Antimicrobial-resistant *Staphylococcus aureus* and MRSA prevalence among Korean families and household items

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Abstract One-hundred-and-seventy-nine *Staphylococcus aureus* strains, collected from a total of 825 resident and household item samples in Korean homes nationwide, were tested, to survey the spread of antimicrobial-resistant *S. aureus* including the methicillin-resistant *S. aureus* (MRSA) strain. Antimicrobial resistance to sulfamethoxazole–trimethoprim (0.0%), teicoplanin (0.0%), tetracycline, vancomycin (0.0%), chloramphenicol (0.0%), ciprofloxacin (1.1%), clindamycin (1.1%), amikacin (2.2%), gentamicin (8.4%), oxacillin (17.9%), erythromycin (21.8%), ampicillin (96.6%) and penicillin (96.6%), was detected by disk diffusion method and 7.3% (13/179) of the antimicrobial-resistant isolates, were multi-drug resistant (resistant to ≥ 3 antimicrobial classes). Nine of 32 oxacillin-resistant strains, were positive for the *mecA* gene and identified as MRSA, in 4 residents and 4 loafah samples, by polymerase chain reaction. These findings provide public health information and contribute to preventing circulation of antimicrobial-resistant strains in community settings including healthy homes.

Keywords Antimicrobial · Home · MRSA · Resistant · *Staphylococcus aureus*

Introduction

Staphylococcus aureus, primarily present on the skin and in the nasal carriage of humans, is a source of skin, soft tissue, bone, joint, respiratory, and gastrointestinal infection and produces enterotoxins that cause foodborne illness [12, 26]. Over recent decades, *S. aureus* has commonly been a critical human pathogen and become a health threat due to the acquisition of antimicrobial resistance [27].

Treatment of nosocomial *S. aureus* infections with penicillin, has caused *S. aureus* to develop β -lactam resistance. By 1946, after penicillin was commercialized, only about 6% *S. aureus* strains were resistant to penicillin [13]. However, by 1948, 50% were resistant and this proportion has increased to approximately 80–90% [13]. Methicillin was introduced in 1959, for the treatment of penicillin-resistant *S. aureus*. However, as observed with the introduction of penicillin, methicillin-resistant *S. aureus* (MRSA) emerged immediately [18].

MRSA isolates were once largely confined to hospitals, other healthcare environments, and patients frequenting these facilities. Until the mid-1990s, MRSA infections were confined to nosocomial infection in the elderly, very young, and patients with weakened immune systems or undergoing surgery [18]. However, community-associated MRSA (CA-MRSA) infections were reported in healthy individuals, without connection to healthcare institutions [2, 18]. In recent years, the incidence of CA-MRSA infections has increased and, consequently, MRSA infections in communities, are a growing public health concern worldwide [14, 27]. According to the Center for Disease Control and Prevention (CDC), the incidence of CA-MRSA increased by 1.57% in 2016 compared to 2013, while the incidence of hospital-associated MRSA (HA-MRSA), decreased by 5.36% [17]. In Denmark, a low

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prevalence of MRSA (<1%) has been achieved, by a strict practice of isolation of all hospitalized patients suspected or confirmed MRSA-positive and restricted use of broad-spectrum antimicrobial agents [4]. However, clones causing CA-MRSA infections, have emerged during the last decades [4]. In Korea, Moon et al. [16] analyzed 3594 *S. aureus* isolates from routine culture over 4 years and reported 33, 54, and 73% of MRSA isolates were CA-MRSA, health care-associated community onset, and health care-associated, respectively. According to the authors, CA-MRSA had increased over the 4 years, whereas, the proportion of HA-MRSA confirmed strains had remained the same [16].

In contrast to HA-MRSA, CA-MRSA strains producing Panton-Valentine leucocidin toxin, are more virulent and lead to severe necrotizing fasciitis and necrotizing pneumonia, with mortality rates of 20 and 75%, respectively [3, 23]. Furthermore, two previous studies found that these strains were circulated between the hospital and other settings including the home and transferred from person-to-person, through contaminated surfaces [2, 3].

The present study was conducted, to survey the spread of antimicrobial-resistant *S. aureus* including MRSA, from residents and contaminated items in Korean homes.

Materials and methods

Sample collection

The 138 households that voluntarily participated in this study, were located in seven Korean metropolises: Seoul, Gyeonggi province, Incheon, Busan, Daejeon, Daegu, and Gwangju. Data regarding the health condition of the family members, current or recent (within the past 3 months of the interview) antimicrobial therapy and possession of pets, was collected through face-to-face interviews with all of the homemakers. A total of 549 household samples from the dishcloth ($n = 135$), sink surface ($n = 138$), kitchen sponge ($n = 138$) and bathroom loofah ($n = 138$), as well as from 138 residents living in two ($n = 276$) of the seven metropolises examined, were tested between February and October 2011, to isolate *S. aureus*.

Isolation and identification of *S. aureus*

All samples were obtained by sterile cotton swabs (e-Swab, 3 M, St. Paul, MN, USA). These samples were transferred to tryptic soy broth (Oxoid, Basingstoke, Hampshire, England) containing 10% NaCl and incubated at 37 °C for 18 h, for enrichment culture. After incubation, 1 loopful of enriched broth was streaked to Baird-Parker agar supplemented with rabbit plasma fibrinogen (Biomérieux, Marcy

l'Etoile, France) and further incubated at 37 °C for 18 h. The gray/black colonies, surrounded by an opaque halo, were identified as *S. aureus*, using the Vitek-2 Compact system (Biomérieux).

Antimicrobial susceptibility testing

The antimicrobial agents tested were amikacin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin (10 µg), sulfamethoxazole–trimethoprim (23.75/1.25 µg), teicoplanin (30 µg), tetracycline (30 µg), and vancomycin (30 µg). The antimicrobial susceptibility was performed by disk diffusion assay, according to the Clinical and Laboratory Standards Institute (CLSI, 2011) guideline. The confirmed *S. aureus*-positive isolates, were cultured in Mueller–Hinton broth (Oxoid) at 37 °C for 2–6 h and adjusted to 0.5 McFarland turbidity standard. With a sterile cotton swab, suspended *S. aureus* was uniformly spread on Mueller–Hinton agar (Oxoid). After the agar surface was dried, the antimicrobial disks were placed on the agar plate, using a disk dispenser. The plates were incubated at 37 °C for 24 h. After incubation, the antimicrobial susceptibility was determined (CLSI, 2011). *S. aureus* ATCC25923 was used for quality control.

Multiple antimicrobial resistance (MDR) index

The MDR index (a/b), was defined as the ratio of the number of antimicrobial agents the isolates were resistant against (a) to the total number of antimicrobial agents the isolates were tested against (b), as described by Kilonzo-Nthenge et al. [10].

Detection of *mecA* gene

Polymerase chain reaction (PCR) (Applied Biosystems, Waltham, MA, USA) was performed to detect the *mecA* gene, among the isolates concurrently resistant to ampicillin, oxacillin and penicillin, using forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3' and reverse primer 5'-AGTTCTGCAGTACCGGATTTGC-3' [25]. The PCR thermocycler conditions, consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. A final extension of the reaction was performed at 72 °C for 1 min. PCR products were analyzed using 1.5% agarose gel (Merk, Darmstadt, Germany) electrophoresis and visualized by a gel document image system (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Data were analyzed by Fisher's exact test or Chi square test, using SPSS (version 18, SPSS, Chicago, IL, USA). Differences were considered significant at $p < 0.05$.

Results and discussion

Isolation of *Staphylococcus aureus* from Korean homes

In order to evaluate the extent of *S. aureus* contamination in the home environment, 825 residents and household item samples, selected based on direct contact with humans and prepared food, were tested. A total of 179 *S. aureus* isolates were collected from 84 homes, in which at least one sample tested, was contaminated. Table 1 presents the frequency of *S. aureus* occurrence. Seventy-four isolates were collected from the residents (hand; $n = 51$ and nasal carriage; $n = 23$). *S. aureus* is commonly found on human skin. Among the household item samples, the most common isolates were collected from the loofah ($n = 37$). The loofah is an item shared among family members and regularly makes direct contact with the skin. Consequently, this item showed a high level of *S. aureus* contamination compared to the other household items examined.

Antimicrobial resistance of *S. aureus* isolates

Table 2 shows the antimicrobial susceptibility of the 179 *S. aureus* isolates, obtained from residents and household items. Thirteen antimicrobial agents were tested. All of the *S. aureus* isolates were susceptible to chloramphenicol, teicoplanin, sulfamethoxazole–trimethoprim and vancomycin. A total 3.4% (6/179) of the *S. aureus* isolates were susceptible to all the antimicrobial agents tested and, among those six isolates, four isolates were recovered from three residents. The highest prevalence of resistance was for ampicillin (96.6%) and penicillin (96.6%). All the

isolates that were resistant to ampicillin, were also resistant to penicillin. The high prevalence of *S. aureus* resistance to ampicillin and penicillin might be because the penicillin class is the most useful and commonly used antimicrobial agent, to treat staphylococcal infections. Other common antibiotic drugs that the *S. aureus* isolates were resistant to included erythromycin; 21.8% ($n = 39$), oxacillin; 17.9% ($n = 32$), gentamicin; 8.4% ($n = 15$), tetracycline; 3.9% ($n = 7$), amikacin; 2.2% ($n = 4$) and clindamycin and ciprofloxacin; 1.1% ($n = 2$), respectively. Among the *S. aureus* isolates obtained from residents, 94.6, 94.6, and 21.6% were resistant to ampicillin, penicillin and erythromycin, respectively. For the *S. aureus* isolates obtained from household items, resistance to ampicillin, penicillin and erythromycin was 98.1, 98.1, and 21.9% respectively. There was no significant difference between residents and household items, regarding the proportion of *S. aureus* isolates with antimicrobial resistance ($p > 0.05$).

In this study, the proportion of *S. aureus* isolates resistant to erythromycin and tetracycline, was less than isolates from tertiary hospitals in Korea (erythromycin; 66.5%, tetracycline; 55.1%) and China (erythromycin; 62.7%, tetracycline; 47.5%) but more prevalent than isolates from healthy students at the University Putra Malaysia (erythromycin and tetracycline; 11.7%, respectively) [1, 27]. The isolates with antimicrobial resistance to erythromycin and tetracycline, showed no distinction between residents and household items ($p > 0.05$). The presence of tetracycline-resistant isolates from the sink and sponge, was 10 and 14.8%, respectively, whereas, no tetracycline-resistant isolates from dishcloth and loofah, were found. The sink and sponge are commonly associated with food preparation and handling of food derived from animals. A previous study reported various food items that cause bacterial contamination of the sink, kitchen sponge and other kitchen surfaces [6]. In several recent studies, tetracycline-resistant *S. aureus* strains were isolated at high frequency, from various food of animal origin. In Taiwan, 66.7% of the *S. aureus* strains in pork and chicken carcasses, were resistant to tetracycline [12]. Among the *S. aureus* isolates from

Table 1 Distribution of *Staphylococcus aureus* isolates according to residents and household items

Samples	Samples (n)	<i>S. aureus</i> isolates (n)	<i>S. aureus</i> isolates (%)
Household item			
Dishcloth	135	31	12.0
Sink	138	10	7.2
Sponge	138	27	19.6
Loofah	138	37	26.8
Resident			
Hand	138	51	37.0
Nasal carriage	138	23	16.7
Total	825	179	100

Table 2 Prevalence of antimicrobial-resistant *Staphylococcus aureus* isolates obtained from residents and household items

Antimicrobial agent	Prevalence of antimicrobial resistance (%)							
	Dishcloth (n = 31)	Sink (n = 10)	Sponge (n = 27)	Loofahs (n = 37)	House-hold items (n = 105) ^a	Hand (n = 51)	Nasal carriage (n = 23)	Residents (n = 74) ^b
Amikacin	3.2	0.0	3.7	0.0	1.9	2.0	4.3	2.7
Ampicillin	96.8	100.0	96.3	100.0	98.1	94.1	95.7	94.6
Chloramphenicol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	0.0	0.0	3.7	2.7	1.9	0.0	0.0	0.0
Clindamycin	6.5	0.0	0.0	0.0	1.9	0.0	0.0	0.0
Erythromycin	25.8	10.0	29.6	16.2	21.9	19.6	26.1	21.6
Gentamicin	3.2	0.0	7.4	10.8	6.7	9.8	13.0	10.8
Oxacillin	16.1	20.0	22.2	16.2	18.1	15.7	21.7	17.6
Penicillin	96.8	100.0	96.3	100.0	98.1	94.1	95.7	94.6
Sulfamethoxazole– trimethoprim	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Teicoplanin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline	0.0	10.0	14.8	0.0	4.8	2.0	4.3	2.7
Vancomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^aTotal of dishcloth, sink, sponge and loofah samples^bTotal of hand and nasal carriage samples

USA retail meats, 69% were resistant to tetracycline, 26% to penicillin, 17% to ampicillin, and 8% to erythromycin [9]. In Korea, 72.7% of the *S. aureus* strains isolated from livestock and marine products and processed food, were resistant to ampicillin and penicillin, respectively, 37.6% were tetracycline-resistant and 20.6% were erythromycin-resistant [19]. In particular, the kitchen surface acts as a common intermediate for the transmission of antimicrobial-resistant *S. aureus* strains from pork products to human skin [24]. Based on these reports, it is reasonable to suggest that the high prevalence of tetracycline resistance found among the isolates from sink and sponge samples, in the current study, is associated with the handling of meat products.

According to the interview conducted in this study, of the 138 homes, at least one family member in 27 homes was on antimicrobial therapy within the 3 months prior to the interview, and 29 homes had pets (24 dogs; 82.8%, 3 hamsters; 10.3%, 2 cats; 6.9%). Twenty-one *S. aureus* isolates were collected from 27 homes with residents on antimicrobial therapy, and 33 were collected from 29 homes with pets. Table 3 shows the antimicrobial susceptibility of these isolates.

All of the 21 isolates from 27 homes with residents on antimicrobial therapy, were resistant to ampicillin and penicillin (100%, respectively). Although the proportion of isolates showing resistance to ampicillin and penicillin was higher in the homes with residents on antimicrobial therapy

than non-antimicrobial therapy homes (96.2%), no significant difference ($p > 0.05$) was found. These isolates were susceptible to 9 of the 13 antimicrobial agents tested, except penicillin classes. The percentage of isolates resistant to erythromycin (0.0%) and oxacillin (14.3%) was lower for the homes with residents on antimicrobial therapy than the non-antimicrobial therapy homes (24.7 and 18.4%, respectively). However, only the proportion of isolates with antimicrobial resistance to erythromycin, was significantly different ($p < 0.05$).

Pets can also be colonized and contaminated with *S. aureus* including MRSA and cause disease in humans. Therefore, pets are an indispensable factor in the study of pathogen transmission in households [6]. All of the 33 isolates from 29 homes with pets, were resistant to ampicillin and penicillin (100%, respectively). The percentage of isolates with resistance to erythromycin, oxacillin, clindamycin and tetracycline, was 21.2, 12.1, 3.0, and 3.0%, respectively. Although there were more isolates showing resistance to ampicillin and penicillin, in homes with pets, compared to non-pet homes, there was no significant difference in the prevalence of resistant isolates, to any of the antimicrobial agents tested ($p > 0.05$). The prevalence of antimicrobial resistance, among the isolates from residents in homes with pets and non-pet homes, was consistent with the overall prevalence of resistance to antimicrobial agents in these homes. Although no association was found between the prevalence of antimicrobial

Table 3 Prevalence of antimicrobial-resistant *Staphylococcus aureus* isolates obtained from homes classified by traits

Antimicrobial agent	Prevalence of antimicrobial-resistant isolates in homes (%)			
	Resident undergoing antimicrobial therapy		Pet possession	
	Yes (n = 21)	No (n = 158)	Yes (n = 33)	No (n = 146)
Amikacin	0.0	2.5	0.0	2.7
Ampicillin	100.0	96.2	100.0	95.9
Chloramphenicol	0.0	0.0	0.0	0.0
Ciprofloxacin	0.0	1.3	0.0	1.4
Clindamycin	0.0	1.3	3.0	0.7
Erythromycin	0.0	24.7	21.2	21.9
Gentamicin	0.0	9.5	0.0	10.3
Oxacillin	14.3	18.4	12.1	19.2
Penicillin	100.0	96.2	100.0	95.9
Sulfamethoxazole–trimethoprim	0.0	0.0	0.0	0.0
Teicoplanin	0.0	0.0	0.0	0.0
Tetracycline	0.0	4.4	3.0	4.1
Vancomycin	0.0	0.0	0.0	0.0

resistance and the presence of pets at home, a previous study reported that pets were probably carriers of *S. aureus* and MRSA, within households, and cause transmission, either directly by human hand and mouth contact or indirectly through contact with the bed [6].

Multidrug resistance of *S. aureus* isolates and multiple antibiotic resistance (MAR) index

Multidrug resistance (MDR), defined as resistant to ≥ 3 antimicrobial classes, as categorized by the CLSI, of *S. aureus* has been a serious concern worldwide [15]. Infections caused by MDR organisms show 2–5 times higher mortality rates compared to infections related to antimicrobial susceptible bacteria delaying in effective antimicrobial therapy [7]. A total of seven antimicrobial resistance patterns of multidrug was observed in 7.3% (13/179) of the *S. aureus* isolates (Table 4). The contamination of MDR *S. aureus* isolated from home in this study was consistent to the previous study, which reported MDR *S. aureus* contamination of hand touched surfaces in University campus in U.S (7.9%, 6/76) [29]. However, the prevalence of MDR *S. aureus* in this study was less than other studies including 15.4% from raw cow milk, 14.3% from traditional cheese in Iran, 32.0% from pork, 11.7% from chicken, 10.2% from beef in USA supermarkets and retail chains [8, 28].

The most common MDR patterns observed were the penicillin class (AMP-P-OX), macrolide class (E), and tetracycline class (TE), which were isolated from sponge

(n = 2) and residents (n = 2). Among the 13 MDR isolates, the MAR index ranged from 0.31 to 0.46, and the highest values were obtained from 2 isolates, from dishcloth and sponge. A MAR index > 0.2 , suggests the microorganisms are resistant to several antimicrobial agents [1]. Therefore, the MAR indices > 0.46 , indicated the isolates from dishcloth and sponge, were in the environment that antimicrobial agents were often exposed to.

Prevalence of MRSA strains

Thirty-two of the 179 isolates, were concurrently resistant to ampicillin, oxacillin and penicillin, and these isolates were tested for the *mecA* gene. The β -lactam resistant gene *mecA*, located in the staphylococcal chromosomal cassette *mec*, confers resistance via penicillin-binding protein 2a (PBP2a), the protein product of *mecA* that has very low affinity for all β -lactam antibiotics [13, 17]. Therefore, screening for the presence of the *mecA* gene, can avoid misclassifying MRSA [1].

Nine of 32 isolates were positive for the *mecA* gene, therefore 5.0% (9/179) strains were determined as MRSA strains. In USA population, colonized with MRSA, is estimated 1.5 and 5.3% of hand touched surfaces in university campus were contaminated by MRSA [28, 29]. However, other studies related to the communities reported higher prevalence of MRSA compared to this study. The prevalence of MRSA from University student home surfaces, football and wrestling locker room of National

Table 4 Multiple antibiotic resistance (MAR) patterns and indices of *Staphylococcus aureus* isolated from residents and household items

Sample	Resistant pattern (no. antimicrobial classes) ^a	<i>S. aureus</i> isolates (n)	MAR index
Household item			
Dishcloth	AMP, P-E-DA (3)	2	0.38
	AMP, OX, P-AK, CN-E (3)	1	0.46
Sponge	AMP, P-CN-E (3)	1	0.31
	AMP, P-E-CIP (3)	1	0.31
	AMP, OX, P-E-TE (3)	2	0.38
	AMP, OX, P-AK, CN-E (3)	1	0.46
Loofah	AMP, P-CIP-CN-E (4)	1	0.38
Resident			
Hand	AMP, OX, P-E-TE (3)	1	0.38
Nasal carriage	AMP, P-CN-E (3)	1	0.31
	AMP, OX, P-CN-E (3)	1	0.38
	AMP, OX, P-E-TE (3)	1	0.38
Total		13	

^aAK amikacin, AMP ampicillin, CIP ciprofloxacin, CN gentamicin, DA clindamycin, E erythromycin, OX oxacillin, P penicillin, TE tetracycline

Collegiate Athletic Association Division school in U.S were 11.8, 31.0, 10.0% respectively [29].

The nine MRSA strains isolated in this study, were derived from 4 residents and 4 loofahs (Table 5). Of the 4 MRSA stains from residents, the MRSA strain coexisted on the hand and in the nasal carriage, simultaneously, of one subject. Numerous recent studies have reported that households colonized with MRSA, play a key role in the spread of these strains [2, 6, 22]. Transmission of MRSA occurs from household-to-person bi-directly, via skin contact including hand contact and sharing contaminated items. Therefore, in this study, the loofahs contaminated with MRSA strains, which have skin-to-skin contact and are shared among the family members, can be a vehicle for transfer. The spread of MRSA in a community, is linked to the interactions among humans, within households, between households and with community sites (e.g. schools, sports clubs, daycare facilities, travel, farms) [11, 21]. In seven homes with MRSA stains, the risk factors

for MRSA acquisition, were not found among the family members (no pets, no current antimicrobial prescriptions, no antimicrobial agents used within the past 3 months of the interview, no recent hospitalization and no MRSA infection).

As a large number of studies on antimicrobial resistance have been based on healthcare settings worldwide, the prevalence of *S. aureus* for nosocomial infections has been primarily reported [5]. In order to control the rate of antimicrobial resistance and improve public health, not only prudent use of antimicrobial agents but also minimizing the circulation of antimicrobial resistance in the community, healthcare setting and veterinary sectors, are required [20]. In addition, monitoring the prevalence of antimicrobial resistant strains including MRSA strains, in residents and households in healthy homes, must be constantly implemented.

In summary, this study found that *S. aureus* strains isolated from households, were commonly resistant to ampicillin, penicillin, oxacillin and erythromycin, and spread throughout the homes. Moreover, nine MRSA strains were recovered from residents, and loofahs likely acted as vehicles of the pathogen. A limitation of this study is that the origin of contamination with MRSA strains, does not discriminate, directly. However, in various communities, humans are mediators of CA-MRSA that is transferred into their home.

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Table 5 Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains with the *mecA* gene isolated from residents and household items

Sample	MRSA strains (n)	MRSA strains (%)
Household item		
Loofah (n = 37)	4	10.8
Resident		
Hand (n = 51)	1	2.0
Nasal carriage (n = 23)	4	17.4
Total (n = 179)	9	5.0

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