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Characterization of Multidrug-resistant *Salmonella enterica* Serovar Typhimurium Isolated from Swine Sources

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A total of 28 *Salmonella enterica* serovar Typhimurium isolated from diseased pigs and swine carcasses between 2001 and 2003 were characterized by the antimicrobial resistance profiles, PCR for detection of *S.* Typhimurium DT104 and pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Xba*I. All but one isolate presented multidrug resistance (MDR) to more than two antibiotics tested. A total of 11 resistance profiles were observed, and two phenotypes, ST and ASSuTG, were the most common among them. Two isolates were found to be *S.* Typhimurium DT104 isolates by PCR, and their resistance profile did not show the DT104 typical resistance type ACSSuT, but ACSSuTGK instead. PFGE identified 11 banding patterns in dendrogram, and three main clusters (designated A to C) were represented. Interestingly, sixteen of 19 *S.* Typhimurium isolates belonging to cluster B showed an identical band pattern.

Key Words: *Salmonella* Typhimurium, DT104, Antimicrobial resistance, PFGE

INTRODUCTION

Non-typhoidal salmonellosis is an important food-borne disease worldwide, and the main source of infections has been contaminated food of animal origin (D'Aoust, 1994). The genus *Salmonella* comprises more than 2,400 serotypes, most of which are considered potential human pathogens, but only a limited number of serotypes have been associated with human and swine infections (Baggesen et al., 2000; schwartz 1997). *Salmonella enterica* serovar Typhimurium is a common cause of salmonellosis in many countries (Kariuki et al., 1999; David, 2001; Mmolawa et al., 2002). It represented the most common serotype isolated from humans and animals in the United States and the second most common cause of human salmonellosis in the United Kingdom (Bender et al., 2001). In Korea, *S.* Typhimurium has also been one of the most frequent isolates of *Salmonella* serovars (Park et al., 2002).

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The frequency of antimicrobial resistance among food-borne pathogens has been increased dramatically due to their abusive use in human and veterinary medicine (McEwen and Fedorca-Cray, 2002). Furthermore, resistance to combinations of several classes of antimicrobials has led to the emergence of multidrug-resistant (MDR) strains that may pass from food animals to humans. One notable MDR *Salmonella* strain is *S.* Typhimurium DT104 resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (resistance profile ACSSuT). It was first recognized in the United Kingdom (Threlfall et al., 1994) and since has been reported in many parts of the world (Besser et al., 1997; Glynn et al., 1998; Metzer et al., 1998 kariuki et al., 1999; Lai-King et al., 1999).

The frequency of DT104 among total *S.* Typhimurium isolated from human has also been continuously observed since 1997 in Korea (Kim et al., 2004). Despite the importance of *S.* Typhimurium causing human salmonellosis in Korea, there have been a limited number of epidemiological studies of this organism isolated from animals. This study has applied a combination of phenotypic and genotypic methods for *S.* Typhimurium isolated from swine sources, including pulsed-field gel electrophoresis (PFGE), which has been recognized as a powerful tool and the gold stan-

dard of molecular typing methods (Olive and Bean, 1999) in the analysis of *Salmonella* isolates.

MATERIALS AND METHODS

1. Bacterial strains and PCR

A total of 28 isolates of *S.* Typhimurium from diseased pigs of field cases or swine carcasses from Youngnam province between 2001 and 2003 were used in this study (Fig. 3). Identification and serotyping of *Salmonella* isolates were performed by the standard microbiological culture and serum agglutination methods (Ewing, 1986). Also, PCR was performed for detection of *S.* Typhimurium DT104 among isolates as described by Bolton et al. (1999).

2. Antimicrobial susceptibility test

All isolates were tested by antibiotic disc test on Muller-Hinon agar (Difco, USA) according to the agar diffusion method (Bauer et al., 1966). The antimicrobial disks used were amikacin (AK), amoxicillin (AX), ampicillin (A), cephalothin (CT), chloramphenicol (C), ciprofloxacin (CF), colistin (CL), gentamicin (G), kanamycin (K), penicillin (P), streptomycin (S), sulfamethoxazole (Su) and tetracycline (T).

3. PFGE

PFGE procedure was performed using a CHEF bacterial genomic DNA plug kit (Bio-Rad, USA) with minor modifications. Colonies grown on Trytic soy agar (Difco, USA) were suspended in cell suspension buffer (100 mM Tris HCl, 100 mM EDTA, pH 8.0) and adjusted to a range of turbidity 18% T 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. After treated with lysozyme buffer (10 mg/ml) and proteinase K lysis buffer (10 mg/ml), the plugs were washed four times in 1× washing buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and digested with 50 U of *Xba*I restriction enzyme for 5 h at 30℃. The plugs were run on a 1% SeaKem Gold agarose gel using CHEF-mapper system (Bio-Rad, USA) in 0.5× TBE buffer with pulse times of 2.16 to 63.8 s. The gel was stained in ethidium bromide, and DNA bands were visualized with UV transilluminator. A dendrogram was constructed with Analysis software program (Biometra, Germany). The patterns were compared by means of the Dice coefficient of band-based similarity by unweighted pair group method using averages (UPGMA).

Table 1. Antimicrobial resistance profiles of *S.* Typhimurium isolates in this study

Drug resistance no.	Resistance profiles	No. (%) of isolates	
7	ASSuTCGK	2	2 (7.1)
5	ASSuTG	5	6 (21.4)
	SSuTCK	1	
4	SSuTG	2	5 (17.9)
	ASSuT	1	
	ASTC	2	
3	AST	3	5 (17.9)
	STC	1	
	STG	1	
2	ST	9	9 (32.1)
1	T	1	1 (3.6)
Total		28	28 (100)

RESULTS

Results of antimicrobial susceptibility test showed that all but one isolate of *S.* Typhimurium (96.4%, 27/28) expressed more than two-drug resistance (MDR) (Table 1). All isolates expressed the resistance to tetracycline, 96.4% (27 of 28) to sulfamethoxazole and 46.4% (13 of 28) to ampicillin. Also, a total of 11 resistance profiles for *S.* Typhimuium isolates were found, and ST (32.1%) and ASSuTG (17.9%) resistance profiles were the most common. PCR amplification of DNA extracted from each isolate indicated that two isolates produced the predicted 215-bp *S.* Typhimurium DT104 specific product (Fig. 1). Those two isolates did not show the DT104 typical resistance type, ACSSuT, but ACSSuTGK type instead. We found no resistance to amikacin, amoxicillin, cephalothin, ciprofloxacin, colistin and penicillin.

We analyzed a total of 28 *S.* Typhimurium isolates by PFGE digested with *Xba*I. The band pattern consisted of 12~14 bands with fragment sizes in the range of 30~728 kb (Fig. 2). It showed that most of isolates had an identical band pattern in fragment sizes of more than 194 kb except three isolates, including ST08 and ST10, which were found to be DT104 and contained an additional 48 kb band (land

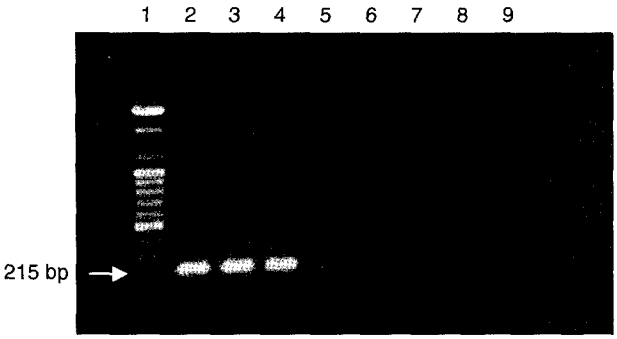

Fig. 1. PCR products of template DNA from *S.* Typhimurium DT104 isolates for detection of flo gene
Lane 1, 100 bp ladder; Lane 2, *S.* Typhimurium DT104; Lane 3~4, ST08 and ST10, respectively; Lane 5~8, 4 representative *S.* Typhimurium isolates; *S.* Lane 9, negative control (distilled water).

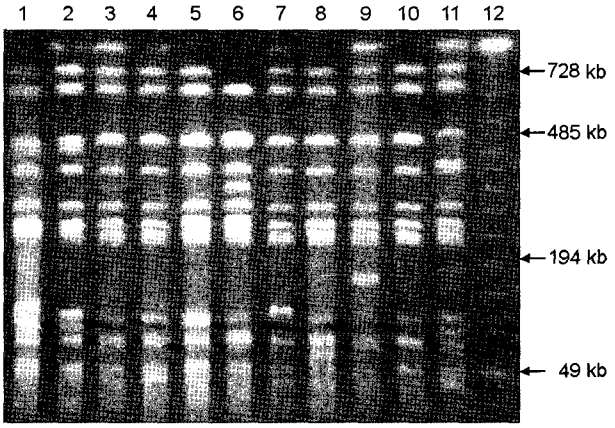

Fig. 2. Representative 11 PFGE profiles of MDR *S.* Typhimurium isolates with restriction enzyme *XbaI*.
Lane 1, ST10; Lane 2, ST08; Lane 3, ST04; Lane 4, ST02; Lane 5, ST01; Lane 6, ST03; Lane 7, ST28; Lane 8, ST27; Lane 9, ST20; Lane 10, ST15; Lane 11, ST19; Lane 12, lambda ladder size marker.

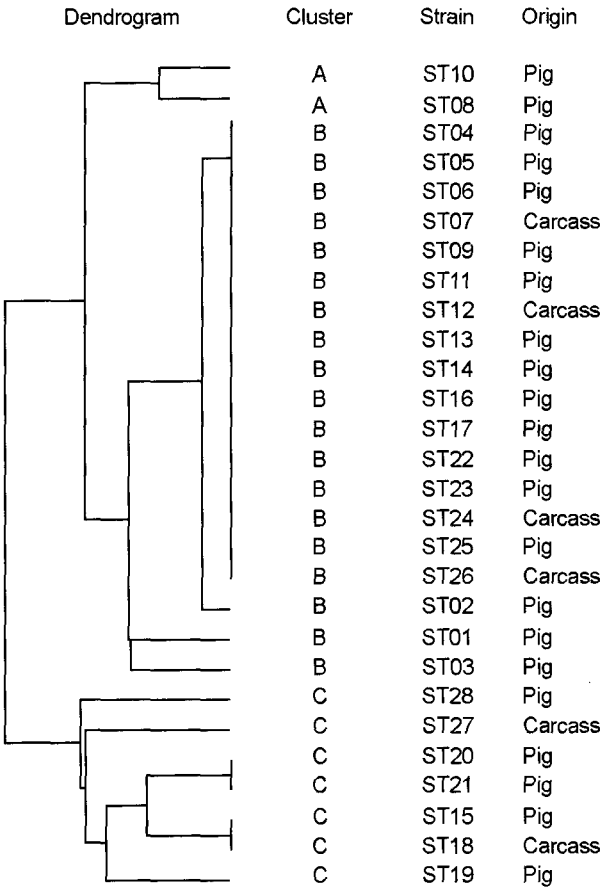

Fig. 3. Dendrogram based on PFGE profiles of 28 *S.* Typhimurium isolates.

1 and 2). One isolate, ST03 contained a 340 kb band (lane 6). Genetic diversities among *S.* Typhimurium isolates were mainly detected in band sizes of between 30 kb and 194 kb. PFGE results identified 11 individual PFGE banding patterns in dendrogram (Fig. 3). They were divided into three main clusters, designated A, B and C; clusters A (7.1%; n = 2), cluster B (67.9%; n = 19), and C (25%; n = 7). Interestingly, sixteen of 19 *S.* Typhimurium isolates belonging to cluster B showed an identical band pattern.

DISCUSSION

This study has utilized a combination of phenotypic and genotyping methods to observe relationship among *S.* Typhimurium isolates from swine source. The data reported here demonstrated a high level of MDR (96.4%) *S.* Typhimurium, a result correlates with those of other studies (Gebreyes et al., 2000; Gorman and Adley, 2004). Gorman and Adley found 88% of *S.* Typhimurium isolated from human and animal sources were MDR with predominant resistance file of ACSSuT in Ireland. Gebreyes et al. (2000) also reported that 47.6% of all *Salmonella* isolates from swine farm were MDR with 67.8% of AT resistance file, but serovar *S.* Typhimurium constituted 96% (170 of 176) of MDR isolates in USA. Another report in Korea described that all of *S.* Typhimurium isolated from animals were MDR, and SSuT resistance type was the most prevalent among them (Yang et al., 2002). We found the resistance to tetracycline and sulfamethoxazole to be most common among these isolates. Isolates were also commonly resistant to β-lactam antibiotic, ampicillin. We further found two most common resistance profiles, ST and ASSuTG. The former resistance

type coincided somewhat with a domestic result by Yang et al. (2002).

In the 1990s, a new *S.* Typhimurium phage type DT104 expressing ACSSuT penta-resistance profile emerged in many countries including USA and European Union (Baggesen et al., 2000; Glynn et al., 1998; Poppe et al., 2002). Izumiya et al. (2001) reported that 47.3% of *S.* Typhimurium isolated from animal source was identified as DT104 in Japan and that ACSSuT resistance type was the most predominant among *S.* Typhimurium, followed by ACSSuTK and ACSSuTN type. Gebreyes and Altier (2002) also found from a longitudinal study of antimicrobial resistance among *S.* Typhimurium isolated from USA swine farms that 38.6% of all *S.* Typhimurium isolates were phage type DT104, and 96.3% of DT104 strains showed the ACSSuT resistance pattern. Yang et al. (2002), however, found only 9.1% of those isolates were *S.* Typhimurium DT104, all showing ACSSuT resistance type. Two isolates in this study were found to be *S.* Typhimurium DT104, showing resistance to gentamicin and kanamycin in addition to ACSSuT type, ACSSuTGK. In general, resistance pattern of *S.* Typhimurium isolates from domestic swine source seemed to be different from that of foreign isolates. Resistance such as this may be associated with the use gentamicin and kanamycin to combat swine infection in Korea. Incidence of DT104 among *S.* Typhimurium isolates in Korea seems to be low when combining two results by Yang et al. (2002) and this study, though small numbers of the isolates were included.

Previous reports on the relationships of serovar Typhimurium from humans and various other sources showed that isolates of this serovar were highly homogeneous and clustered within a similarity of 70% (On and Baggesen, 1997; Heir et al., 2002). In spite of their tight genetic relationship among them, it was possible to divide all isolates into 11 subclusters in this study. More investigations are on the way whether these diverse patterns we found were due to recent genetic events such as deletions, insertions, or mutations of one or a few loci or by the acquisition of plasmids. Interestingly, 57.1% of the isolates had an indistinguishable pattern, suggesting that at least one clonal line of *S.* Typhimurium might be prevalent on the farms tested. Moreover, there was no clear differentiation with regard to their sources between diseased pigs and swine carcasses. Due to the high degree of clonality of *S.* Typhimurium, the use of multiple typing techniques is considered the best approach for discriminating among isolates (Liebana et al., 2002). DT 193 isolates have been among the most common MDR strains isolated from swine in USA (Gebrey and Altier, 2002). Moreover, Yang et al. (2002) found 80% of *S.* Typhimurium isolates from animal sources to be untypable in Korea. Genetic characterization of all isolates using phage typing would also be needed to determine the genetic linkages among them, and to identify a predominant phage type in swine herd in Korea.

Baggesen et al. (2000) reported from a study of *S.* Typhimurium DT104 isolates from Denmark, Europe and the USA that 98.5% of those strains (134 of 136) had the same PFGE profiles regardless of their sources when the *Xba*I restriction enzyme was used. They suggested that MDR *S.* Typhimurium DT104 has been highly homogeneous and spread clonally in those countries. Kim et al. (2004) found 4 PFGE profiles from six *S.* Typhimurium DT104 isolates from human and swine in Korea. In this study, we observed a distinct PFGE profile between two DT104 isolates belonging to cluster A and other non-DT104 *S.* Typhimurium isolates, even those two isolates did not show the same pattern. Characteristic PFGE patterns of different phage types have been previously demonstrated, including DT104 (Baggesen et al., 2000). Our results also indicated that *S.* Typhimurium DT104 strains were continuously detected since the first detection of DT104 isolates from human and animal in 1997 in Korea (Yang et al., 2002; Kim et al., 2004). Further studies would be needed for future surveillance and monitoring of the cross contamination of these strains between food and animal sources.

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