Molecular Typing of *Salmonella enterica* serovar Typhi Strains Isolated in Busan by Pulsed-Field Gel Electrophoresis

Sang-Kee Min*, Ju-Hyun Lee, Eun-Hee Park, Jung-Ah Kim and Kyu-Won Kim[1]

Division of Microbiology, Busan Institute Of Health and Environment, Busan 613-104,
[1]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Received April 6, 2006 / Accepted May 25, 2006

We obtained 424 *Salmonella enterica* serovar Typhi isolates from sporadic cases of infection in Busan during 1996 to 2005. We investigated the trend of antimicrobial resistance and molecular typing by pulsed-field gel electrophoresis (PFGE). Of the total 424 isolates, 6 strains (1.4%) were multi-drug-resistant (MDR) *S. enterica* serovar Typhi isolates, 2 strains (0.5%) were resistant to only nalidixic acid, and the remaining 416 strains (98.1%) were fully susceptible to the 18 antimicrobial agent. PFGE of *Xba*I-digested chromosomal DNA was performed on 50 sporadic *S. enterica* serovar Typhi isolates with the objective of investigating the extent of genetic diversity of these isolates in our region. We could find that these isolates were much more heterogeneous and at least 32 different PFGE patterns were generated according by dice coefficient, between 0.69 and 1.0. Restriction fragment patterns consisted of 13 to 18 fragments ranged in size from 20 to 630 kb. The results confirmed that PFGE would be an useful tool for investigating surveillance of sporadic or outbreak case and assessing clonality for *S. enterica* serovar Typhi in Busan area. Our finding will be valuable in developing rational strategies to control this pathogen and setting the basis of an effective PulseNet system in Korea.

Key words – *Salmonella enterica* serovar Typhi, antimicrobial susceptibility, MDR, PFGE

Typhoid caused by *Salmonella enterica* serovar Typhi belongs to the category of legislated communicable disease group I in Korea as it spreads rapidly and poses a high level of health risk to national health, requiring immediate control measures at the development or onset of an outbreak. It is a significant infectious disease in developing countries with an annual incidence of 16 million case and approximately 600,000 deaths worldwide[7]. The incidence of typhoid fever results from mainly consuming foods or water contaminated by feces of patients or carriers. Thus, government adopted various measures to prevent public health from this disease including purification of water supplies, sewage control, treatment of chronic carriers, and sanitary and hygiene education especially among food handlers in our nation.

It is known that human is exclusively the only infectious reservoir of *S. enterica* serovar Typhi in nature, so eradication of this etiological agent could be possibly achieved by effective antimicrobial chemotherapy or adequate vaccines against patient and chronic carrier. Notwithstanding, typhoid fever continues to be a public health problem in developed country and remains epidemic disease in most parts of Central America[8,24], Southeast Asia[15,20,21], the Indian subcontinent[26,28], Africa[12,18], Turkey[11]. Moreover, the emergence of multidrug-resistant (MDR) *S. enterica* serovar Typhi strain have become a serious problem globally in recent year[28]. In Korea, *S. enterica* serotype Typhi is endemic pathogen occurring by sporadic or rarely outbreak case in recent year. According to annual report of communicable disease published by Korea Center for Disease Control and Prevention (KCDC), its incidental case appears to be as follows: 265 case in 1997, 380 case in 1998, 308 case in 1999, 234 case in 2000, 401 case in 2001, 221 case in 2002, 199 case in 2003, and 174 case in 2004 by year[36]. After MDR *S. enterica* serovar Typhi strains was firstly confirmed in 1992, which was isolated from a patient who returned from Southeast Asia travel, National Institute of Health of Korea (NIHK) reported additionally 10 more MDR strains during 1996 to 1997 through the national laboratory communicable disease surveillance system, but the origins of the strains were not clear[5,29].

As entitled to the public referral laboratory in our region, we collected most of the *S. enterica* serovar Typhi isolated from local health center and hospital in Busan.

We conducted antimicrobial agent susceptibility test

***Corresponding author**
Tel : +82-51-757-6936, Fax : +82-51-757-2879
E-mail : girin@bs21.net

over all the collected strains to survey the drug resistance change by year. Particularly we selected a panel of 50 isolates comprising the representative strains of each year and 6 MDR strains, which are all obtained from sporadic cases of typhoid fever and performed a molecular typing by pulse-field gel electrophoresis (PFGE).

The study of epidemiologic marker is important in an attempt to trace the source of infection. Although various typing methods, such as the antibiogram, plasmid profiles, phage typing, PFGE, ribotyping, PCR-based amplification fragment length polymorphism, single-strand conformation polymorphism, and nucleotide sequence analysis has been used for the subtyping of *S. enterica* serovar Typhi, PFGE has proved to be the most effective method because of its reliable, reproducible results and high discriminatory ability[3,21,22]. Also, PFGE is able to differentiate between clonally related strains and strains which represent independent clones[17].

The objective of this study was to investigate the extent of genetic diversity using PEGE and the trend of antimicrobial resistance among *S. enterica* serovar Typhi isolates during 1996 to 2005 in Busan, Korea.

Materials and Methods

Bacterial isolates

We collected 424 *S. enterica* serovar Typhi strains which were isolated from local health center and hospital during 1996 to 2005 in Busan metropolitan city, Korea. The number of *S. enterica* serovar Typhi isolates collected per year were as followed : 67 isolates in 1996, 50 in 1997, 77 in 1998, 53 in 1999, 42 in 2000, 35 in 2001, 40 in 2002, 34 in 2003, 13 in 2004, 13 in 2005, respectively.

The identification *S. enterica* serovar Typhi isolates was based on conventional methods, including the use of selenite broth, MacConkey agar, salmonella-shigella agar and kligler-iron agar and confirmed with the API 20E system (BioMerieux, France). O-group antigen was serotyped using agglutination antisera produced by the National Institute of Health of Korea (NIHK) or commercial antisera (Difco, Detroit, Mich.). Isolates were stored at -70℃ deep freezer until analyzed.

Antimicrobial susceptibility test

S. enterica serovar Typhi isolates were tested for susceptibility to antimicrobials by the disc diffusion method (Bauer *et al.* 1966: National Committee for clinical Laboratory Standards 1998). The 18 antimicrobials were used: ampicillin (10 μg), amikacin (30 μg), ampicillin/sulbactam (10/10 μg), cephalothin (30 μg), cefazolin (30 μg), cefepime (30 μg), cefotetan (30 μg), cefotaxim (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), ticarcillin (75 μg), sulfamethoxazol/ trimethorim (23.75/1.25 μg), kannamycin (30 μg) and amoxicillin/clavulanic acid (20/10 μg). Test strains were cultured in TSB broth at 37℃ for 2-8 h. The culture was adjusted to 0.5 McFarland turbidity. The bacterial cells were then spread evenly on a Mueller-Hinton agar plate. The test antimicrobial discs (BBL Sensi-Disc Becton Dickenson, Cockeysville, MD, USA) were placed on the agar. After 18 h of incubation at 35℃, the diameter of antibiotic zone was measured. *Escheichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as controls for the potency of antibiotics. Interpretation of inhibitory zone was made in accordance with the interpretative standards provided by the manufacturer.

Restriction endonuclease digestion and PFGE

Chromosomal DNA for PFGE analysis was prepared by the method of Gautom with modifications[9].

Bacterial cells were suspended in cell suspension TE buffer (100 mM Tris, 100 mM EDTA, pH 7.5), and the cell density was adjusted to a turbidity of transmittance rate 10% to 15% (Vitek colorimeter, Hach company, USA). Ten microliter of proteinase K (20 mg/ml) and 200 μl of cell suspension was added to 200 μl of 1.2% SeaKem Gold agarose (Cambrex, Rockland, ME, USA). The agarose mixture was pipetted into disposable plug molds (Bio-Rad Laboratories, Hercules, CA). Solidified agarose plugs were transferred to a tube containing 1.5 ml of ES lysis buffer (0.5 M EDTA, pH 9.0, 1% sodium-lauroylsarcosine) and 40 μl of proteinase K (20 mg/ml). and plugs were incubated in a shaking water bath at 55℃ for 1 hr. Plugs were washed one time with deionized distilled water (D.D.W) for 15 min and two times with TE buffer (10 mM Tris, 10 mM EDTA, pH 7.5) for 15 min, each time in a shaking water bath at 55℃. Agarose plugs containing DNA were cut to slice of 1 mm thickness and restricted with 30 U of *Xba*I (New England Biolabs, Beverlu, MA, USA) at 37℃ for 90 min. The digested DNA plugs were loaded on the comb, and a 1% SeaKem gold agarose gel was prepared using

0.5× TBE (Tris-Borate-EDTA) buffer (Bioneer, Daejeon, Korea) and electrophoresed using CHEF Mapper (Bio-Rad) with switch times: an initial 2.16 sec and a final 63.8 sec, field strength: 6 V cm^{-1}, and run time: 18 hours. Gels were stained with ethidium bromide (0.5 μg/ml: Bioneer) for 30 min, destained in distilled water for 30 min, and photographed under UV illumination (Vilber Lourmat, France).

Salmonella serovar Branderup H9812 (ATCC BAA-664) was used as a reference standard to normalize gels.

Data analysis

Patterns generated by PFGE were assessed visually and the band differences were interpreted by the method of Tenover *et al.*[30]. By this criteria, it has been proposed that isolates with similar PFGE band patterns were described as genetically indistinguishable isolates with fewer than four band shift were assumed to be closely related (which caused by a single genetic event, e.g, a point mutation resulting in loss or gain of a restriction site, insertion, deletion, or chromosomal inversion), isolates with differences in four to six bands might be possibly related, and isolates showing a differences in more than seven bands were considered to be epidemiologically unrelated.

The similarity of fragment length patterns between two strains is scored by the Dice similarity coefficient. This coefficient, F, was calculated by the formular $F = 2n_{xy}/(n_x + n_y)$, where n_x is the total number of DNA fragments from isolate X, n_y is the total number from isolate Y, and n_{xy} is the number of fragments identical in the two isolates. An F value of 1.0 indicates that the two isolates have identical PFGE patterns. The original F value multiplied by 100 gives similarity percentages[34]. Dendrogram was created by setting dice similarity coefficient and clustered by the method of unweighted pair group average (UPGMA), performed with BioNumerics (Applied Maths, Belgium). Each unique PFGE pattern initial name was assigned as X based on the restriction endonuclease *Xba*I.

Results and Discussion

Antibiotic susceptibility testing

Of the total 424 *S. enterica* serovar Typhi isolates, only 8 (1.9%) strains showed three resistance types (Table 1). The first type (A) included two isolates that were resistant to 5 antimicrobial agents: ampicillin, chloramphenicol, trimethoprim-sulfamethoxazol (co-trimoxazole), tetracycline and nalidixic acid.

The second type (B) included four isolates that showed the same resistant pattern with A type except tetracycline, and the last type (C) included two isolates that showed resistance to only nalidixic acid. Besides 8 isolates, the remaining 416(98.1%) were fully susceptible to the 18 antimicrobial agents, Although antimicrobial susceptibility test was also used traditionally as epidemiologic marker, in this study only three types of antibiogram were found. Therefore, results of antimicrobial susceptibility could not be useful to determine the transmission route of the *S. enterica* serovar Typhi strains in this area.

Since emergence of chloramphenicol resistant *S. enterica* serovar Typhi was reported firstly in the early 1970s[2], antimicrobial resistant *S. enterica* serovar Typhi had not been found before 1997 in Korea. Recently, chloramphenicol resistant *S. enterica* serovar Typhi isolates were appeared in this country as following resistant rates according to the reports of KCDC : 4.7% in 1999, 6.8% in 2000, 11.7% in 2001, 3.3% in 2002, 6.6% in 2003, 3.0% in 2004[36]. Since chloramphenicol has been the drugs most frequently used to treat typhoid fever, serovar Typhi isolates resistant to this drug and two more first-line drugs (ampicillin and co-trimoxazole) were defined as MDR *S. enterica* serovar Typhi[12]. Acquisition of resistance to chloramphenicol, ampicillin, trimethoprim, sulfonamides, and tetracyclines is usually associated with an incompatibility group HI plasmid[27,28]. These plasmids are large (~180 kb) and conjugative and originated from Southeast Asia[10].

Table 1. Types of antimicrobial susceptibility for *S. enterica* serovar Typhi isolates

Type	No. of strains	resistant drug pattern[a)]	isolates(yr)	PFGE pattern
A	2	AM, C, SXT, Te, NA	1998/1, 1999/2	X30
B	4	AM, C, SXT, NA	1998/2, 1999/1 2001/1, 2003/3	X26, X29, X28
C	2	NA	2002/1, 2005/2	X27, X6
Others	416	susceptible to all drugs tested		

a) AM; ampicillin, C; chloramphenicol, SXT; trimethoprim-sulfamethoxazol, Te; tetracycline, NA; nalidixic acid

Recently, it was reported that MDR serovar Typhi also resistant to nalidixic acid and the fluoroquinolones was revealed in Bangladesh[4], India[23], Thailand, Vietnam[6,14], and Tajikistan[19]. Although MDR serovar Typhi has been raised a global problem, the incident rate has been decreased recently in our nation because the condition of environmental and personal sanitation have been improved substantially. Also we found that the spread and emergence rate of MDR serovar Typhi was not increased in our region. Although the frequency of drug resistant strains are rarer comparing to other *Salmonella* serovars, it is needed to continue evaluation of antimicrobial resistance patterns because resistance may be easily spread by genetic exchange between *S. enterica* serovar Typhi and other members of the family *Enterobacteriaceae* in normal gut flora.

PFGE patterns for *S. enterica* serovar Typhi isolates

When chromosomal DNA of all 50 *S. enterica* serovar Typhi isolates were restricted with *Xba*I and analysed by PFGE, a total of 32 different PFGE subtype patterns was generated according by dice coefficient, between 0.69 and 1.0. Patterns produced by PFGE consisted of 13 to 18 fragments ranging in size from 20 to 630 kb. Electrophoresis on an agarose gel of representative PFGE pattern is shown in Fig. 1. The number of fragments was shown to be 13 in two type patterns, 14 in 12 type patterns, 15 in 4 type patterns, 17 in 6 type patterns and 18 in 8 type patterns.

Thong *et al.* reported that PFGE produced by restriction endonuclease analysis (REA) pattern consisted of 11 to 24 DNA fragments[33]. Also they reported that PFGE following digestion with restriction endonuclease *Xba*I (5'-TCTAGA-3'), *Spe*I (5'-ACTAGT-3'), and *Avr*II (5'-CCTAGG-3') was able to differentiate isolates of *S. enterica* serovar Typhi chromosomal DNA. And that *Xba*I is the most useful restriction enzyme for PFGE of serovar Typhi because it is more discriminatory and cheaper than *AvrII* or *SpeI*. 10 subtypes (F = 1.0) were shared by two or more strains: X3 (n=2), X4 (n=7), X6 (n=4), X7 (n=2), X12 (n=2), X22 (n=3), X23 (n=2), X24 (n=2), X26 (n=2), X30 (n=2). Of these, X4 type was the major subtype and shared by seven strains, of which five strains were isolated sporadically in 2004. These strain may be the same or at least, clonally highly related strains. The dendrogram of the 50 PFGE patterns is shown in Fig. 2. At a similarity of 81.4%, there are two major cluster designated A and B arbitrarily. The

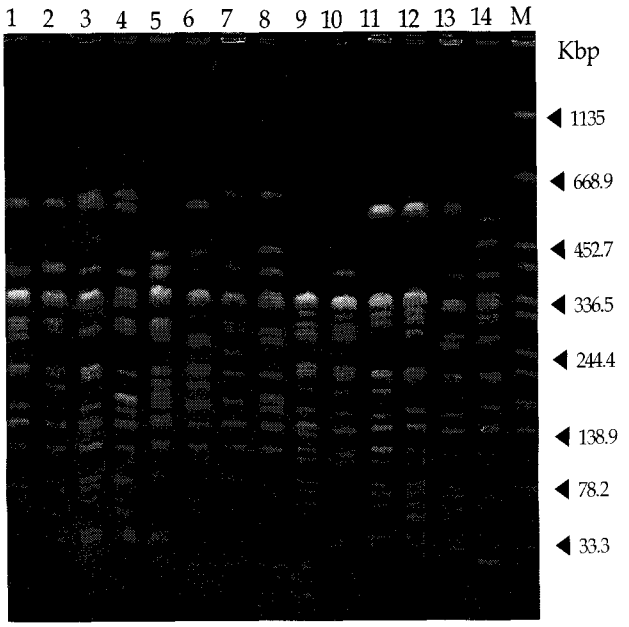

Fig. 1. PFGE (*Xba*I digested) patterns of representative *S. enterica* serovar Typhi isolates from sporadic case in Busan from 1996 to 2005. Lane 1, 2005/13 (X3); Lane 2, 2005/6 (X6); Lane 3, 1999/4 (X11); Lane 4, 2000/1 (X12); Lane 5, 2004/6 (X17); Lane 6, 2005/12 (X18); Lane 7, 1997/4 (X20); Lane 8, 2005/1 (X21); Lane 9, 2004/12 (X22); Lane 10, 1998/3 (X24); Lane 11, 1999/1 (X26); Lane 12, 1998/1 (X30); Lane 13, 1997/6 (X31); Lane 14, 2005/9 (X32); Lane 15, molecular size marker, *Sallmonella* serovar Branderup H9812 reference standards restricted with *Xba*I.

A cluster contained significant number of 33 strains and was the major cluster. Among 26 strains isolated in 2004 and 2005, 21 (80.7%) isolates were amongst this predominant pattern

Interestingly, all of 6 MDR serovar Typhi isolates had similar PFGE pattern (F=0.88 to 1.0) grouped to B cluster. By the criteria of Tenover *et al.*[30], isolates differing in one to four bands (F=0.88 to 0.97) assumed to be closely related. Maslow *et al.* also pointed out that strains with one or two bands' shift in PFGE analysis have been considered to be clonally related [17]. From this suggestion, these MDR serovar Typhi strains can be designated closely related strains. Because MDR serovar Typhi include the plasmid DNA ranging from 40 to 220 kbp[28], it seemed that the restriction fragment pattern of these isolates were somewhat different from susceptible serovar Typhi isolates in band position and number especially below 100 kb. (Fig. 1)

Kariuki *et al.*[12] found that there was no correlation between the antimicrobial susceptibility phenotype and the

PFGE restriction fragment pattern of serovar Typhi isolated in Kenya. However, our observations seemed to be in contrast to these findings since the MDR serovar Typhi strains (ampicillin, chloramphenicol, tetracycline, nalidixic acid, co-trimoxazole) shared similar PFGE patterns.

Some patterns such as X3 and X12, although belonging to the same PFGE pattern, were isolated at different time (1997 *vs* 2005 and 1997 *vs* 2000). Thus, recirculation of certain infectious strains from sporadic case may be possibile, suggesting that certain genotype of *S. enterica* serovar

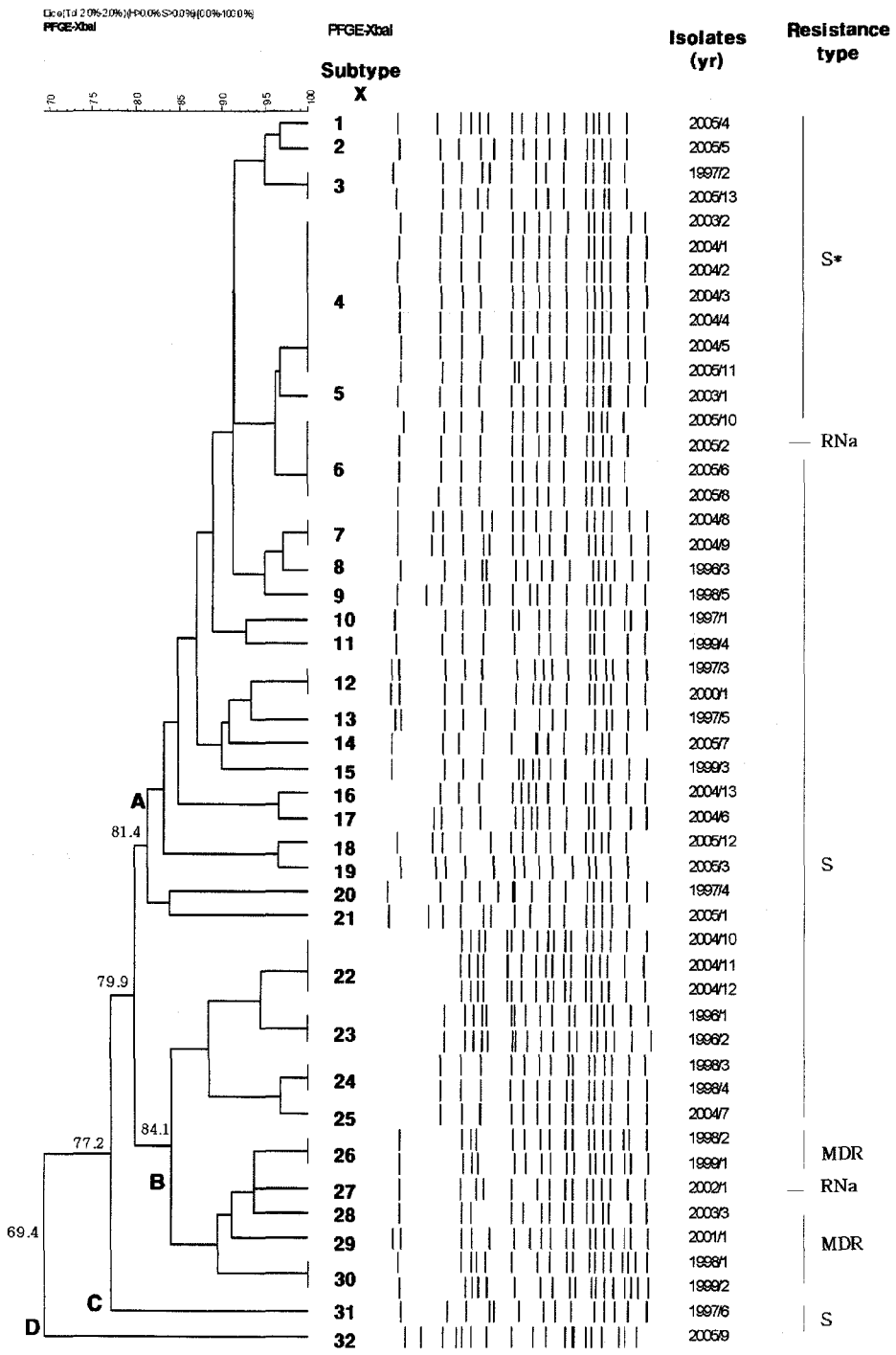

Fig. 2. Dendrogram showing similarities among the *Xba*I PFGE patterns of the 50 sporadic case *S. enterica* serovar Typhi strains. *S, susceptible; RNa, resistance to only nalidixic acid; MDR, multidrug-resistant

Typhi are stably maintained and persist over this considerable time period.

In Asia including Malaysia, Indonesia, Thailand, Taiwan, and Papua New Guinea, there have been reports describing many PFGE types in circulation[31,32,33,34,35]. In Malaysia, PFGE suggested that individual outbreaks were associated with closely related strains, whereas isolates of serovar Typhi from sporadic case were very diverse. Forty-six *Xba*I digestion patterns were found for the 60 strains of sporadic serovar Typhi from Malaysia, and nine for 10 serovar Typhi strains obtained in Thailand. Kubota *et al.* showed that *Xba*I digested genomic DNA produced 79 unique patterns associated with international travel to 31 different country. Our findings are similar to those of Thong *et. al.*[33]who found a significant genetic diversity as demonstrated PFGE fingerprint patterns within the same region.

We could find that multiple genetic variants of *S. enterica* serovar Typhi are simultaneously present in our region.

These results confirmed that PFGE is utilized in assessing clonality of *S. enterica* serovar Typhi and proved to be an useful tool for investigating local epidemics and for surveillance of sporadic case in Busan area.

In Korea, PulseNet (The national molecular subtyping network) has recently been set to develop a standardized PFGE protocol including *S. enterica* serovar Typhi isolates.

Despite the limited geographic region and sample size, our findings would be valuable in developing rational strategies to control this pathogen and setting the basis of an effective PulseNet system in our nation.

Reference

1. Ackers, M. L., N. D. Puhr, R. V. Tauxe, and E. D. Mintz. 2000. Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. *JAMA* **283**, 2668-2673.
2. Anderson, E. S., and H. R. Smith. 1972. Chloramphenicol resistance in the typhoid bacillus. *Br. Med. J.* **3**, 329-331.
3. Arbeit, R. D. 1999. Laboratory procedure for the epidemiologic analysis of microorganism, P. 116-137. *In* P. R. Murray, E. J. Barron, M. A. Pfaller, ed. American Society for Microbiology, Washington, D. C.
4. Asna, S. M., J. A. Haq, and M. M. Rahman. 2003. Nalidixic acid resistant *Salmonella enterica* serovar Typhi with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. *Jpn. J. Infect. Dis.* **56**, 32-33.
5. Chong, Y. S., K. W. Lee, D. E. Yong, J. H. Yum, Y. S. Lim, H. S. Kim, B. K. Lee. 2004. Emergence of multidrug resistant *Salmonella enterica* serovar Typhi in Korea. *Antimicrob. Agents Chemother.* **48**, 4130-4135.
6. Connerton, P., J. Wain, T. T. Hien, T. Ali, C. Parry, N. T. Chinh, H. Vinh, V. A. Ho, T. S. Diep, N. P. J. Day, N. J. White, G. Dougan, and J. J. Farrar. 2000. Epidemic Typhoid in Vietnam: Molecular Typing of multiple antibiotic resistant .*Salmonella enterica* serovar Typhi from four outbreaks. *J. Clin. Microbiol.* **38**, 895-897.
7. Edleman, R., and M. M. Levine. 1986. Summary of an international workshop on typhoid fever. *Rev. Infect. Dis.* **8**, 329-335.
8. Fica, A. E., S. Prat-Miranda, A. Fernandez-Ricci, K. D'Ottone, and F. C. Cabello. 1996. Epidemic typhoid in Chile: analysis by molecular and conventional methods of *Salmonella typhi* strain diversity in epidemic(1997 and 1981) and nonepidemic 1990 years. *J. Clin. Microbiol.* **34**, 1701-1707.
9. Gautom, R. K. 1997. Rapid pulse-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram negative organisms in 1 day. *J. Clin. Microbiol.* **35**, 2977-2980.
10. Hermans, P. W. M., S. K. Saha, W. J. van Leeuwen, H. A. Verbrugh, A. van Belkum, and W. H. F. Goessens. 1996. Molecular typing of *Salmonella typhi* strains from Dhaka (Bangladesh) and development of DNA probes identifying plasmid encoded multidrug resistant isolates. *J. Clin. Microbiol.* **34**, 1373-1379.
11. Hosoglu, S., M. Loeb, M. F. Geyik, H. Ucmak, and P. Jayaratne. 2002. Molecular epidemiology of invasive *Salmonella typhi* in southeast Turkey. *Eur. J. Clin. Microbiol. Infect. Dis.* **9**, 727-730.
12. Kariuki, S., G. Revathi, T. Muyodi, J. Mwituria, A. Munyalo, S. Mirza, and C. A. Hart. 2004. Characterization of multidrug-resistant typhoid outbreak in Kenya. *J. Clin. Microbiol.* **42**, 1477-1482.
13. Kubota, K., T. J. Barrett, M. L. Ackers, P. S. Brachman, and E. D. Mintz. 2005. Analysis of *Salmonella enterica* serotype Typhi pulsed-field gel electrophoresis patterns associated with international travel. *J. Clin. Microbiol.* **43**, 1205-1209.
14. Le, T. A. H., M. L. Collin, P. A. D. Grimont, T. L. Hoang, T. V. Nguyen, F. Grimont, and M. R. Scavizzi. 2004. Endemic, epidemic clone of *Salmonella enterica* serovar Typhi harboring a single multidrug resistant plasmid in Vietnam between 1995 and 2002. *J. Clin. Microbiol.* **42**, 3094-3099.
15. Ling, J. M., N. W. Lo, Y. M. Ho, K. M. Kam, N. T. Hoa, L. T. Phi, and A. F. Cheng. 2000. Molecular methods for the epidemiological typing of *Salmonella enterica* serotype Typhi from Hong Kong and Vietnam. *J. Clin. Microbiol.* **38**, 292-300.
16. Litwin, C. M.,A. L. Storm, S. Chipowsky, and K. J. Ryan. 1991. Molecular epidemiology of *Shigella* infections : plasmid profiles, serotype corelation, and restriction endonuclease analysis. *J. Clin. Microbiol.* **29**, 104-108.
17. Maslow, J. N., A. M. Slutsky, and R. D. Arbeit. 1993.

Application of pulsed field gel electrophoresis to molecular epidemiology, p. 563-572. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed), Diagnostic molecular microbiology: principle and applications-1993. American society for Microbiology, Washington, D.C.

18. Mills-Robertson F., M. E. Addy, P. Mensah, and S. S. Crupper. 2002. Molecular characterization of antibiotic resistance in clinical *Salmonella typhi* isolated in Ghana. *FEMS Microbiol. Lett.* **215**, 249-253.
19. Murdoch, D. A., N. A. Banatvala, A. Bone, B. I. Shoismatulloev, L. R. Ward, and J. E. Threlfall. 1998. Epidemic of ciprofloxacin resistant *Salmonella typhi* in Tajikistan. *Lancet* **351**, 339.
20. Mirza, S. H., N. J. Beeching, and C. A. Hart. 1996. Multidrug resistant typhoid: a global problem. *J. Med. Microbiol.* **44**, 317-319.
21. Mirza, S., S. Kariuki, K. Z. Mamum, N. J. Beeching, and C. A. Hart. 2000. Analysis of plasmid and chromosomal DNA of multidrug resistant *Salmonella enterica* serovar Typhi from Asia. *J. Clin. Microbiol.* **38**, 1449-1452.
22. Navarro, F., T. LLovet, M. A. Echeita, P. Coll, A. Aladuena, M. A. Usera, and G. Prats. 1996. Molecular typing of *Salmonella enterica* serovar Typhi. *J. Clin. Microbiol.* **34**, 2831-2834.
23. Nath, G., A. Tikoo, H. Manocha, A. K. Tripathi, and A. K. Gulati. 2000. Drug resistance in *Salmonella typhi* in North India with special reference to ciprofloxacin. *J. Antimicrob. Chemother.* **46**, 149-150.
24. Olarte, J., and E. Galindo. 1973. *Salmonella typhi* resistant to chloramphenicol, ampicillin and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. *Antimicrob. Agents Chemother.* **4**, 597-601.
25. Phipps, M., T. Pang, C. L. Koh, and S. Puthucheary. 1991. Plasmid incidence rate and conjugative chloramphenicol and tetracycline resistance plasmids in Malaysian isolates of *Salmonella typhi*. *Microbiol. Immunol.* **35**, 157-161.
26. Rahman, M., A. Ahmad, and S. Shoma. 2002. Decline in epidemic of multi drug resistant .*Salmonella typhi* is not associated with increased incidence of antibiotic-susceptible strain in Bangladesh. *Epidemiol. Infect.* **129**, 29-34.
27. Rowe, B., L. R. Ward, and E. J. Threlfall. 1997. Multidrug-resistant *Salmonella typhi:* a worldwide epidemic. *Clin. Infect. Dis.* 27(Suppl. 1): S106-S109.
28. Shanahan, P. M., K. A. Karamat, C. J. Thomson, and S. G. Ameyes. 1998. Molecular analysis and identification of antibiotic resistance genes in clinical isolates of *Salmonella typhi* from India. *J. Clin. Microbiol.* **36**, 1595-1600.
29. Shin, Y. H., J. S. Yoo, K. S. Oh, J. K. Lee, and K. S. Kim. 1999. Plasmid profile and β-lactamase type of multidrug resistant *Salmonella typhi* isolated from Korea, 1997. *J. Korean Soc. Microbiol.* **34**, 445-452.
30. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swamminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**, 2232-2329.
31. Thong, K. L., A. Cordano, R. M. Yassin, and T. Pang. 1996. Molecular analysis of environmental and human isolates of *Salmonella typhi*. *Appl. Environ. Microbiol.* **62**, 271-274.
32. Thong, K. L., Y. L. Goh, R. M. Yasin, M. G. Lau, M. Passey, G. Winston, M. Yoannes, T. Pang, and J. C. Reeder. 2002. Increasing genetic diversity of *Salmonella enterica* serovar Typhi isolates from Papua New Guinea over the period from 1992 to 1999. *J. Clin. Microbiol.* **40**, 4156-4160.
33. Thong, K. L., Y. M. Cheong, S. D. Puthucheary, C. L. Koh, and T. Pang. 1994. Epidemiological analysis of sporadic *Salmonella typhi* isolates and those from outbreaks by pulsed field gel electrophoresis. *J. Clin. Microbiol.* **32**, 1135-1141.
34. Thong, K. L., Puthucheary, R. M. Yassin, P. Sudarmono, M. Padmidewi, E. Soewandojo, I. Handojo, S. Sarasombath, and T. Pang. 1995. Analysis of *Salmonella typhi* isolates from Southeast Asia by pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **33**, 1938-1941.
35. Tsen. H. Y., J. S. Lin, H. H. Hu, P. R. Liu, and T. K. Wang. 1999. Use of pulsed field gel electrophoresis as an epidemiological tool for analysis of sporadic associated strains of *Salmonella typhi* isolated in Taiwan. *J. Applied Microbiol.* **86**, 761-768.
36. http://karms.nih.go.kr, January (2006).

초록 : 부산지역에서 분리된 *Salmonella enterica* serovar Typhi균에 대한 PFGE를 이용한 Molecular typing

민상기* · 이주현 · 박은희 · 김정아 · 김규원[1]
(부산광역시보건환경연구원, [1]서울대학교 약학대학)

1996년부터 2005년까지 부산지역에서 분리된 *Salmonella enterica* serovar Typhi 균주에 대한 항균제 내성 변화양상 및 Pulsed-field gel electrophoresis(PFGE)를 이용한 분리주의 분자역학적 형별을 분석하였다. 전체 424주에 대한 항균제 감수성 시험결과 multidrug-resistant (MDR) 6주(1.4%)와 nalidixic acid에만 내성을 보이는 2주를 제외한 나머지 416주(98.1%)가 시험 항균제 18종 모두에 감수성을 보였다. 부산지역 분리 장티푸스균의 유전적 이질성을 확인하고자 실시한 산발 분리 50주의 PFGE/*Xba*I 시험결과, 최소 32종의 다양한 패턴이 나타났다. 각 패턴별로 제한효소 절편 수는 13개에서 18개까지였고, 절편크기는 약 20 kb에서 630 kb 범위였다. 본 시험결과 부산지역의 장티푸스의 산발 또는 집단발생시 PFGE는 유용한 역학적 지표로 사용가능함을 알 수 있었으며 또한 전국적 PulseNet 구축의 기초 자료로서 활용도가 높을 것으로 사료된다.