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Antimicrobial resistance of *Staphylococcus aureus* isolated from domestic and imported raw meat in Korea

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Abstract : The rapid evolution of antibiotic resistance in *Staphylococcus* (*S.*) *aureus* is of considerable concern. Methicillin-resistant *S. aureus* (MRSA) strains are especially one of the greatest public concerns since the treatment of infections is more difficult when encountering resistance. In this study, we conducted a nationwide survey on the antimicrobial resistance of *S. aureus* isolated from raw meat samples collected from 16 countries, including Korea, and investigated the prevalence of MRSA as a possible source of human infection. Of 1,984 meat samples, *S. aureus* was isolated from 218 (11.0%) samples consisting of 23 (12.1%) from domestic meat and 195 (10.9%) from imported meat. The isolation rates of poultry meat, pork and beef were 12.8%, 7.0% and 10.0%, respectively. With regard to imported meat, the incidence varied from 4.8% to 16.6% from 13 countries, with the exception of Austria and Poland. In a resistance test to 20 antimicrobial agents, one hundred and eighty-four isolates (84.4%) were resistant to one or more antimicrobial agents tested. Especially, 17 (7.8%), 124 (56.9%) and 28 (12.8%) isolates showed a resistance to 3, 2 and 1 drugs, respectively. One isolate originating from domestic beef was resistant to 7 drugs. Another isolate originating from imported poultry meat showed resistance to oxacillin and methicillin by the disk diffusion test and minimal inhibition concentration methods, but showed negative for detection of the *mecA* gene.

Keywords : antimicrobial resistance, MRSA, *S. aureus*

Introduction

Staphylococcus (*S.*) *aureus* causes severe animal diseases such as suppurative disease, mastitis, arthritis, and urinary tract infections, that are associated with numerous virulence factors [2, 22]. In humans, this organism is a major cause of food poisoning, pneumonia, postoperative wound infections, and nosocomial bacteremia [20]. The rapid evolution of antibiotic resistance in *S. aureus* is of considerable concern. Multidrug-resistant *S. aureus* strains are especially one of the greatest public concerns since the treatment of infections is more difficult when encountering resistance.

Methicillin-resistant *S. aureus* (MRSA) is known to be one of the most prevalent nosocomial pathogens throughout the world and is capable of causing a wide range of hospital-linked infections. The β-lactam resistance of MRSA is determined by the function of the penicillin-binding protein (PBP) 2a, which is encoded by the methicillin resistant gene, *mecA* [6]. The prevalence of methicillin resistance is known to be more than 70% among *S. aureus* isolates from hospitals in Korea [12]. The isolation of MRSA from non-human sources, including foodstuffs and animals has also been reported [7, 8, 11]. Such organisms are frequently resistant to the majority of commonly used antimicrobial agents, including aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones [13]. In addition, MRSA strains are considered to be resistant to all cephalosporins, cephems, and other β-lactams regardless of the in vitro test results obtained with those agents [5].

Although the USA and some European countries have strict regulations on antibiotic uses for animal growth and therapy, there is a lack of data regarding the prevalence and antimicrobial resistance profiles of

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foodborne pathogens present in imported verses domestic foods in Korea. In this study, we conducted a nationwide survey on the antimicrobial resistance of *S. aureus* isolated from raw meat samples collected from 16 countries including Korea, and investigated the prevalence of MRSA as a possible source of human infection.

Materials and Methods

Collection of samples

From September 2003 to September 2005, poultry meat, pork and beef samples were collected from 16 domestic slaughter houses and cold storage warehouses containing raw meat samples imported from 15 countries.

Isolation of *S. aureus*

S. aureus from meat was isolated and identified according to 'Processing Standard and Ingredient Specifications for Livestock Products of the Republic of Korea' [20]. Twenty-five g of samples were weighed into sterile stomacher bags and diluted with 225 ml sterile butterfield's phosphate buffered dilution water (BPD). All samples were homogenized in a stomacher for about 1 min. Ten ml BPD homogenized was inoculated into tryptic soy broth (TSB; Difco, USA) with 10% NaCl and incubated at 37°C for 18 h. One loopful of the TSB was streaked on Baired-Parker agar (Oxoid, UK) supplemented with egg yolk-tellurite emulsion (Oxoid, UK), and incubated at 37°C for 24 h. From each plate, typical colonies of *S. aureus* were isolated and cultured separately on brain-heart infusion (BHI; Difco, USA) agar. The identification was carried out using the following tests; gram staining, production of coagulase, and fermentation of mannitol. If several colonies were isolated from one meat sample, only one isolate was randomly chosen. The organisms were finally confirmed by PCR [6] and kept in freezing at −70°C before use.

Disk diffusion method

All *S. aureus* isolates were investigated for their antimicrobial resistance by the agar disk diffusion test using the following antibiotics: amikacin (An), amoxicillin/clavulanic acid (Amc), ampicillin (Am), chloramphenicol (C), ciprofloxacin (Cip), clindamycin (Cc), enrofloxacin (Eno), erythromycin (E), gentamicin (Gm), linezolid (Lzd), nitrofurantoin (F/M), oxacillin (Ox), penicillin (P), quinupristin/dalfopristin (Syn), ripampin (Ra), sulfamethoxazole/trimethoprim (Sxt) and tetracycline (Te) of BBL, and gatifloxacin (Gtx), methicillin (Met) and teicoplanin (Tec) of Oxoid. Mueller-Hinton agar (MHA; Difco, USA) was dispensed into a plastic culture plate to yield a uniform depth of 4 mm. The density of all isolates tested was adjusted to 0.5 McFarland turbidity ($1\sim2 \times 10^8$ CFU/ml) by using a spectrophotometer, and inoculated over the agar surface by flooding. Antimicrobial disks were applied by a dispenser within 15 min after inoculation. Inhibition zone diameters were measured after 16-18 h of incubation at 35°C, but 24 h for Ox and Met. *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control strains of this test. The results were evaluated according to NCCLS [17].

Minimal inhibitory concentrations (MICs)

The MICs for Ox and Met were examined by the agar dilution method. Antimicrobial agents were added to autoclaved MHA (added 2% NaCl for Ox and Met) cooled to 50°C to the final concentration from 0.25 to 512 μg/ml by two fold diluting, and the medium were dispensed to 20 μl after gently mixing. The isolates suspension, which was adjusted to 10^4 CFU/ml, was applied to the surfaces of the agar plates containing a series of concentrations of antimicrobials with a steer's replicator device that delivered about 3 μl of suspension. The MIC was defined as the lowest concentration of antimicrobials inhibiting visible growth after 24 h incubation at 35°C. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains. Breakpoints published by the NCCLS [16] were used: Ox susceptible (S) $\leq$2 μg/ml and resistant (R) $\geq$4 μg/ml; Met S $\leq$8 μg/ml and $\geq$R 16 μg/ml.

DNA extraction

DNA extraction was performed according to the protocol described in the G-spin genomic DNA extraction kit (iNtRON, Korea) as follows. Cells incubated 24 h on BHI agar plates were suspended in 1 ml of phosphate buffered saline (Sigma, USA), centrifuged at 13,000 rpm for 1 min and removed supernatant. Fifty μl of pre-buffer and 3 μl of lysozyme solution were added and then incubated at 37°C for 15 min. Two hundred and fifty μl of G-buffer solution was added

and incubated at 65°C for 15 min. Two hundred and fifty µl of binding buffer solution was added again and vortexed. Cell lysates were loaded on column and centrifuged at 13,000 rpm for 1 min. After washing two times, 100 µl of elution buffer was added onto the membrane. After incubation at room temperature for 1 min, the column was centrifuged at 13,000 rpm for 1 min. The elute were used for PCR assay and stored at −20°C prior to use.

Amplification of *mecA* gene

The presence of the *mecA* gene was verified by means of PCR. Amplification of the *mecA* gene was performed by using the method reported by Kearns *et al.* [6]. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as negative control strains and *S. aureus* ATCC 43300, *S. aureus* ATCC 3300, *S. aureus* ATCC 3287, *S. aureus* ATCC 3299, *S. aureus* ATCC 9179 and *S. aureus* ATCC 2246 were used as positive control strains.

Results

Isolation rate of *S. aureus* from meat samples is shown in Table 1. Of 1,984 meat samples, *S. aureus* was isolated from 218 (11.0%) samples consisting of 23 (12.1%) from domestic meat and 195 (10.9%) from imported meat. Each isolation rate of poultry meat, pork and beef samples were 12.8%, 7.0% and 10.0%, respectively. In imported meat, the frequency of isolation varied from 4.8% to 16.6% from 13 countries, with the exceptions of Austria and Poland.

The resistance of *S. aureus* to 20 antimicrobial agents and antimicrobial resistance pattern are shown in Table 2 and Table 3, respectively. All isolates showed absolute susceptibility to Amc, Gtx, F/M, Syn, Ra, Tec and An. One hundred and eighty-four isolates (84.4%) were resistant to one or more antimicrobial agents tested. Especially, 17 (7.8%), 124 (56.9%) and 28 (12.8%) isolates showed a resistance to 3, 2 and 1 drugs, respectively. One isolate originating from domestic beef was resistant to 7 drugs.

One isolate originating from imported poultry meat showed resistance to Ox and Met by the disk diffusion method and the MICs for this isolate were 4 µg/ml for Ox and 16 µg/ml for Met which are breakpoints according to the resistance-criteria of NCCLS (Table 4). However, this isolate tested negative for detection of the *mecA* gene.

Table 1. Isolation rate of *S. aureus* from domestic and imported meat

Origin	Country	No. of isolates/No. of samples (%)			
		Poultry meat	Pork	Beef	Total
Domestic	Korea	14/90 (15.6)	5/50 (10.0)	4/50 (8.0)	23/190 (12.1)
Imported	Netherlands	–*	2/18 (11.1)	–	2/18 (11.1)
	New Zealand	–	-	21/141 (14.9)	21/141 (14.9)
	Denmark	98/579 (16.9)	1/18 (5.6)	–	99/597 (16.6)
	USA	17/225 (7.6)	3/75 (4.0)	3/24 (12.5)	23/324 (7.1)
	Belgium	–	7/57 (12.3)	–	7/57 (12.3)
	Spain	–	2/18 (11.1)	–	2/18 (11.1)
	UK	7/81 (8.6)	–	–	7/81 (8.6)
	Austria	–	0/9 (0.0)	–	0/9 (0.0)
	Chile	–	4/48 (8.3)	–	4/48 (8.3)
	Canada	–	2/42 (4.8)	–	2/42 (4.8)
	Thailand	3/63 (4.8)	–	–	3/63 (4.8)
	Poland	–	0/24 (0.0)	–	0/24 (0.0)
	France	7/99 (7.1)	0/12 (0.0)	–	7/111 (6.3)
	Hungary	–	3/33 (9.1)	–	3/33 (9.1)
	Australia	–	1/24 (4.2)	14/204 (6.9)	15/228 (6.6)
	Subtotal	132/1,047 (12.6)	25/378 (6.6)	38/369 (10.3)	195/1,794 (10.9)
Total		146/1,137 (12.8)	30/428 (7.0)	42/419 (10.0)	218/1,984 (11.0)

*Not sampled.

Table 2. Percentage of resistant *S. aureus* isolates to 20 antimicrobial drugs

Origin	Meat type	No. of isolates	Antimicrobial drugs[a]																		
			P	Am	Te	E	Cc	Gm	Lzd	C	Sxt	Cip	Eno	Ox	Met	Amc	Gtx	F/M	Syn	Ra	Tec	An
Domestic	Poultry	14	50.0	50.0	92.9	28.6	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pork	5	80.0	80.0	60.0	40.0	20.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Beef	4	75.0	75.0	50.0	50.0	25.0	50.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Subtotal	23	60.9	60.9	78.3	34.8	8.7	8.7	8.7	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Imported	Poultry	132	77.3	77.3	15.2	3.8	0.0	0.8	0.8	0.8	0.0	0.0	0.0	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Pork	25	60.0	60.0	36.0	28.0	12.0	4.0	0.0	0.0	8.0	4.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Beef	38	60.5	60.5	2.6	18.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Subtotal	195	71.8	71.8	15.4	9.7	1.5	1.0	0.5	0.5	1.0	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total		218	70.6	70.6	22.0	12.4	2.3	1.8	1.4	0.9	0.9	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]P, penicillin; Am, ampicillin; Te, tetracycline; E, erythromycin; Cc, clindamycin; Gm, gentamicin; Lzd, linezolid; C, chloramphenicol; Sxt, sulfamethoxazole/trimethoprim; Cip, ciprofloxacin; Eno, enrofloxacin; Ox, oxacillin; Met, methicillin; Amc, amoxicillin/clavulanic acid; Gtx, gatifloxacin; F/M, nitrofurantoin; Syn, quinupristin/dalfopristin; Ra, ripampin; Tec, teicoplanin; An, amikacin.

Table 3. Antimicrobial resistance pattern of *S. aureus* isolates

No. of resistant antimicrobial drugs	Resistant pattern [a]	Domestic				Imported				Total (n = 218)
		Poultry meat (n = 14)	Pork (n = 5)	Beef (n = 4)	Subtotal (n = 23)	Poultry Meat (n = 132)	Pork (n = 25)	Beef (n = 38)	Subtotal (n = 195)	
7	CcCEGmPAmTe	0[b]	0	1	1	0	0	0	0	1 (0.5)[c]
6	CcEGmPAmTe	0	0	0	0	0	1	0	1	3 (1.4)
	CcSxtEPAmTe	0	0	0	0	0	1	0	1	
	CEGmPAmTe	0	0	0	0	1	0	0	1	
5	CcEPAmTe	0	0	0	0	0	1	0	1	2 (0.9)
	EGmPAmTe	0	0	1	1	0	0	0	0	
4	CcEPAm	0	1	0	1	0	0	0	0	9 (4.1)
	EPAmTe	4	1	0	5	0	1	0	1	
	EnoCipPAm	0	0	0	0	0	1	0	1	
	OxMetPAm	0	0	0	0	1	0	0	1	
3	EPAm	0	0	0	0	1	0	2	3	17 (7.8)
	LzdPAm	0	1	0	1	1	0	0	1	
	PAmTe	2	1	0	3	4	4	1	9	
2	ETe	0	0	0	0	3	0	0	3	124 (56.9)
	LzdTe	1	0	0	1	0	0	0	0	
	PAm	1	0	1	2	92	6	20	118	
1	E	0	0	0	0	0	3	5	8	28 (12.8)
	Sxt	0	0	0	0	0	1	0	1	
	Te	6	1	0	7	11	1	0	12	
Total		14 (100)	5 (100)	3 (75.0)	22 (95.7)	114 (86.4)	20 (80.0)	28 (73.7)	162 (83.1)	184 (84.4)

[a]Cc, clindamycin; C, chloramphenicol; E, erythromycin; Gm, gentamicin; P, penicillin; Am, ampicillin; Te, tetracycline; Sxt, sulfamethoxazole/trimethoprim; Eno, enrofloxacin; Cip, ciprofloxacin; Ox, oxacillin; Met, methicillin; Lzd, linezolid.
[b]Number of isolates included.
[c]Number (%) of isolates included.

Table 4. Distribution of minimal inhibitory concentrations to antimicrobial drugs

Origin	Meat type	No. of isolates tested	No. of isolates for which the MIC (μg/ml) was:								
			Oxacillin					Methicillin			
			4[a]	2	1	0.5	0.25	16[a]	4	2	1
Domestic	Poultry	14	–	–	3	7	4	–	6	8	–
	Pork	5	–	–	3	2	–	–	1	3	1
	Beef	4	–	–	1	–	3	–	2	1	1
	Subtotal	23	–	–	7 (30.4)[b]	9 (39.1)	7 (30.4)	–	9 (39.1)	12 (52.2)	2 (8.7)
Imported	Poultry	132	1	3	41	61	26	1	79	49	3
	Pork	25	–	–	2	7	16	–	5	20	–
	Beef	38	–	–	3	18	17	–	9	27	2
	Subtotal	195	1 (0.5)	3 (1.5)	46 (23.6)	86 (44.1)	59	1 (0.5)	93	96	5
Total		218	1 (0.5)[c]	3 (1.4)	53 (24.3)	95 (43.6)	66	1 (0.5)[c]	102 (46.8)	108 (49.5)	7 (3.2)

[a]The MIC (μg/ml) as oxacillin-resistant or methicillin-resistant *S. aureus* according to resistance-criteria of NCCLS was shown in shaded area.
[b]No. of isolates (%).
[c]One isolate showed resistance to oxacillin and methicillin by disk diffusion method and MICs, but negative for detection of the *mecA* gene.

Discussion

Recently, MRSA has been a widespread problem in Korea. The rate of Met resistance among human *S. aureus* isolates is over 50% in Korea [10]. The existence of MRSA in non-human sources has aroused curiosity as to whether these MRSA isolates originated from foodstuffs, including milk, ice cream and raw meat. But there are only a limited number of publications on the epidemiological relatedness of MRSA isolation in foodstuffs [7, 8, 11]. The prevalence of antimicrobial resistant *S. aureus*, including MRSA, are considerably different among individual countries [19, 23]. First of all, this study attempted to compare the nationwide tendency regarding the antimicrobial resistance of *S. aureus* isolated from raw meat samples of 16 countries, including Korea. *S. aureus* was isolated from 15.6% of poultry meat, 10% of pork and 8% of beef samples. There was no significant difference as to the frequency of isolation in imported verses domestic raw meat samples. However, the incidence for poultry meat was slightly higher than other meats. Lee [11] reported that the frequency of isolation of *S. aureus* from beef, pork and chicken meat samples collected from slaughter houses, meat processing facilities and food stores in Korea were 9.3%, 24.2% and 13%, respectively. Therefore, it is suggested that there may be a great variation in incidences from plant to plant, depending on the level of sanitation in the processing plant.

The number of resistant isolates from imported meat (71.8%) samples was higher than from domestic meat samples (60.9%). However, 15.4% of *S. aureus* isolated from imported meat samples were resistant to Te. Otherwise, nearly four-fifths of *S. aureus* originating from domestic meat samples (78.3%) were resistant to Te. As is known from many reports, Te has been used as a feed additive in Korea for a long time, therefore there is extensive resistance to this antibiotic.

In our survey, only 1 isolate from poultry meat imported from the UK showed resistance to Ox and Met by the disk diffusion method and MICs. However, this isolate did not possess the *mecA* gene by the PCR method. The identification of Met-resistant staphylococci in the laboratory is sometimes complicated by the heterogenous expression of resistance and variables that influence this express (i.e., pH, temperature, and salt concentrations). The NCCLS provides a breakpoint MIC for resistance, but many investigators advocate recognition of degrees of resistance that are clinically important. Borderline methicillin resistance varies from study to study to include MICs of Ox from 1 to 16 ug/ml with disk diffusion zone diameters of 6 to 13 mm [3, 4, 15, 21]. Lee *et al.* [9] reported that the *mecA* gene was not detected in more than 50% MRSA of isolates showing low-level Ox MICs (4 or 8 μg/ml).

Tomasz *et al.* [21] reported that 12 of 17 *S. arueus* selected for low-level methicillin resistance (MIC of Met, 2 to 4 μg/ml; MIC of Ox, 0.5 to 8 μg/ml) strains did not react with the *mec*-specific DNA probe and suggested that staphylococci with borderline resistance may contain at least three different classes of mechanisms; non-PBP 2a-dependent mechanisms such as the hyperproduction of β-lactamase [3, 15, 21], the presence of other low-affinity PBPs [1, 21], or production of other methicillinase [14].

Many studies of antimicrobial resistance to *S. aureus* have been conducted by comparing a specific phenotype and genotype. But only sporadic cases of *S. aureus* originating from foodstuffs such as raw meat and bovine milk were reported in Korea. We investigated the nationwide tendency on antimicrobial resistance of *S. aureus* isolated from a large number of raw meats and this result has generated a lot of useful information regarding public health.

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