ciprofloxacin for *Salmonella* bacteremia in acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1989;110:1027-9.
7. Hooper DC, Wolfson JS. Fluoroquinolone antimicrobial agents. *N Engl J Med* 1991;324:384-94.
8. Olsen SJ, DeBess EE, McGivern TE, et al. A nosocomial outbreak of fluoroquinolone-resistant salmonella infection. *N Engl J Med* 2001;344:1572-9.
9. Piddock LJ, Griggs DJ, Hall MC, Jin YF. Ciprofloxacin resistance in clinical isolates of *Salmonella typhimurium* obtained from two patients. *Antimicrob Agents Chemother* 1993;37:662-6.
10. Herikstad H, Hayes P, Mokhtar M, Fracaro ML, Threlfall EJ, Angulo FJ. Emerging quinolone-resistant *Salmonella* in the United States. *Emerg Infect Dis* 1997;3:371-2.
11. Frost JA, Kelleher A, Rowe B. Increasing ciprofloxacin resistance in salmonellas in England and Wales, 1991-1994. *J Antimicrob Chemother* 1996;37:85-91.
12. Griggs DJ, Gensberg K, Piddock LJV. Mutations in *gyrA* gene of quinolone-resistant *Salmonella* serotypes isolated from humans and animals. *Antimicrob Agents Chemother* 1996;40:1009-13.
13. Mølbak K, Baggesen DL, Aarestrup FM, et al. An outbreak of multi-drug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. *N Engl J Med* 1999;341:1420-5.
14. Chiu CH, Lin TY, Ou JT. Predictors for extraintestinal infections of non-typhoidal *Salmonella* in patients without AIDS. *Int J Clin Pract* 1999;53:161-4.
15. Farmer JJ III. *Enterobacteriaceae*: introduction and identification. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, eds. *Manual of clinical microbiology*. 6th ed. Washington, D.C.: American Society for Microbiology, 1995:438-49.
16. Performance standards for antimicrobial disk susceptibility tests. 7th ed. Wayne, Pa.: National Committee for Clinical Laboratory Standards, 2000:1-18. (NCCLS document no. M2-A7)
17. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 5th ed. Wayne, Pa.: National Committee for Clinical Laboratory Standards, 2000:1-32. (NCCLS document no. M7-A5.)
18. Chu C, Chiu CH, Wu WY, Chu CH, Liu TP, Ou JT. Large drug resistance virulence plasmids of clinical isolates of *Salmonella enterica* serovar Choleraesuis. *Antimicrob Agents Chemother* 2001;45:2299-303.
19. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981;145:1365-73.
20. Chiu CH, Ou JT. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture-multiplex PCR combination assay. *J Clin Microbiol* 1996;34:2619-22.
21. Gulig PA, Danbara H, Guiney DG, Lax AJ, Norel F, Rhen M. Molecular analysis of *spv* virulence genes of the *Salmonella* virulence plasmids. *Mol Microbiol* 1993;7:825-30.
22. Su LH, Leu HS, Chiu YP, et al. Molecular investigation of two clusters of hospital-acquired bacteraemia caused by multi-resistant *Klebsiella pneumoniae* using pulsed-field gel electrophoresis and infrequent restriction site PCR. *J Hosp Infect* 2000;46:110-7.
23. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233-9.
24. Chiu CH, Lin TY, Ou JT. Prevalence of the virulence plasmids of non-typhoid *Salmonella* in the serovars isolated from humans and their association with bacteremia. *Microbiol Immunol* 1999;43:899-903.
25. Giraud E, Brisabois A, Martel JL, Chaslus-Dancla E. Comparative studies of mutations in animal isolates and experimental *in vitro*- and *in vivo*-selected mutants of *Salmonella* spp. suggest a counterselection of highly fluoroquinolone-resistant strains in the field. *Antimicrob Agents Chemother* 1999;43:2131-7.
26. Wiuff C, Madsen M, Baggesen DL, Aarestrup FM. Quinolone resistance among *Salmonella enterica* from cattle, broilers, and swine in Denmark. *Microb Drug Resist* 2000;6:11-7.
27. Bender JB, Hedberg CW, Boxrud DJ, et al. Use of molecular subtyping in surveillance for *Salmonella enterica* serotype typhimurium. *N Engl J Med* 2001;344:189-95.
28. Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. *APMIS* 1998;106:606-22.
29. White DG, Zhao S, Sudler R, et al. The isolation of antibiotic-resistant salmonella from retail ground meats. *N Engl J Med* 2001;345:1147-54.
30. Gorbach SL. Antimicrobial use in animal feed — time to stop. *N Engl J Med* 2001;345:1202-3.
31. McDonald LC, Chen MT, Lauderdale TL, Ho M. The use of antibiotics critical to human medicine in food-producing animals in Taiwan. *J Microbiol Immunol Infect* 2001;34:97-102.

Copyright © 2002 Massachusetts Medical Society.