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Methicillin (Oxacillin)-Resistant *Staphylococcus aureus* Strains Isolated from Major Food Animals and Their Potential Transmission to Humans

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From May 2001 to April 2003, various types of specimens from cattle, pigs, and chickens were collected and examined for the presence of methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA). *S. aureus* was isolated and positively identified by using Gram staining, colony morphology, tests for coagulase and urease activities, and an API Staph Ident system. Among 1,913 specimens collected from the animals, 421 contained *S. aureus*; of these, 28 contained *S. aureus* resistant to concentrations of oxacillin higher than 2 μg/ml. Isolates from 15 of the 28 specimens were positive by PCR for the *mecA* gene. Of the 15 *mecA*-positive MRSA isolates, 12 were from dairy cows and 3 were from chickens. Antimicrobial susceptibility tests of *mecA*-positive MRSA strains were performed by the disk diffusion method. All isolates were resistant to members of the penicillin family, such as ampicillin, oxacillin, and penicillin. All isolates were also susceptible to amikacin, vancomycin, and trimethoprim-sulfamethoxazole. To determine molecular epidemiological relatedness of these 15 animal MRSA isolates to isolates from humans, random amplified polymorphic DNA (RAPD) patterns were generated by arbitrarily primed PCR. The RAPD patterns of six of the isolates from animals were identical to the patterns of certain isolates from humans. The antibiotypes of the six animal isolates revealed types similar to those of the human isolates. These data suggested that the genomes of the six animal MRSA isolates were very closely related to those of some human MRSA isolates and were a possible source of human infections caused by consuming contaminated food products made from these animals.

Staphylococcus aureus causes severe animal diseases, such as suppurative disease, mastitis, arthritis, and urinary tract infection, that are associated with numerous virulence factors, such as the production of extracellular toxins and enzymes (5, 33). For humans, this organism is an important cause of food poisoning, pneumonia, postoperative wound infections, and nosocomial bacteremia (8). Human isolates of *S. aureus*, unlike animal isolates, are frequently resistant to the penicillinase-resistant penicillins (12, 23, 28). An organism exhibiting this type of resistance is referred to as methicillin (oxacillin)-resistant *S. aureus* (MRSA). Such organisms are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (14). In addition, MRSA strains should be considered to be resistant to all cephalosporins, cephems, and other β-lactams (such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam, and the carbapenems) regardless of the in vitro test results obtained with those agents (19).

MRSA is known to be one of the most prevalent nosocomial pathogens throughout the world and to be capable of causing a wide range of hospital-linked infections. MRSA was first reported in the United Kingdom in 1961 (soon after the introduction of methicillin) and by the mid-1970s had become endemic in many countries (32). Some strains of MRSA have been designated epidemic strains; these are associated with a higher prevalence and have been shown to spread within hospitals, between hospitals, and between countries (1, 11, 17, 25, 26). MRSA has become a widespread problem in Korea. The rate of methicillin resistance among human *S. aureus* isolates in Korea is over 50% (13). MRSA has now become established outside the hospital environment and is appearing in community populations without identifiable risk factors (7). To control the spread of the infections, sources of contamination and mechanisms of transmission must be identified. Transmission of MRSA is thought to occur primarily from colonized or infected persons to other persons (3, 16, 22). While the environment contributes to MRSA transmission (31), transmission through food products has not been thoroughly investigated.

There is a limited number of publications on the epidemiological aspects of MRSA infections in animals; a few veterinary reports have been published on MRSA infections in dairy herds with mastitis and in companion animals (2, 4, 27, 30). The present report provides data on the isolation of MRSA from 12 dairy cow and 3 chicken specimens collected over a 2-year period. To investigate food animal MRSA isolates as a possible source of human infections, genetic relatedness among the isolates from food animals and humans was determined by random amplified polymorphic DNA (RAPD) patterns generated with arbitrarily primed PCR (AP-PCR).

MATERIALS AND METHODS

Isolation and processing of MRSA. Feces, milk, feed material, joint, trachea, uterus, and meat specimens of beef cattle, dairy cattle, pigs, and chickens were collected between May 2001 and April 2003 at monthly intervals from 15 slaughterhouses, seven meat processing facilities, 58 feedlots, and 11 food stores located throughout Korea, including the provinces of Chungcheong, Gyeongsang, and Jeolra. One specimen per animal was collected from the various sites. For

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TABLE 1. Results for MRSA isolates from major food animals (sampled during May 2001 to April 2003)

Animal and site or source[a]	Total no. of specimens	No. of specimens			
		Positive for *S. aureus*	Resistant to oxacillin at a concn (μg/ml) of:		*mecA* positive
			2–8[b]	>8[c]	
Beef cattle					
Meat	54	5	0	0	0
Feces	28	1	0	0	0
Joint	25	2	0	0	0
Trachea	19	1	0	0	0
Dairy cattle					
Feces	40	1	0	0	0
Milk	894	265	7[d]	10	12
Feed	32	3	0	0	0
Joint	26	4	0	0	0
Trachea	30	4	0	0	0
Pig					
Meat	161	39	2	0	0
Feces	68	2	0	0	0
Feed	37	2	0	0	0
Joint	116	17	0	0	0
Trachea	72	17	1	0	0
Uterus	15	5	0	0	0
Chicken					
Meat	69	9	2	1	1
Feces	35	2	0	0	0
Feed	36	4	0	0	0
Joint	119	33	3	2	2
Trachea	37	5	0	0	0
Total	1,913	421	15	13[e]	15

[a] Most of the *S. aureus* meat samples were isolated from suppurative regions in the meat.

[b] Numbers of samples that contained *S. aureus* resistant to 2 to 8 μg of oxacillin/ml.

[c] Numbers of samples that contained *S. aureus* resistant to more than 8 μg of oxacillin/ml.

[d] Two of seven oxacillin (MICs, 2 to 8 μg/ml)-resistant *S. aureus* strains from milk samples of dairy cattle were positive for the *mecA* gene by PCR, while the other oxacillin-resistant strains within this range of MICs were negative for *mecA*.

[e] All of the oxacillin (MICs, >8 μg/ml)-resistant *S. aureus* strains were positive for *mecA*.

joint, trachea, and uterus specimens, surface areas of at least 10 by 10 cm were swabbed with staphylococcus broth (Difco, Detroit, Mich.). The total number of specimens collected was 1,913 (Table 1). During each sampling occasion, two to five randomly selected samples were collected per feedlot and food store and 5 to 15 samples were collected per slaughterhouse and meat processing facility. All samples were immediately transported to the laboratory in ice-cooled containers. Approximately 10 g of each specimen of feces, feed material, and homogenized meat and 10 ml of each specimen of milk and of the swabbed specimens of joint, trachea, and uterus were inoculated into 100 ml of staphylococcus broth or Trypticase soy broth (Difco) with 70 mg of NaCl/ml and incubated at 35°C for 20 h with shaking. The inoculum was spread onto Baird-Parker agar and incubated at 35°C for 24 to 48 h. The colonies were tested (using conventional methods that included Gram staining, colonial morphology, and coagulase and urease assays) for *S. aureus* levels. They were also tested with an API Staph Ident system (Biomerieux, Lyon, France). Phenotypic oxacillin resistance of *S. aureus* was determined by an agar screen test performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (20, 34) with Mueller-Hinton agar (Difco) containing 4% NaCl and 2, 4, or 8 μg of oxacillin (Sigma, St. Louis, Mo.) per ml. Oxacillin-resistant colonies were stored at −70°C in freezer vials pending further analysis.

To examine the genetic relatedness of the animal MRSA isolates to human isolates and the possibility of transmission, during the investigation period a total of 38 MRSA isolates originating from humans were collected from several hospitals located throughout the nation and examined. Resistance to oxacillin and the presence of the *mecA* gene were confirmed for these isolates.

Preparation of whole-cell DNA for PCR and RAPD. A previously described method (15) was used (with modifications) for whole-cell DNA extraction. Cells grown in 1.5 ml of Trypticase soy broth at 35°C for 20 h were harvested and centrifuged at 16,000 × *g* for 3 min. The pellet was washed with 1.0 ml of sterile distilled water, resuspended in 50 μl of Triton X-100 lysis buffer (100 mM NaCl, 10 mM Tris-HCl [pH 8], 1 mM EDTA [pH 9], 1% Triton X-100), boiled for 10 min, and then centrifuged at 16,000 × *g* for 3 min. The suspension was cooled at room temperature for 5 min and centrifuged at 16,000 × *g* for 3 min. A total of 2 ml of the supernatant was used as the template.

Amplification of the *mecA* gene. The presence of the *mecA* gene was verified for the oxacillin-resistant isolates by means of PCR. Amplification of the *mecA* gene was performed using the primers *mec*A1 (5′-AAAATCGATGGTAAAG GTTGGC) and *mec*A2 (5′-AGTTCTGCAGTACCGGATTTGC), yielding a PCR product of 533 bp (18). PCR was performed in a 25-μl volume with a PCR buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, a 200 μM concentration of each deoxynucleoside triphosphate (Promega, Madison, Wis.), 2.5 U of *Taq* polymerase (Promega), and a 0.2 μM concentration of each primer. Amplification was carried out using 40 cycles of amplification at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; this reaction was followed by 5 min of an additional extension at 72°C. PCR products were electrophoresed on a 1.5% agarose gel. A positive result was inferred by detection of a 533-bp band, which represented a part of the *mecA* gene.

Antimicrobial susceptibility testing. The susceptibilities of all *mecA*-positive MRSA isolates to different antimicrobial agents were tested by the disk agar method as standardized by the NCCLS (21). The isolates were tested with a panel of 15 antibiotics (amikacin, ampicillin, cefoxitin, cephalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, kanamycin, norfloxacin, ofloxacin, tetracycline, and trimethoprim-sulfamethoxazole) (Biomerieux). The results were recorded after 24 h of incubation at 35°C and interpreted according to the guidelines of the NCCLS. Antibiotypes were considered identical when the results of susceptibility tests with all agents tested were identical. The MICs of oxacillin and vancomycin were also examined according to NCCLS recommendations (20) by an agar dilution method using an inoculum of 10^4 CFU/spot on Mueller-Hinton agar containing 2% NaCl as well as oxacillin and vancomycin in concentrations ranging from 0.5 to 128 and 2 to 128 μg/ml, respectively.

AP-PCR. The RAPD patterns generated by AP-PCR of the MRSA isolates were determined. Approximately 5 ng of DNA was included per PCR mixture. The PCR mixture consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 M $MgCl_2$, 0.01% gelatin, and 0.1% Triton X-100. Deoxyribonucleotide triphosphate (0.2 mM) and *Taq* DNA polymerase (0.5 U) were present. A 0.2 μM concentration of each of two arbitrary oligonucleotide primers, M13 (5′-GAG GGTGGCGGTTCT-3′) and H12 (5′-ACGCGCATGT-3′), was used (10). The amplification was performed using a program consisting of 40 repeated cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C. Prior to cycling, the mixture was denatured at 94°C for 3 min; postcycling, the mixture was incubated at 72°C for an additional 10 min. PCR products were analyzed by 2% agarose gel electrophoresis. The AP-PCR analyses of these isolates were performed in duplicate.

RAPD patterns were analyzed both visually and by computer-aided methods. Visual interpretation of banding patterns was done according to a previously reported guideline (29). The results for each primer were indexed by numbering, thereby defining the number of different patterns. Isolates with one or two minor band differences between two patterns derived from different isolates were assigned the same type. The overall types were defined by a combination of the results obtained with the two primers. Patterns were digitized with a Hewlett-Packard Scanjet IIcx/T scanner and stored as TIFF files. Banding patterns generated with each primer were integrated and analyzed using GelCompar software (version 4.0; Applied Maths, Kortrijk, Belgium) to calculate Dice coefficients of correlation and to generate a dendrogram by the unweighted pair group method using arithmetic average clustering.

RESULTS

Isolation of MRSA from food animal specimens. *S. aureus* from food animal specimens was identified by conventional methods and the API Staph Ident system as described above. Of 1,913 animal specimens collected, *S. aureus* was isolated

TABLE 2. Summary of results for *mecA*-positive MRSA isolates from major food animals

Isolate	Origin	Date of isolation (mo/yr)	Antibiotype[a]	MIC (μg/ml)		RAPD pattern[b] as determined with primer(s):		
				Oxacillin	Vancomycin	M13	H12	M13 and H12 combined
1	Chicken	05/2001	A	16	<2	I	I	I
2	Cattle	07/2001	B	32	<2	II	I	II
3	Chicken	08/2001	A	32	<2	I	I	I
4	Chicken	08/2001	A	32	<2	I	I	I
5	Cattle	10/2001	B	32	<2	II	I	II
6	Cattle	03/2002	C	16	<2	III	II	III
7	Cattle	05/2002	D	32	<2	IV	III	IV
8	Cattle	06/2002	E	32	<2	V	IV	V
9	Cattle	06/2002	F	128	<2	V	V	VI
10	Cattle	06/2002	G	64	<2	II	I	II
11	Cattle	08/2002	H	8	<2	II	I	II
12	Cattle	09/2002	H	16	<2	II	I	II
13	Cattle	10/2002	F	>128	<2	V	V	VI
14	Cattle	03/2003	C	64	<2	VI	VI	VII
15	Cattle	04/2003	I	4	<2	VII	VII	VIII
16	Human	05/2002	J	64	<2	VIII	VIII	IX
17	Human	05/2002	D	>128	<2	IV	III	IV
18	Human	07/2002	K	32	<2	IX	IX	X
19	Human	10/2002	B	64	<2	II	I	II

[a] Antibiotype results refer to Table 3 data.
[b] AP-PCR patterns are shown in Fig. 1.

from 421 (Table 1). The majority of the isolates identified were from milk specimens. The presence of MRSA isolates was determined by an agar screen test with various concentrations of oxacillin (i.e., 2, 4, and 8 μg/ml). *S. aureus* isolates from 28 specimens were resistant to levels of oxacillin higher than 2 μg/ml. Many isolates were resistant to 2 to 8 μg of oxacillin/ml in the initial culture and became sensitive to the oxacillin after multiple subculturing. Isolates from the 28 specimens were tested for the presence of the *mecA* gene. Isolates from 15 specimens were positive for *mecA* and were consistently resistant to oxacillin. Among the 15 *mecA*-positive MRSA isolates, 12 were from cattle and 3 were from chicken specimens. All 12 cattle isolates originated from milk. Nine of the milk specimens that harbored MRSA were from cows that showed signs of mastitis, as indicated by results of the somatic cell count assays (data not shown). Among three MRSA strains from chickens, one was isolated from a suppurative region in chicken meat. The other two isolates were from joints of the chicken, which had signs of arthritis. No *mecA*-positive MRSA strains were isolated from any specimens of pigs during this investigation.

The MRSA isolates were detected from March to October during the study period (Table 2).

Antimicrobial susceptibility. The *mecA*-positive MRSA strains isolated in this study showed the following frequencies of resistance to the 15 antibiotics (Table 3). All MRSA isolates from the animals were resistant to penicillin and ampicillin. Among the 15 isolates, 11, 10, 10, 8, 6, 8, 10, 4, 3, 2, and 1 were resistant to erythromycin, gentamicin, kanamycin, clindamycin, tetracycline, cefoxitin, cephalothin, imipenem, ciprofloxacin, ofloxacin, and norfloxacin, respectively. All isolates were susceptible to amikacin and trimethoprim-sulfamethoxazole. All MRSA strains showed a multidrug resistance phenotype with increased susceptibility to fluoroquinolones. The results from the susceptibility profiles indicated that the animal isolates from this study were within nine different antibiotypes. Oxacillin resistance was confirmed by determining the MICs for all MRSA isolates. The range of the oxacillin MICs for the animal MRSA isolates was between 4 and greater than 128 μg/ml (Table 2). The MICs of vancomycin for the MRSA isolates were all less than 2 μg/ml.

RAPD patterns. To determine the genetic relatedness of the animal MRSA isolates to human isolates, RAPD patterns were generated. Among the 15 animal MRSA isolates, eight visually different RAPD patterns (types I to VIII) were generated by AP-PCR based on combinations of primers M13 and H12 (Fig. 1 and Table 2). Among all 38 human MRSA isolates during this study, four different patterns were visually identified. A total of 4 isolates were selected from the 38 human MRSA isolates. Each of the four isolates was representative of one of the four different RAPD patterns determined for the human MRSA isolates. These isolates were designated isolates 16, 17, 18, and 19 (Fig. 1 and Table 2). Among the MRSA isolates from dairy cattle the most frequent pattern was RAPD type II, which was characteristic of five isolates (41.7%), including isolates 2, 5, 10, 11, and 12. One of the human isolates, isolate 19, showed a RAPD pattern (type II) identical to that of these isolates. RAPD type II, which was shared by 14 of the 38 human MRSA isolates collected in this study, was also the most prevalent pattern in human isolates. In the results of a GelCompar analysis, the isolates showing a RAPD type II pattern grouped together with 95% genetic similarity (Fig. 2). Isolates 9 and 13, which possessed type VI RAPD patterns, shared 91% similarity with the isolates of type II. One of the dairy cattle isolates (isolate 7) showed a pattern identical to that of a human isolate (isolate 17) that exhibited type IV characteristics. However, isolate 6, a dairy cattle isolate, showed RAPD patterns (type III) distinctly different from those of the other animal and human isolates. As determined

TABLE 3. Antibiotic susceptibility profiles determined by the agar diffusion method for *mecA*-positive MRSA isolates

Isolate[a]	Susceptibility[b]															Antibiotype
	AN	AM	FOX	CF	CIP	CC	E	GM	IM	K	NOR	OFX	P	TE	SXT	
1	S	R	R	R	S	R	R	S	S	S	S	S	R	S	S	A
2	S	R	S	R	S	S	S	R	S	R	S	S	R	R	S	B
3	S	R	R	R	S	R	R	S	S	S	S	S	R	S	S	A
4	S	R	R	R	S	R	R	S	S	S	S	S	R	S	S	A
5	S	R	S	R	S	S	S	R	S	R	S	S	R	R	S	B
6	S	R	R	R	R	R	S	R	R	R	S	S	R	R	S	C
7	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	D
8	S	R	R	S	S	R	R	R	S	R	S	R	R	S	S	E
9	S	R	R	R	S	R	R	R	R	R	S	S	R	S	S	F
10	S	R	S	R	S	S	R	R	S	R	S	S	R	S	S	G
11	S	R	S	S	S	S	R	R	S	R	S	S	R	S	S	H
12	S	R	S	S	S	S	R	R	S	R	S	S	R	S	S	H
13	S	R	R	R	S	R	R	R	R	R	S	S	R	S	S	F
14	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	C
15	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	I
16	S	R	R	R	S	R	S	S	S	S	S	S	R	R	S	J
17	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	D
18	S	R	R	R	S	S	R	R	S	R	S	S	R	R	S	K
19	S	R	S	R	S	S	S	R	S	R	S	S	R	R	S	B

[a] Isolate numbers are in accordance with those in Table 2.
[b] Abbreviations: AN, amikacin; AM, ampicillin; FOX, cefoxitin; CF, cephalothin; CIP, ciprofloxacin; CC, clindamycin; E, erythromycin; GM, gentamicin; IM, imipenem; K, kanamycin; NOR, norfloxacin; OFX, ofloxacin; P, penicillin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; S, susceptible; R, resistant.

on the basis of GelCompar analysis, the data showing the genetic relatedness of this isolate to the other isolates revealed only 65% similarity (Fig. 2). Three MRSA isolates from chicken specimens were isolated during this study. AP-PCR showed that all three chicken isolates had identical RAPD patterns (all type I) and detected genetic homogeneity at 97% similarity. These isolates were as much as 83% genetically related to the other animal and human isolates (Fig. 2).

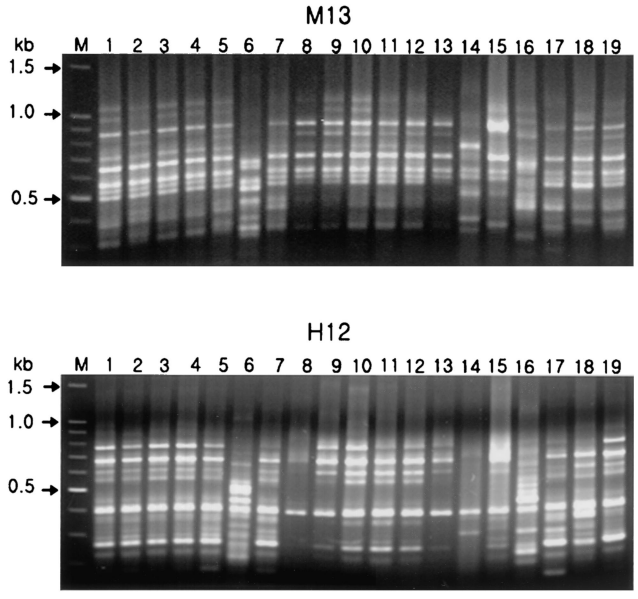

FIG. 1. RAPD patterns of MRSA isolates generated by AP-PCR with primers M13 (upper panel) and H12 (lower panel). Isolate numbers (in accordance with the data presented in Table 2) are indicated above the lanes, and molecular size markers (M) are indicated to the left of the panels. RAPD isolate patterns generated by each primer are designated I to IX (shown in Table 2) on the basis of the visual interpretation of the RAPD results generated by the two primers.

DISCUSSION

This study was designed to determine the prevalence of MRSA isolates among *S. aureus* isolates from major food animal-related specimens and to determine the antimicrobial susceptibilities of these isolates and their genetic relatedness to human MRSA isolates. This was done to evaluate whether animal MRSA isolates are a possible source of human infections.

Among the specimens from cattle, pigs, and chickens examined in this study, 421 contained *S. aureus.* Of the isolates from these specimens, 15 (3.6%) were resistant to oxacillin and were positive for the *mecA* gene. The level of oxacillin resistance of these 15 isolates ranged between 4 and greater than 128 μg/ml (Table 2). The other antimicrobial susceptibility tests revealed that the isolates had the characteristics of general multidrug resistance. All MRSA isolates were resistant to penicillin and ampicillin and were less susceptible to erythromycin, gentamicin, and kanamycin. Such profiles of antibiotic resistance occur rather frequently in many of the MRSA isolates from other countries (1, 10, 15, 26, 27, 32). The isolates from this study, however, were more susceptible to fluoroquinolones, such as ciprofloxacin, ofloxacin, and norfloxacin, and all of the isolates were susceptible to vancomycin, amikacin, and trimethoprim-sulfamethoxazole. The majority (12 of 15) of the MRSA isolates were identified in milk specimens of dairy cattle. Among 12 MRSA isolates from milk specimens, 9 showed signs of mastitis, as indicated by the results of somatic cell count assays. In Korean dairy farms with livestock with mastitis problems, antibiotics (including members of the penicillin family such as ampicillin and penicillin) are largely used as a dry-cow treatment, although oxacillin and methicillin are rarely used in this

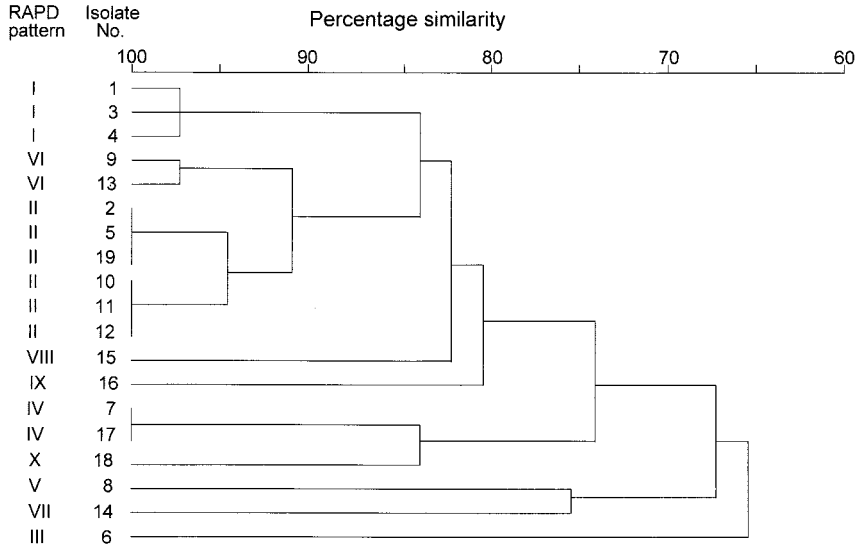

FIG. 2. Dendrogram showing the levels of similarity between RAPD patterns of 19 isolates of *mecA*-positive MRSA isolates as determined by AP-PCR with primers M13 and H12 and subsequent GelCompar analysis of digitized photographs. The scale indicates levels of genetic relatedness within this set of isolates. Each pattern of isolates is designated type I to X based on visual interpretation of the combined RAPD results shown in Fig. 1. Isolate numbers 1 to 19 are in accordance with the results shown in Table 2.

veterinary field. This practice may contribute to the increasing incidence of MRSA strains in cows associated with mastitis.

RAPD analysis has been extensively applied for epidemiological typing and taxonomic study of microbial isolates (9, 25, 29, 31). RAPD patterns were generated to determine the genetic relatedness between the animal and human MRSA isolates in a community. Eight visually different RAPD patterns (types I to VIII) from the 15 animal MRSA isolates were generated (on the basis of combinations of primers M13 and H12) (10) by AP-PCR. Techniques using antibiotypes are also useful tools for the epidemiological study of bacterial isolates (9, 10, 15, 22, 27). The results of determinations of the susceptibility profiles of the 15 isolates revealed nine different antibiotypes. RAPD patterns assigned to isolates were compared with antibiotypes of isolates (Table 2). General agreement was found between the two types of data but with some discrepancies. Isolates with the same RAPD types were within the same antibiotype categories, although isolates with RAPD type II patterns revealed three different antibiotypes (Table 2). This finding indicates that categorization of the MRSA isolates by RAPD pattern was concordant with antibiotype categorization. The most frequent RAPD pattern was type II, which was characteristic of five isolates from different milk specimens of dairy cattle. One of the human isolates generated a type II RAPD pattern identical to that of these isolates. RAPD type II was also the most prevalent pattern among the 38 human MRSA isolates in this study. These results indicated that the MRSA strain generating RAPD pattern type II is most likely the dominant MRSA strain in the community environment. Of the dairy cattle isolates, one (isolate 7 [type IV]) also showed a pattern identical to that of a human isolate (Fig. 1 and 2). There are several study reports that suggest that the transfer of *S. aureus* between humans and cattle is possible (6, 24, 35), although successful transfer of these bacteria between humans and cattle is not a frequent event. The present data also indicated that infection of humans by transmission through food products contaminated with animal MRSA is very plausible. The animal MRSA isolates may have originally come from humans, considering that the rate of methicillin resistance among human *S. aureus* isolates in Korea is over 50% (13) and that the incidence of MRSA in animals (3.6% in this study) is relatively low. Once interspecies transfer has occurred, these isolates can become widespread in the veterinary environment of Korea, where antibiotics such as vancomycin, amikacin, and fluoroquinolones (which are more effective against this organism) are rarely used for animals. This can lead to an increasing prevalence of MRSA strains in humans.

In the GelCompar analysis, the six isolates generating the type II RAPD pattern in this study were also grouped together at 95% genetic similarity (Fig. 2), suggesting a shared genomic background. Isolates 9 and 13, which generated the type VI RAPD pattern, also shared 91% similarity with the isolates generating the type II RAPD pattern. Three MRSA isolates from chicken specimens were isolated during this study. To our knowledge, this is the first report on isolation of MRSA from chicken. All these three isolates showed the same RAPD pattern (type I) and had a genetic homogeneity of 97% similarity among the isolates. These isolates were 83% genetically related to the animal and human isolates that generated RAPD pattern types II and VI. All of this may indicate that these isolates are a closely related and coherent group that may have diverged from a common ancestor.

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REFERENCES

1. **Aires de Sousa, M., I. S. Sanches, M. L. Ferro, M. J. Vaz, Z. Saraiva, T. Tendeiro, J. Serra, and H. de Lencastre.** 1998. Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. J. Clin. Microbiol. **36:**2590–2596.
2. **Cefai, C., S. Ashurst, and C. Owens.** 1994. Human carriage of methicillin-resistant *Staphylococcus aureus* linked with pet dog. Lancet **344:**539–540.
3. **Crossley, K., B. Landseman, and D. Zaske.** 1979. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides II. Epidemiologic studies. J. Infect. Dis. **139:**280–287.
4. **Devriese, L. A., and J. Hommez.** 1975. Epidemiology of methicillin-resistant *Staphylococcus aureus* in dairy herds. Res. Vet. Sci. **19:**23–27.
5. **Foster, T. J.** 1991. Potential for vaccination against infections caused by *Staphylococcus aureus.* Vaccine **9:**221–227.
6. **Fox, L. K., M. Gershman, D. D. Hancock, and C. T. Hutton.** 1991. Fomites and reservoirs of *Staphylococcus aureus* causing intramammary infections as determined by phage typing: the effect of milking time hygiene practices. Cornell Vet. **81:**183–193.
7. **Herold, B. C., L. C. Immergluck, M. C. Maranan, D. S. Lauderdale, R. E. Gaskin, S. Boyle-Vavra, C. D. Leitch, and R. S. Daum.** 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA **279:**593–598.
8. **Horan, T., D. Culver, W. Jarvis, et al.** 1988. Pathogens causing nosocomial infections: preliminary data from the national nosocomial infections surveillance system. Antimicrob. Newsl. **5:**65–67.
9. **Hsueh, P.-R., L.-J. Teng, S.-W. Ho, W.-C. Hsieh, and K.-T. Luh.** 1996. Clinical and microbiological characteristics of *Flavobacterium indologenes* infections associated with indwelling devices. J. Clin. Microbiol. **34:**1908–1913.
10. **Hsueh, P.-R., L.-J. Teng, P.-C. Yang, H.-J. Pan, Y.-C. Chen, L.-H. Wang, S.-W. Ho, and K.-T. Luh.** 1999. Dissemination of two methicillin-resistant *Staphylococcus aureus* clones exhibiting negative staphylase reactions in intensive care units. J. Clin. Microbiol. **37:**504–509.
11. **Humphreys, H., J. D. Carroll, C. T. Keane, M. T. Cafferkey, H. M. Pomeroy, and D. C. Coleman.** 1990. Importation of methicillin-resistant *Staphylococcus aureus* from Baghdad to Dublin and subsequent nosocomial spread. J. Hosp. Infect. **15:**127–135.
12. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282–298. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
13. **Lee, H. J., J. T. Suh, Y. S. Kim, W. Lenz, G. Bierbaum, and K. P. Schaal.** 2001. Typing and antimicrobial susceptibilities of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated in a hospital in Korea. J. Korean Med. Sci. **16:**381–385.
14. **Mandell, G., J. Douglas, and R. Bennett (ed.).** 1995. Principles and practices of infectious diseases, 4th ed. Churchill Livingstone, Ltd., Edinburgh, United Kingdom.
15. **Melter, O., I. Santos Sanches, J. Schindler, M. Aires de Sousa, R. Mato, V. Kovarova, H. Zemlickova, and H. de Lencastre.** 1999. Methicillin-resistant *Staphylococcus aureus* clonal types in the Czech Republic. J. Clin. Microbiol. **37:**2798–2803.
16. **Muder, R. R., C. Brennen, M. M. Wagener, R. M. Vicker, J. D. Rihs, G. A. Hancock, Y. C. Yee, J. M. Miller, and V. L. Yu.** 1991. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **Mulligan, M. E., K. A. Murray-Leisure, B. S. Ribner, H. C. Standiford, J. F. John, J. A. Korvick, C. A. Kauffman, and V. L. Yu.** 1993. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am. J. Med. **94:**313–327.
18. **Murakami, K., W. Minamide, K. Wada, E. Nakamura, H. Teraoka, and S. Watanabe.** 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J. Clin. Microbiol. **29:**2240–2244.
19. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standards. NCCLS document M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. **National Committee for Clinical Laboratory Standards.** 2001. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
21. **National Committee for Clinical Laboratory Standards.** 2001. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, Pa.
22. **Opal, S. M., K. H. Mayer, M. J. Stenberg, J. E. Blazek, D. J. Mikolich, D. L. Dickensheets, L. W. Lyhte, R. R. Trudel, and J. M. Musser.** 1990. Frequent acquisition of multiple strains of methicillin-resistant *Staphylococcus aureus* by healthcare workers in an endemic hospital environment. Infect. Control Hosp. Epidemiol. **11:**479–485.
23. **Peacock, J. E., Jr., F. J. Marsik, and R. P. Wenzel.** 1980. Methicillin-resistant *Staphylococcus aureus*: introduction and spread within a hospital. Ann. Intern. Med. **93:**526–532.
24. **Roberson, J. R., L. K. Fox, D. D. Hancock, J. M. Gay, and T. E. Besser.** 1994. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J. Dairy Sci. **77:**3354–3364.
25. **Roberts, R. B., A. de Lencastre, W. Eisner, E. P. Severina, B. Shopsin, B. N. Kreiswirth, A. Tomasz, and the MRSA Collaborative Study Group.** 1998. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in 12 New York hospitals. J. Infect. Dis. **178:**164–171.
26. **Saroglou, G., M. Cromer, and A. L. Bisno.** 1980. Methicillin-resistant *Staphylococcus aureus*: interstate spread of nosocomial infections with emergence of gentamicin-methicillin resistant strains. Infect. Control **1:**81–87.
27. **Seguin, J. C., R. D. Walker, J. P. Caron, W. E. Kloos, C. G. George, R. J. Hollis, R. N. Jones, and M. A. Pfaller.** 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J. Clin. Microbiol. **37:**1459–1463.
28. **Tenover, F. C., and R. P. Gaynes.** 2000. The epidemiology of *Staphylococcus* infections, p. 414–421. *In* V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy, and J. I. Rood (ed.), Gram-positive pathogens. American Society for Microbiology, Washington, D.C.
29. **Tenover, F. C., R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hébert, B. Hill, R. Hollis, W. R. Jarvis, B. Kreiswirth, W. Eisner, J. Maslow, L. K. McDougal, J. M. Miller, M. Mulligan, and M. A. Pfaller.** 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus.* J. Clin. Microbiol. **32:**407–415.
30. **Tomlin, J., M. J. Pead, D. H. Lloyd, S. Howell, F. Hartmann, H. A. Jackson, and P. Muir.** 1999. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. Vet. Rec. **16:**60–64.
31. **Udo, E. E., I. A. al-Obaid, L. E. Jacob, and T. D. Chugh.** 1996. Molecular characterization of epidemic ciprofloxacin- and methicillin-resistant *Staphylococcus aureus* strains colonizing patients in an intensive care unit. J. Clin. Microbiol. **34:**3242–3244.
32. **Voss, A., and B. N. Doebbeling.** 1995. The worldwide prevalence of methicillin-resistant *Staphylococcus aureus.* Int. J. Antimicrob. Agents **5:**101–106.
33. **Waldvogel, F. A.** 1990. *Staphylococcus aureus*, p. 1754–1777. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practices of infectious disease, 3rd ed. Churchill Livingstone, New York, N.Y.
34. **Working Party of the Hospital Infection Society and British Society for Antimicrobial Chemotherapy.** 1986. Guidelines for the control of epidemic methicillin resistant *Staphylococcus aureus.* J. Hosp. Infect. **7:**193–201.
35. **Zadoks, R., W. van Leeuwen, H. Barkema, O. Sampimon, H. Verbrugh, Y. H. Schukken, and A. van Belkum.** 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J. Clin. Microbiol. **38:**1931–1939.