Genotyping, Phage Typing, and Antimicrobial Resistance of Salmonella Typhimurium Isolated from Pigs, Cattle, and Humans

Min-Seok Ju1, Zheng-Wu Kang, Ji Hun Jung2, Seongbeom Cho3, Sung Hun Kim4, Young-Ju Lee5, Chong Hae Hong, Son-Il Pak, and Tae-Wook Hahn*

College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon 200-701, Korea
1National Veterinary Research and Quarantine Service, Incheon Regional Office, Incheon International Airport, Incheon 400-715, Korea
2Seoul Metropolitan Government Research Institute of Public Health and Environment, Seoul 137-734, Korea
3National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA
4Division of Enteric Bacterial Infection, Center for Infectious Disease, National Institute of Health, Seoul 122-701, Korea
5College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea

Abstract

Salmonella enterica serovar Typhimurium (ST) is one of the most common serovars isolated from humans and animals. It has been suggested that ST infections in Koreans are largely due to the consumption of contaminated pork and beef. To investigate the genotypes, phage types, and antimicrobial resistance patterns for ST isolates of different origins, a total of 70 ST strains, including 19 isolates from humans, 44 isolates from pigs, and 6 isolates from cattle, were analyzed using pulsed-field gel electrophoresis (PFGE), phage typing, and antimicrobial susceptibility tests. Forty-three distinct PFGE patterns were generated from 70 ST isolates, which were grouped into 14 PFGE groups (from A to N) at the level of 75% similarity. The most prevalent group was the A (A1-A17 subtypes) group, encompassing 54.5% (38/70) of ST isolates. ST isolates from pigs and cattle mostly belong to groups A and L, whereas ST isolates from humans mostly belong to groups F and C. Antimicrobial susceptibility tests using 11 antimicrobial agents showed that resistance to tetracycline (TE) (81.4%) was highly prevalent, followed by streptomycin (S) (64.3%) and nalidixic acid (NA) (31.4%) resistance. A total of seventeen antimicrobial resistance patterns were observed. Only 8.6% of isolates, including a reference strain, were susceptible to all antimicrobial agents tested. The most prevalent resistance pattern was TE-S (37.1%), which was seen in 66.6% of bovine, 40.8% of swine and 21.1% of human isolates. Three ST isolates from humans (15.9%) showed resistance to 7-8 antimicrobials. The most predominant phage type (PT) was U302 (64.3%), followed by DT170 (10.0%). PFGE types did not coincide with antimicrobial resistance patterns and phage types; therefore, the combination of those types allowed for further differentiation between tested ST isolates.

Key words: Salmonella Typhimurium, pulsed-field gel electrophoresis, antimicrobial susceptibility test, phage typing

Introduction

More than 2,500 Salmonella serotypes have been reported (Popoff et al., 2004). Among them, Salmonella enterica serovar Typhimurium (ST) is one of the most frequently isolated from humans and animals, primarily cattle, pigs, goats, sheep and poultry (Bender et al., 2001; Doran et al., 2005; Gross et al., 1998). The most common clinical manifestations of ST in those hosts are enteric disease and septicemia (Best et al., 2007). Most Salmonella serovars contaminate meats, eggs and their by-products via feces and intestinal contents in farms or slaughter houses. ST is mainly associated with meat contamination with being the most predominant serovar isolated from pork. ST is difficult to control in food animal environments because carrier animals may exhibit asymptomatic fecal shedding. These carrier animals likely play an important role in the spread of infection between herds or flocks, and consequently serve as source of food.
contamination and transmission to humans (Yang et al., 2002). In Korea, ST has been one of the most frequently identified Salmonella serovars (Kim et al., 2004).

Numerous phenotypic and genotypic methods have been described to subtype ST and to investigate any relatedness among strains in animals and humans. These methods include phage typing (Corbett-Feeney and Riai, 1998), plasmid profiling, ribotyping, and amplified fragment length polymorphism. Phage typing is widely used for subtyping ST but is useful only in a limited number of serotypes. Plasmid profiling is also useful but may be confounded by the instability of certain plasmids; it is also complicated by its requirement of special reagents as well as training and experience in its performance and in the interpretation of results (Doran et al., 2005).

In recent years, standardized pulsed-field gel electrophoresis (PFGE) has been applied as an additional typing method in Salmonella reference laboratories. PFGE has been known as “gold standard” of genotypic typing of Salmonella and other foodborne pathogens for outbreak identification and source identification (Bessa et al., 2007; Best et al., 2007; Corbett-Feeney and Riai, 1998; Doran et al., 2005). In the absence of timely phage typing, PFGE is a simple way to detect and monitor multi-drug-resistant strains (Bender et al., 2001).

Antimicrobial resistance in ST isolates has been increasing dramatically in recent years. Multi-drug resistant ST isolates have been isolated from animals and humans suffering from diarrhea. The detection and monitoring of multi-drug resistant ST isolates is important for the selection of antibiotics to treat clinical salmonellosis and to assess the risk of the dissemination of the multi-drug resistant strains (Yang et al., 2002). Antimicrobial susceptibility tests and genotyping are important epidemiological tools to determine potential sources of infection. Moreover, data on the distribution of ST in different animals is useful for understanding the epidemiology of antimicrobial resistance and the spread of particular clonal genotypes.

Analysis of ST isolates from different origins, including humans and animals, indicates the spread of certain genetically identical or similar clones of ST in humans and animals. It is interesting to ask whether the clonal identity or similarity of ST isolates between humans and animals may be due to the limited diversity of ST isolates or the spreading of certain types of isolates between humans and animals. Comparison of subtypes for unrelated ST strains isolated from different origins, such as humans, pigs, cattle and other animals may help to answer this question. Such comparisons may also identify the most prevalent subtypes in ST infection in both humans and animals in Korea.

Previously, we have used the PFGE method with XbaI to analyze 155 S. Enteritidis isolates from humans and chickens and found that A5 was the major subtype (Kang et al., 2009). In this study, 70 ST isolates from different origins were analyzed by PFGE, antimicrobial susceptibility tests and phage typing.

**Materials and Methods**

**Bacterial strains**

A total of 70 ST isolates (44 from swine, 6 from bovines, 19 from humans and one type strain, ATCC14028) were analyzed. All swine and bovine ST isolates were from Gangwon and Gyeonggi provinces from 2000 to 2007. All human ST isolates were from Seoul from 2003 to 2005. ST isolates were taken from the feces of pigs and cattle regardless of clinical symptoms, while ST isolates from humans were isolated from diarrheal patients. All isolates were identified by the API kit (bioMérieux, Montalieu Vercieu, France) and Salmonella specific PCR and were serotyped with the Kauffman method (Popoff et al., 2004).

**Antimicrobial susceptibility test**

All ST isolates were tested for antimicrobic susceptibility on Muller-Hinton agar plates by the disk diffusion method (NCCLS, 2000). The media and the disks were purchased from BBL (Becton Dickinson Microbiology systems, Cockeysville, USA). A total of 11 antimicrobial agents were used: tetracycline (TE) 30 µg, streptomycin (S) 10 µg, kanamycin (K) 30 µg, gentamicin (GM) 12 µg, neomycin (N) 30 µg, ampicillin (AM) 10 µg, ticarcillin (TIC) 75 µg, sulfamethoxazole/trimethoprim (SXT) 23.75 µg, cephalothin (CF) 30 µg, nalidixic acid (NA) 30 µg, ciprofloxacin (CIP) 5 µg. The inhibition zones were interpreted as recommended by the supplier except that the intermediate and sensitive isolates were grouped together.

**Preparation of DNA for PFGE**

PFGE was performed according to the protocol used by the ‘Pulse Net’ system of the Center for Disease Control and Prevention. Salmonella embedded in agarose plugs was prepared by the previously described method (Seo et al., 2006). ST was cultured on LB agar plates and incubated overnight at 37°C. Colonies on agar plates were harvested and suspended in TE cell suspension buffer (100 mM Tris and 100 mM EDTA, pH 7.5). The turbidity of the bacterial cell suspension was adjusted to 20%