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Prevalence of Enterotoxigenic *Staphylococcus aureus* in Retail Ready-to-eat Korean *Kimbab* Rolls

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Abstract *Staphylococcus aureus* in Korean *kimbab* rolls was monitored seasonally in 4 major cities of Korea to investigate the risk of *S. aureus* in a pre-prepared meal. Thirty-five (28.6%) of 105 *kimbab* rolls purchased in winter were contaminated with *S. aureus* with an average level of 2.6 log CFU/g. Thirty-six (33.0%) of 109 *kimbab* rolls purchased in summer and autumn were contained *S. aureus* with an average level of 2.9 log CFU/g. *Kimbab* purchased in snack bars showed higher *S. aureus* contamination rates with the maximum level of 4.7 log CFU/g than that purchased in convenience stores. Of the raw materials in *kimbab*, uncooked perilla leaf had the highest contamination rate of *S. aureus*. Less than 50% of *S. aureus* isolated from *kimbab* produced enterotoxin and most of the staphylococcal enterotoxin produced by *S. aureus* in *kimbab* was type A.

Keywords: *Staphylococcus aureus*, staphylococcal enterotoxin, ready-to-eat meal, *kimbab*

Introduction

Food poisoning has recently occurred on a large scale with the increasing consumption of foods from catering outlets and restaurants. In Korea, *Staphylococcus aureus* is the third most common microorganism involved in food poisoning after *Salmonella* spp. and *Vibrio* spp. Food poisoning outbreaks caused by ready-to-eat meals, such as *kimbab* and packed lunch boxes, have accounted for 26.7% of these in 2003 (1). The major causative food-poisoning bacteria in *kimbab* in Korea have been *S. aureus* and *Salmonella* spp. (2).

Staphylococcal food poisoning occurs when enterotoxin causes vomiting, nausea, acute prostration, and abdominal cramps. *S. aureus* is present in the nasal passages, throat, hair, and skin of humans. Food is contaminated with *S. aureus* from materials such as meat and vegetables and by food handlers (3, 4). Staphylococcal food poisoning is dependent on the toxin production during the growth of *S. aureus* in food. The optimum temperature for enterotoxin production is in the 40-45°C range, although it can be produced in the lower temperature (5). The staphylococcal enterotoxins are 28 kDa single-chain polypeptides, and A to Q types of enterotoxins have been identified (6-8). The major illness-causing types of enterotoxins are types A and D (6).

Kimbab is a fully cooked, ready-to-eat Korean roll made of cooked meat, egg, seafood, and vegetables inside rice and seaweed. Warm rice is easy to roll during the preparation of *kimbab*, but contributes to the growth of bacteria. However, there is no hazard-reduction process after the *kimbab* is rolled. *Kimbab* rolls are prepared at home and are sold in many places in Korea such as department stores, convenience stores, *kimbab* stores, roadside stands, and snack bars.

Although other studies related to enterotoxigenic *S. aureus* in various ready-to-eat foods have been reported (1, 9-14), the consumption rate of pre-prepared meals is increasing. Recently, the prevalence of *S. aureus* and the characterization of staphylococcal enterotoxins have been reported in Korea (15, 16).

In this study, we investigated the prevalence and levels of *S. aureus* and the production of staphylococcal enterotoxin in Korean *kimbab* rolls in order to prepare a management plan to reduce the potential hazards of pre-prepared meals in Korea.

Materials and Methods

***Kimbab* preparation** *Kimbab* rolls were randomly purchased from snack bars, department stores, and convenience stores in 4 major cities (Seoul, Busan, Daejeon, and Gwangju) of Korea. *Kimbab* sampling was conducted according to Table 1. Approximately 400-500 g of the samples were immediately transferred to an icebox in the laboratory and analysed within 24 hr. Further samples (200-250 g) of the *kimbab* were also separated into each of their components using sterile forceps to investigate the source materials of *S. aureus* contaminations.

Isolation and quantification of *S. aureus* in *kimbab* *S. aureus* in *kimbab* was quantified using the selective agar-plating method. A sample (25 g) of each *kimbab* roll or *kimbab* constituent was diluted 10-fold in 0.85% NaCl and homogenized for 1 min with a stomacher (Seward Medical UAC House, London, UK). An aliquot (1 mL) of each homogenate was serially diluted and plated onto Baird-Parker agar (Difco, Becton Dickinson Co., Sparks, MD, USA) or mannitol salt agar plus egg yolk (Difco) (17). The samples were plated in triplicate and incubated at 37°C for 48 hr. Black colonies in white circles on Baird-

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Table 1. Number of samples collected from 4 regions depending on the selling place and the season

Season	Region	No. of *kimbab* sample			
		Snack bar	Convenience store	Department store	Subtotal
Winter (Jan-Feb)	Seoul	13	6	6	25
	Daejeon	16	6	6	28
	Gwangju	17	4	5	26
	Busan	17	5	4	26
	Subtotal	63	21	21	105
Summer (July-Aug)	Seoul	18	3	5	26
	Busan	23	5	0	28
	Subtotal	41	8	5	54
Autumn (Sep-Oct)	Daejeon	21	5	3	29
	Gwangju	16	7	2	25
	Subtotal	37	12	5	54

Parker agar and yellow colonies in white circles on mannitol salt agar plus egg yolk were counted. *S. aureus* ATCC 13565, *S. aureus* ATCC 19095, and *S. aureus* ATCC 26664 were used as reference strains. The suspected colonies were transferred to brain-heart infusion (BHI) agar (Difco) and cultured at 37°C for 18-24 hr. Gram staining was used to confirm Gram-positive cocci. Biochemical tests were carried out using API Staph (Biomerieux, Marcy l'Etoile, France), and coagulase plasma with EDTA (BBL, Sparks, MD, USA) was used for the coagulase test. Confirmation was according to the instructions of the Bacteriological Analytical Manual of the FDA (18). Detection limit in this study was 100 CFU/g.

Staphylococcal enterotoxin test To identify the staphylococcal enterotoxin, positive *S. aureus* colonies were challenged with a staphylococcal enterotoxin detection kit using reversed passive latex agglutination (SET-RPLA, Denka Seiken, Japan). The confirmed *S. aureus* colonies were cultured in BHI broth for 18 hr, after which the cultures were centrifuged at 8,000×g for 20 min. The supernatants were serially diluted and 25 μL of diluted solution was applied to 5 rows of wells in a microplate. Latex A, B, C, D, or a latex control were loaded into the wells and the mixtures were shaken for 10 min before incubation at 25°C for 18-20 hr. The toxin type was determined from the agglutination pattern.

Results and Discussion

S. aureus* contamination in *kimbab To monitor the prevalence of *S. aureus* in *kimbab*, 105 *kimbab* rolls purchased in winter, 55 *kimbab* rolls purchased in summer, and 54 *kimbab* rolls purchased in autumn on sale in 4 major cities (Seoul, Busan, Daejeon, and Gwangju) in Korea were analysed qualitatively. In winter Seoul had the lowest *S. aureus* contamination rate (28.0%) relative to the contamination rates of Daejeon (35.7%), Gwangju

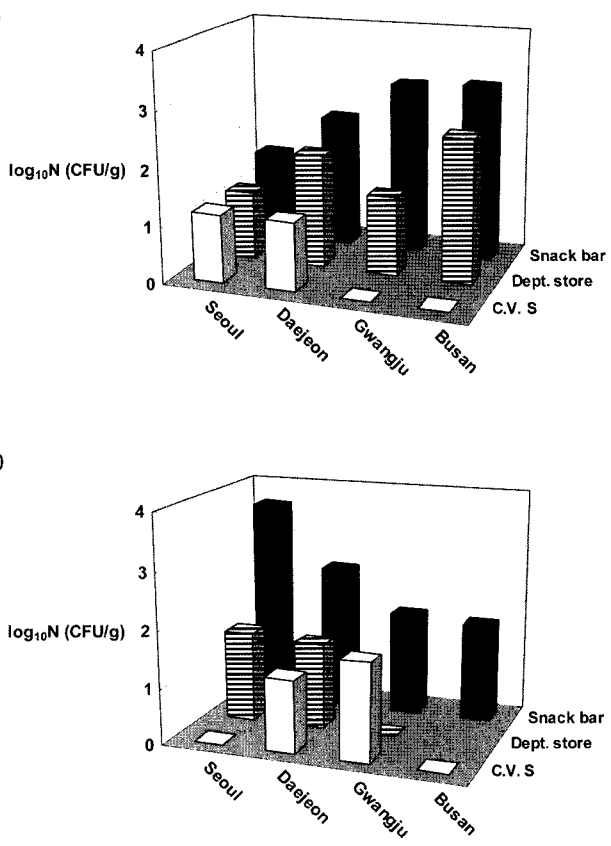

Fig. 1. Average cell numbers of *S. aureus* detected in *kimbab* sold in department stores, snack bars, and convenience stores (C.V. S) in South Korea according to the season of winter (a) and summer/autumn (b).

(38.5%), and Busan (34.6%), while in summer Seoul had the highest *S. aureus* contamination rate (50.0%) relative to the contamination rates of Busan (21.4%), although Seoul is located in the north (Table 2). According to the place of sale, snack bars (35.1-44.4%) had a higher contamination rate than that of department stores (20.0-28.6%) or convenience stores (0.0-25.0%) regardless to the season. More than 4 log CFU/g of *S. aureus* contaminants were observed in *kimbab* sold in snack bars (Table 3). The data were divided to those of winter and those of summer/autumn in this study not only because there was not much difference between the data of summer and that of autumn about the prevalence and the level of *S. aureus* in *kimbab* (Table 2), but also because the atmospheric temperature of Gwangju and Daejeon in autumn was similar to Busan in summer (Table 4). Snack bars in Seoul had the highest contamination level (more than 3 log CFU/g) in summer, and convenience stores in Busan had the lowest level (0%) both in winter and in summer (Fig. 1).

S. aureus is a pyogenic food-borne bacterium. Although food poisoning occurs most frequently in summer, food poisoning by *S. aureus* occurs regardless of the season because *S. aureus* is present on the skins of humans and animals and in the air and soil (4). *Kimbab* was monitored to determine how much the seasons affect the risk of *S. aureus* (Table 2 and Table 4). The qualitative detection rate

Table 2. Number and level of contamination with *S. aureus* of *kimbab* purchased in four regions of South Korea

Season	City	No. of *kimbab* sample	No. of positive sample (%)	Range of log CFU/g
Winter (Jan-Feb)	Seoul	25	7 (28.0)	<2.0
	Daejeon	28	10 (35.7)	2.0-3.2
	Gwangju	26	10 (38.5)	2.0-3.9
	Busan	26	9 (34.6)	2.0-4.1
	Subtotal	105	36 (34.2)	<4.1
Summer (July-Aug)	Seoul	26	13 (50.0)	2.0-4.7
	Busan	28	6 (21.4)	2.0-2.9
	Subtotal	55	19 (34.5)	2.0-4.7
Autumn (Sep-Oct)	Daejeon	29	11 (37.9)	2.0-3.8
	Gwangju	25	6 (24.0)	2.0-2.9
	Subtotal	54	17 (31.5)	2.0-3.8

Table 3. Number and level of *S. aureus* detected in *kimbab* sold in department stores, snack bars, and convenience stores in South Korea

Season	Selling place	No. of sample	No. of positive sample (%)	Range of log CFU/g
Winter (Jan-Feb)	Snack bar	63	28 (44.4)	2.0-4.1
	Convenience store	21	2 (9.5)	<2.0
	Department store	21	6 (28.6)	2.0-3.1
Summer (July-Aug)	Snack bar	41	17 (41.2)	2.0-4.7
	Convenience store	8	0 (0.0)	ND[1)]
	Department store	5	2 (40.0)	<2.0
Autumn (Sep-Oct)	Snack bar	37	13 (35.1)	2.0-2.9
	Convenience store	12	3 (25.0)	2.0-2.4
	Department store	5	1 (20.0)	<2.0

[1)]Not detected.

was the same (around 34%) in both January-February (average temperature of the experimental day: -1.4°C) and July-October (average temperature: 19.1°C). However, the cell level in winter (2.6 log CFU/g) was slightly less than that in summer (2.9 log CFU/g) (Table 4). In winter, the highest *S. aureus* level of 3.0 log CFU/g (at the average atmospheric temperature of 4.2°C) in *kimbab* purchased in Busan and the least of 1.4 log CFU/g (-7.8°C) in Seoul was detected. On the contrary, in summer, *S. aureus* level of 3.5 log CFU/g (26.7°C) in Seoul and 1.7 log CFU/g (15.5°C) in Busan may be attributed to oceanic climate of Busan.

The average cell numbers detected in *kimbab* sold in snack bars (more than 2 log CFU/g) were more than those detected in convenience stores (approximately 1 log CFU/g) (Fig. 2). The low level of *S. aureus* in the *kimbab* sold in convenience stores is attributed to the low ambient temperature (10°C) during distribution and display after the *kimbab* is manufactured compared with the ambient room temperature during the rolling and selling of *kimbab* in department stores or snack bars. The average cell number in the more than 200 *kimbab* rolls tested was in the range of 2.6-2.9 log CFU/g (10^2-10^3 CFU/g) (Table 4). This study demonstrates that the type of retail outlet and the ambient temperature during the preparation and serving of the *kimbab* are more important factors affecting *S. aureus* levels than are the region in which it is

Table 4. The average cell numbers of *S. aureus* detected in *kimbab* purchased in four regions of South Korea and the atmospheric temperature

Season	Region	Average temperature (°C)	*S. aureus* number $Log_{10}N$ (CFU/g)
Winter (Jan-Feb)	Seoul	-7.8	1.4
	Daejeon	0.6	2.2
	Gwangju	-2.4	2.9
	Busan	4.2	3.0
	Average	-1.4	2.6
Summer/ Autumn (July-Oct)	Seoul	26.7	3.5
	Daejeon	15.6	2.4
	Gwangju	18.4	1.7
	Busan	15.5	1.7
	Average	19.1	2.9

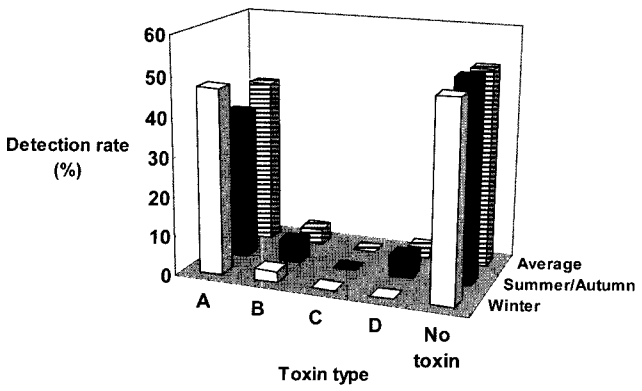

Fig. 2. Detection rate according to staphylococcal enterotoxin type of *S. aureus* isolated from *kimbab*.

purchased or the season in which it is prepared.

***S. aureus* contamination of *kimbab* constituents** The 15 kinds of constituents of *kimbab* were separated and the *S. aureus* contamination level of each material was analysed. Perilla leaf showed the highest level of contamination (10%) and both seaweed and rice showed 4.7% contamination (Table 5). Generally, raw materials were more contaminated than cooked materials in *kimbab*. The contamination in raw materials can spread to the cooked rice and grow easily there, because there are no competitive bacteria in rice after it is cooked, and therefore it is an aspect of the process requiring special care (19). To decrease the levels of *S. aureus*, it is necessary that approved sanitizers for washing be used to wash vegetables. Short heating of materials such as perilla leaf, cucumber, and seaweed can also reduce the contamination of *kimbab* by *S. aureus*.

Table 5. Isolation rate of *S. aureus* in *kimbab* constituents

Material	Isolation rate (%)
Burdock	3.0
Carrot	1.0
Crab meat	2.2
Cucumber	1.1
Egg	0.0
Fried bean curd	0.0
Fish meat	0.0
Ham	0.0
Laver	4.6
Meat	0.0
Picked radish	0.9
Rice	4.6
Scallion	0.0
Perilla leaf	10.0
Spinach	0.0

Staphylococcal enterotoxin identification in *kimbab* Of the 36 cases of *S. aureus* contamination in 105 *kimbab* samples purchased in winter and the 37 cases of in 109 *kimbab* samples purchased in summer/autumn, enterotoxin type A was detected the most up to 40%. There was no difference in toxin type depending on the season. In total, enterotoxin types A, B, C, and D were produced by nearly 50% of the *S. aureus* isolates collected from *kimbab* (Fig. 2). Thus, the majority of enterotoxin isolated from *S. aureus* collected from *kimbab* in this study was type A. This supports data indicating that the most common enterotoxin expressed by *S. aureus* isolated from food in the USA is type A, followed by type D and B (9, 10). However, these results are not consistent with the fact that enterotoxin type B is most commonly reported in food in Europe, especially that made from raw milk (13, 20, 21). In Korea, the major staphylococcal enterotoxin type in *S. aureus* isolated from strawberries was also type A (16). In that research, enterotoxigenic type A was also isolated from the employees' hands, clothes, and scissors. These data suggest that contamination with *S. aureus* expressing enterotoxigenic type A in Korea may be attributable to the food handlers' hands. Therefore, hand washing with soap before handling, preparing, or cooking is required. Polyvinyl gloves must also be changed regularly because some handlers use these gloves over long periods, putting them on when they roll *kimbab* and taking them off when they serve.

It has been reported that *S. aureus* enterotoxin is not degraded by proteases such as trypsin, chymotrypsin, renin, or papain (22, 23) and is heat stable even at 100°C for 180 min or 120°C for 20 min (3, 24, 25). The result that nearly 50% of *S. aureus* isolated from *kimbab* can produce enterotoxin regardless to the type (Fig. 2) emphasizes that the use of hygienic food materials and proper cooking over 75°C (5) are necessary to reduce *S. aureus* contamination in food. Short storage times and prompt consumption, to minimize cell growth and enterotoxin production, are very important in pre-prepared meals. Food handlers' personal hygiene while handling the foodstuffs is necessary to prevent cross-contamination, thus reducing staphylococcal food-borne illness. Good manufacturing practices should be taught to food handlers in all kitchen and serving units.

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