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Genotypic Characterization of *Salmonella enterica* Serotype Enteritidis Isolated from Food-Poisoning Cases and Chickens by Pulsed Field Gel Electrophoresis

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A total of 22 *Salmonella enterica* serotype Enteritidis (*S.* Enteritidis) isolates from human and chicken sources were analyzed by pulsed field gel electrophoresis (PFGE) using *Xba*I restriction enzyme to assess the genetic relationships between strains from different sources. PFGE permitted the resolution of *Xba*I restriction fragments of the 22 *S.* Enteritidis into 6 distinct PFGE types (PFT), designated PFT1 to PFT6, and 2 subtypes within PFT2, and allowed to detect between 9 and 10 bands with fragments sizes in the range of 25~635 kb. Four of twelve isolates from human showed an identical PFGE patterns with 2 isolates from chickens. Also, another one isolate from human showed an identical PFGE patterns with other 5 isolates from chickens. Only one isolate from chicken, however, showed a different pattern compared to other PFTs. These results suggested that sporadic human food-poisoning cases infections caused by *S.* Enteritidis in this study were due to the consumption of contaminated chicken meats and that a clonally highly similar strains exist and spread between human and chicken sources.

Key Words: *Salmonella* Enteritidis, Pulsed field gel electrophoresis (PFGE), Food poisoning, Chickens

INTRODUCTION

Non-typhoidal *Salmonella enterica* are of increasing public health concern as food poisoning worldwide in humans and are widespread in animals as well. Also, infections caused by these organisms can produce symptoms ranging in severity from intestinal disturbances to death, especially in neonates and immunocompromised patients (Miller et al., 1995). *Salmonella* serotype Enteritidis (*S.* Enteritidis) and *Salmonella* Typhimurium (*S.* Typhimurium) are two major etiologic agents of food-borne salmonellsis in human. Also, *S.* Enteritidis is the most commonly isolated *Salmonella* serotype in many countries, which was found to be mainly implicated with the consumption of eggs and egg products (Boonmar et al., 1998; Molbak et al., 2002).

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Bacterial strain typing at the subspecific level is an essential tool for public health and the basic research of its molecular epidemiology and evolutionary biology (Johnson and O'bryan, 2000). Several molecular subtyping methods, including ribotyping (Olsen et al., 1994), phage typing (Laconcha et al., 1997), repetitive sequence (rep)-PCR (Weigel et al., 2004) and Pulsed field gel electrophoresis (PFGE) (Thong et al., 1998) have been used to characterize the molecular epidemiology of *Salmonella enterica*. PFGE has recently been applied to epidemiological investigation of *S.* Enteritidis, and provided a useful indicator of the genotypic diversity level between strains from food-born outbreaks (Tsen and Lin, 2001). It involves the use of rare-cutter restriction enzymes to generate a limited number of high molecular weight restriction fragments. These fragments are then separated by agarose gel electrophoresis with programmed variations in both the direction and the duration of the electric field. This study describes the genotypic characterization of *S.* Enteritidis using PFGE isolated from humans and chickens in Korea.

MATERIALS AND METHODS

1. Bacterial strains

A total of 22 *S.* Enteritidis strains isolated in the two-year period from 2001 to 2002 were studied (Table 1). Twelve isolates were isolated from patients or samples collected from food-poisoning cases. Ten non-human isolates were recovered from the caecum of slaughtered chickens. All isolates had been identified as *S.* Enteritidis (Miller et al., 1995).

2. Preparation of genomic DNA

Intact genomic DNA from *S.* Enteritidis isolates was prepared in agarose plugs using a CHEF bacterial genomic DNA plug kit (Bio-Rad, USA) with some modifications. Briefly, bacteria were grown on tryptic soy agar plates at 37℃ for 24 h. Cells were suspended in cell suspension buffer, and the cell density was adjusted to within a range of 7 using colorimeter (bioMerieux, France). An equal volume of molten 1.2% SeaKem Gold agarose (FMC bioproducts, USA) was mixed with 100 μl of bacterial cells, and the mixture was dispensed into 1.5-mm thick disposable molds (Bio-Rad, USA). The agarose plugs were treated with 1 mg of lysozyme sol per ml for 2 h at 37℃. The plugs were transferred to lysis buffer containing 1 mg of proteinase K per ml and 1 mM EDTA (pH 9.0) after rinsing once with sterile water, and the plugs were incubated overnight at 50℃ without agitation. After proteolysis, the plugs were washed four times in 1x washing buffer, 1 h each at room temperature with gentle agitation. Unless used immediately, the plugs were stored at 4℃.

3. PFGE

Before digestion with restriction enzyme, the plugs were washed once again with 0.1x wash buffer for 1 h. This last wash reduces the EDTA concentration, allowing for faster buffer equilibration with restriction enzyme buffers. High-molecular-weight *S.* Enteritidis DNA in 1.5 mm plug slices were digested with *Xba*I (Takara, Japan) in buffer solutions according to the manufacturer's instructions. Restriction was done at an enzyme concentration of 50 U per plug for 5 h at 30℃. The samples were run on an 1% SeaKem Gold agarose gel (FMC bioproducts, USA) using CHEF-mapper system (Bio-Rad, USA) under the following conditions: temperature 14℃; initial switch time, 2.16 s; final switch time, 63.8 s; run time, 18 h; angel 120°; gradient, 6.0 V/cm; ramping factor, linear. Lambda ladder I (Bio-Rad, USA) was used as fragment size marker. After electrophoresis the gels were stained for 15 min in 250 ml of deionized water containing 25 μl of ethidium bromide (10 mg/ml) and destained by two washes of 20~30 min each using 500 ml of deionized water.

Table 1. Characteristics and PFGE analysis of *Xba*I-digested genomic DNA from 22 *Salmonella* Enteritidis strains

Strain	Sources	PFGE types (PFTs)
SH01	Human	6
SH02	Human	6
SH03	Human	6
SH04	Human	6
SH05	Human	2b
SH06	Human	6
SH07	Human	6
SH08	Human	2b
SH09	Human	3
SH10	Human	2b
SH11	Human	2a
SH12	Human	2b
SC01	Chicken	2b
SC02	Chicken	2a
SC03	Chicken	2a
SC04	Chicken	2a
SC05	Chicken	5
SC06	Chicken	1
SC07	Chicken	2a
SC08	Chicken	4
SC09	Chicken	2b
SC10	Chicken	2a

4. Pattern analysis

A dendrogram was constructed with Analysis software (Biometra, Germany). The patterns were compared by means of the Dice coefficient of band-based similarity by unweighted pair group method using averages (UPGMA); a tolerance of 5% in the band position was applied. Any nonidentity in the presence, absence, or apparent mobility of a band was considered one difference from the pattern of the strain. Variation in band intensity was not counted as a difference.

RESULTS

PFGE permitted the resolution of *Xba*I restriction fragments of the 22 *S.* Enteritidis into 6 distinct PFGE types (PFT), designated PFT1 to PFT6, and 2 subtypes within PFT2 (Fig. 1). *Xba*I allowed them to detect between 9 and 10 bands with fragments sizes in the range of 25~635 kb. Two PFTs, PFT2 and PFT6, predominated (Fig. 2 and 3). PFT2 has been identified in isolates from both food-poisoning cases and chickens. PFT2 was divided into two subtypes, PFT2a and PFT2b. PFT2a could be distinguished from PFT2b by an additional fragment of approximately 696 kb. PFT2a comprised 6 isolates, 5 from chickens and 1 from human outbreaks, and PFT2b comprised 6 isolates, 4 from human outbreaks and 2 from chickens, respectively. PFT6, however, comprised only isolates from humans (Table). Other 4 PFTs were represented by single isolate each, of which PFT1 was most remote related with others.

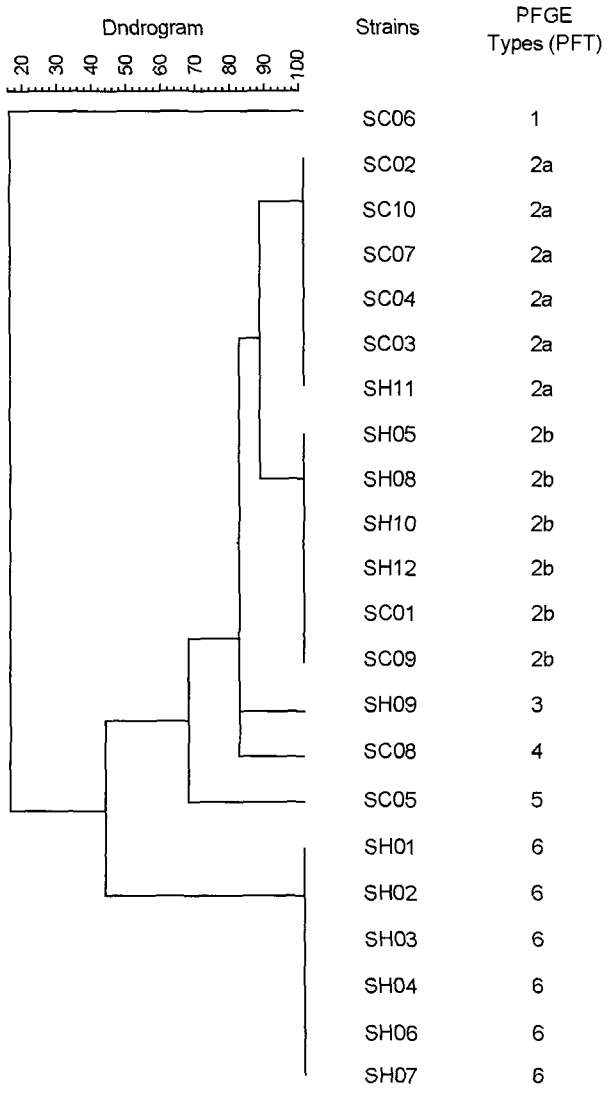

Fig. 1. Dendrogram of *Xba*I-digested PFGE patterns obtained from 22 *Salmonella* Enteritidis strains.

DISCUSSION

In this study, PFGE has been used to fingerprint isolates of *S.* Enteritidis from human food-poisoning cases and chi-

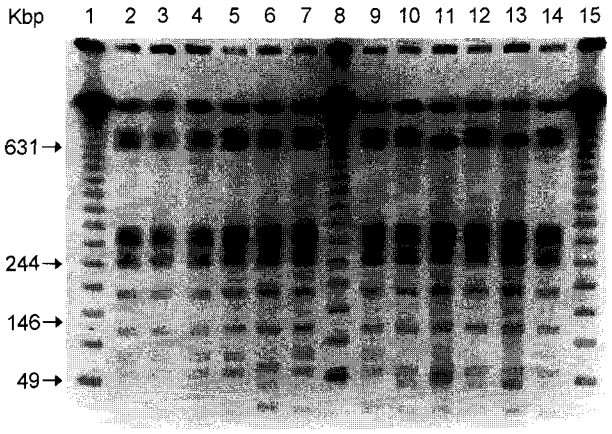

Fig. 2. PFGE profiles of *Xba*I-digested genomic DNA from 12 *Salmonella* Enteritidis strains isolated from food-poisoning cases. Lane 1, 8 and 15, lambda ladder marker; Lane 2~7, SH01, 02, 03, 04, 05 and 06, respectively; Lane 9~14, SH07, 08, 09, 10, 11 and 12, respectively.

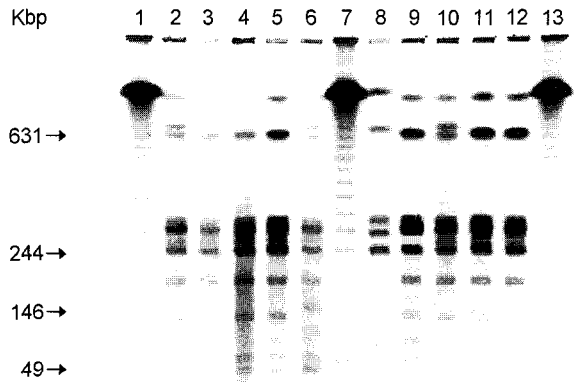

Fig. 3. PFGE profiles of *Xba*I-digested genomic DNA 10 *Salmonella* Enteritidis strains isolated from chicken feces. Lane 1, 7 and 13, lambda ladder marker; Lane 2-6, SC01, 02, 03, 04 and 05 respectively; Lane 8~12, SC06, SC07, 08, 09 and 10, respectively.

cken feces of slaughterhouse between 2001 and 2002. Macrorestriction patterns with usually 10 fragments ranging in size from 25 to 635 kb in this study were a few small compared to those with 13 to 16 resoluble bands ranging from 40 to 600 kb by Ridley et al. (1998). These differences were attributed to the PFGE electrophoresis condition. We changed pulse switch time 2.16~63.8 s from 4~40 s to view the band sizes of between 240 and 630 kb more clearly because the data by the authors above were barely differentiable in that size bands between strains. Six PFTs and two subtypes within one of the two common PFTs have been identified. Twelve isolates from human outbreaks were clu stered into 3 PFTs, PFT2, PFT3 and PFT6, respectively. Interestingly, all four isolates belonged to PFT2b were from the food poisoning outbreaks occurred on August 2002. These isolates showed an identical PFGE patterns with 2 isolates from chickens. Also, one isolate belonging to PFT-2a showed an identical PFGE patterns with 5 isolates from chickens. Powell et al. (1994) reported that 30 of 39 *S.* Enteritidis strains were shared by one profile by PFGE analysis using *Xba*I enzyme. Boonmar et al. (1998) also found that 84.9% of 53 *S.* Enteritidis isolates from human and broiler chickens showed indistinguishable PFGE patterns with *Bln*-digested DNA. Maslow et al. (1993) have reported that strains with a one or two band-shift in PFGE analysis can be considered as clonally related. The results in this study suggested that some of the sporadic human food-poisoning cases infections caused by *S.* Enteritidis were mainly due to the consumption of contaminated chicken meats. One isolate from chicken belonging to PFT1 showed a different pattern compared to other PFTs. We have no information on epidemiological data about this isolate. Further studies with more samples from different sources as well as from foreign countries would elucidate the genetic relation between strains.

Other studies with *Salmonella* serotypes other than Enteritidis, however, showed different results that subtyping of 55 *S.* Typhi strains from food-poisoning cases resulted in 41 PFGE patterns (Tsen et al., 1999). Also, 26 PFGE patterns were obtained from 45 *S.* Typhimurium strains from food-poisoning cases in Taiwan (Tsen et al., 2000). Although PFGE allows good strain discrimination for *Salmonella* serotypes, most of *S.* Enteritidis strains share one or two major PFGE patterns even when several restriction enzymes have been used due to their high genetic homogeneity (Landeras et al., 1996; Millermann et al., 1995).

Weigel et al. (2004) reported the greater discriminative ability of repetitive-element PCR (rep-PCR) for genotyping of *Salmonella* serotypes when compared with PFGE given the equal high reliability of both genotyping methods. Rep-PCR uses as primers oligonucleotides homologous to defined repetitive sequences, which are present in multiple copies in the bacterial genomes (Lupski, 1992). Fingerprinting of the 22 *S.* Enteritidis isolates with rep-PCR using ERIC and BOX primers generated an identical pattern between isolates from human and even chicken sources except one isolate (data not shown). The isolate corresponded to that belonged to PFT1, which was the most remote related with other PFTs in this study. It, therefore, is suggested that PFGE can provide the better discriminating ability than rep-PCR for genotypic and epidemiological investigations of *S.* Enteritidis in this study.

In consideration of the facts mentioned above, it could be concluded that a genetically identical, or at least clonally highly similar strains might exist and spread between human and chicken sources. Furthermore, it was suggested that standard protocols and procedures for computerized data analysis of *S.* Enteritidis be developed, so that national and international databases could be set up to facilitate tracing of this organism for international distribution across the world for veterinary and human public health in the future.

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