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Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* in Raw Meat in Korea

Lim, Suk-Kyung*, Hyang-Mi Nam, Hyun-Jung Park, Hee-Soo Lee, Min-Jung Choi, Suk-Chan Jung, Ji-Yeon Lee, Young-Cho Kim, Si-Wook Song, and Sung-Hwan Wee

National Veterinary Research and Quarantine Service, Anyang, Gyeonggi-do 430-824, Korea

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A total of 2,858 meat samples collected during 2003–2008 in Korea were investigated, and methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were isolated from 1.0% (9/890) of beef, 0.3% (4/1,055) of pork, and 0.3% (3/913) of chicken meat samples, respectively. MRSA isolates showed the two sequence types (STs), ST72 from beef and pork and ST692 from chicken meat. MRSA isolates from beef and pork were Panton–Valentine leukocidin-negative, staphylococcal cassette chromosome *mec* type IVa strain with ST72, which is the most prevalent type of community-acquired MRSA in Korea. An identical pulse-field gel electrophoresis pattern was detected among 10 of 16 MRSA isolates: 9 strains from beef (n=5) and pork (n=4) in 2008, and one strain from beef in 2005.

Keywords: MRSA, meat, SCC*mec*, MLST

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as an important pathogen in humans, and increased community-acquired MRSA (CA-MRSA) infection has also been reported in recent years [3]. Recent reports have documented MRSA infections in animals, and it is now considered as one of the most important zoonotic pathogens [22].

In Korea, MRSA has been isolated from various non-human sources such as bovine milk, pets, and chicken meats and joints [10, 13, 17], suggesting the potential of human infection with MRSA from non-human sources. Food-related MRSA infection has been reported from the United States [5] and The Netherlands [7]. In spite of the significance of MRSA infection, most studies on MRSA in Korea were conducted during 2000–2003 within limited sampling regions [9, 10, 17]. The purpose of this study was, therefore, to investigate the prevalence of MRSA in meat samples collected from slaughterhouses and retail markets throughout Korea during the recent 5 years from 2003 to 2008. The MRSA isolates were also characterized with staphylococcal cassette chromosome *mec* (SCC*mec*) typing, multilocus sequence typing (MLST), and pulse-field gel electrophoresis (PFGE).

A total of 2,858 meat samples (890 beef, 1,055 pork, and 913 chicken meats) were collected from 298 slaughterhouses and 25 retail markets throughout Korea during 2003–2008. *Staphylococcus aureus* (*S. aureus*) was isolated from each carcass sample using sterile gauze wetted with 1% buffered peptone water (Becton Dickinson and Co., Cockeysville, MD, U.S.A.). All the isolates were confirmed as *S. aureus* by conventional methods including coagulase test and the Vitek system (bio-Merieux, Marcy I'Etoile, France). Antimicrobial susceptibility was determined by performing disk diffusion testing according to the Clinical Laboratory Standard Institute guidelines [1]. Cartridges of antimicrobial-containing discs were obtained from Becton Dickinson (BBL Sensi-Disk). The minimum inhibition concentrations (MICs) of suspected MRSA isolates were tested using the E-test strips (AB BioDisk, Solona, Sweden). *S. aureus* ATCC 25923 and *S. aureus* ATCC 29212 were used as quality control strains. To confirm the MRSA, polymerase chain reaction (PCR) for the *S. aureus*-specific gene and *mecA* gene [18] was performed. All MRSA isolates were then characterized by Panton–Valentine leucocidin (PVL) gene detection [11] by PCR, SCC*mec* typing [14, 15], PFGE with *Sma* I digestion [12], and MLST [4].

A total of 681 (23.8%) *S. aureus* isolates were isolated from 2,858 meat samples: Of 2,748 raw meat samples taken from 298 slaughterhouses and 110 samples collected from 25 retail markets, 9.7% (86/890) of beef, 19.0% (200/1,055) of pork, and 43.3% (395/913) of chicken meat samples contained *S. aureus*. MRSA was recovered from 0.4% (4/847) of beef and 0.3% (3/902) of chicken meat samples

**Corresponding author*
Phone: +82-31-467-1770; Fax: +82-31-467-1778;
E-mail: imsk@nvrqs.go.kr

Table 1. Prevalence of MRSA in raw meat from slaughterhouses and retail markets.

Meat type	Slaughterhouse			Retail market			Total
	No. of Slaughterhouses	No. of samples	No. of positive samples (%)	No. of retail markets	No. of samples	No. of positive samples (%)	No. of positive samples/ No. of tested samples (%)
Beef	97	847	4 (0.5)	10	43	5 (11.6)	9/890 (1.0)
Pork	104	999	0 (0.0)	11	56	4 (7.1)	4/1,055 (0.4)
Chicken meat	97	902	3 (0.3)	4	11	0 (0.0)	3/913 (0.3)
Total	298	2,748	7 (0.3)	25	110	9 (8.2)	16/2,858 (0.6)

from slaughterhouses and 11.6% (5/43) of beef and 7.1% (4/56) of pork from retail markets (Table 1). The prevalence of MRSA in raw meats observed in this study was somewhat higher than those of comparable studies from other countries and Korea: MRSA were isolated from 0.05% (2/444) of chicken meats in Japan [6], 2.5% (2/79) of pork and beef in The Netherlands [23], and 5.6% of pork and 3.3% of beef samples in the United States [19]. In Korea, MRSA were recovered from 0.5% (2/430) of chicken meats but none from beef and pork [8]. To our knowledge, this is the first report of MRSA isolated from pork and beef in Korea. As such, the prevalence of MRSA in raw meats has been low so far. In a recent study, however, MRSA were isolated from 11.9% (264/2,217) of meat samples in The Netherlands [2], where a more specific and sensitive detection method was applied to enhance the isolation rate.

In this study, a much higher prevalence was observed in meats collected from retail markets (8.2%) than meats from slaughterhouses (0.3%), which is in agreement with the result of a previous report from Korea [9]. In the case of pork, 4 (7.1%) MRSA were isolated from retail markets but none from slaughterhouses. This may have been due to the fact that the chance of exposure to a potential vector for spread of MRSA, such as personnel, equipment surfaces, and kitchenwares, was higher at retail markets than in slaughterhouses.

By SCC*mec* typing, all MRSA isolates in this study were identified as SCC*mec* typeIV that is usually associated with CA-MRSA. Two types of ST, ST72 and ST692, were identified by MLST analysis (Table 2). The distribution of ST varied according to the sources: beef and pork isolates all belonged to ST72, whereas chicken isolates belonged to ST692. Meanwhile, all MRSA originated from beef and pork were a ST72-SCC*mec* IVa–PVL negative, and 7 of the 13 (53.8%) beef and pork isolates showed resistance only to erythromycin in this study. These findings are in agreement with those of previous studies on CA-MRSA in humans in Korea [16], which reported that a specific clone,

Table 2. Characteristics of MRSA isolates from raw meats.

Strains	Years	Sample	Site[a]	MICs (μg/ml)[b]		*mecA*[c]	PVL[c]	Antimicrobial resistance[b]	SCC*mec*	ST
				FOX	OX					
B-S01-52	2005	Beef	S1	16	24	+	-	Em	IV_A	ST72
B-S01-59	2005	Beef	S2	12	12	+	-	-	IV_A	ST72
B-S01-60	2005	Beef	S2	12	16	+	-	-	IV_A	ST72
B-S01-61	2005	Beef	S2	8	8	+	-	-	IV_A	ST72
B-S01-93	2008	Beef	R1	24	48	+	-	-	IV_A	ST72
B-S01-94	2008	Beef	R1	24	32	+	-	-	IV_A	ST72
B-S01-97	2008	Beef	R2	24	24	+	-	Em	IV_A	ST72
B-S01-98	2008	Beef	R2	24	24	+	-	Em	IV_A	ST72
B-S01-99	2008	Beef	R2	24	24	+	-	-	IV_A	ST72
P-S02-225	2008	Pork	R2	24	24	+	-	Em	IV_A	ST72
P-S02-234	2008	Pork	R2	24	24	+	-	Em	IV_A	ST72
P-S02-235	2008	Pork	R2	16	48	+	-	Em	IV_A	ST72
P-S02-236	2008	Pork	R2	24	64	+	-	Em	IV_A	ST72
C-S03-200	2006	Chicken	S3	6	10	+	-	CL, CIP, TE, Em	IV	ST692
C-S03-237	2006	Chicken	S3	12	6	+	-	CL, CIP, TE, Em	IV	ST692
C-S03-238	2006	Chicken	S3	12	32	+	-	CL, CIP, TE, Em	IV	ST692

[a]S, slaughterhouse; R, retail market.
[b]Antimicrobials are abbreviated as follows: FOX, cefoxitin; OX, oxacillin, Em, erythromycin; CL, clindamycin; CIP, ciprofloxacin; TE, tetracycline.
[c]+, detected; -, not detected.

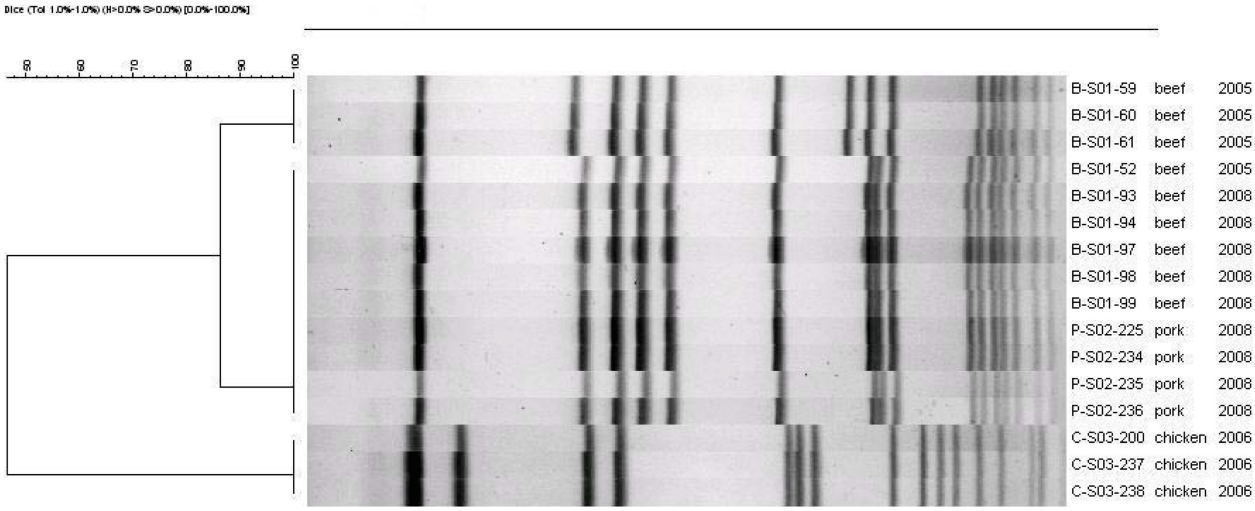

Fig. 1. *Sma*I macrorestriction patterns and cluster analysis of MRSA from raw meats.

ST72-SCC*mec* IV a without PVL toxin was the most common and unique type, differing in several characteristics from those of other countries [16]. In Korea, ST5-SCC*mec* type II and ST5-SCC*mec* type III, known to be generally associated with hospital-acquired MRSA (HA-MRSA), were reported in hospitalized dogs and chickens [9], respectively. Moreover, ST5-SCC*mec* IVg was detected in bovine milk in Korea [8]. However, ST72-SCC*mec* IVa, the most prevalent type of CA-MRSA detected from humans in Korea, has never been reported from non-human sources in Korea.

The *Sma* I macrorestriction patterns and cluster analysis of the MRSA isolates are presented in Fig. 1. Of 9 beef and 4 pork isolates, an identical PFGE type was observed among 1 beef isolate collected from a slaughterhouse (S1) in 2005, 2 and 3 beef isolates collected from retail markets 1 (R1) and 2 (R2) in 2008, respectively, and 4 pork isolates collected from R2, which is the same place that the 3 beef isolates were detected in 2008.

The presence of a human epidemic MRSA clone in raw meats was also reported recently from the United States [19], in which 19 and 3 MRSA isolates from pork and beef were identified to be of two unique human epidemic clones, USA100 and USA300, respectively. Although we have no clue about the source for the CA-MRSA in the beef and pork in this study, there was a possibility that these strains might be introduced onto raw meats during food processing, considering the fact that a significant part of the MRSA isolates in this study did not belong to the livestock-associated ST398.

In this study, an identical PFGE type was observed among 5 beef isolates and 4 pork isolates, some of which have been originated from a different place and year. No specific information is available about the slaughterhouses and retail markets from which the MRSA strains were isolated, except that the two retail markets are located in the same area. Further study on the relatedness between the markets and the slaughterhouses is needed to identify the source of MRSA, including transportation system, processing area, and personnel. However, clonal relatedness between strains that originated from different type of sources in the same place indicates the potential for cross-contamination of the meats at some point during food processing. In previous studies, MRSA strains were isolated from food handlers [21] and also from various kinds of household surfaces [20]. Although further studies are needed to identify whether the PFGE pattern observed commonly in beef and pork isolates is an epidemic clone in Korea or not, our finding suggests a potential persistence of a specific MRSA clone in the food chain system in this country.

Previous studies have also suggested that cattle, horse, and swine farms were significant sources for CA-MRSA [23, 24]. Therefore, a strict surveillance system on foodstuffs is urgently needed, and the carrier and transmission route should be identified to prevent the contamination and spread of MRSA.

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