Resistance to fluoroquinolones in CMY-2 AmpC enzyme has been reported most often (1–3). Salmonella enterica Detroit, MI, USA). The 4 most common serotypes of isolates were serotyped with commercial antisera (Difco, centers and 14 district hospitals throughout Taiwan; these isolates from 585 patients were obtained from 5 medical

The Study

We report the prevalence and characteristics of Salmonella strains resistant to ciprofloxacin and extended-spectrum cephalosporins in Taiwan from January to May 2004. All isolates resistant to extended-spectrum cephalosporins carried bla<sub>CMY-2</sub>, and all ciprofloxacin-resistant Salmonella enterica serotype Choleraesuis isolates were genetically related.

Resistance to extended-spectrum cephalosporins (ESCs) or fluoroquinolones in Salmonella enterica has become a global concern (1). ESC resistance in Salmonella strains is usually due to the production of plasmid-mediated extended-spectrum β-lactamas (ESBLs) or AmpC β-lactamases, and among these β-lactamases, the CMY-2 AmpC enzyme has been reported most often (1–3). Resistance to fluoroquinolones in Salmonella strains is usually due to the accumulation of mutations in the quinolone resistance–determining regions (QRDRs) of DNA gyrase genes (1,4,5). Resistance to both ESCs and fluoroquinolones remains extremely rare in salmonellae.

In Taiwan, increasing resistance to fluoroquinolones and the emergence of CMY-2–producing ESC-resistant strains in salmonellae have been noted (3–6). The emergence of Salmonella strains resistant to both ceftriaxone and ciprofloxacin was reported more recently in Taiwan and may pose a serious therapeutic problem (7,8). We conducted the present study to investigate the prevalence and characteristics of Salmonella strains resistant to ciprofloxacin and ESCs in Taiwan.

The Study

From January to May 2004, a total of 600 Salmonella isolates from 585 patients were obtained from 5 medical centers and 14 district hospitals throughout Taiwan; these isolates were serotyped with commercial antisera (Difco, Detroit, MI, USA). The 4 most common serotypes of Salmonella enterica (Enteritidis, Typhimurium, Stanley, and Choleraesuis) accounted for 66.8% of all isolates. Two isolates were untypeable, and the remainder were typed into 42 serotypes (data not shown), which were each represented by 1 to 23 isolates.

MICs of antimicrobial agents were determined by the agar dilution method (9). Resistance to ciprofloxacin (MIC >4 µg/mL) was seen in 50 (8.3%) isolates (Table 1); 20 (3.3%) were resistant (MICs ranging from 8 to >64 µg/mL) to ceftazidime, ceftriaxone, cefotaxime, or aztreonam (Table 2); 6 isolates showed decreased susceptibilities to 1 or 2 of the 4 ESCs (MICs 0.5–2 µg/mL); 10 (1.7%) isolates were resistant to both ciprofloxacin and ESCs. S. Choleraesuis had high rates of resistance to ciprofloxacin (84.4%), ESCs (17.8%), and both (17.8%). None of the 26 Salmonella isolates with resistance or decreased susceptibility to ESCs produced ESBL, according to the double-disk synergy method (10). Among the 20 ESC-resistant isolates, 10 isolates were ciprofloxacin-resistant, 4 isolates showed decreased susceptibility to ciprofloxacin (MIC 0.25–1 µg/mL) and resistance to nalidixic acid, and 6 isolates were susceptible to ciprofloxacin and nalidixic acid (Table 2). All 20 ESC-resistant isolates were susceptible to ceftazidime (MIC <0.03 µg/mL) and imipenen (MIC <1 µg/mL), and 17 isolates were resistant to >1 non–β-lactam agent.

All 20 ESC-resistant isolates expressed a β-lactamase of pl 9.0 by isoelectric focusing (3,11); 11 of these isolates expressed an additional pl 5.4 β-lactamase (Table 2). bla<sub>CMY-2</sub> was detected in all ESC-resistant isolates. bla<sub>TEM</sub> was detected in the 11 isolates with the pl 5.4 β-lactamase by polymerase chain reaction (PCR) and sequence analyses with the primers for the entire bla<sub>TEM</sub>-related and bla<sub>CMY-2</sub>-related structural genes (2,3).

The QDR sequences of gyrA, gyrB, parC, and parE of the 20 ESC-resistant Salmonella isolates were determined by PCR and sequence analyses (5). All 10 ciprofloxacin-resistant isolates showed 2 mutations at the Ser-83 and Asp-87 codons in gyrA and a single mutation at the Ser-80 codon in parC (Table 2). Four isolates with decreased susceptibility to ciprofloxacin had a single mutation at either the Ser-83 or the Asp-87 codon in gyrA. All 20 ESC-resistant isolates showed no mutations in the QDRDs of gyrB and parE.

ESC resistance was transferred from 18 of the 20 ESC-resistant Salmonella isolates to Escherichia coli C600 in the liquid mating-out assay (3,12). All transconjugants showed decreased susceptibilities to the 4 ESCs tested (MICs 16–64 µg/mL) and cefoxitin (MIC 64–128 µg/mL) and were susceptible to all non–β-lactam agents tested. A pl 9.0 vz β-lactamase and bla<sub>CMY-2</sub> were detected by isoelectric focusing and PCR assays, respectively, in all transconjugants. Restricted by the endonuclease EcoRI, the 18 transferred plasmids produced 9 major restriction
patterns (Figure 1 and Table 2). Patterns E and I were further divided into 4 and 2 subtypes, respectively. \( \text{bla}_{\text{CMY}-2} \) on the transferred plasmids was demonstrated by Southern hybridization with the \( \text{bla}_{\text{CMY}-2} \) probe.

The 38 ciprofloxacin-resistant \( S. \) \textit{Choleraesuis} isolates were genotyped by pulsed-field gel electrophoresis on a CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the PulseNet protocol (13). Banding patterns generated by \( XbaI \) restriction were compared with BioNumerics software (Applied Maths, Kortrijk, Belgium). The 38 isolates showed a close relationship (Dice correlation coefficient of 90%) and had only 1 pulsotype, based on Tenover criteria (Figure 2) (14). The pulsotype was divided into 7 pulsosubtypes, among which

| Table 1. Resistance to ciprofloxacin, extended-spectrum cephalosporins, and both in \textit{Salmonella enterica} serotypes, by region and pulsotype, Taiwan, January—May 2004 |
|---------------------------------------------------------------|
| Resistance and region* | No. of resistant \textit{S. enterica} isolates/total isolates (%) | Pulsotypes of \textit{Choleraesuis} isolates (no. of isolates) |
|---------------------------------------------------------------|
| Ciprofloxacin resistance | | | |
| Northern | 0/96 (0) | 0/38 (0) | 0/14 (0) | 1/3 [8.1] | 6/88 (8.8) | 19/252 (7.5) | A (11), D (1), E (1) |
| Central | 1/32 (3.1) | 0/34 (0) | 0/14 (0) | 6/88 (7.5) | 4/37 (10.8) | 11/125 (8.8) | A (5), C (1) |
| Southern | 0/25 (0) | 0/60 (0) | 0/24 (0) | 18/20 (90.0) | 1/65 (1.5) | 19/194 (9.8) | A (14), B (1), C (1), F (1), G (1) |
| Eastern | 0/8 (0) | 0/10 (0) | 0/1 (0) | 1/1 (100) | 0/9 (0) | 1/29 (3.4) | A (1) |
| ESC resistance | | | | | | | |
| Northern | 0/96 (0) | 0/38 (0) | 3/53 (5.7) | 8/45 (17.8) | 9/199 (4.5) | 20/600 (3.3) | |
| Central | 0/32 (0) | 0/34 (0) | 1/14 (7.1) | 1/16 (6.3) | 7/68 (10.0) | 9/252 (3.6) | |
| Southern | 0/25 (0) | 0/60 (0) | 1/24 (4.2) | 7/20 (35.0) | 1/65 (1.5) | 9/194 (4.6) | |
| Eastern | 0/8 (0) | 0/10 (0) | 0/1 (0) | 0/9 (0) | 0/29 (0) | |

*Includes 197 isolates of 42 uncommon serotypes and 2 untypeable isolates.

The 38 ciprofloxacin-resistant \( S. \) \textit{Choleraesuis} isolates were genotyped by pulsed-field gel electrophoresis on a CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the PulseNet protocol (13). Banding patterns generated by \( XbaI \) restriction were compared with BioNumerics software (Applied Maths, Kortrijk, Belgium). The 38 isolates showed a close relationship (Dice correlation coefficient of 90%) and had only 1 pulsotype, based on Tenover criteria (Figure 2) (14). The pulsotype was divided into 7 pulsosubtypes, among which

Table 2. Characteristics of 20 \textit{Salmonella} isolates resistant to extended-spectrum cephalosporins

| Serotype | Specimen type | pl (s) | Resistance pattern* | gyrA at position‡ | parC at position $\beta$ | Isolate (restriction pattern of transferred \( \text{bla}_{\text{CMY}-2} \)) |
|-----------|---------------|-------|---------------------|------------------|-------------------------|---------------------------------|
| Albany    | Urine         | 9.0   | Am ESC Fx Cm Na Sxt Tc Tc† | TTC (Phe) | AAC (Asn) | NC04.001 (H1), NC04.002 (H1), NC04.003 (H1) |
| Cairo     | Stool         | 9.0, 5.4 | Am ESC Fx Cm Na Gm Km Sxt Tc Tc† | TTC (Phe) | AAC (Asn) | NC04.001 (H1), NC04.002 (H1), NC04.003 (H1) |
| Chester   | Stool         | 9.0   | Am ESC Fx | TTC (Phe) | AAC (Asn) | NC04.016 (G) |
| Choleraesuis Wound | Stool | 9.0, 5.4 | Am ESC Fx Cm Cps Na Gm Km Sxt Tc | TTC (Phe) | AAC (Asn) | NC04.005 (F), SG04.006 |
| Blood     | Blood         | 9.0, 5.4 | Am ESC Fx Cm Cps Na Gm Km | TTC (Phe) | AAC (Asn) | NC04.042 (E2), SG04.047 (E4) |
| Blood     | Blood         | 9.0, 5.4 | Am ESC Fx Cm Cps Na Gm Km Sxt Tc | TTC (Phe) | AAC (Asn) | NC04.042 (E2), SG04.047 (E4) |
| Joint fluid | Joint fluid | 9.0, 5.4 | Am ESC Fx Cm Cps Na Gm Km Sxt Tc | TTC (Phe) | AAC (Asn) | NC04.004 (H2), NC04.005 (F), SG04.006 |
| Kaduna    | Tissue        | 9.0, 5.4 | Am ESC Fx Cm Cps Na Sxt Tc | TTC (Phe) | AAC (Asn) | NC04.015 (I) |
| Saintpaul | Stool         | 9.0   | Am ESC Fx Gm | TTC (Phe) | AAC (Asn) | NC04.011 (G), NC04.011 (G) |
| Stanley   | Stool         | 9.0, 5.4 | Am ESC Fx Cm Sxt Tc | TTC (Phe) | AAC (Asn) | NC04.039 (D) |

*Am, ampicillin; ESC, extended-spectrum cephalosporins; Fx, cefoxitin; Cm, chloramphenicol; Cps, ciprofloxacin; Na, nalidixic acid; Gm, gentamicin; Km, kanamycin; Sxt, trimethoprim-sulfamethoxazole; Tc, tetracycline.
‡The S. Albany isolate and the S. Cairo isolates showed decreased susceptibilities to ciprofloxacin (MIC 0.25–1 \( \mu \)g/mL).
§For each isolate, the first letter indicates region (C, central region; N, northern region; S, southern region), and the second letter represents hospital.
Isolates NC04.001, NC04.002, and NC04.003 were from the same patients; all other isolates were from different patients.
were 1–4 band differences. Five ESC-resistant isolates displayed the same pulsosubtypes (IA or IC) as ESC-susceptible isolates (Table 1 and Figure 2).

Conclusions

We describe the prevalence of resistance to ciprofloxacin and ESCs among salmonellae isolated from January to May 2004 in Taiwan. We found widespread resistance of *Salmonella* isolates to both ESCs and ciprofloxacin; high prevalence of resistance to ciprofloxacin, ESCs, and both in *S. Choleraesuis*; and widespread prevalence of CMY-2–producing *Salmonella* isolates of various serotypes in Taiwan.

The prevalence of *Salmonella* isolates resistant to both ceftriaxone and ciprofloxacin may pose a therapeutic problem. CMY-2 is one of the AmpC enzymes, which are usually less active against cepefmepi and ceftiraxone than ESBLs (15). Accordingly, we have used ceftiraxone to successfully treat several patients infected with CMY-2–producing and ciprofloxacin-resistant *S. Choleraesuis* (8). Therefore, AmpC-producing strains should be differentiated from ESBL-producing strains by phenotypic or genotypic methods when ESC-resistant *Salmonella* strains are isolated in the clinical microbiology laboratory (15).

The ciprofloxacin-resistant rate in *S. Choleraesuis* in Taiwan has been >60% since 2001; the high prevalence was mainly due to clonal spread of resistant strains (4–6). The ciprofloxacin-resistant rate in *S. Choleraesuis* in this report (84.4%) was higher than those reported previously (≤70%) (4–6). *bla* _CMY-2_ in *Salmonella* in Taiwan was first reported in 2 *S. Typhimurium* strains isolated in 2000 (3). The first reported *S. Choleraesuis* strain with *bla* _CMY-2_ was a ciprofloxacin-resistant strain isolated in 2002 (7). All our 38 ciprofloxacin-resistant *S. Choleraesuis* isolates, including 8 ESC-resistant isolates, were genetically related. Moreover, we found possibly unrelated *bla* _CMY-2_-positive plasmids (lanes 3, 4, 7, 8, 10, and 11 in Figure 1) among closely related isolates (Figure 2). These data together suggest that the development and rapidly increasing prevalence of ESC and ciprofloxacin resistance in *S. Choleraesuis* in Taiwan might result from the extremely high prevalence of ciprofloxacin resistance followed by

![EcoRI restriction patterns of transferred CMY-2-encoding plasmids of 18 Salmonella isolates. The result of the hybridization assay with the bla*CMY-2* probe labeled with digoxigenin (Roche Molecular Biochemicals, Mannheim, Germany) is shown below the gel, and arrowheads indicate the locations of the restriction fragments that were hybridized. Lanes 2-21, plasmids from transconjugants of *Salmonella* isolates NB04.022, SB04.003, NL04.050, SA04.028, CG04.039, SG04.039, SG04.042, SE04.006, SG04.047, SE04.005, NG04.011, NG04.016, NG04.018, NC04.001, NC04.002, NC04.003, NC04.004, and CE04.015; lanes 1 and 22, molecular marker II (Roche Molecular Biochemicals); lanes 12 and 13, a 1-kb molecular marker (Promega Co., Madison, WI, USA)](image)

**Figure 1.** EcoRI restriction patterns of transferred CMY-2-encoding plasmids of 18 *Salmonella* isolates. The result of the hybridization assay with the *bla* _CMY-2_ probe labeled with digoxigenin (Roche Molecular Biochemicals, Mannheim, Germany) is shown below the gel, and arrowheads indicate the locations of the restriction fragments that were hybridized. Lanes 2-21, plasmids from transconjugants of *Salmonella* isolates NB04.022, SB04.003, NL04.050, SA04.028, CG04.039, SG04.039, SG04.042, SE04.006, SG04.047, SE04.005, NG04.011, NG04.016, NG04.018, NC04.001, NC04.002, NC04.003, NC04.004, and CE04.015; lanes 1 and 22, molecular marker II (Roche Molecular Biochemicals); lanes 12 and 13, a 1-kb molecular marker (Promega Co., Madison, WI, USA)

![Dendrogram (left) obtained from cluster analysis of XbaI-generated macrorestriction patterns of 38 ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis (right). Asterisks indicate extended-spectrum cephalosporin-resistant isolates. A percent scale of similarity is shown above the dendrogram. Pulsed-field gel electrophoresis (PFGE) types are shown between the gel and the dendrogram. H9812, *S. enterica* serotype Braenderup strain H9812, which was used as reference size marker.](image)

**Figure 2.** Dendrogram (left) obtained from cluster analysis of XbaI-generated macrorestriction patterns of 38 ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis (right). Asterisks indicate extended-spectrum cephalosporin-resistant isolates. A percent scale of similarity is shown above the dendrogram. Pulsed-field gel electrophoresis (PFGE) types are shown between the gel and the dendrogram. H9812, *S. enterica* serotype Braenderup strain H9812, which was used as reference size marker.
the horizontal transfer of \( \text{bla}_{\text{CMY-2}} \) into ciprofloxacin-resistant epidemic strains rather than from the spread of a clone that had been resistant to ciprofloxacin and ESCs.

All our ciprofloxacin-resistant Salmonella isolates tested had mutations in the QRDRs of \( \text{gyrA} \) and \( \text{par} \), a finding consistent with previously reported results (1,4,5). The rates of ciprofloxacin resistance in the 3 most common serotypes, Enteritidis, Typhimurium, and Stanley, remained very low (0%–0.6%). Six of 11 ciprofloxacin-resistant isolates in the group of uncommon serotypes belonged to serotype Schwarzengrund and accounted for 42.9% of all serotype Schwarzengrund isolates. Thus, the high rate (5.5%) of ciprofloxacin resistance in this group was in part due to the high prevalence of ciprofloxacin resistance in serotype Schwarzengrund.

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