Ciprofloxacin-resistant Salmonella enterica Typhimurium and Choleraesuis from Pigs to Humans, Taiwan

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We evaluated the disk susceptibility data of 671 nontyphoid Salmonella isolates collected from different parts of Taiwan from March 2001 to August 2001 and 1,261 nontyphoid Salmonella isolates from the National Taiwan University Hospital from 1996 to 2001. Overall, ciprofloxacin resistance was found in 2.7% (18/671) of all nontyphoid Salmonella isolates, in 1.4% (5/347) of Salmonella enterica serotype Typhimurium and in 7.5% (8/107) in S. enterica serotype Choleraesuis nationwide. MICs of six newer fluoroquinolones were determined for the following isolates: 37 isolates of ciprofloxacin-resistant (human) S. Typhimurium (N = 26) and Choleraesuis (N = 11), 10 isolates of ciprofloxacin-susceptible (MIC <1 mg/mL) (human) isolates of these two serotypes, and 15 swine isolates from S. Choleraesuis (N = 13) and Typhimurium (N = 2) with reduced susceptibility to ciprofloxacin (MIC >0.12 µg/mL). Sequence analysis of the gryA, gryB, parC, parE, and acrR genes, ciprofloxacin accumulation, and genotypes generated by pulsed-field gel electrophoresis with three restriction enzymes (SpeI, XbaI, and BlnI) were performed. All 26 S. Typhimurium isolates from humans and pigs belonged to genotype I. For S. Choleraesuis isolates, 91% (10/11) of human isolates and 54% (7/13) of swine isolates belonged to genotype B. These two genotypes isolates from humans all exhibited a high-level of resistance to ciprofloxacin (MIC 16–64 mg/mL). They had two-base substitutions in the gyrA gene at codons 83 (Ser83Phe) and 87 (Asp87Gly or Asp87Asn) and in the parC gene at codon 80 (Ser80Arg, Ser80Ile, or Ser84Lys). Our investigation documented that not only did these two S. enterica isolates have a high prevalence of ciprofloxacin resistance nationwide but also that some closely related ciprofloxacin-resistant strains are disseminated from pigs to humans.

Infections caused by nontyphoid Salmonella in humans are increasingly frequent in developed and developing countries (1,2). The increasing rates of resistance to traditional anti-Salmonella agents (i.e., ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) and extended-spectrum cephalosporins among these isolates have made treatment of invasive salmonellosis a clinical dilemma (3–6). Of particular concern is the emergence of fluoroquinolone resistance among nontyphoid Salmonella and the occurrence of outbreaks caused by some resistant clones, since this class of antimicrobial agents constitutes the drug of choice for treating potentially life-threatening Salmonella infections caused by the multidrug-resistant strains in adult persons (7–16). Moreover, cases of treatment failure due to fluoroquinolone resistance in Salmonella strains have been reported (17–19). Researchers have increasingly reported that widespread use of fluoroquinolones in food animals leads to the rapid emergence and dissemination of resistant Salmonella infections to humans, particularly in developing countries (4,8,20–23). In Taiwan, Chiu et al. reported that resistance to ciprofloxacin among S. enterica Choleraesuis isolates first appeared in 2000 and ≤60% of the isolates recovered from two hospitals in northern Taiwan in the third quarter of 2001 were resistant to ciprofloxacin (14). Molecular investigation clearly demonstrated that the primary sources of these resistant strains were herds of pigs.

To better understand the prevalence of nationwide resistance and the probable dissemination of ciprofloxacin-resistant nontyphoid Salmonella isolates, particularly S. Typhimurium and Choleraesuis, we determined the mechanisms of quinolone resistance and the genotypes of ciprofloxacin-resistant isolates from humans and pigs, collected in different parts of Taiwan. This study is part of the Surveillance from Multicenter Antimicrobial...
Resistance in Taiwan (SMART) programs conducted in 2001.

Materials and Methods

Bacterial Isolates
A total of 671 nontyphoid Salmonella isolates were collected for the study. These isolates were recovered from various clinical specimens of patients treated at 11 major hospitals (bed capacities from 800 to 2,000) in different regions of Taiwan. These hospitals included the National Taiwan University Hospital (NTU; hospital A), Taipei; Taipei Veterans General Hospital (hospital B), Taipei; Mackay Memorial Hospital (hospital C), Taipei; Tri-service General Hospital (hospital D), Taipei; Taichung Veterans General Hospital (hospital E), Taichung; China Medical College Hospital (hospital F), Taichung; National Cheng-Kung University Hospital (hospital G), Tainan; Chi-Mei Medical Center (hospital H), Tainan; Kaohsiung Veterans General Hospital (hospital I), Kaohsiung; and Tzu-Chi General Hospital (hospital J), Tainan. Of the 671 isolates tested, 429 (64%) were recovered from stool samples, 141 (21%) from blood, and the rest from various body fluids.

Disk diffusion susceptibility results on these isolates were also provided by the hospitals and evaluated. Organisms were categorized as susceptible or resistant (including intermediate isolates) to the antimicrobial agents tested on the basis of the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS) (24). Isolates of Salmonella serogroups B and C resistant to ciprofloxacin (by the disk diffusion method) were further identified to the serotype level by the Kauffman and White scheme, by using somatic and fla- agents tested on the basis of the guidelines provided by the hospitals.

Antimicrobial Susceptibility Testing
Of the 671 isolates collected from 11 medical centers in 2001, 37 ciprofloxacin-resistant (by the disk diffusion method) S. Typhimurium (N = 26) and S. Choleraesuis (N = 11) isolates and 10 randomly selected ciprofloxacin-sus-
ceptible (by the disk diffusion method) isolates of these two serotypes were tested for susceptibility to six fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, trovafloxacin, gatifloxacin, and garenoxacin) to determine their MICs by using the agar dilution method according to the guidelines established by NCCLS (26). The 37 ciprofloxacin-resistant isolates included 13 isolates from the 2001 SMART program (5 S. Typhimurium isolates and 8 S. Choleraesuis isolates) and 24 recovered from 1996 to 2000 at hospital A (21 S. Typhimurium isolates and 3 S. Choleraesuis isolates). These ciprofloxacin-resistant isolates were recovered from 29 patients. Six patients (patients 9, 10, 11, 15, 29, and 32) had isolates that were recovered after >7 days from various clinical specimens. The patients’ ages ranged from <1 year to 84 years (mean 31 years); those <2 years of age were predominant (47%) among patients with S. Typhimurium isolations. None of the patients with S. Choleraesuis bacteremia were ≤16 years. Among the 37 human isolates of ciprofloxacin-resistant nontyphoid Salmonella isolates, 13 (3 of S. Typhimurium and 10 of S. Choleraesuis) were recovered from blood specimens of 12 patients with bloodstream infections. The rest of the isolates were recovered from stool or urine specimens.

Dilution susceptibilities to the aforementioned fluoroquinolones were also performed for the 15 isolates from pig herds, according to the NCCLS guidelines (26).

**PCR Amplification and DNA Sequencing of gyrA, gyrB, parC, parE, and acrR**

The sequences of the primers for the polymerase chain reaction (PCR) amplification of gyrA, gyrB, parC, parE, and acrR have been previously described (27–30). The preparation of the template DNA and the determination of sequences of each gene followed the procedures described previously (27–29). The sequences of the quinolone resistance-determining regions (QRDRs) were determined to be between amino acids 54 and 171 of gyrA, 397 and 520 of gyrB, 12 and 130 of parC, and 421 and 524 of parE.

**Ciprofloxacin Accumulation**

The accumulation of ciprofloxacin, with or without 100 mM carbonyl cyanide m-chlorophenylhydrazone, was determined for two ciprofloxacin-resistant strains and one ciprofloxacin-susceptible S. Typhimurium (ciprofloxacin MIC = 0.06 µg/mL) as described previously (28,29). These experiments were performed twice to ensure reproducibility.

**Molecular Typing**

Genotyping of the human ciprofloxacin-resistant S. Typhimurium (N = 26) and Choleraesuis (N = 11) isolates, the 10 human ciprofloxacin-susceptible isolates of the two serotypes, and the 15 isolates from pigs was determined by the pulsotypes generated by pulsed-field gel electrophoresis (PFGE). The DNA extraction and purification were also carried out as described previously (31,32). The DNA was digested by the restriction enzymes SpeI, XbaI, and BlnI (9,16,23,32), and the restriction fragments were separated in a CHEF-DRIII unit (Bio-Rad, Hercules, CA). Interpretation of the PFGE profiles followed the description by Tenover et al. (33). Isolates belonging to the similar pulsotypes (within six band differences) by each of the three restriction enzymes were defined as the same genotypes (closely related clusters). Isolates with identical pulsotypes (no band differences) by the three restriction enzymes were defined as the same genosubtypes (clones).

**Results**

**Nationwide Resistance in 2001**

The rates of ciprofloxacin resistance among isolates of nontyphoid Salmonella, S. Typhimurium, and S. Choleraesuis from the 11 hospitals, stratified by region of Taiwan, is shown in Table 1. Overall, ciprofloxacin resistance was found in 2.7% (18/671) of all nontyphoid Salmonella isolates from humans, 1.4% in S. Typhimurium and 7.5% in S. Choleraesuis nationwide (Table 1). Among S. Choleraesuis isolates, the highest rate of ciprofloxacin resistance was found in hospital B (28.5%) and southern region of Taiwan (9.5%). Among S. Typhimurium isolates, the highest rate of ciprofloxacin resistance was found in hospital D (25.0%) and in the northern region of Taiwan (3.5%). Nontyphoid Salmonella isolates recovered from patients in eastern region of Taiwan were all susceptible to ciprofloxacin. Rates of resistance to ampicillin and chloramphenicol were higher in eastern Taiwan than those from other regions of Taiwan. Resistance to cefotaxime (three hospitals tested ceftriaxone instead of cefotaxime) among all nontyphoid Salmonella isolates was low (<1%). However, 6% and 4% of S. Choleraesuis isolates recovered from central and southern Taiwan, respectively, were resistant to cefotaxime (ceftaxime).

**Prevalence of Ciprofloxacin Resistance at Hospital A**

The annual number of nontyphoid Salmonella isolates (Salmonella group B and Salmonella group C) ranged from 294 in 1996 (182 and 46, respectively) to 90 in 2001 (76 and 8, respectively). Overall, the rate of ciprofloxacin resistance among nontyphoid Salmonella isolates was 2.1%. For Salmonella group B isolates, the rates of ciprofloxacin resistance were high (6% to 9%) during 1996 and 1997, reached a trough in 1999 (3%), and increased gradually in the following 2 years (4% in 2000 to 5% in 2001). Annual rates of resistance to ciprofloxacin among Salmonella group C isolates fluctuated during the same 6-
year period (data not shown). In 1996, 1998, and 2001, none of the isolates were resistant to ciprofloxacin, and the highest rate of ciprofloxacin resistance was found in 2000 (13%).

The annual rates of resistance to ampicillin, cefotaxime, chloramphenicol, and trimethoprim-sulfamethoxazole among all nontyphoid Salmonella isolates, Salmonella group B, and Salmonella group C at hospital A from 1996 to 2001 were evaluated. Overall, the prevalence of resistance to cefotaxime among these isolates was low (0% to 4%). Rates of resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole among all nontyphoid Salmonella isolates declined gradually from 1996 (64%, 64%, and 42%, respectively) to 2000 (47%, 52%, and 34%, respectively). In 2001, however, rates of resistance to ampicillin (73%) and chloramphenicol (76%) increased but that of trimethoprim-sulfamethoxazole (28%) continued to decrease. A similar scenario was found among Salmonella group B isolates. For Salmonella group C isolates, rates of resistance to these agents also fluctuated during the study period.

Antimicrobial Susceptibilities of Human Ciprofloxacin-resistant Isolates

All of the human ciprofloxacin-resistant isolates were highly resistant to ampicillin (MIC ≥128 μg/mL), chloramphenicol (MIC ≥128 μg/mL), and trimethoprim-sulfamethoxazole (≥128 μg/mL) but susceptible to cefotaxime (MIC 0.06–8 μg/mL). These ciprofloxacin-resistant isolates from humans all exhibited high-levels of resistance to ciprofloxacin (MIC 8 to 64 μg/mL), levofloxacin (MIC 32–64 μg/mL), moxifloxacin (MIC 32–64 μg/mL), gatifloxacin (MIC 16–32 μg/mL), and trovafloxacin (MIC 8–64 μg/mL).

Fluoroquinolone Susceptibilities among Swine S. Choleraesuis Isolates

All 15 isolates of S. Typhimurium and Choleraesuis from pigs had reduced susceptibility to ciprofloxacin (MIC ≥0.125 μg/mL). Eight of the 15 isolates (53%) were susceptible or intermediate to ciprofloxacin according to the NCCLS breakpoint recommendation (MIC ≤2 μg/mL). Seven (47%) isolates had high ciprofloxacin MICs (MIC ≥64 μg/mL); these seven isolates were also highly resistant to five other newer fluoroquinolones: levofloxacin (MIC 32–64 μg/mL), moxifloxacin (MIC 32–128 μg/mL), trovafloxacin (MIC 64 μg/mL), gatifloxacin (MIC 32–64 μg/mL), and garenoxacin (MIC 32–64 μg/mL).

Nucleotide Sequence Analysis

Of human ciprofloxacin-resistant S. Typhimurium isolates, all were associated with two-base substitutions in the QRDR of gyrA at codon 83 (Ser83Phe) (TCC→TTC) and 87 (Asp87Gly) (GAC→GGC), and either Ser80Arg or Glu84Lys in the QRDR of the parC gene (Table 2). One base substitution in the QRDR of gyrA (Ser83Tyr or Ser83Phe) was found in ciprofloxacin-susceptible isolates. One isolate had a mutation in the QRDR of the gyrB gene, but none had mutations in the QRDR of the parE gene. None of the S. Typhimurium isolates, including ciprofloxacin-susceptible or -resistant isolates, had mutations in the acrR genes.

Of human ciprofloxacin-resistant S. Choleraesuis, all were associated with two-base substitutions in the QRDR of the gyrA gene at codon 83 (Ser83Phe) and 87 (Asp87Asn), Ser80Ile in the QRDR of the parC gene, and Gln78Stop in the QRDR of the acrR gene (Table 3). None of these isolates had mutations in the QRDR of the gyrB or parE genes. One base substitution in the QRDR of the

Table 2. Characteristics of Salmonella enterica serotype Typhimurium isolates from humans^a^ and pigs^b^, Taiwan

| Ciprofloxacin susceptibility (N) | MIC (μg/mL) | gyrA gene | parC gene | acrR gene | Genotype: genosubtype |
|---------------------------------|-------------|-----------|-----------|-----------|----------------------|
|                                 |             | Ser83Phe  | Ser83Phe  | Ser83Phe  | Ser83Tyr  | Ser80Arg | Glu84Lys | Gln78Stop | Arg107Cys | Genosubtype          |
| Humans                          |             | Asp87Gly  | Asp87Asn  | Asp87Gly  | (N)       | (N)       | (N)       | (N)       | (N)       |                       |
| Resistant (26)                  | 16–64       | 24        | 2         | 0         | 0         | 24        | 2         | 0         | 0         | I (26), Ia (4), Ic (5), Id (6), Ie (1), If (1), Ig (2), Ii (1) |
| Susceptible (5)                 | 0.03–0.25   | 0         | 0         | 1         | 2         | 0         | 0         | 0         | 0         | IIa (1), IIb (1), IIc (1) |
| Pigs                            | 128         | 2         | 0         | 0         | 0         | 2         | 0         | 0         | 0         | I (2), Ic (1), Im (1)  |

^aN = 31.
^bN = 2.
gyrA \text{(Asp87Asn)} was found in the ciprofloxacin-susceptible isolates.

Of pig herd ciprofloxacin-resistant \textit{S. Typhimurium} isolates (N = 2), both had a mutation in the QRDR of the \textit{gyrA} and \textit{parC} genes, respectively (Table 2). None of these isolates had mutations in the QRDR of the \textit{gyrB}, \textit{parE}, or \textit{arcR} genes. Among pig herd ciprofloxacin-resistant \textit{S. Choleraesuis} isolates, all had two mutations in the QRDR of \textit{gyrA} \text{(Ser83Phe plus Asp87Asn or Asp87Gly)} and one mutation in \textit{parC} \text{(Ser80Ile)} and \textit{arcR} \text{(Gln78Stp)} (Table 3).

**PFGE Analysis and Genotypes**

All of the ciprofloxacin-resistant \textit{S. Typhimurium} isolates from humans had the same pulsotype (pulsotype S) when the SpeI restriction enzyme was used. Figures 1 and 2 illustrate the pulsotypes and pulsosubtypes of \textit{S. Typhimurium} (Figures 1A and 1B) and \textit{S. Choleraesuis} (Figures 2A, 2B, and 2C) isolates by \textit{XbaI} and \textit{BlnI}. Using \textit{XbaI} and \textit{BlnI}, we observed six and eight pulsosubtypes, respectively, for \textit{S. Typhimurium} isolates. Among \textit{S. Choleraesuis} isolates, one pulsotype \text{(x)} and one pulsosubtype \text{(x-1)} were observed when the \textit{XbaI} restriction enzyme was used, and two pulsotypes \text{(a and b)} with six pulsosubtypes \text{(b-1 to b-6)} were observed when the \textit{BlnI} restriction enzyme was used. Using the three restriction enzymes, we found that all ciprofloxacin-susceptible isolates of \textit{S. Typhimurium} and \textit{Choleraesuis} had different genosubtypes (clones).

Among human ciprofloxacin-resistant \textit{S. Typhimurium} isolates, all were closely related (genotype I) and belonged to 11 genosubtypes (genosubtypes Ia to Ik). Among the 11 genosubtypes, Ia (4 isolates), Ic (5 isolates), and Id (6 isolates) predominated. The five ciprofloxacin-susceptible isolates belonged to four genotypes (II–V) (Table 2). None of the genotypes among the \textit{S. Typhimurium} isolates studied were identical to those of DT104.

Of human \textit{S. Choleraesuis} isolates, 91% (10 of the 11 isolates) belonged to genotype B, which was different from those of the five ciprofloxacin-susceptible isolates from humans (genotypes C to G). None of the six genosubtypes (B1 to B6) of the genotype B isolates was predominant. Two isolates collected within 7 days of one another from patient 4 had identical genosubtypes (B2), but those from patient 7 had differing genosubtypes (B4 and B5) (Table 3).

Seven (54%) of the 13 swine \textit{S. Choleraesuis} isolates belonged to genotype B (Table 3). Among the six genosubtypes of genotype B, two genosubtypes (B2 and B5) were also found in human isolates. Two swine isolates that showed decreased susceptibility to ciprofloxacin (MICs, 0.5 \text{µg/mL} and 2 \text{µg/mL}, respectively) also belonged to genotype B (genosubtypes B7 and B10, respectively).

**Evidence for Active Efflux**

Ciprofloxacin uptake appeared to be remarkably low in the two ciprofloxacin-resistant genotypes (genosubtypes Ia and B1) (Figure 3). A rapid increase in cell-associated ciprofloxacin uptake among isolates belonging to the genosubtypes was evident after addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP), a proton motive force uncoupler.

**Discussion**

This report describes the rates of antimicrobial resistance among nontyphoid \textit{Salmonella} isolates in a universi-
hospital during a 6-year period. Furthermore, it characterizes the nationwide dissemination of ciprofloxacin-resistant strains of \textit{S. Typhimurium} and \textit{Choleraesuis}. Our observations in Taiwan suggest five important facets: First, ciprofloxacin resistance among our nontyphoid \textit{Salmonella} from humans was high (2.7%), particularly among human \textit{S. Choleraesuis} isolates. Furthermore, a previous study found a remarkably high incidence (57%, 12 of 21 isolates) of \textit{S. Choleraesuis} at two major teaching hospitals in northern Taiwan in the second to third quarters of 2001 (14). This observation contrasts greatly with our findings (7.7%, 2 of 25 isolates, from four hospitals in northern Taiwan, or 7.5% nationwide from March 2001 to August 2001) (14).

Second, nearly all ciprofloxacin-resistant \textit{S. Choleraesuis} isolates from humans and pigs collected in 1999–2002 were closely related to one another (genotype B) and differed from those found in pigs in 1997–1998 (these isolates had highly diverse genotypes). These find-
ings suggest that nationwide dissemination of S. Choleraesuis isolates from pigs to humans occurred from 1999 to 2002. Two isolates (AC-6 and AC-10) of S. Choleraesuis from pigs had reduced susceptibility to ciprofloxacin (MICs, 0.5 µg/mL and 2 µg/mL, respectively). They had an identical genotype (genotype B) to that of most of the epidemic strains found in humans and pigs. These strains had high-level ciprofloxacin resistance (MIC 16 to 64 µg/mL). This indicates that the swine isolates with reduced susceptibility to ciprofloxacin might be an ancestor (a unique clone line) of the isolates that are highly resistant to ciprofloxacin and which have spread among herbs and humans nationwide (23,27).

Third, the S. Typhimurium strains (genotype I) with high-level fluoroquinolone resistance have been widely disseminated in humans in Taiwan since 1996. Strains belonging to genotype I and the other genotypes found in this study were domestically acquired and were not related to the clones of DT104, which were already disseminated throughout Europe and the United States (15,23). In 1998, one isolate exhibiting genotype I (genosubtype Ic) was isolated from a pig from southern Taiwan. Further studies on S. Typhimurium isolates from animals should be conducted to identify the primary source of the epidemic genotype strains.

Fourth, an increasing prevalence of resistance to ampicillin and chloramphenicol over time was observed in human S. Typhimurium isolates at hospital A. The spread of third-generation cephalosporin-resistant isolates harboring plasmid-mediated CMY-2 like cephalosporinase among S. Typhimurium isolates has been previously reported in Taiwan (6). Although all of the highly ciprofloxacin-resistant isolates in our study were susceptible to cefotaxime, according to NCCLS guidelines (24), five isolates from four patients with high cefotaxime MICs (MIC 4–8 µg/mL) is noteworthy. The emergence of decreased susceptibility to cefotaxime, along with the pre-existing ciprofloxacin resistance among nontyphoid Salmonella isolates, particularly those causing bloodstream infection, makes antimicrobial therapy more complicated.

Finally, rates of resistance varied geographically; higher rates of resistance to ampicillin and chloramphenicol were found in the eastern region of Taiwan. However, none of the nontyphoid Salmonella isolates collected in the eastern region of Taiwan was resistant to ciprofloxacin.

S. Typhimurium and S. Choleraesuis isolates with high levels of resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, cipro-floxacin, and other newer fluoroquinolones were rarely previously reported (2,19,28,30,34). In most gram-negative bacteria, including Salmonella, a high-level of fluoroquinolone resistance is related to the presence of multiple mutations in the QRDRs of the genes, particularly in the gyrA and parC genes (8,13,14,19,27,28,35).

Additional resistance mechanisms, such as decreased cell envelope permeability (loss of outer membrane porins or alterations of the lipopolysaccharide), decreased cellular accumulation of quinolones involving the major multidrug active efflux pump (AcrAB), or the presence of integrons, can also be responsible for fluoroquinolones resistance and resistance to a wide range of antimicrobial agents (31,35–39). Mutations in the acrR (regulator/repressor) gene are partly responsible for fluoroquinolone resistance in Escherichia coli (29). In our study, the two major genotypes (genotypes I and B) of ciprofloxacin-resistant isolates had both mutations in the gyrA (at least two mutations) and parC (at least one mutation) genes. The addition of CCCP, resulted in an increase in cell-associated ciprofloxacin uptake. This indicated that an active efflux contributed to fluoroquinolone resistance (28–31). In our study, acrR mutations were found in ciprofloxacin-resistant S. Choleraesuis, but not in Typhimurium, isolates. This finding is consistent with that of previous reports (30). Further studies are warranted to add clarification to the complexity of the mechanisms of high-level resistance among S. Typhimurium and Choleraesuis isolates.

PFGE analysis using restriction enzyme XbaI is a well-established method for epidemiologic typing of the Salmonella species (9,16,23,32). However, PFGE patterns by XbaI for most ciprofloxacin-resistant isolates investigated, including S. Choleraesuis isolates from human and animal origins, were indistinguishable. This scenario was also found in PFGE patterns for human S. Typhimurium isolates. When BlnI was added, the discriminatory power of pulotyping improved among these ciprofloxacin-resistant isolates. Genotyping by using pulotypes generated by
quinolones in animal husbandry and active surveillance of resistant isolates, with reduced susceptibility to cefotaxime. However, ciprofloxacin-salmonellosis and other invasive infections include only ceftriaxone-resistant Salmonella enterica Typhimurium and other serovars. The remaining therapeutic options available to veterinarians might develop full resistance (two gyrA mutations or multiple mutations in the QRDRs of other genes) in animals or humans and could probably jump from animals to humans (14,27). Our observations and findings from Chiu et al. indicate that outbreak-associated human Salmonella strains with high-level ciprofloxacin resistance might have emerged several years ago, similar to strains with antibiotic resistance, or some clones with reduced susceptibility (a single gyrA mutation) to quinolones, might develop full resistance (two gyrA mutations or multiple mutations in the QRDRs of other genes) in animals or humans and could probably jump from animals to humans (14,27).

In conclusion, our investigation documented that S. Typhimurium and S. Choleraesuis isolates, which are highly fluoroquinolone-resistant and multidrug-resistant, have become widespread pathogens in Taiwan. The recent occurrence of ciprofloxacin resistance among Salmonella in animals, and its nationwide spread, is of particular concern. The remaining therapeutic options available to veterinarians and physicians for treatment of extraintestinal salmonellosis and other invasive infections include only third-generation cephalosporins. However, ciprofloxacin-resistant isolates, with reduced susceptibility to cefotaxime, have already emerged in Taiwan. Restricted use of quinolones in animal husbandry and active surveillance of quinolone resistance among Salmonella are crucial mitigation efforts to reduce selection and clonal spread of quinolone-resistant Salmonella.

This work was partly supported by a research grant NSC91-2314-B002-171 from the National Science Council, Taiwan.

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References
1. Tauxe RV. Emerging food-borne diseases: an evolving public health challenge. Emerg Infect Dis 1997;3:425–34.
2. Ho YH, Hsueh PR, Lu CY, Chen RT, Yang TY, Yang SC, et al. Serogroups and antimicrobial susceptibility of non-typhoid Salmonella isolates in a medical center. J Microbiol Immunol Infect 2003. In press.
3. 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.
4. Angulo FJ, Johnson KR, Tauxe RV, Cohen ML. Origins and consequences of antimicrobial-resistant non-typhoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microb Drug Resist 2000;6:77–83.
5. Hsueh PR, Liu CY, Luh KT. Current status of antimicrobial resistance in Taiwan. Emerg Infect Dis 2002;8:132–7.
6. Yan JJ, Ko WC, Chiu CH, Tsai SH, Wu HM, Wu JJ. Emergence of ceftriaxone-resistant Salmonella isolates and the rapid spread of plasmid-encoded CMY-2-like cephalosporinase, Taiwan. Emerg Infect Dis 2003;9:323–8.
7. Threlfall EJ, Ward LR, Skinner JA, Graham A. Antimicrobial drug resistance in non-typhoidal salmonellas from humans in England and Wales in 1999: decrease in multiple resistance in Salmonella enterica serotypes Typhimurium, Virchow, and Hadar. Microb Drug Resist 2000;6:319–25.
8. Carnevale R, Molbak K, Bager F, Aarestrup FM. Fluoroquinolone resistance in Salmonella: a Web discussion. Clin Infect Dis 2000;31:128–30.
9. Molbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydenhahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype Typhimurium DT104. N Engl J Med 1999;341:410–5.
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. Hakanen A, Siitonen A, Kotilainen P, Huovinen P. Increasing fluoroquinolone resistance in salmonella serotypes in Finland during 1995–1997. J Antimicrob Chemother 1999;43:145–8.
12. Leegaard TM, Caugnat DA, Froholm LO, Hoiby EA, Lassen J. Emerging antibiotic resistance in Salmonella Typhimurium in Norway. Epidemiol Infect 2000;125:473-80.
13. Hakanen A, Kotilainen P, Huovinen P, Helenius H, Siitonen A. Reduced fluoroquinolone susceptibility in Salmonella enterica serotypes in travelers returning from Southern Asia. Emerg Infect Dis 2001;7:996–1003.
14. Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, et al. The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype Choleraesuis. N Engl J Med 2002;346:413–9.
15. Glynn MK, Bopp C, Dewitt W, Dahney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant Salmonella enterica serotype Typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333–8.

16. Olsen SJ, DeBess EE, McGiven TE, Marano N, Eby T, Mauvais S, et al. A nosocomial outbreak of fluoroquinolone-resistant Salmonella infection. N Engl J Med 2001;344:1527–9.

17. Piddock LJV, 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.

18. Heisig P. High level fluoroquinolone resistance in Salmonella Typhimurium isolates due to alterations in both gyrA and gyrb genes. J Antimicrob Chemother 1993;32:367–77.

19. Nakaya H, Yasuhara A, Yoshimura K, Oshihoi Y, Izumiya H, Watanahe H. Life-threatening diarrhea from fluoroquinolone-resistant Salmonella enterica Typhimurium with mutations in both gyrA and parc. Emerg Infect Dis 2003;9:255–7.

20. Witte W. Medical consequences of antibiotic use in agriculture. Science 1998;279:996–7.

21. Thréfalle EJ. Antimicrobial drug resistance in Salmonellas: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev 2002 26:141–8.

22. Kiessling CR, Cutting JH, Loftis M, Kiessling WM, Datta AR, Sofos JN. Antimicrobial resistance of food-related Salmonella isolates, 1999–2000. J Food Protect 2002;65:603–8.

23. Baggesen DL, Sandvang D, Aarestrup FM. Characterization of Salmonella enterica serovar Typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J Clin Microbiol 2000;38:1581–6.

24. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standards. 5th edition. M7-A4. Wayne (PA); The National Committee for Clinical Laboratory Standards; 2000.

25. Ross B, Arenas F, Estados-Gonzalez J, Mayorga H, Munk OL. Characterization of Salmonella enterica serovar Typhimurium DT104 isolated from domestic animals in England and Wales. J Clin Microbiol 2002;40:1481–6.

26. Tenover FC, Arlet R, Goering RV, Mickelsen PA, Murray BE, Persing DH, 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.

27. Wang H, Dzink-Fox JL, Chen M, Levy SB. Genetic characterization of highly fluoroquinolone-resistant clinical Escherichia coli strains from China: role of acrR mutation. Antimicrob Agents Chemother 2001;45:1515–21.

28. Giraud E, Chaslus-Dancla E. Comparative studies of mutations in animal isolates and experimental in vitro- and in vivo-selected mutants of Salmonella spp. suggest a counter selection of highly fluoroquinolone-resistant strains in the field. Antimicrob Agents Chemother 1999;43:2131–7.

29. Wang H, Dzink-Fox JL, Chen M, Levy SB. Genetic characterization of highly fluoroquinolone-resistant clinical Salmonella enterica serotype Typhimurium. Antimicrob Agents Chemother 2000;44:1223–8.

30. 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 counter selection of highly fluoroquinolone-resistant strains in the field. Antimicrob Agents Chemother 1999;43:2131–7.