The Journal of Microbiology, December 2007, p. 590-592

Vol. 45, No. 6

NOTE

Isolation of Multidrug-Resistant *Salmonella typhimurium* DT104 from Swine in Korea

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(Received August 14, 2007 / Accepted November 7, 2007)

We report the isolation of *Salmonella enterica* serotype Typhimurium phage type DT104 (CCARM 8104) from swine in Korea. The CCARM 8104 isolate was resistant to nalidixic acid and showed reduced susceptibility to quinolones. The CCARM 8104 isolate had a missense mutation, Asp87Asn, in the quinolone resistance-determining region in *gyrA* and produced PSE-1. The CCARM 8104 isolate carried two different class 1 integrons, and the PSE-1 β-lactamase gene was inserted into a 1,200 bp class 1 integron. The presence of DT104 with *pse-1* in an integron located in a plasmid and reduced susceptibility to quinolone in swine pose a significant threat of possible horizontal spread between swine and humans.

Keywords: antimicrobial resistance, integron, PSE-1, quinolone, Salmonella DT104

Multidrug-resistant (MDR) *Salmonella* spp. cause significant therapeutic problems in animal and human health care and raise further questions regarding the association between antimicrobial resistance, antimicrobials used in animals, and the transfer of MDR *Salmonella* spp. between animals and humans (Weill *et al.*, 2006). The situation is of particular concern because it presents the danger of extended spectrum β-lactamase (ESBL) spread among pathogens circulating in livestock and the community (Makanera *et al.*, 2003).

Salmonella spp. have been responsible for 20.7% of foodborne diseases in Korea (Lee *et al.*, 2001), and 85.4% of *Salmonella* spp. isolated from foods showed antimicrobial resistance (Chung *et al.*, 2003). TEM-52 and OXA-type ESBL-producing non-typhoidal Salmonella (NTS) have been reported in a clinical environment (Lee *et al.*, 2003), while only TEM-producing *S. typhimurium* has been isolated from animals in Korea (Yang *et al.*, 2002). In this study, we report for the first time the isolation of PSE-1-producing MDR *S. typhimurium* from animals in Korea.

Intestinal contents were obtained from 152 pigs from a slaughterhouse in Kyung-gi province near Seoul. The samples (5 g/pig) were mixed with 45 ml of buffered peptone water (BPW) and incubated for 20 h at 37°C. After incubation, 0.1 ml of sample was inoculated into 10 ml Rappaport Vassiliadis R10 broth (RV, Merck, Germany) and then incubated for 24 h at 42°C. One loop of RV culture, which changed in color from blue to discolored or green, was streaked onto the surface of XLD agar (Difco, USA) and Salmonella-Shigella agar (Difco) plates, and the suspected colonies were tested with Salmonella antisera (Denka Seiken, Japan). Phenotypic identification was performed using the VITEK GNI (bioMériux, France), Easy 24E plus (KOMED, Korea) or API 20E (bioMériux) identification system, and genotypic identification was carried out by PCR amplification of 16S rRNA and the subsequent sequencing. Serotyping was performed according to the standardized methodology (Ewin, 1986). Phage typing was performed with 31 phage suspensions in accordance with the method described by the Public Health Laboratory Service (PHLS, United Kingdom). PCR was performed with specific primers for DT104 (Leon-Velarde *et al.*, 2004) and *flo* (Bolton *et al.*, 1999). Quinolone resistance-determining regions in *gyrA* and *gyrB* were amplified and sequenced as previously described (Jung *et al.*, 2002). Class 1 integron was amplified as previously described (Levesque *et al.*, 1995). Both strands of the purified amplicon were sequenced by Genome Express (Bionics, Korea) using an ABI 100 DNA sequencer (Applied Biosystems, USA). A database search was performed using the BLAST program of NCBI (http://www.ncbi.nlm.nih.gov/).

The antimicrobial resistance of the isolates was screened by the disk diffusion method using disks impregnated with an appropriate amount of each antimicrobial agent, including ampicillin (10 μg), chloramphenicol (30 μg), streptomycin (10 μg), sulfonamide (250 μg), and tetracycline (30 μg). Antimicrobial susceptibility was determined according to the Clinical Laboratory Standard Institute (CLSI) guidelines for veterinary microorganisms (CLSI, 2003a). Minimal inhibitory concentrations (MICs) were determined by agar dilution with amoxicillin/clavulanic acid, ampicillin, cefotaxime, cephalothin, chloramphenicol, florfenicol, piperacillin, ticarcillin, kanamycin, streptomycin, sulfisoxazole, tetracycline, ciprofloxacin, enrofloxacin, gatifloxacin, gemifloxacin, levo-

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Table 1. MIC (μg/ml) of *S. typhimurium* DT104 from swine in Korea

	Amo/Cla	Amp	Cef	Cep	Chl	Flo	Pip	Tic	Kan	Str	Sul	Tet	Cip	Enr	Gat	Gem	Lev	Mox	Nal	Nor	Ofl
CCARM 8104	16/8	>128	0.25	8	>128	64	>128	>128	1	32	>1024	>128	0.25	1	0.5	0.5	0.5	1	>128	1	1
E. coli ATCC 25922	4/2	4	<0.12	4	2	2	1	2	1	2	16	2	0.008	0.06	0.008	0.008	0.008	0.016	2	0.032	0.032

Amo/Cla, Amoxicillin/clavulanic acid; Amp, Ampicillin; Cef, Cefotaxime; Cep, Cephalothin; Chl, Chloramphenicol; Flo, florfenicol; Pip, Piperacillin; Tic, Ticarcillin; Kan, Kanamycin; Str, Streptomycin; Sul, Sulfisoxazole; Tet, Tetracycline; Cip, Ciprofloxacin; Enr, Enrofloxacin; Gat, Gatifloxacin; Gem, Gemifloxacin; Lev, Levofloxacin; Mox, Moxifloxacin; Nal, Nalidixic acid; Nor, Norfloxacin; Ofl, Ofloxacin

floxacin, moxifloxacin, nalidixic acid, norfloxacin, and ofloxacin (CLSI, 2003b). *Escherichia coli* ATCC 25922 was used as the control strain. Double-disk synergy test (DDST) and isoelectric focusing (IEF) were used to detect ESBLs. IEF was performed with the cell lysate according to the method described by Mathew *et al.* (1975) in a Mini IEF cell system (Bio-Rad, USA). The *blaPSE* gene was amplified using primers (5'-GCA GTC GCC CTA AAA CAA CG-3' and 5'-ACT TGA TGG CCT TGT TAG CC-3') with 30 cycles of denaturation at 95°C for 1 min, annealing at 47°C for 1 min, extension at 72°C for 1 min, and a 975 bp PCR product was sequenced.

Ten *Salmonella* spp. were isolated from 152 swine in 2004. One isolate (CCARM 8104) was resistant to ampicillin, chloroamphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT type), and it was serotyped as B and phage typed as DT104L. CCARM 8104 produced a 162 bp DNA fragment by PCR with specific primers for DT104, and the amplicon sequences showed 100% homology with a PCR product from *S. typhimurium* phage type DT104 (GenBank accession no. AF275268). CCARM 8104 also produced a 215 bp DNA fragment by PCR for *flo*, which is responsible for florfenicol and chloramphenicol resistance. CCARM 8104 was resistant to nalidixic acid and showed reduced susceptibility to the various quinolones tested (Table 1). Since growth inhibition zones formed by cephalothin only increased 2 mm by DDST, CCARM 8104 could not be classified as an ESBL-producer. However, IEF showed a β-lactamase with pI 5.6 of PSE, and the sequence of the PCR product covering the complete gene (979 bp) showed 100% homology with that of *S. typhimurium* (GenBank accession no. Z18955.1). The CCARM 8104 isolate had a missense mutation, Asp87Asn (GAC to AAC), in *gyrA*, but no mutation in *gyrB*. The CCARM 8104 isolate carried two different class 1 integrons, with sizes of 1,000 bp and 1,200 bp, on plasmids. The PSE-1 β-lactamase gene was inserted as a gene cassette of the 1,200 bp class 1 integron.

Ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol have been frequently used to treat severe Salmonella infections in clinical environments, but the increasing prevalence of resistance to these agents has significantly reduced their efficacy in clinical settings. Therefore, fluoroquinolones and expanded-spectrum cephalosporins have been recommended for the treatment of invasive Salmonella infections in humans. However, MDR ESBL-producing *Salmonella* spp. have already been reported in clinical environments worldwide (Gupta *et al.*, 2003). The *Salmonella* spp. that have been isolated from clinical specimens in Korea include (listed in order of decreasing incidence) *S. enteritidis*, and *S. typhimurium*, and *S. typhi.* The Korea Food and Drug Administration reported that 12.3% of *S. enteritidis* and 80.7% of *S. typhimurium* isolated from humans were resistant to antimicrobial agents (http://www.kfda.go.kr). *S. enteritidis* was the most frequently detected serotype (29.3%) in a study of food isolates between 1993 and 2001. Approximately 85% of *Salmonella* spp. were resistant to at least one antimicrobial agent, and 65.9% of the isolates were MDR *Salmonella* spp. (Chung *et al.*, 2003). Recent studies have presented differences in the prevalent species between the animal industry and the clinical environment. *S. typhimurium* (51.8%) was the most common isolate in animals, followed by *S. enteritidis* (20.5%), *S. agona* (13.3%), and *S. rissen* (8.4%). *Salmonella* spp. isolates from animals showed a 44.6% rate of resistance to at least one antimicrobial agent, and MDR isolates were detected in 15.7% of *Salmonella* spp., which was much lower than the rate of detection from clinical isolates mentioned above (Lee *et al.*, 2001; Lee *et al.*, 2003). The resistance rates of *S. typhimurium* isolates from animals were lower than those of human clinical isolates, whereas the resistance rates of *S. enteritidis* isolates from animals were twice those of human clinical isolates. In contrast to the prevalence of resistance to third- and fourth-generation cephalosporins and fluoroquinolones among *Salmonella* spp. from humans (Gupta *et al.*, 2003), ESBL-producing *Salmonella* spp. or fluoroquinolone-resistant *Salmonella* spp. from animals have been rarely reported (Lee *et al.*, 2004). Of the *S. typhimurium* isolates obtained from animals between 1983 and 1999, two DT104 isolates had TEM-type β-lactamases, but were susceptible to florfenicol (Yang *et al.*, 2002). The presence of ESBL genes in the non-ESBL producers categorized according to the CLSI guidelines suggested that these genes may produce enough enzymes in animals to cause an increased incidence of severe food- borne diseases. Especially, the *blaPSE-1* gene detected in class 1 integrons in this study will be easily transferred to human isolates.

In summary, we showed the presence of a super-bacterium in food-producing animals. The MDR *S. typhimurium* DT104 CCARM 8104 isolate carried the *blaPSE-1* gene in an integron, and this is the first reported detection of β-lactamase (PSE-1) in *Salmonella* spp. in Korea. In addition, CCARM 8104 isolates showed reduced susceptibility to various quinolones. Therefore, the induction of ESBLs should be prevented in animal isolates. The emergence of the MDR *S. typhimurium* DT104 CCARM 8104 isolate in Korea suggests an urgent need to establish a strict hazard

analysis of the critical control point program (HACCP).

Authors thank Dr. Won Jun Whang for assistance with sampling. This study was supported by a grant from Seoul Women's University (2007).

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