

## Changes of Multi-Drug Resistance Pattern in *Salmonella enterica* Subspecies *enterica* Serovar Typhimurium Isolates from Food-Producing Animals in Japan

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**ABSTRACT.** A total of 153 isolates of *Salmonella* Typhimurium derived from food-producing animals in Japan between 2002 and 2005 were investigated for antimicrobial resistance and phage types related to definitive phage type 104 (DT104). The predominant resistance type was resistance to ampicillin, dihydrostreptomycin, kanamycin, and oxytetracycline in bovine (45.2%, 48/104) and resistance to dihydrostreptomycin and oxytetracycline in porcine isolates (58.7%, 27/48). DT104-related phage type was found in 32 of 104 bovine isolates, two of 48 porcine isolates, and one of eight isolates from poultry, showing that the proportion of the phage type in *S. Typhimurium* isolates from cattle and pigs significantly ( $P < 0.01$ ) decreased from 71.9% and 31.4% in 1999-2001 to 30.8% and 4.1% in 2002-2005, respectively.

**KEY WORDS:** antimicrobial resistance, food-producing animal, *Salmonella* Typhimurium.

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*Salmonella enterica* subspecies *enterica* serovar Typhimurium is a well-known zoonotic bacterium causing diarrhea, pyrexia and septicemia in humans and animals. Following *S. Enteritidis* and *S. Infantis*, *S. Typhimurium* is the third commonest serovar causing human food poisoning cases in Japan and most of the *S. Typhimurium* strains obtained from humans and domestic animals showed resistance to a variety of antimicrobial agents [13]. *S. Typhimurium* phage type DT104 commonly exhibits resistance to five antimicrobial agents: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline. In Japan, DT104 strains were found in diseased cattle in the early 1990's, and high frequencies (64%) of *S. Typhimurium* DT104 were demonstrated in adult dairy cattle infected with *S. Typhimurium* from 1991 to 1998 [17]. Between 1999 and 2001, 71.9% and 31.4% of *S. Typhimurium* originated from cattle and pig, respectively, were identified as DT104 or 104B [10]. Recently, change of resistance phenotype or phage type in multidrug-resistant (MDR: resistance to two or more antimicrobials) *S. Typhimurium* of animal origins has been reported in Denmark [2-5, 7, 8, 11] and the United States [6, 19]. The present report shows the changing proportion of MDR *S. Typhimurium* in food-producing animals in Japan.

A total of 153 clinical isolates of *S. Typhimurium*, consisting of 104 bovine isolates, 48 porcine isolates and eight isolates of poultry origin, were derived from diagnostic submissions throughout Japan between 2002 and 2005. The minimum inhibitory concentration for *S. Typhimurium* iso-

lates was determined by an agar dilution method according to the Clinical and Laboratory Standards Institute (formerly, the National Committee for Clinical Laboratory Standards [NCCLS]) guidelines [14], and quality control strains were routinely used: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The following 11 antimicrobials were tested: ampicillin (ABPC), cefazolin (CEZ), colistin (CL), chloramphenicol (CP), dihydrostreptomycin (DSM), gentamycin (GM), kanamycin (KM), oxytetracycline (OTC), nalidixic acid (NA), enrofloxacin (ERFX) and trimethoprim (TMP). The breakpoints of ABPC, CEZ, CP, GM, KM, NA and TMP were adopted to the same breakpoints by NCCLS guidelines [15]. The breakpoints of CL, DSM, OTC and ERFX have been described in our previous report [9]. Bacteriophage typing was performed according to the methods of the Public Health Laboratory Service (PHLS), London, United Kingdom [1].

The predominant resistance type has changed from ABPC-CP-DSM-OTC resistance to ABPC-DSM-KM-OTC resistance in bovine isolates and to DSM-OTC resistance in porcine isolates (Table 1). The data of resistance type between 1999 and 2001 was also shown in Table 1 for comparison. *S. Typhimurium* isolates from cattle that exhibited ABPC-DSM-KM-OTC resistance increased from 1.9% (1/54) in 1999-2001 to 45.2% (48/104) in 2002-2005, resulting in decrease in CP resistance and increase in KM resistance. DSM-OTC-resistant isolates from pigs increased from 34.3% (12/35) in 1999-2001 to 58.7% (27/46) in 2002-2005. The changing resistance type in porcine isolates has resulted in low frequencies of ABPC (12.5%, 6/48) and CP resistance (18.8%, 9/48). Antimicrobial use is likely to lead

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Table 1. Antimicrobial resistance types of *S. Typhimurium* isolates from food-producing animals

Resistance type*	No. of isolates (DT104 related)					
	Cattle		Pig		Poultry	
	1999–2001	2002–2005	1999–2001	2002–2005	1999–2001	2002–2005
ABPC, CP, DSM, GM, OTC, NA, ERFX, TMP		1				
ABPC, CP, DSM, GM, KM, OTC, TMP	2	1				
ABPC, CP, DSM, KM, OTC, TMP	1(1)		1			
CP, DSM, OTC, NA, TMP		1				
ABPC, CP, DSM, KM, OTC	1(1)	2(1)		1		
ABPC, CP, DSM, OTC, NA	6(5)					
ABPC, CP, DSM, OTC, TMP			1(1)	3		
ABPC, DSM, KM, OTC, TMP		5	2			
DSM, OTC, NA, TMP				1		
ABPC, CP, DSM, OTC	40(36)	31(30)	13(10)	2(2)		1(1)
ABPC, DSM, KM, OTC	1	48				
ABPC, DSM, OTC, TMP			2	1		
CP, DSM, OTC, TMP				1		
DSM, GM, OTC, TMP				1		
ABPC, DSM, NA		1(1)				
ABPC, DSM, OTC		1		2		
ABPC, DSM, KM	1					
DSM, OTC, TMP				2		
CP, DSM, OTC				2		
ABPC, NA		1				
DSM, OTC		2	12	27		
DSM	2(2)	3				1
OTC	1	1	3	2		
ABPC	1(1)					
Susceptible	8	6	1	3	8	6
Total	54(46)	104(32)	35(11)	48(2)	8	8(1)

\* ABPC: Ampicillin, CP: Chloramphenicol, DSM: Dihydrostreptomycin, GM: Gentamicin, KM: Kanamycin, OTC: Oxytetracycline, NA: Nalidixic acid, ERFX: Enrofloxacin, TMP: Trimethoprim.

Table 2. Number of isolates identified as DT104 and its related phage types from food-producing animals in Japan (2002–2005)

Isolation year	Cattle				Pig		Poultry	
	Typhimurium	Phagetype			Typhimurium	Phagetype	Typhimurium	Phagetype
		104	104B	U302				
2002	13	5	0	0	11	0	4	0
2003	24	8	2	0	8	0	0	0
2004	25	3	0	2	8	1	0	0
2005	42	12	0	0	21	1	4	1
Total	104	28	2	2	48	2	8	1

to selection of antimicrobial-resistant bacteria. In Japan, veterinary use of CP for food-producing animals was prohibited in 1998, but thiamphenicol and florfenicol have been approved for treatment of bacterial diseases in both animal species. In Japan, penicillin antibiotics have been used frequently in swine practice. Thus, changing proportion of antimicrobial resistance pattern in MDR isolates may not be due to overall use of antimicrobial drugs. In the United States, MDR *S. Typhimurium* DT104 isolates with ABPC-CP-DSM-OTC resistance increased in the middle of the 1990's, but decreased recently [6, 19]. In addition, increased MDR *S. Typhimurium* isolates with ABPC-DSM-KM-OTC resistance in substitution for those with ABPC-

CP-DSM-OTC resistance belonged to a clonal group by pulsed-field gel electrophoresis (PFGE) analysis [19]. Further investigations on farm-level use of antimicrobial drugs and molecular epidemiological analysis are needed to clarify the reason for the change in types of MDR strains in cattle and pigs.

Of the 104 bovine isolates, 28 (26.9%) were DT104, 2 (1.9%) were 104B, and 2 (1.9%) were U302 (Table 2). The proportion of DT104-related isolates significantly decreased from 71.9% in 1999–2000 [10] to 30.8% in 2002–2005 (chi-square test:  $P < 0.01$ ). In the United Kingdom, the proportion of DT104-related isolates from cattle decreased from the 1990's to 2000's [16]. In Denmark, DT104 accounted for

about 30% of bovine isolates of *S. Typhimurium* in 1998-2000 and then declined to 10-20% in 2001-2004 [2-5, 7, 8, 11]. Only 2 (4.1%) of the 48 porcine isolates were DT104, showing the decreased proportion of DT 104 in *S. Typhimurium* isolates from 1999-2001 [10] to 2002-2005 ( $P < 0.01$ ). During the periods studied, a strain of DT104 was isolated from poultry. Although DT104 was not isolated from poultry in our previous study [10], a few researchers have reported the presence of DT104 in poultry in Japan [17, 18]. The decreased proportion of DT104-related isolates contributes to the changing proportion of MDR isolates of *S. Typhimurium* from cattle and pigs.

Moreover, only one isolate from cattle showed a high level of resistance (16 mg/L) to ERFX and resistance to ABPC, CP, DSM, GM, NA, OTC and TMP (Table 1). It was identified as DT12. Fluoroquinolone drugs as well as third-generation cephalosporin antibiotics are commonly used in the treatment of severe gastrointestinal infection in humans. An increase in *S. Typhimurium* resistant to fluoroquinolones may cause treatment failure. Izumiya *et al.* [12] reported that a fluoroquinolone-resistant isolate of *S. Typhimurium* from cattle in 2000 was phagetyped as DT12. However, previous and present strains were originated from cattle in different districts and years.

The present results provide information on selecting an appropriate antimicrobial agent for empiric therapy for *S. Typhimurium* infection in cattle and pigs. In addition, continuous investigations at the national level for antimicrobial-resistant *Salmonella* in food-producing animals remain a high priority for prudent use of antimicrobials.

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