Emergence of Multidrug-Resistant Salmonella enterica Serovar Typhi in Korea

Kyungwon Lee1,2, Dongeun Yong1, Jong Hwa Yum3, Young Sik Lim3, Hyun Sook Kim1, Bok Kwon Lee4 and Yunsop Chong1*

Department of Laboratory Medicine,1 Research Institute of Bacterial Resistance,2 and Brain Korea 21 Project for Medical Sciences,3 Yonsei University College of Medicine, and National Institute of Health,4 Seoul, Korea

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A chloramphenicol-resistant strain of Salmonella enterica serovar Typhi was first noted in Korea in 1992, when a resistant isolate was detected in a returned traveler. Continued isolation of multidrug-resistant (MDR) strains thereafter in other settings prompted a retrospective analysis of laboratory records and phenotypic and genotypic analyses of 12 chloramphenicol-resistant serovar Typhi isolates. Among these, one isolate was resistant only to chloramphenicol, and the other isolates were also resistant to ampicillin and co-trimoxazole. MDR was transferred by conjugation from 9 of the 11 isolates. PCR showed that all isolates had an incompatible group HI1 plasmid, and oriT was detected in 10 isolates, which included strains with an unsuccessful transfer of resistance. All of the ampicillin-resistant isolates had a β-lactamase band of pl 5.4 and blaTEM alleles. A PCR amplicon from an isolate showed that the sequences were identical to those of blaTEM-1, suggesting that all isolates had a TEM-1 β-lactamase. All isolates had class 1 integrons: 10 isolates had integrons of ca. 1.2 kb with dhfr7 gene cassettes, and 1 isolate had an integron of ca. 2.3 kb with aacA4 and blaOXA-1-like gene cassettes. The pulsed-field gel electrophoresis patterns of 7 of 11 MDR isolates were identical and indistinguishable from those reported for isolates in India and Indonesia. In conclusion, some of the MDR strains in Korea are related to those in other Asian countries. Susceptibility testing became necessary for selection of antimicrobial agents for the optimal treatment of patients with the emergence of MDR Salmonella serovar Typhi in Korea.

Typhoid fever, a systemic infectious disease caused by Salmonella enterica serovar Typhi, affected an estimated 16 million people in the 1990s, with 600,000 deaths reported annually worldwide (10). It has become a rare imported disease in developed countries (9), but massive outbreaks still occur in some countries, as was shown in Tajikistan due to the consumption of contaminated municipal water (17). The rate of typhoid fever in Korea has decreased markedly over the last decade, but sporadic cases and small outbreaks still occur.

Antimicrobial therapy is generally not required for the treatment of gastrointestinal infections caused by nontyphoidal Salmonella strains. However, effective antimicrobial therapy is required for typhoid fever to reduce morbidity and mortality (18). Historically, the drugs of choice were chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole (co-trimoxazole). However, antimicrobial-resistant Salmonella serovar Typhi isolates emerged in the 1970s in Latin America (21) and Asia (3). A large number of Salmonella serovar Typhi strains have been isolated in Korea, but no resistant strain was documented until 1992, when a chloramphenicol-, ampicillin-, and co-trimoxazole-resistant strain was isolated from a patient who returned from a Southeast Asian country (K. Lee, D. Yong, J. H. Yum, Y. S. Lim, Y. Chong, and B.-K. Lee, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-865, 2003). We isolated two additional multidrug-resistant (MDR) strains in 1995, and subsequently, Shin et al. (27) reported a chloramphenicol resistance rate of 15% among isolates in Korea in 1997.

MDR Salmonella serovar Typhi isolates commonly harbor a plasmid of incompatibility group HI1. A 365-bp region of the RepHI1A region was detected in MDR strains of Salmonella serovar Typhi isolated in India (26). oriT is located within the TraI region of the plasmid and contains the nic site, which is one of many genes required for conjugal transfer of IncHI1 plasmid (12). The emergence of MDR Salmonella serovar Typhi in Korea was possibly due to the acquisition of the resistance by endemic strains from other resistant gram-negative bacilli or to the spread of resistant strains from other countries to which there had been recent increases in international travel. To verify these assumptions, comparisons of the phenotypic and genotypic characteristics of both our susceptible and our resistant isolates are required.

The aims of this study were to analyze retrospectively the trends in the isolation and susceptibilities of Salmonella serovar Typhi strains from stool specimens at a Korean hospital and to determine the phenotypic and genotypic characteristics of recent isolates of chloramphenicol-resistant Salmonella serovar Typhi strains, including the first three strains isolated.

MATERIALS AND METHODS

Isolation and antimicrobial susceptibility trends for Salmonella serovar Typhi. Stool culture data obtained from 1969 to 1998 at a medical college-affiliated hospital in Seoul, Korea, were used for retrospective analysis. During that period, the methods of isolation and identification of Salmonella serovar Typhi remained essentially similar and were based on conventional methods, including the use of selenite broth, MacConkey agar, and salmonella-shigella agar for isolation and the use of triple sugar iron agar and other biochemical tests (6). The O-group antigen was determined by the slide agglutination method with commercial antisera (Difco, Detroit, Mich.) or antisera produced by the National
Institute of Health of Korea (NIHK). Antimicrobial susceptibility was determined with commercial disks (Difco or Becton Dickinson, Cockeysville, Md.) by the Kirby-Bauer disk-diffusion method (2) until the NCCLS method replaced it in 1978 (20).

Antimicrobial susceptibilities of recent isolates. Strains isolated from 1992 to 1998 were used for the characterization of chloramphenicol-resistant Salmonella serovar Typhi: three strains were isolated at two hospitals affiliated with a medical college in Seoul, and nine strains were isolated by NIHK. Chloramphenicol-susceptible isolates (9 and 13 strains isolated in 1989 and 1998, respectively) were used for comparison. Antimicrobial susceptibility was tested by the agar dilution method (20). The antimicrobial agents used were ampicillin, cephalothin, tetracycline, and nalidixic acid (Sigma Chemical, St. Louis, Mo.; cefotaxime [Aventis, Frankfurt, Germany]; cefoxitin [Merck Sharp & Dohme, Rahway, N.J.]; sulbactam [Pfizer Korea, Seoul, Korea]; chloramphenicol [Chong Kun Dang, Seoul, Korea]; sulfamethoxazole and trimethoprim [Ampire Pharmaceutical, Seoul, Korea]); and ciprofloxacin (Bayer Korea, Seoul, Korea). Escherichia coli strain ATCC 25922 was used for quality control.

Conjugation and plasmid preparation. Resistance transfer was tested by an agar mating method with nalidixic acid-resistant recipient E. coli RG 176. The plasmid was isolated by the alkaline lysis method (25), and the plasmid size was estimated by comparing it to those of plasmids from E. coli strain V517 and Klebsiella pneumoniae ATCC 700603.

PCR. DNAs were extracted by boiling whole cells and were used as templates. The alleles for blaTEM and the class 1 integron were detected by methods reported previously (4, 13). A 365-bp region of the RepH1A replicon was amplified by PCR, which was based on previous studies (7, 26), with primer HI1A-R (5'-CAC GGA AAG AAA TCA CAA C-3' and primer HI1A-F (5'-GTT CCA ACC CAT TGC TTT AC-3'). A 285-bp oriT region was detected with primer F (5'-ATA TGG TAC CGG TTA TTG CTA CTT AAT GCC GA-3') and primer R (5'-ATA TGG TAC CGG TTA TTG CTA CTT AAT GAC GA-3'), which were designed to amplify approximately 0.9 for possibly related patterns (a six-fragment difference), as recommended by Tenover et al. (29).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this work have been assigned to the GenBank database under accession numbers AY245101 (the dfrA7-carrying class 1 integron) and AY348316 (aacA4 and blaOXA-1-like gene encoding the class 1 integron).

RESULTS

Typhoid fever used to be a highly endemic disease in Korea. The mean annual number of Salmonella serovar Typhi isolates recovered from stool specimens from 1969 to 1988 at a medical college-affiliated hospital was more than 40 (Table 1). In the following two 5-year periods, the mean number of isolates recovered annually decreased to 8.8 and 2.8, respectively. During the period from 1974 to 1983 at the same hospital, the annual mean numbers of patients harboring Salmonella serovar Typhi and S. enterica serovar Paratyphi A, as determined from blood cultures, were 217 and 61, respectively (17.2 and 4.8% of all culture-positive patients, respectively). In 1991, the number of patients at the same hospital whose blood harbored Salmonella serovar Typhi was 22 (0.6% of culture-positive patients); and this number then decreased further, but the organism was still occasionally isolated (data not shown).

Isolation of the first MDR Salmonella serovar Typhi strain in 1992 at one of two hospitals affiliated with a medical college, from a patient who had returned from a Southeast Asian country, prompted an analysis of the records. However, among the 985 strains isolated from 1969 to 1993 at the main hospital of the same medical college, none were resistant to chloramphenicol, ampicillin, or co-trimoxazole. In 1995, we isolated two more MDR strains from a 52-year-old male patient and a
A 59-year-old female patient with no history of overseas travel (Table 1).

The antimicrobial susceptibilities of chloramphenicol-resistant and -susceptible recent clinical isolates were compared. Among the 12 chloramphenicol-resistant Salmonella Typhi strains isolated from 1992 to 1998, 11 also showed resistance to ampicillin and co-trimoxazole (Table 2). The ranges of MICs of nalidixic acid and ciprofloxacin were 4 to 8 and 0.015 g/ml, respectively, for both chloramphenicol-resistant isolates, and the isolates had bands for a chromosomal resistance gene cassette, and a short unknown open reading frame (Fig. 1B).

PCR was used to determine the incompatibility group of the plasmid. A 365-bp region of the RepHI I A replicon was detected in all 11 isolates, including 2 conjugation-negative isolates. The oriT allele was detected in all isolates except one (strain 11).

blaTEM alleles were detected in all of the ampicillin-resistant isolates, and the isolates had bands for a β-lactamase of pI 5.4. A PCR amplicon from an isolate (strain 2) had sequences identical to those of blaTEM. This suggests that all isolates had the TEM-1 β-lactamase. In our study, class 1 integrons were detected by PCR in all 11 MDR isolates. They were ca. 1.2 kb in 10 isolates and ca. 2.3 kb in 1 isolate. Sequencing of the 1.2-kb integron from one isolate (strain 2) showed carriage of a dhfr7 gene cassette (Fig. 1A). The strain carrying a 2.3-kb integron had a β-lactamase band of ca. pI 7.4. Sequencing showed that the integron carried aacA4, blaoxa-1-like resistance gene cassettes, and a short unknown open reading frame (Fig. 1B).

The PFGE patterns of XbaI-digested genomic DNA of 12 and 19 chloramphenicol-resistant and -susceptible Salmonella Typhi isolates, respectively, were compared. The pattern of the first MDR isolate in Korea (type A) was different from those of the other MDR isolates. The remaining 10 MDR

### Table 2. Comparison of susceptibilities of chloramphenicol-susceptible and -resistant Salmonella serovar Typhi isolates

<table>
<thead>
<tr>
<th>Isolate type (yr isolated)</th>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>Susceptibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Chloramphenicol resistant</td>
<td>Ampicillin</td>
<td>1.2–2.0</td>
<td>&gt;128</td>
</tr>
<tr>
<td>(1992–1998; n = 12)</td>
<td>Ampicillin-sulbactam</td>
<td>0.5–1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cephalothin</td>
<td>2.0–4.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2.0–4.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Co-trimoxazole</td>
<td>0.06/1.14</td>
<td>0.12/2.28</td>
</tr>
<tr>
<td></td>
<td>Tetracycline</td>
<td>0.5–1</td>
<td>0.5</td>
</tr>
<tr>
<td>Chloramphenicol susceptible</td>
<td>Ampicillin</td>
<td>0.5–1</td>
<td>0.5</td>
</tr>
<tr>
<td>(1989–1998; n = 21)</td>
<td>Ampicillin-sulbactam</td>
<td>0.5–1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Cephalothin</td>
<td>2.0–4.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2.0–4.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Co-trimoxazole</td>
<td>0.06/1.14</td>
<td>0.12/2.28</td>
</tr>
<tr>
<td></td>
<td>Tetracycline</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Therapeutically nonrelevant drugs were also included to compare the antibiograms of chloramphenicol-susceptible and -resistant isolates. All of the isolates were inhibited by ≤0.12 µg of cefotaxime per ml, ≤0.015 µg of ciprofloxacin per ml, and ≤8 µg of nalidixic acid per ml.

### Table 3. Characteristics of MDR Salmonella serovar Typhi isolates

<table>
<thead>
<tr>
<th>YMC strain (no.)</th>
<th>Conjugation result</th>
<th>PCR result for:</th>
<th>β-Lactamase pI</th>
<th>PFGE type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>orIT</td>
<td>IncHII</td>
<td>Integron</td>
</tr>
<tr>
<td>(1) 92/8/5479Y</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
</tr>
<tr>
<td>(2) 95/6/4405S</td>
<td>+</td>
<td>+</td>
<td>1.2`</td>
<td>+`</td>
</tr>
<tr>
<td>(3) 95/12/2488S</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td>+</td>
</tr>
<tr>
<td>(4) 98/K101N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(5) 98/O102N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(6) 98/H103N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(7) 98/H104N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(8) 98/H105N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(9) 98/Y106N</td>
<td>–</td>
<td>+</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>(10) 98/S109N</td>
<td>+</td>
<td>+</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(11) 98/P110N</td>
<td>+</td>
<td>+</td>
<td>2.3`</td>
<td></td>
</tr>
</tbody>
</table>

* A 365-bp region of the RepHI IA replicon.

b Class 1 integron. Data represent approximate sizes (in kilobases).

c The nucleotides of integrons and blaTEM were sequenced.
isolates clustered together: the second MDR strain, isolated in 1995 (Fig. 2, lane 3, type C), showed a pattern identical to those of the seven NIHK strains isolated in 1998 (Fig. 2, lane 6).

One strain that was isolated in 1998 and that was resistant only to chloramphenicol-resistant Salmonella serovar Typhi clustered together with susceptible strains isolated in 1989 (types K, L, N, O, and P). All nine antimicrobial-susceptible strains isolated in 1998 clustered together (types F to I).

**DISCUSSION**

Until the 1980s typhoid fever was an endemic disease in Korea and Salmonella serovar Typhi was frequently isolated from stool specimens (Table 1). Typhoid fever is a serious systemic infection requiring proper antimicrobial therapy. The present retrospective analysis showed that antimicrobial-resistant Salmonella serovar Typhi was absent until 1992, when it was detected in a hospital in Korea (Lee et al., 43rd ICAAC), although MDR Salmonella serovar Typhi became prevalent in Southeast Asia (8, 30), the Middle East, and northeastern Africa (1, 19, 23).

Among the chloramphenicol-resistant Salmonella serovar Typhi strains isolated in the 1990s, 1 chloramphenicol-resistant isolate was susceptible to ampicillin and co-trimoxazole, but the remaining 11 were also resistant to ampicillin and cotrimoxazole (Table 2). Some of the chloramphenicol-resistant isolates were either intermediate or resistant to cephalothin or tetracycline (Table 2), but all 11 MDR isolates were susceptible to ciprofloxacin and nalidixic acid. Ciprofloxacin is recommended as the drug of choice for the treatment of multidrug-resistant Salmonella serovar Typhi infections (18). However, an NIHK study suggested that the clinical failure of empirical fluoroquinolone treatment might occur, as the nalidixic acid resistance rate rapidly increased from 3.5% in 2001 to 23.3% in 2002, with resistance detected in 138 and 58 isolates, respectively (data not shown). Low-level fluoroquinolone resistance can be detected by testing only for nalidixic acid resistance (5). It is a concern that ceftriaxone remains the only drug of choice for the treatment of infections due to ampicillin- and fluoroquinolone-resistant isolates.

It was reported that most MDR Salmonella serovar Typhi isolates have a conjugative plasmid of the IncHI1 type. This plasmid has been implicated as a significant factor in the persistence and reemergence of Salmonella serovar Typhi (11, 19). The reported sizes of the plasmids were quite variable: 220 and 290 kb in isolates from Bangladesh (8); 91.2 MDa in isolates from Tehran, Iran (1); and 110 to 120 MDa in isolates from India (30). The type of β-lactamase reported was mostly TEM-1. Most of our MDR strains also had similar characteristics: they had a ca. 120-MDa conjugative plasmids of the IncHI1 type, they transferred MDR en bloc, and they had the TEM-1 β-lactamase. However, a few of our isolates showed heterogeneity. Repeated attempts to transfer the resistance by conjugation from the first chloramphenicol-resistant strain isolated in Korea (strain 1 in Table 3) failed, even at 28°C; one isolate was PCR negative for the oriT allele.

Integrons are a major vehicle for the spread of multiple-antibiotic resistance (14). Class 1 integrons were detected in all 11 MDR isolates. Their sizes were ca. 1.2 kb in all except one isolate, in which it was ca. 2.3 kb. The smaller integron contained only a dfr7 resistance gene cassette, but the larger one contained aacA4 and bhox, like gene cassettes (Fig. 1). Ploy et al. (24) reported that all 18 of their resistant Salmonella serovar Typhi isolates had class 1 integrons with dfr7II gene cassettes. Pai et al. (22) reported that an MDR strain isolated in Korea in 1999 had a class 1 integron with the aacA4b, catB8, adaA1, dfrA1, and aac(6’)-Iia gene cassettes and a novel bhox2 cassette. bhox2 is a carbencillinase gene. These results obtained from studies performed in Korea indicate the existence of various β-lactamase genes in ampilcin-resistant Salmonella serovar Typhi isolates.

In a comparison of various molecular characteristics, Ling et al. (15) reported that isolates obtained in Hong Kong in 1985 to 1997 differed from those obtained in Vietnam in 1989 and 1990. They reported that 37% of the Vietnamese isolates belonged to two predominant clones. It was reported that the PFGE pattern of XbaI-digested genomic DNA of Salmonella serovar Typhi was stable (29). Interestingly, 7 of our 11 MDR isolates had identical PFGE patterns (Fig. 2, type C), and that pattern was also apparently identical to that of the MDR strains isolated in 1993 and 1994 in India (Fig. 2, lanes 7, 8, and 10, in reference 26) and to that of 1 of 5 Indonesian isolates recovered in 1992 and 1994 (Fig. 1A, lane 14, in reference 29). These results indicate that our MDR strains are related to those in other countries. Ispahani and Slack (9) reported that from 1980 to 1997 all 21 typhoid fever patients at a hospital in the United Kingdom were of Asian descent, and some had a history of travel to Asia. Some of these isolates were MDR strains. With increasing international travel, the further spread of MDR strains can be anticipated. Seven of our eight MDR strains with identical PFGE types were isolated from patients in five cities in one province, suggesting clonal spread. Routine antimicrobial susceptibility testing of Salmonella serovar Typhi isolates became crucial for the optimal treatment of typhoid fever patients in Korea.

In conclusion, the characteristics of chloramphenicol-resistant strains isolated in Korea from 1992 to 1998 were mostly similar to those reported for isolates in other countries: they
were MDR, carried large conjugative IncHI1 plasmids, had the TEM-1 β-lactamase, and had class 1 integrons. Some strains had subtle differences in characteristics: the nontransferability of resistance or an integron with a blaOXA gene cassette. With the emergence of MDR Salmonella serovar Typhi in Korea, susceptibility testing became necessary for selection of the antimicrobial agents that could be used for the optimal treatment of patients.

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REFERENCES


