

## High Rate of Reduced Susceptibility to Ciprofloxacin and Ceftriaxone among Nontyphoid *Salmonella* Clinical Isolates in Asia<sup>∇</sup>

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**This multinational study from Asia revealed that reduced susceptibility to ciprofloxacin (MIC, 0.125 to 1 µg/ml) in nontyphoid *Salmonella* isolates was common in Taiwan (48.1%) and Thailand (46.2%) and in *S. enterica* serotype Choleraesuis (68.8%) and *S. Virchow* (75.0%) from all countries. Reduced susceptibility to ceftriaxone (MIC, 2 to 8 µg/ml) remained uncommon in Asia, except in Taiwan (38.0%) or in *S. Typhimurium* (25.0%) from all countries.**

Nontyphoid *Salmonella* bacteria, with more than 2,500 serotypes, usually cause diarrheal diseases in humans that may be complicated by extraintestinal infections, such as bacteremia, meningitis, and osteomyelitis (11). Resistance to antimicrobial agents, including fluoroquinolones and expanded-spectrum cephalosporins, has been a serious problem worldwide. Nontyphoid salmonellosis has been rampant in Asia (7); however, data on the antimicrobial susceptibilities, as well as the prevalence, of various serotypes in many Asian countries after 2000 have been lacking.

During 2003 to 2005, 400 clinical isolates of nontyphoid *Salmonella* bacteria were randomly collected from 11 medical centers in seven Asian countries (Table 1) and transported to the central laboratory in Samsung Medical Center, Seoul, Korea, for serogrouping and serotyping using O and H antisera, respectively (Difco Laboratories, Detroit, MI). Susceptibilities to ciprofloxacin, tetracycline, ceftriaxone, ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole were determined by a broth microdilution method, and the results were interpreted according to the breakpoints for MICs suggested by the NCCLS (10). For statistical analysis, isolates in the “intermediate” category were deemed “resistant” in this study. Concomitant resistance to at least three of the six antibiotics tested was defined as multidrug resistance (MDR). Isolates

with ciprofloxacin MICs of 0.125 to 1 µg/ml were defined as having “reduced susceptibility” to ciprofloxacin (9). Similarly, “reduced susceptibility” to ceftriaxone was defined as isolates showing ceftriaxone MICs of 2 to 8 µg/ml (14). These definitions were proposed in previous reports to reflect the clinical therapeutic responses (9, 14). The  $\chi^2$  test and Student's *t* test were used to determine the significance of differences, and a *P* value of <0.05 was considered statistically significant.

A total of nine serogroups (B, C1, C2, D, E, G, I, K, and M) and 82 serotypes were identified among the isolates tested. Serogroups B (34.8%) and D (25.0%) were the two leading serogroups in all areas except Philippines, where serogroup E (25 isolates, 53.2%) was most prevalent. The distribution of major serotypes among the countries is shown in Table 1. Overall, *S. enterica* serotype Enteritidis and *S. Typhimurium* were the two most-prevalent serotypes except in Philippines, Singapore, and Thailand, where *S. Weltevreden* was either the most- or second-most-prevalent serotype. In Sri Lanka and Thailand, the most-prevalent serotypes were *S. Agona* and *S. Stanley*, respectively. *S. Choleraesuis* was relatively more prevalent in Taiwan than in other countries.

Resistance to at least one antimicrobial agent was found in 227 (56.8%) isolates, including 93 (65.9%) serogroup B, 54 (54.0%) serogroup D, 35 (71.4%) serogroup C1, 23 (65.7%) serogroup C2, and 20 (28.6%) serogroup E isolates. The resistance to ceftriaxone (3.0%) and ciprofloxacin (4.5%) was much lower than that to the four traditional antibiotics (chloramphenicol, 27.7%; trimethoprim-sulfamethoxazole, 29.0%; ampicillin, 34.5%; and tetracycline, 45.7%). When isolates from different countries were compared (Fig. 1A), resistance to the four traditional antibiotics was relatively higher in isolates from Taiwan or Thailand, while isolates from Korea demonstrated significantly higher ciprofloxacin resistance (13.5%

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TABLE 1. Serotype distribution of the 400 nontyphoid *Salmonella* isolates among the countries

Serotype	No. (%) of isolates from:							Total
	Philippines	Hong Kong	Singapore	Sri Lanka	Korea	Thailand	Taiwan	
Enteritidis	4 (8.5)	10 (35.7)	24 (49.0)	1 (4.0)	22 (42.3)	5 (5.5)	17 (15.7)	83 (20.8)
Typhimurium	9 (19.2)	6 (21.4)	4 (8.2)	3 (12.0)	5 (9.6)	1 (1.1)	28 (25.9)	57 (14.3)
Weltevreden	24 (51.1)		6 (12.2)	1 (4.0)		9 (9.9)	1 (0.9)	41 (10.3)
Stanley	1 (2.1)		1 (2.0)	1 (4.0)		13 (14.3)	1 (0.9)	17 (4.3)
Choleraesuis	2 (4.3)					1 (1.1)	13 (12.0)	16 (4.0)
London	1 (2.1)				9 (17.3)		1 (0.9)	11 (2.8)
Agona				4 (16.0)		4 (4.4)	3 (2.8)	11 (2.8)
Rissen		1 (3.6)			2 (3.9)	6 (6.6)		9 (2.3)
Anatum						7 (7.7)	1 (0.9)	8 (2.0)
Panama						6 (6.6)	2 (1.9)	8 (2.0)
Virchow		1 (3.6)	1 (2.0)			1 (1.1)	5 (4.6)	8 (2.0)
Derby	1 (2.13)	3 (10.7)				2 (2.2)	2 (1.9)	8 (2.0)
Hadar			1 (2.0)			4 (4.4)		5 (1.3)
Newport				2 (8.0)		3 (3.3)		5 (1.3)
Others	5 (10.6)	7 (25.0)	12 (24.5)	13 (52.0)	14 (26.9)	29 (31.9)	34 (31.5)	114 (28.5)
Total	47	28	49	25	52	91	108	400

versus 3.2%,  $P < 0.005$ ). Similar situations were found when the MIC<sub>50</sub>s, MIC<sub>90</sub>s, and geometric MICs of the six antibiotics were compared among isolates from the seven countries (Table 2).

MDR was found in 124 (31.0%) isolates, including 63

(58.3%) from Taiwan, 38 (41.8%) from Thailand, and less than 10 in the other five countries (Fig. 1A). All MDR isolates, except two from Singapore, were also resistant to tetracycline. MDR was more frequent among serogroup B (48.9%) and C1 (46.9%) isolates than those in serogroups C2 (22.9%), D

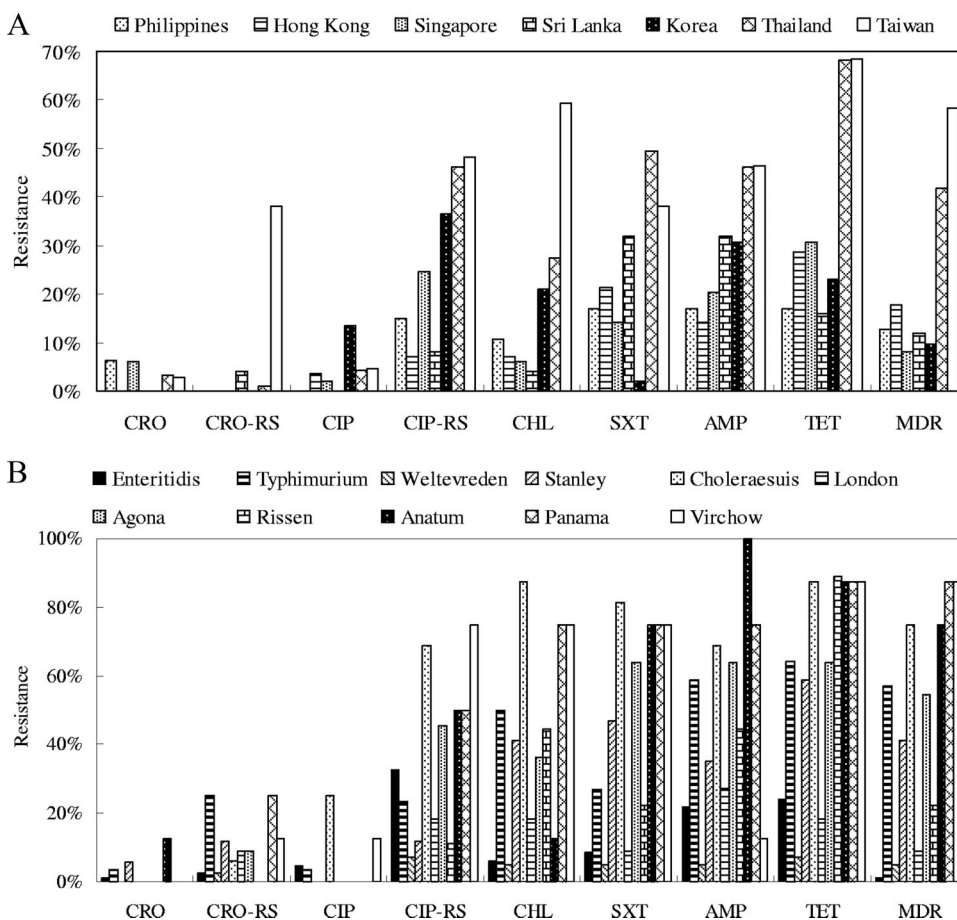


FIG. 1. Comparison of resistance to ceftriaxone (CRO), ciprofloxacin (CIP), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), and tetracycline (TET) and reduced susceptibility to ceftriaxone (CRO-RS) and ciprofloxacin (CIP-RS) of the 400 nontyphoid *Salmonella* isolates from the seven Asian countries (A) and 11 major serotypes (B). MDR, multidrug resistance to at least three of the six antibiotics.

TABLE 2. MIC<sub>50</sub>s, MIC<sub>90</sub>s, and geometric MICs of various drugs for the 400 nontyphoid *Salmonella* isolates among the countries

Country	MIC <sub>50</sub> /MIC <sub>90</sub> /geometric MIC (μg/ml) <sup>a</sup>					
	CRO	CIP	CHL	SXT	AMP	TET
Philippines	≤0.12/0.25/0.19	≤0.06/0.5/0.08	4/>128/5.87	0.5/>4/0.61	0.5/>128/1.36	2/>64/1.70
Hong Kong	≤0.12/0.25/0.13	≤0.06/1/0.08	8/8/6.56	≤0.12/>4/0.26	2/>128/2.63	2/>64/2.38
Singapore	≤0.12/≤0.12/0.18	≤0.06/0.25/0.10	4/8/5.70	0.5/>4/0.51	1/>128/2.69	2/>64/3.42
Sri Lanka	≤0.12/0.25/0.16	≤0.06/≤0.06/0.07	8/8/6.23	1/>4/1.36	1/>128/4.23	2/>64/1.89
Korea	≤0.12/0.25/0.13	0.12/4/0.18	8/>128/9.51	0.25/1/0.33	2/>128/4.57	2/>64/3.84
Thailand	≤0.12/≤0.12/0.16	0.12/1/0.17	4/>128/9.04	1/>4/1.64	1/>128/7.41	>64/>64/16.49
Taiwan	0.25/4/0.57	0.12/1/0.18	>128/>128/31.80	1/>4/0.96	2/>128/9.10	>64/>64/16.63

<sup>a</sup> CRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; AMP, ampicillin; TET, tetracycline.

(11.0%), or E (17.1%) (all *P* values were <0.005). MDR was also relatively more prevalent in isolates of serotypes *S. Panama* (87.5%), *S. Virchow* (87.5%), *S. Choleraesuis* (75.0%), *S. Anatum* (75.0%), *S. Typhimurium* (57.1%), *S. Agona* (54.5%), and *S. Stanley* (41.2%) (Fig. 1B). Concomitant resistance to the four traditional antibiotics was found in 52 (13.0%) isolates and was the most-common MDR pattern found among the isolates. Thirty-eight (75%) of the 52 isolates also showed resistance or reduced susceptibility to ciprofloxacin. The majority of such isolates were from Taiwan (*n* = 23), followed by Thailand (*n* = 21) and Philippines (*n* = 5).

The rates of reduced susceptibility to ciprofloxacin were high in isolates from Taiwan (48.1%), Thailand (46.2%), and Korea (36.5%) and were relatively lower in isolates from Singapore (24.5%), Philippines (14.9%), Hong Kong (7.1%), and Sri Lanka (8.0%) (Fig. 1A). Reduced susceptibility to ciprofloxacin was more common in isolates from serogroups C1 (29 isolates, 59.2%) and C2 (20 isolates, 57.1%) and serotypes *S. Choleraesuis* (11 isolates, 68.8%) and *S. Virchow* (6 isolates, 75.0%) (Fig. 1B). In contrast, higher rates of reduced susceptibility to ceftriaxone were only found in isolates from Taiwan (38.0%). Reduced susceptibility to ceftriaxone was more prevalent in isolates from serogroups B (27 isolates, 19.4%) and C1 (8 isolates, 16.3%) and serotypes *S. Typhimurium* (14 isolates, 25.0%) and *S. Panama* (2 isolates, 25.0%) (Fig. 1B).

Among isolates from the seven Asian countries, isolates from Taiwan appeared to demonstrate the highest antimicrobial resistance, similar to the situation reported in 1998 to 2002 from Taiwan (9). It is worrisome to find that reduced susceptibility to fluoroquinolones increased from 27.9% during that period (9) to 34.3% in the present study. Ceftriaxone resistance also increased significantly, from 0.8% in 1999 to 1.5% in 2003 (11) and further, to 10.8%, in this study. The high rate (38.0%) of reduced susceptibility to ceftriaxone demonstrated in the present study also adds to the complexity of the problem. In Thailand, the rates of antimicrobial resistance were almost as high as those in Taiwan. All these results serve as an alarming message to medical and public health professionals in both countries.

In comparison to a previous report from Korea, although the resistance to tetracycline and ampicillin was decreased in the present study, significant increases were found in the resistance to ciprofloxacin (from 0% to 13.5%), chloramphenicol (from 5.5% to 21.2%), and trimethoprim-sulfamethoxazole (from 0% to 1.9%) (3). The incidence of nalidixic acid resistance, an

indicator for reduced susceptibility to ciprofloxacin, also increased from the previous 19.4% to 36.5% in this study (3).

*S. Enteritidis*, accounting for 65% of all *Salmonella* isolates globally (7), was the most-common serotype in most areas of the world except North America (2, 7) and was so in Hong Kong, Singapore, and Korea in the present study. *S. Typhimurium* was the leading serotype in North America (7), followed by *S. Enteritidis*, as also in Taiwan. *S. Choleraesuis* and *S. Virchow*, the third- and fourth-most-common serotypes in Taiwan, demonstrated the highest rates of reduced susceptibility to ciprofloxacin (68.8% and 75%, respectively), as well as high rates of MDR (75% and 85.5%, respectively). We previously reported a sudden upsurge of ciprofloxacin resistance, to 69%, in *S. Choleraesuis* in Taiwan (4, 5). Although the rate was lower (30.1%) in this study, reduced susceptibility to ciprofloxacin was still found in 61.5% of the *S. Choleraesuis* isolates in Taiwan. In contrast, ciprofloxacin resistance was low (<8%) in *S. Weltevreden*, a phenomenon that explains the low ciprofloxacin resistance observed in Philippines, where *S. Weltevreden* was most prevalent. *S. Weltevreden* was also the leading serotype in Thailand during 1993 to 2002 (1) but was replaced by *S. Stanley* in this study. Since *S. Stanley* bears a high rate of MDR (41.2%), the prevalence of the serotype may contribute to the high rates of antimicrobial resistance among *Salmonella* isolates in Thailand.

Resistance to traditional antibiotics has limited therapeutic choices for *Salmonella* infection. Hence, an increase of incomplete susceptibility to fluoroquinolones and extended-spectrum cephalosporins would further exacerbate the problem. Some studies defined isolates with a MIC of ciprofloxacin of ≥0.125 μg/ml as having “decreased susceptibility” to ciprofloxacin (14), including both resistance and reduced susceptibility to ciprofloxacin. Furthermore, isolates with a MIC of ≥2 μg/ml were defined as having “decreased susceptibility” to extended-spectrum cephalosporins (14). There were only 13 isolates among a total of 14,043 isolates of nontyphoid *Salmonella* (0.1%) that expressed decreased susceptibility to both drug classes in the United States during 1996 to 2004 (14). In the present study, although there were only limited numbers of isolates collected from the participating countries, 37 (9.3%) isolates showed concurrent decreased susceptibility to ciprofloxacin and ceftriaxone. Resistance to any one of the traditional antibiotics was found to be positively correlated with decreased susceptibility to either ciprofloxacin or ceftriaxone (all *P* values were <0.05). Another alarming message for pub-

lic health in Taiwan is that 30 such isolates were derived from Taiwan, i.e., approximately 27.8% of the nontyphoid *Salmonella* isolates in Taiwan may express such dual decreased susceptibility. *S. Typhimurium* was the most-common serotype (nine isolates, 24.3%) expressing concurrent decreased susceptibility to the two drugs in this study.

Infections caused by nontyphoid *Salmonella* isolates that are resistant or have reduced susceptibility to antibiotics were associated with an increased rate of hospitalization or death compared with infections caused by susceptible isolates (6, 8, 12, 13). Thus, continual surveillance of antimicrobial resistance and restricted use of antibiotics in food animals are crucial to reduce the selection and spread of resistant *Salmonella* bacteria in Asian countries. In view of the high rate of reduced susceptibility to ciprofloxacin, ceftriaxone is suggested as the drug of choice in the treatment of invasive nontyphoid *Salmonella* infections.

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