TÜBİTAK

Original Article

Turk J Med Sci
2010; 40 (3): 447-458
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0904-9

Molecular analyses of *Salmonella* serotype Typhi and *Salmonella* serotype Paratyphi B strains isolated in Turkey

İştar DOLAPÇI, Birsel ERDEM, Alper TEKELİ, Ebru US, Mehseti BAYRAMOVA, Begüm SARAN, Fikret ŞAHİN

Aim: To investigate the characteristics of *Salmonella* serotype Typhi and *Salmonella* serotype Paratyphi B, which still remain significant in Turkey.

Materials and methods: Eighteen *S.* Typhi and 42 *S.* Paratyphi B strains isolated from clinical samples were investigated by plasmid profile analysis, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), and pulsed field gel electrophoresis (PFGE) methods.

Results: Plasmid profile analysis showed that 10 out of 18 (56%) *S.* Typhi isolates harbored 1 to 3 plasmids, and 11 out of 42 (26.2%) *S.* Paratyphi B isolates harbored 1 to 4 plasmids. PFGE performed with restriction enzyme *Avr*II grouped both *S.* Typhi and *S.* Paratyphi B isolates into 14 clusters; with restriction enzyme *Xba*I, *S.* Typhi and *S.* Paratyphi B isolates were grouped into 13 and 12 clusters, respectively.

Conclusion: These results showed that plasmid profile analysis and PFGE are reliable and discriminative methods complementing the phenotypic characters of *S.* Typhi and *S.* Paratyphi B isolates, and may contribute to the epidemiologic investigation of typhoid or paratyphoid diseases.

The study presented here is the first report on *S.* Typhi and *S.* Paratyphi B isolates from Turkey investigated by plasmid profile analysis, ERIC-PCR, and PFGE methods.

Key words: *Salmonella* serotype Typhi, *Salmonella* serotype Paratyphi B, plasmid profiles, ERIC-PCR, PFGE, Turkey

Türkiye'de izole edilen *Salmonella* serotip Typhi ve *Salmonella* serotip Paratyphi B suşlarının moleküler yöntemlerle incelenmesi

Amaç: Bu çalışmada amacımız Türkiye'de halen önemini korumakta olan *Salmonella* serotype Typhi ve *Salmonella* serotype Paratyphi B suşlarının özelliklerini ortaya koymaktır.

Yöntem ve gereç: Klinik örneklerden izole edilen 18 *S.* Typhi ve 42 *S.* Paratyphi B izolatı, plasmid profil analizi, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) ve pulsed field gel electrophoresis (PFGE) yöntemleri kullanılarak incelenmiştir.

Bulgular: Plazmid profil analizi sonuçları 18 *S.* Typhi izolatından 10'unun (% 56) 1-3 adet ve 42 *S.* Paratyphi B izolatının 11'inin (% 26,2) 1-4 adet plasmid taşıdığını göstermiştir.

*Avr*II kesim enzimi ile yapılan PFGE ile hem *S.* Typhi hem de *S.* Paratyphi B izolatları 14 gruba ayrılırken, *Xba*I enzimi *S.* Typhi izolatlarını 13, *S.* Paratyphi B izolatlarını ise 12 gruba bölmüştür.

Sonuç: Çalışma sonuçları hem plasmid profil analizi hem de PFGE'nin *S.* Typhi ve *S.* Paratyphi B izolatlarının karakteristiklerini ortaya koymada fenotipik özelliklerini tamamlayıcı, uygun ve ayırıcı yöntemler olduğunu göstermiştir. Aynı zamanda tifoid ve paratifoid hastalıkların epidemiyolojik araştırılmalarına katkıda bulunabilecekleri ortaya konulmuştur.

Received: 22.04.2009 – Accepted: 26.10.2009

Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Ankara University, Ankara - TURKEY

Correspondence: *İştar DOLAPÇI, Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Ankara University, Ankara - TURKEY*
E-mail: dolapci@medicine.ankara.edu.tr

Bu çalışma Türkiye'de *S.* Typhi ve *S.* Paratyphi B izolatlarının plasmid profil analizi, ERIC-PCR ve PFGE yöntemleriyle araştırıldığı ilk çalışma olma özelliğindedir.

Anahtar sözcükler: *Salmonella* serotip Typhi, *Salmonella* serotip Paratyphi B, plasmid profili, ERIC-PCR, PFGE, Türkiye

Introduction

Salmonella infections are an important public health issue in Turkey as well as in many other parts of the world. Although *Salmonella* serotype Enteritidis and *Salmonella* serotype Typhimurium are the most common serotypes isolated from clinical samples in Turkey, the incidence of *Salmonella* serotype Typhi and *Salmonella* serotype Paratyphi B is gradually increasing (1,2). *S.* Typhi and *S.* Paratyphi B are the etiologic agents of typhoid and paratyphoid fever, very severe diseases with characteristic symptoms and complications in humans (3).

Despite the high morbidity of typhoid and paratyphoid fevers in Turkey, statistical and epidemiological data of *S.* Typhi and *S.* Paratyphi B isolated in Turkey are minimal (1). All information about *Salmonella* strains isolated in Turkey is based on the findings of a few individual studies (4,5). *S.* Typhi and *S.* Paratyphi B isolates are usually defined phenotypically by serotype and antimicrobial resistance patterns in the studies in Turkey. In epidemiological studies, when the majority of isolates show similar antibiograms, this phenotyping method is not sufficiently discriminative for the subtyping of *Salmonella* strains.

In order to understand the spread of infection and identify the sources of the bacteria and the transmission chains, a few molecular typing methods have been developed. Molecular techniques such as plasmid profiling and DNA fingerprinting provide data that complement traditional methods, but their use is limited by their cost and technical difficulties (6,7). Among these molecular techniques, pulsed field gel electrophoresis (PFGE) has been widely recommended for differentiation of *Salmonella* (8) because of its discriminatory power and successful application in epidemiological investigations (9-11).

The aim of the present study was to investigate the molecular epidemiological characteristics of *S.* Typhi and *S.* Paratyphi B strains isolated in Turkey by plasmid profile, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), and PFGE patterns, and to compare the value of these methods in the differentiation of these *Salmonella* isolates.

Materials and methods

Isolates

A total of 18 *S.* Typhi and 42 *S.* Paratyphi B strains were selected from the culture collection of the Enterobacteria Laboratory of Ankara University Medical School's Department of Microbiology and Clinical Microbiology. These strains were isolated, serotyped, and tested for antimicrobial susceptibility using the agar dilution method in a previous study (2).

In the previous study, *S.* Typhi strains were isolated from various clinical samples [from blood (12), stool (4), bone marrow (1), and pus (1)] of sporadical cases in 6 Turkish provinces [Ankara (5), Eskişehir (2), İstanbul (1), İzmir (1) Kayseri (5), and Konya (4)], and *S.* Paratyphi B strains were also isolated from various clinical samples [blood (19), stool (22), and bone marrow (1)] of sporadical cases in 6 Turkish provinces [Ankara (32), Bursa (2), Eskişehir (1), İzmir (3), Konya (2), and Trabzon (2)], all after the year 2000, using standard procedures.

Plasmid extraction and profile analysis

Plasmids, extracted by the method of Kado and Liu (12) with modifications, were separated by electrophoresis on 0.7% horizontal agarose gel (Serva, Heidelberg, Germany) containing 0.5 μg mL^{-1} of ethidium bromide with 0.5× Tris-boric acid-EDTA (TBE) buffer at 100 V for 2 h. Plasmid sizes were determined by coelectrophoresis with plasmids of known sizes from *Escherichia coli* standard strain V517 (53.7, 7.2, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kb), *S.* Typhimurium strain 020255-Ankara (90 kb), and *S.* Enteritidis strain 006956-Ankara (57, 5.8, and 4.8 kb). A GeneRuler™ 1 kb DNA Ladder (Fermentas Life Sciences, Vilnius, Lithuania) was used for

determining plasmid sizes. DNA bands were visualized on an ultraviolet transilluminator (TFX 20M; Vilber Lourmat, Marne-la-Vallée, France). Control strains (*S.* Typhimurium 020255-Ankara and *S.* Enteritidis 006956-Ankara) were obtained from the Communicable Diseases Research Department, Refik Saydam National Hygiene Center, Ankara, Turkey.

ERIC-PCR

Chromosomal DNA was obtained using a commercial DNA extraction kit (NucleoSpin extraction kit; Macherey-Nagel, Düren, Germany) according to the manufacturer's guidelines. The PCR was performed using the enterobacterial repetitive intergenic consensus primer 2, ERIC-2 (AAG TAA GTG ACT GGG GTG AGC G), as described previously (13) with minor modifications. The PCR products were analyzed by electrophoresis on 1% agarose gel (Serva, Heidelberg, Germany) containing 0.5 mg mL^{-1} of ethidium bromide with 0.5× TBE buffer, visualized on an ultraviolet transilluminator (TFX 20M; Vilber Lourmat, Marne-la-Vallée, France), and photographed. The GeneRuler^{TM} 100 bp DNA Ladder Plus (MBI Fermentas, St. Leon-Rot, Germany) was used as a DNA size marker.

PFGE

PFGE was performed according to World Health Organization protocols (14). In this method, each isolate was separately digested with *Avr*II and *Xba*I macrorestriction enzymes (Fermentas, Lithuania).

Briefly, all isolates were grown overnight at 37 °C. Agarose plugs were prepared with 1.3% low melting point agarose. Genomic DNA was digested with 20 U of restriction endonucleases, *Avr*II and *Xba*I, for 4 h each, according to the manufacturer's recommendations. PFGE was performed with 1.3% agarose gel using the CHEF-DR II system (Bio-Rad, Hercules, CA, USA) at 6 V cm^{-1} and 14 °C, in 0.5× TBE for 18 h with switch times of 2.2-63.8. The gels were stained with ethidium bromide (0.2 mg mL^{-1}) and photographed under UV light. PFGE patterns were analyzed both visually as described previously by Tenover et al. (9) and with computer-assisted analysis with Gene Directory software (Syngene, Cambridge, UK). A similarity index was determined by using Dice's coefficient and the unweighted pair-group method average (UPGMA) method with 1% band tolerance.

Results

Plasmid profile analysis

The plasmid DNA of *S.* Typhi and *S.* Paratyphi B isolates was identified according to its molecular weight in kilobase pairs (kb). Plasmid profile analysis showed that 10 out of 18 (56%) *S.* Typhi isolates harbored 1 to 3 plasmids ranging in size from 1.5 to 120 kb (Table 1), and 11 out of 42 (26.2%) *S.* Paratyphi B isolates harbored 1 to 4 plasmids ranging from 1.5 to 150 kb (Table 2). Eight (44%) *S.* Typhi and 31 (73.8%) *S.* Paratyphi B isolates did not reveal any plasmids.

ERIC-PCR analyses

After ERIC-PCR with ERIC-2 primers, the 18 isolates of *S.* Typhi generated the same band patterns, with 6-10 major bands between 150 and 6000 bp. With ERIC-2 primers, all of the 42 *S.* Paratyphi B strains displayed similar band models, with 3-6 major bands between 150 and 6000 bp. It was not possible to discriminate *S.* Typhi or *S.* Paratyphi B strains with ERIC-PCR analysis.

PFGE

PFGE of chromosomal DNA from 18 *S.* Typhi isolates digested by *Avr*II and *Xba*I displayed stable and reproducible patterns consisting of 9-15 fragments between 48.5 and 1018 kb and 12-16 fragments between 48.5 and 824 kb, respectively (Figures 1a, 1b, 2a, and 2b). Fourteen different PFGE patterns of *S.* Typhi strains were observed after digestion by *Avr*II (AT1-AT14), with a range of similarity between 53% and 95% (Table 1 and Figure 1a). Using *Xba*I enzymes, *S.* Typhi isolates were grouped into 13 clusters (XT1-XT13) with a range of similarity between 68% and 95% (Table 1 and Figure 2a).

Digestion of *S.* Paratyphi B strains by *Avr*II displayed 10-15 DNA fragments ranging from approximately 48.5 to 970 kb (Figures 3a and 3b). PFGE profiles for the *S.* Paratyphi B strains using *Xba*I enzyme revealed 9-14 fragments with sizes between 48.5 and 921 kb (Figures 4a and 4b). *S.* Paratyphi B isolates were grouped into 14 clusters using *Avr*II

Table 1. PFGE patterns after restriction with *Avr*II and *Xba*I enzymes and plasmid profiles of *S.* Typhi isolates.

Isolate no.	Isolation date	City	Sample	Resistance pattern	Plasmid profile (kb)	PFGE (*Avr*II)	PFGE (*Xba*I)
1	12-04-2001	Ankara	Blood	A A/C T	-	AT3	XT8
2	17-08-2001	Kayseri	Stool	A A/C C	120 4.0 3.0	AT13	XT13
3	23-07-2001	Ankara	Blood	Susceptible	57	AT9	XT4
4	18-08-2000	Ankara	Bone marrow	Susceptible	100	AT8	XT8
5	15-04-2001	Ankara	Pus	Susceptible	-	AT7	XT12
6	18-10-2001	Ankara	Blood	Susceptible	3.5	AT11	XT9
7	20-04-2001	Kayseri	Blood	Susceptible	90	AT7	XT7
8	21-04-2001	Kayseri	Blood	Susceptible	90	AT5	XT5
9	22-04-2001	Kayseri	Blood	Susceptible	-	AT5	XT3
10	22-12-2001	Kayseri	Blood	Susceptible	13	AT11	XT9
11	20-012001	Konya	Stool	Susceptible	-	AT1	XT8
12	05-02-2001	Konya	Blood	Susceptible	-	AT1	XT2
13	06-02-2001	Konya	Blood	Susceptible	15 2.5 1.5	AT6	XT1
14	19-02-2001	Konya	Blood	Susceptible	15	AT2	XT1
15	07-01-2001	Eskişehir	Stool	Susceptible	-	AT10	XT10
16	08-01-2001	Eskişehir	Stool	Susceptible	90	AT14	XT6
17	12-09-2000	İstanbul	Blood	Susceptible	-	AT12	XT11
18	30-07-2001	İzmir	Blood	Susceptible	-	AT4	XT5

Susceptible: Susceptible to all studied antimicrobials.

A A/C T: Resistant to ampicillin, amoxicillin/clavulanic acid, and tetracycline.

A A/C C: Resistant to ampicillin, amoxicillin/clavulanic acid, and chloramphenicol.

enzyme (AP1-AP14) with a range of similarity between 30% and 80% (Table 2 and Figure 3a). Using *Xba*I enzyme, the isolates were grouped into 12 clusters (XP1-XP12) with a range of similarity between 24% and 69% (Table 2 and Figure 4a).

Discussion

Salmonellosis is one of the most significant public health problems in developing countries, and despite measures taken for its control it still affects large numbers of people. The importance of this infection has led to the creation of instruments for monitoring and controlling these processes (6).

S. Typhi and *S.* Paratyphi B isolates are usually defined phenotypically by serotype and antimicrobial resistance patterns in studies in Turkey. Since most *S.* Typhi and *S.* Paratyphi B strains isolated in Turkey are susceptible to all tested antimicrobials (15), the phenotypic typing method based on antibiogram (R-typing) is not useful in the discrimination of *S.* Typhi isolates. Epidemiological studies require the use of more efficient methods to trace precisely the spread of the strains (2).

Characterization of *S.* Typhi and *S.* Paratyphi B isolates traditionally depended on phage typing. However, this method is not very discriminating and would not provide useful epidemiological information if there were only a few predominating phage types. Moreover, acquisition or loss of lysogenic phages might result in a change in the phage type. Furthermore, the requirement for specialized phage collections and bacterial strains for their propagation has resulted in a protocol that is routinely practiced

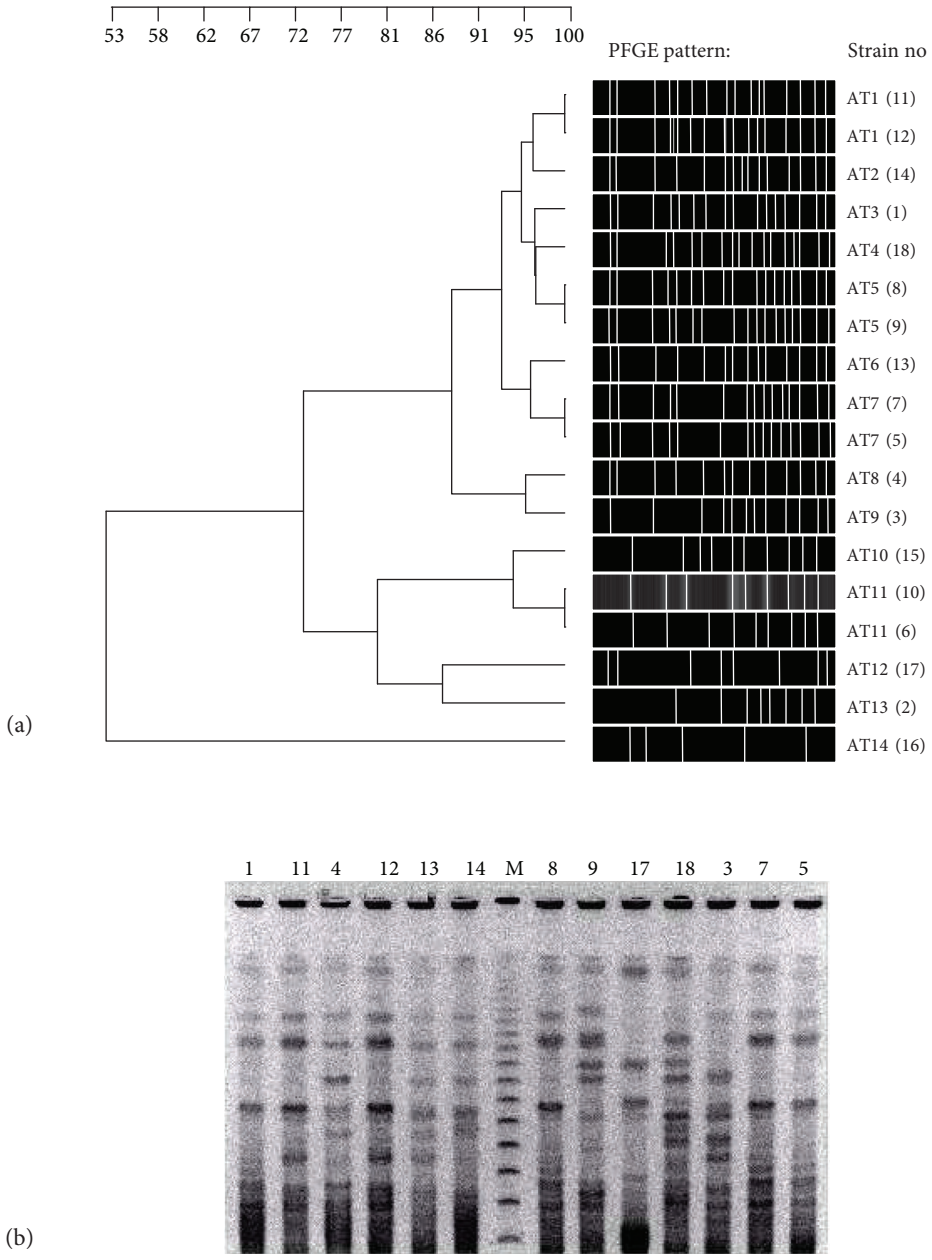
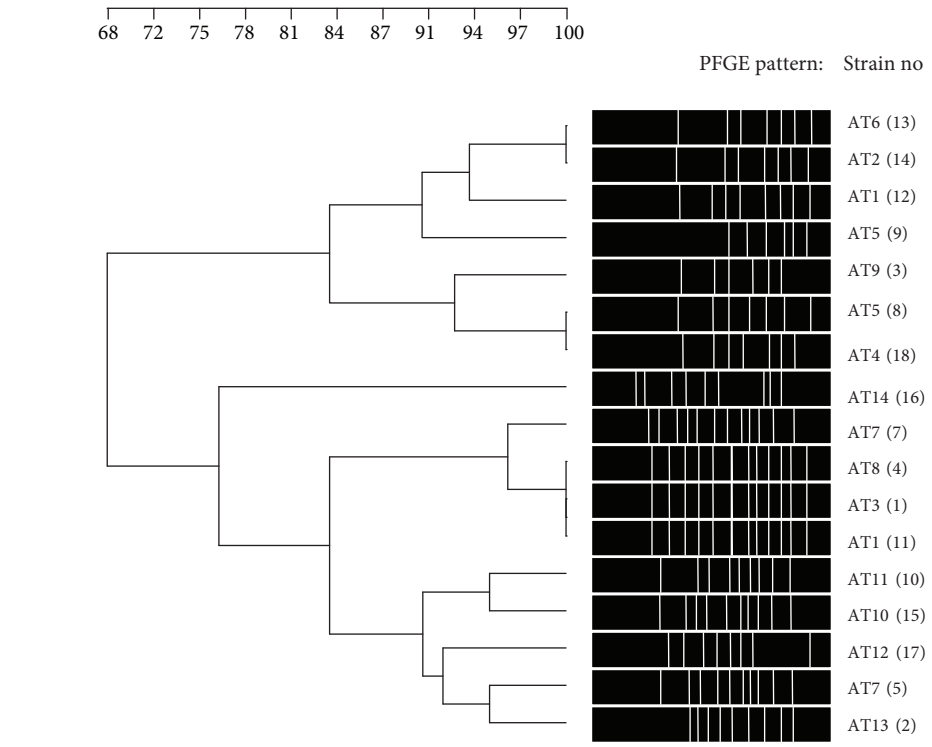


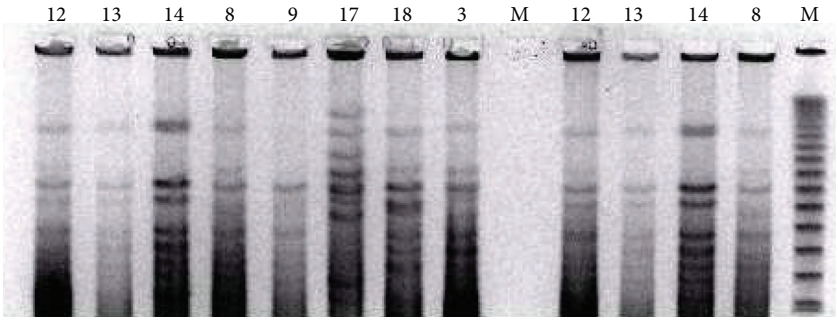
Figure 1. (a) Dendrogram of PFGE *Avr*II band patterns of *S*. Typhi isolates generated by Gene Directory software using the unweighted pair group with arithmetic mean (UPGMA) method.
(b) Examples of representative PFGE fingerprint patterns of *S*. Typhi strains after restriction with *Avr*II enzyme. 1, 11, 4, 12-14, 8, 9, 17, 18, 3, 7, and 5: strain numbers; M: lambda ladder PFGE marker (N0340S, New England Biolabs, Hertfordshire, UK; band size: 48.5-1018.5 kb).

in only a few reference laboratories. There is no reference center for phage typing of *Salmonella* isolates in Turkey.

During the last decade, traditional methods of strain typing have been supplemented or replaced in many laboratories with newer molecular methods (9).



(a)



(b)

Figure 2. (a) Dendrogram of PFGE *Xba*I band patterns of *S*. Typhi isolates generated by Gene Directory software using the unweighted pair group with arithmetic mean (UPGMA) method.
(b)Examples of representative PFGE fingerprint patterns of *S*. Typhi strains after restriction with *Xba*I enzyme. 12-14, 8, 9, 17, 18, 3, 12, 13, 14, and 8: strain numbers; M: lambda ladder PFGE marker (N0340S, New England Biolabs, Hertfordshire, UK; band size: 48.5-1018.5 kb).

In this study, *S*. Typhi and *S*. Paratyphi B clinical isolates from various Turkish provinces were investigated by plasmid profile analyses, ERIC-PCR, and PFGE.

Plasmid profile analysis is based on the numbers and molecular weights of plasmids after extraction and electrophoresis of plasmid DNA. This method is restricted to strains possessing plasmids. Plasmid profiles are not very useful for subtyping *Salmonella* strains when strains do not carry plasmids or possess only serotype-specific plasmids, or when only a few of the strains harbor plasmids (16,17).

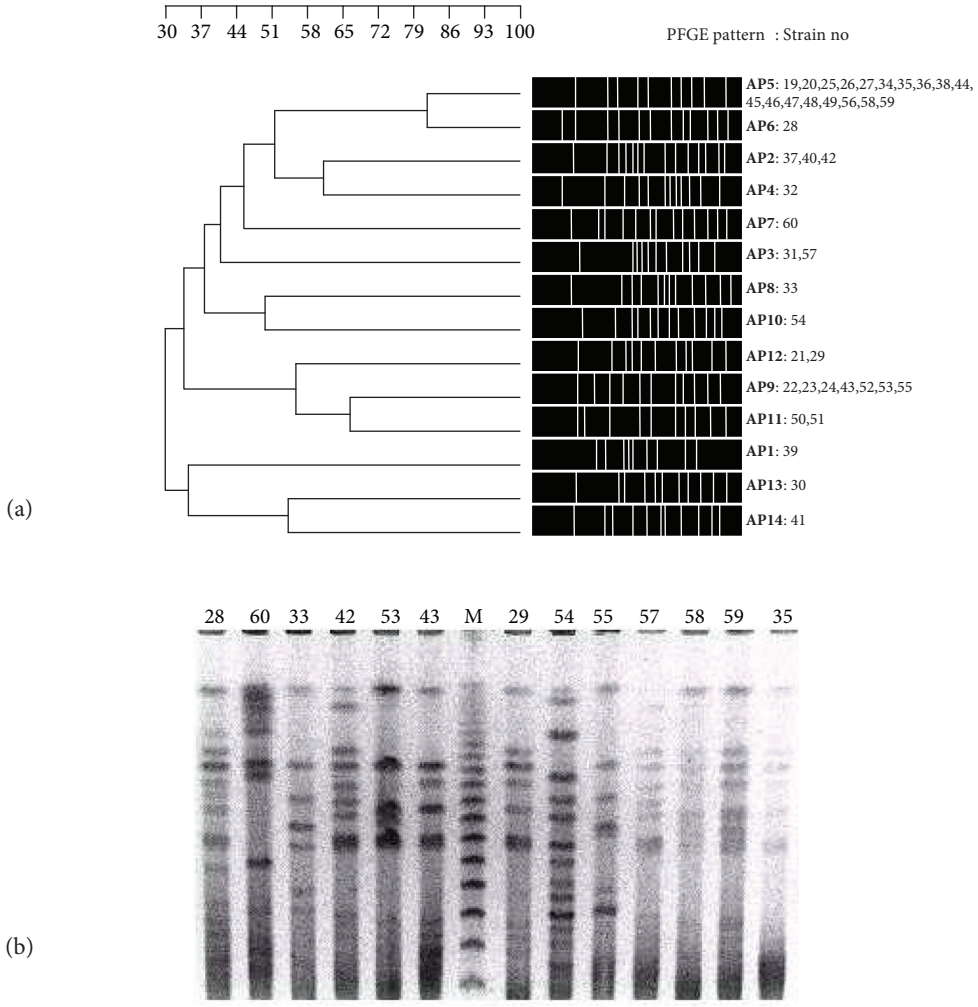


Figure 3. (a) Dendrogram of PFGE *Avr*II band patterns of *S.* Paratyphi B isolates generated by Gene Directory software using the unweighted pair group with arithmetic mean (UPGMA) method.
(b) Examples of representative PFGE fingerprint patterns of *S.* Paratyphi B strains after restriction with *Avr*II enzyme. 28, 60, 33, 42, 53, 43, 29, 54, 55, 57-59, and 35: strain numbers; M: lambda ladder PFGE marker (N0340S, New England Biolabs, Hertfordshire, UK; band size: 48.5-1018.5 kb).

According to Quintaes et al. (18), a total of 36.7% of the 30 *S.* Typhi isolates showed plasmids, and it was possible to distinguish 4 different profiles. Molecular weights of plasmids varied from 4.2 to 105 kb, but plasmids of 16.5 and 105 kb were prevalent. The majority of strains (63.3%) did not show any plasmids, even in a second analysis. According to Le et al. (19), 107 multidrug-resistant (MDR) serotype Typhi isolates were investigated; their transconjugants harbored a large plasmid (184.8 ± 8.2 kb), and 50 of these serotype Typhi isolates contained a second plasmid (94.6 ± 3.5 kb). They also showed that multidrug resistance was associated with 184.8 ± 8.2 kb self-transferable plasmids.

In this study, 22 of 60 (36.6%) strains examined had 1 to 4 plasmids. Plasmid profile analyses showed that 10 of 18 (56%) *S.* Typhi isolates harbored 1 to 3 plasmids ranging in sizes from 1.5 to 120 kb, and 11 of 42 (26.2%) *S.* Paratyphi B isolates harbored 1 to 4 plasmids ranging from 1.5 to 150 kb. Eight (44%) *S.* Typhi and 31 (73.8%) *S.* Paratyphi B isolates did not reveal any plasmids.

Table 2. PFGE patterns after restriction with *Avr*II and *Xba*I enzymes and plasmid profiles of *S.* Paratyphi B isolates.

Isolate No.	Isolation date	City	Sample	Resistance pattern	Plasmid profile (kb)		PFGE (*Avr*II)	PFGE (*Xba*I)
19	05-11-2001	Ankara	Stool	Susceptible	-		AP5	XP7
20	13-11-2001	Ankara	Stool	Susceptible	-		AP5	XP7
21	16-10-2001	Ankara	Stool	Susceptible	-		AP12	XP7
22	04-12-2001	Ankara	Stool	Susceptible	-		AP9	XP7
23	24-09-2002	Ankara	Blood	Susceptible	-		AP9	XP10
24	28-07-2003	Ankara	Blood	Susceptible	-		AP9	XP7
25	31-08-2000	Ankara	Blood	Susceptible	-		AP5	XP7
26	31-08-2000	Ankara	Stool	Susceptible	-		AP5	XP7
27	12-09-2000	Ankara	Blood	Susceptible	-		AP5	XP7
28	18-10-2000	Ankara	Blood	Susceptible	85		AP6	XP7
29	18-09-2001	Ankara	Blood	Susceptible	-		AP12	XP7
30	10-08-2003	Ankara	Blood	Susceptible	-		AP13	XP7
31	28-08-2000	Konya	Stool	Susceptible	57		AP3	XP2
32	26-09-2000	Konya	Stool	Susceptible	-		AP4	XP3
33	06-04-2001	İzmir	Stool	Susceptible	150, 90, 80		AP8	XP5
34	01-09-2000	Eskişehir	Stool	Susceptible	90		AP5	XP7
35	29-08-2001	Ankara	Stool	A	-		AP5	XP7
36	25-09-2001	Ankara	Blood	A		20	AP5	XP7
37	25-12-2001	Ankara	Stool	A	-		AP2	XP7
38	01-02-2005	Ankara	Stool	AA/C	110, 90,	5.0	AP5	XP7
39	04-09-2000	İzmir	Stool	T/S		7.5, 7.0	AP1	XP1
40	09-10-2000	İzmir	Stool	T	90,	20, 5.0, 1.5	AP2	XP3
41	01-02-2005	Ankara	Stool	T		5.0	AP14	XP11
42	21-08-2001	Ankara	Blood	C	110, 90		AP2	XP6
43	17-09-2001	Ankara	Blood	C	-		AP9	XP12
44	01-11-2001	Ankara	Stool	C	-		AP5	XP7
45	30-10-2001	Ankara	Blood	C	-		AP5	XP7
46	30-10-2001	Ankara	Blood	C	-		AP5	XP7
47	09-11-2001	Ankara	Blood	C	-		AP5	XP7
48	13-10-2001	Ankara	Blood	C	-		AP5	XP6
49	13-11-2001	Ankara	Stool	C	-		AP5	XP7
50	27-09-2001	Trabzon	Bone marrow	C	-		AP11	XP9
51	27-09-2001	Trabzon	Stool	C	-		AP11	XP7
52	05-09-2000	Ankara	Blood	AC	-		AP9	XP9
53	28-06-2001	Ankara	Blood	CT	-		AP9	XP7
54	24-04-2001	Bursa	Stool	AT	-		AP10	XP8
55	21-07-2001	Bursa	Stool	AT	120		AP9	XP7
56	27-07-2001	Ankara	Stool	AT	-		AP5	XP7
57	24-08-2001	Ankara	Blood	ACT	-		AP3	XP7
58	28-08-2001	Ankara	Blood	ACT	-		AP5	XP7
59	23-09-2001	Ankara	Blood	ACT	-		AP5	XP7
60	22-11-2000	Ankara	Stool	AA/CCT	-		AP7	XP4

Susceptible: Susceptible to all studied antimicrobials.

A: Resistant to ampicillin, A/C: Resistant to amoxicillin/clavulanic acid,

T/S: Resistant to trimethoprim sulfamethoxazole, T: Resistant to tetracycline, C: Resistant to chloramphenicol.

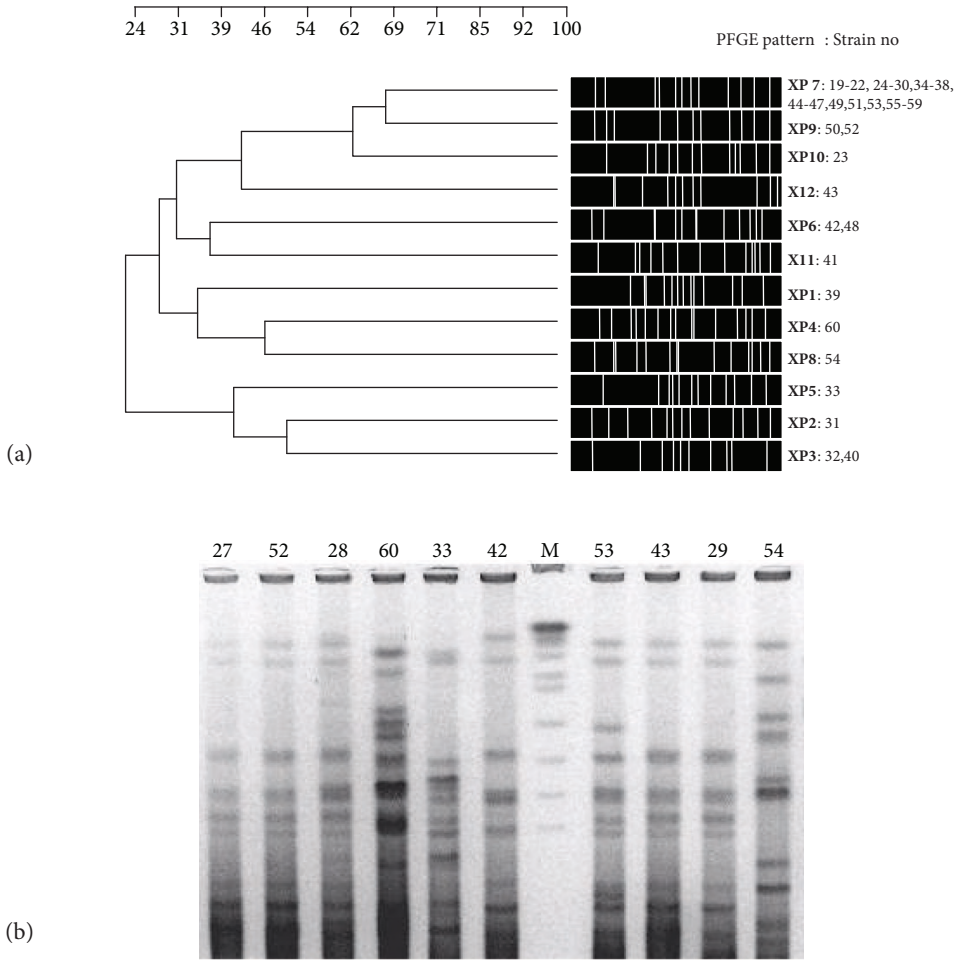


Figure 4. (a) Dendrogram of PFGE *Xba*I band patterns of *S.* Paratyphi B isolates generated by Gene Directory software using the unweighted pair group with arithmetic mean (UPGMA) method.
(b) Examples of representative PFGE fingerprint patterns of *S.* Paratyphi B strains after restriction with *Xba*I enzyme. 27, 52, 28, 60, 33, 42, 53, 43, 29, and 54: strain numbers; M: lambda ladder PFGE marker (N0340S, New England Biolabs, Hertfordshire, UK; band size: 48.5-1018.5 kb).

Our study revealed 8 different plasmid profiles among *S.* Typhi strains, and 7 plasmid profiles were shown among antimicrobial susceptible *S.* Typhi strains. Different plasmid profiles were observed in each of the 11 *S.* Paratyphi B strains carrying plasmids, and the *S.* Paratyphi B isolates with similar antimicrobial resistance patterns also harbored different plasmids. These results indicate that there is no relation between antimicrobial resistance and plasmids. Therefore, for the subtyping of *S.* Typhi and *S.* Paratyphi B, it would be useful to examine antibiotic resistance and plasmid profiles of the isolates together.

In this study, ERIC-PCR was performed using ERIC-2 primers. Since isolates within the Typhi and Paratyphi B serotypes showed similar band models, we came to the conclusion that the technique of ERIC-PCR with ERIC-2 primers is not suitable for differentiating the Turkish strains of *S.* Typhi and *S.* Paratyphi B or for grouping the isolates into clusters.

PFGE, which separates by size DNA fragments produced by digestion with restriction enzymes

targeting specific nucleotide sequences, has been a standard whole genome typing method for bacteria (9). PFGE has been adopted by the Centers for Disease Control as the standard for *Salmonella* genotyping. In comparison with other methods, several authors have specifically recommended using PFGE for *Salmonella* genotyping (10,20-24).

Use of PFGE with endonuclease *Xba*I has been widely recognized as a sensitive means of fingerprinting *Salmonella* serotypes. It was demonstrated that macrorestriction analysis with 2 enzymes was a more discriminatory method for subtyping *Salmonella* (11).

Among 11 *S.* Typhi isolates that were typed with PFGE after digestion by *Xba*I, Le et al. (19) demonstrated that 4 patterns were observed and that the number of DNA fragments obtained by *Xba*I digestion by PFGE ranged between 11 and 13. Navarro et al. (25) showed that 48 sporadic *S.* serotype Typhi isolates, after digestion with *Xba*I, generated 38 different PFGE patterns and 15-21 DNA fragments, whereas, in our study, among 18 isolates, 14 different PFGE patterns of *S.* Typhi strains were observed after digestion by *Avr*II and 13 patterns were observed after digestion by *Xba*I; 9-15 and 12-16 DNA fragments were observed, respectively.

In this study, a total of 60 isolates of *S.* Typhi and *S.* Paratyphi B obtained from various clinical samples of sporadical cases were analyzed by PFGE using *Avr*II and *Xba*I macrorestriction enzymes. *S.* Typhi strains generated 14 different PFGE patterns after digestion with macrorestriction enzyme *Avr*II, while *S.* Paratyphi B strains also generated 14 different PFGE patterns. *S.* Typhi strains generated 13 different PFGE patterns after digestion with macrorestriction enzyme *Xba*I, while *S.* Paratyphi B strains generated 12 different PFGE patterns. Consequently, *S.* Typhi and *S.* Paratyphi B isolates were divided into 17 and 22 distinct PFGE cluster profiles (such as AT3-XT8 and AP5-XP7), respectively, obtained using both enzymes (Tables 1 and 2). Thus, the isolates that have the same resistance type and harbor the same plasmids were differentiated easily by the PFGE patterns of these 2 restriction enzymes.

When antimicrobial resistance patterns, plasmid profiles, and the PFGE patterns of the 2 enzymes were used together, nearly all isolates could be differentiated. This was not an unexpected result, since these strains were obtained from sporadic cases.

While a total of 5 *S.* Typhi strains from Ankara showed 5 different patterns of PFGE with 2 enzymes, 5 isolates from Kayseri revealed 5 different patterns and 4 Konya strains showed 4 different PFGE patterns. These data reveal that *S.* Typhi strains isolated in Turkey show genomic variety.

The 2 strains (numbers 6 and 10) that showed similar PFGE models (AT11-XT9) among *S.* Typhi isolates were isolated from blood cultures in Ankara and Kayseri during a time span of 2 months. Both were susceptible to antimicrobials, and while one of them carried a 3.5 kb plasmid, the other carried a 13 kb plasmid (Table 1).

Goh et al. (26) observed 22 *Xba*I-pulsotypes among 65 D-tartrate-negative (dT-) strains and 17 *XbaI*-pulsotypes among 22 D-tartrate-positive (dT+) strains of *S.* Paratyphi B isolates. According to Weill et al. (27), PFGE, using *Xba*I, subtyped 49 *S.* Paratyphi B strains into 14 pulsotypes, and the *Xba*I patterns were characterized by 10-15 fragments ranging in size from <30 kb to >700 kb. Meanwhile, in our study, among 42 isolates, 14 different PFGE patterns of *S.* Paratyphi B strains were found after digestion by *Avr*II and 12 patterns were found after digestion by *Xba*I; 10-15 and 9-14 DNA fragments ranging from approximately 48.5 to 970 kb and 48.5 to 921 kb were observed, respectively.

Among *S.* Paratyphi B strains, 22 PFGE models were revealed by using 2 enzymes. Among these PFGE models the AP5-XP7 model was the most prominent and 17 isolates shared it. Table 2 shows that 3 of the 17 isolates that showed AP5-XP7 model had plasmids. However, they showed different plasmid profiles. Strain number 34 carried 90 kb sized plasmid, strain number 36 carried 20 kb sized plasmid, and strain number 38 carried 110, 90, and 5.0 kb sized plasmids (Table 2). Furthermore, their antimicrobial resistance models were rather different. Four strains of *S.* Paratyphi B (numbers 22, 24, 53, and 55) showed another PFGE model, AP9-XP7. Their antimicrobial resistance models were also the same. Only one of them (number 55) carried a 120 kb plasmid. Two strains (numbers 21 and 29) that had

the same PFGE model (AP12-XP7) had similar characteristics and could not be separated from each other. These strains were isolated in Ankara during the span of a month, were both susceptible to antimicrobials, and carried no plasmids (Table 2). A total of 32 strains isolated in Ankara were distributed among 16 different PFGE patterns by using 2 enzymes. These findings showed the genetic variety of *S.* Paratyphi strains in Turkey.

These results showed that plasmid profile analysis and PFGE are reliable methods for subtyping *S.* Typhi and *S.* Paratyphi B strains and could be used as discriminative methods complementing antibiograms. Combining these 3 methods would be useful for the investigation of outbreak epidemiology.

Acknowledgements

This study was supported by TÜBİTAK, the Scientific and Technological Research Council of Turkey (Project no: 106S354 SBAG-HD-201).

We thank the members of the Turkish Salmonella Study Group (Coordinator: B. Erdem), along with participating hospitals, as follows: A.D. Aysev, Ankara University School of Medicine; G. Hasçelik, D. Gür, and S. Erciş, Hacettepe University School of Medicine; S. Gedikoğlu, Uludağ University School of Medicine; B. Sümerkan and D. Esel, Erciyes University School of Medicine; I. Tuncer, Selçuk University School of Medicine; M. Tuğrul and M. Tatman-Otkun, Trakya University School of Medicine; A. Tünger, Ege University School of Medicine; Y. Akgün, Osmangazi University School of Medicine; N. Acar, Ankara Teaching Hospital, Ministry of Health; M. Gültekin, Akdeniz University School of Medicine; İ. Köksal, Karadeniz University School of Medicine; and G. Söyletir, Marmara University School of Medicine, for isolating and sending the strains to the coordinating center of the study.

We also thank Dr. B. Esen and B. Levent from the Communicable Diseases Research Department, Refik Saydam National Hygiene Center, Ankara, Turkey, for kindly providing the control strains of *Salmonella* Braenderup (H9812), *Salmonella* Typhimurium (020255-Ankara), and *Salmonella* Enteritidis (006956-Ankara).

References

1. Toreci K, Ang O. Türkiye'de saptanmış olan Salmonella serovarları ve Salmonellozların genel değerlendirilmesi. Türk Mikrobiyol Cem Derg 1991; 21: 1-18.
2. Erdem B, Ercis S, Hasçelik G, Gür D, Gedikoğlu S, Aysev AD et al. Antimicrobial resistance patterns of Salmonella enterica serotypes isolated from humans in Turkey, 2000-2002. Eur J Clin Microbiol Infect Dis 2005; 24: 220-5.
3. Janda JM, Abbott SL. The Enterobacteriaceae. Philadelphia: Lippincott-Raven; 1998.
4. Erdem B, Hasçelik G, Gedikoğlu S, Gür D, Erciş S, Sümerkan B et al. Salmonella enterica serotipleri ve Salmonella enfeksiyonları: Türkiye'de on ili kapsayan cok merkezli bir çalışma. Mikrobiyol Bul 2004; 38: 173-86.
5. Hosoglu S, Loeb M, Geyik MF, Uçmak H, Jayaratne P. Molecular epidemiology of invasive Salmonella typhi in southeast Turkey. Clin Microbiol Infect 2003; 9: 727-30.
6. Ruiz M, Rodriguez JC, Sirvent E, Escribano I, Cebrian L, Royo G. Usefulness of different techniques in the study of the epidemiology of salmonellosis. APMIS 2003; 111: 848-56.
7. Woo YK. Finding the sources of Korean *Salmonella enterica* serovar Enteritidis PT4 isolates by pulsed-field gel electrophoresis. J Microbiol 2005; 43: 424-9.
8. Threlfall EJ, Powell NG, Rowe B. Differentiation of *Salmonellas* by molecular methods. PHLS Microbiol Dig 1994; 11: 1999-202.
9. Tenover FC, Arbeit RD, 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.
10. Liebana E, Garcia-Migura L, Breslin MF, Davies RH, Woodward MJ. Diversity of strains of *Salmonella enterica* serotype Enteritidis from English poultry farms assessed by multiple genetic fingerprinting. J Clin Microbiol 2001; 39: 154-61.
11. Cardinale E, Gros-Claude P, Rivoal K, Rose V, Tall F, Mead GC et al. Epidemiological analysis of *Salmonella enterica* ssp. *enterica* serovars Hadar, Brancaster and Enteritidis from humans and broiler chickens in Senegal using pulsed-field gel electrophoresis and antibiotic susceptibility. J Appl Microbiol 2005; 99: 968-77.
12. Kado CI, Liu ST. Procedure for detection of large and small plasmids. J Bacteriol 1981; 145: 1365-73.
13. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucl Acid Res 1991; 19: 6823-31.
14. Hendriksen RS, editor. Global Salm-Surv: A global *Salmonella* surveillance and laboratory support project of the World Health Organization. 5th ed. 2004.

15. Erdem B, Ercis S, Hascelik G, Gur D, Aysev AD. Antimicrobial resistance of *Salmonella enterica* group C strains isolated from humans in Turkey, 2000-2002. Int J Antimicrob Agents 2005; 26: 33-7.

16. Helmuth R, Stephan R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect Immun 1985; 48: 175-82.

17. Erdem B, Threlfall EJ, Schofield SL, Ward LR, Rowe B. Plasmid profile typing provides a method for the differentiation of strains of *Salmonella enteritidis* phage type 4 isolated in Turkey. Lett Appl Microbiol 1994; 19: 265-7.

18. Quintaes BR, Leal NC, Reis EM, Fonseca EL, Hofer E. Conventional and molecular typing of Salmonella typhi strains from Brazil. Rev Inst Med Trop Sao Paulo 2002; 44: 315-9.

19. Le TA, Lejay-Collin M, Grimont PA, Hoang TL, Nguyen TV, Grimont F et al. Endemic, epidemic clone of Salmonella enterica serovar typhi harboring a single multidrug-resistant plasmid in Vietnam between 1995 and 2002. J Clin Microbiol 2004; 42: 3094-9.

20. Weide-Botjes M, Kobe B, Lange C, Schwarz S. Molecular typing of *Salmonella enterica* subsp. *enterica* serovar Hadar: evaluation and application of different typing methods. Vet Microbiol 1998; 61: 215-27.

21. Garaizar J, Lopez-Molina N, Laconcha I, Baggesen DL, Rementeria A, Vivanco A et al. Suitability of PCR fingerprinting, infrequent-restriction site PCR, and pulsed-field gel electrophoresis, combined with computerized gel analysis, in library typing of *Salmonella enterica* serovar Enteriditis. Appl Environ Microbiol 2000; 66: 5273-81.

22. Punia P, Hampton MD, Ridley AM, Ward LR, Rowe B, Threlfall EJ. Pulsed-field electrophoretic fingerprinting of *Salmonella indiana* and its epidemiologic applicability. J Appl Microbiol 1998; 84: 103-7.

23. Murakami K, Horikawa K, Otsuki K. Epidemiological analysis of *Salmonella enteritidis* from human outbreaks by pulsed field gel electrophoresis. J Vet Med Sci 1999; 61:439-42.

24. Weigel RM, Qiao B, Teferedegne B, Suh DK, Barber DA, Isaacson RE et al. Comparison of pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of genetic diversity and inferring transmission of *Salmonella*. Vet Microbiol 2004; 100: 205-17.

25. Navarro F, Llovet T, Echeita MA, Coll P, Aladueña A, Usera MA et al. Molecular typing of *Salmonella enterica* serovar typhi. J Clin Microbiol 1996; 34: 2831-4.

26. Goh YL, Yasin R, Puthucheary SD, Koh YT, Lim VK, Taib Z et al. DNA fingerprinting of human isolates of *Salmonella enterica* serotype Paratyphi B in Malaysia. J Appl Microbiol 2003; 95: 1134-42.

27. Weill FX, Fabre L, Grandry B, Grimont PA, Casin I. Multiple-antibiotic resistance in *Salmonella enterica* serotype Paratyphi B isolates collected in France between 2000 and 2003 is due mainly to strains harboring *Salmonella* genomic islands 1, 1-B, and 1-C. Antimicrob Agents Chemother 2005; 49: 2793-801.