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RESEARCH ARTICLE

Exposure Assessment of *Staphylococcus aureus* in *Kimbab*, a Ready-to-Eat Korean Food

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Abstract The exposure assessment was carried out for *Staphylococcus aureus* in *kimbab* by predicting growth of *S. aureus* and the production of enterotoxin using Food MicroModel® program. Environmental parameters selected were pH 5.5, Aw 0.999, and storage temperatures in the range of 10 to 30°C. It was predicted that 6.3 hr could be a critical time for enterotoxin production while *kimbab* was stored at 30°C. Mild case scenario analysis showed that enterotoxin could not be produced if *kimbab* was kept at 10°C during preparation and distribution and then left at 25°C for 4 hr before consumption. In the worst case scenario, the keeping time at 25°C was assumed to be 7.0 hr. The level of *S. aureus* in the worst case was predicted to be 6.8×10^6 CFU/g which is lower than the critical level (7.8×10^6 CFU/g) for toxin production.

Keywords: *Staphylococcus aureus,* exposure assessment, *kimbab,* predictive modeling

Introduction

Staphylococcus aureus is recognized as one of the main causative organisms of food poisonings in South Korea, others including *Salmonella* and *Vibrio*. Total patients of food poisoning illness in South Korea, as reported from 2001 to 2005, were 33,353 and among these 10.8% got sickness due to enterotoxins produced by *S. aureus* (1). *S. aureus* is widespread in nature with various routes of contamination, making it difficult to control toxin production in foods. This signifies the need for regular risk assessment of *S. aureus* growth and toxin production for systematic hygiene (2,3).

Risk assessment is defined as the qualitative or quantitative characterization and estimation of potential adverse health effects associated with the exposure of individuals or populations to hazards due to physical, chemical, and/or microbial agents (4,5). Quantitative microbial risk assessment is a useful tool to ensure food safety. It consists of 4 components: hazard identification, dose-response assessment, exposure assessment, and risk characterization, which together provide numerical expressions of risk (6). Predictive studies have been frequently used for risk assessment of food-borne microorganisms. Predictive microbiology deals with the study of models for microbial growth and survival under particular environmental conditions (7) and it has been developed and implemented to predict the occurrence of food-borne pathogens (8). The importance of microbiological quality and safety of food products has stimulated interest in the use of mathematical models for quantifying and predicting microbial behavior (9). Microbial risk assessment is becoming crucial in the safety of food products and it is also an essential requirement of food companies so as to conform to food safety standards set by regulatory agencies (10).

Kimbab is a ready-to-eat food and extensively popular in Korea. It was domestically prepared in the past, however currently it is an extensively commercialized food product. Boiled rice is rolled along with different food materials (ham, egg, seafood, vegetables, etc) in a thin sheet of dried green laver (*Ulva lactuca*), a type of seaweed. With a near neutral pH, higher Aw, and low salt concentration, *kimbab* is quite susceptible to pathogens. The holding temperatures

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of *kimbab* may also vary, depending on the individual retail stores, increasing the microbial risk. Park *et al.* (11) tested 32 samples of *kimbab* from several restaurants and found that 20 samples were contaminated with *S. aureus*. They also documented that 'lunch box' (an assortment of Korean foods commonly served in a plastic box) and *kimbab* together are the primary causes of food-borne illness in Korea, followed by fishery and meat products. It has been reported that *S. aureus* is the third leading causative bacteria for food-borne illness in Korea (1).

This study was carried out to establish the objective exposure assessment procedures and to evaluate the risk of *S. aureus* in commercially available *kimbab*. This study also aims to assist in developing control measures for prevention of *Staphylococcal* food poisoning hence contributing to public health enhancement.

Materials and Methods

Microbial risk assessment approach The stepwise microbial risk assessment of *S. aureus* in *kimbab* was based on contamination studies and surveillance results from *kimbab* manufacturing companies and the contamination and prevalence levels of *S. aureus* were used in this study. Stages or processing steps, not contributing towards the risk exposure, were excluded.

Predictive analysis on *S. aureus* in *kimbab* using Food MicroModel® The prediction of *S. aureus* growth was carried out using Food MicroModel® (FMM) program (v 2.5; Food Microbial Ltd., Surrey, UK). The scenario analysis for the worst case and the mild case was also carried out to estimate the suitable keeping time of *kimbab*. FMM is commercially available software that predicts the growth, survival, and thermal death of the major food pathogens in a wide variety of foods. This program is based on more than 20 individual models, each being organism specific. The growth models for specific micro-organism are drawn by using the physicochemical factors of foods such as Aw, pH, and temperature. This program is extensively used by professionals in order to establish suitable models which can be used to prevent the growth of pathogens (12).

Predictive analysis of growth and survival of *S. aureus* in *kimbab* was carried out by using pH 5.5, Aw 0.999, NaCl concentration 0.22%, and temperature 10-30°C as inputs. These 3 factors have also been emphasized in other studies establishing mathematical models of *S. aureus* growth (13). In order to determine functions of kinetic parameters for growth and death of *S. aureus* in response to growth factors, FMM was based on Gompertz model (14) as follows:

$$\ln (N_t/N_0)=A \exp[-\exp\{\mu_{max}/A_{max} \exp(\lambda\text{-}t)+1\}]$$

where, N_t represents the number of microorganisms at time t, N_0 is the initial number of microorganisms, A_{max} is the maximum level of microorganisms, μ_{max} is the maximum specific growth rate at a specific value of temperature, and λ is lag time at a specific value of temperature.

Scenario analysis for *S. aureus* in *kimbab* The scenarios analysis for the mild and the worst cases were performed. The general *kimbab* consumption patterns in Korea were considered to describe the scenarios and associated *S. aureus* risks. The maximum time till consumption of *kimbab* was determined using the results of predictive model and the contamination levels as described by Yoon *et al.* (15).

Risk assessment for populations using Monte Carlo simulation Parameters such as time, temperature, and pH for *S. aureus* growth were used as input data and the probabilities of food poisoning incidence were predicted. The relationships between the parameters were mathematically modeled and random numbers were used as inputs for Monte Carlo simulation (2). Risk calculation was done using a commercial software program, @Risk (version 4 Industrial ed.; Palisade Corporation, Newfield, ME, USA). This program selected sample distribution from database using randomized numbers which was then used to generalize the risk for the whole population. Statistically, this process was iterated several times because the reliability of the outcomes depends on sample distribution and the credible data from the several samples is critical when proposing the best estimation applicable to the whole population. The sample distribution, generalized using probability distribution, was close to the β-Poisson distribution which was suitable for investigating the contamination level and the distribution of *S. aureus* in *kimbab*. Total contamination level and disease incidence rates were quantitatively expressed.

Results and Discussion

Exposure assessment of *S. aureus* using FMM The interaction between different controlling factors which affect bacterial growth and the probability that a given microorganism will grow, survive, or die under specific conditions can be studied by using microbiological challenge testing, storage testing, and predictive microbiology. The former 2 methods are slow and expensive and applicable to a reduced set of conditions where results can be relied on (16,17). They also lack the important predictive component in assessing the changes in product formulation, processing,

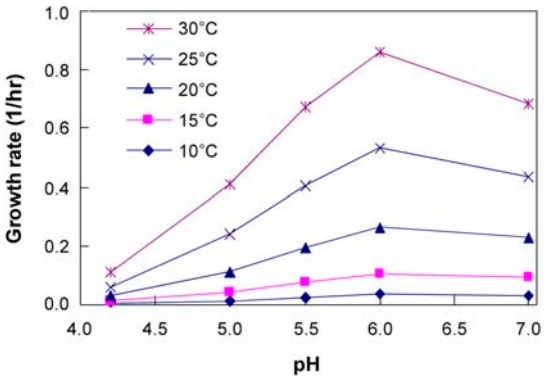

Fig. 1. Growth curves of *S. aureus* at pH 5.5 and Aw 0.999 under different temperatures predicted using Food MicroModel®.

Table 1. Production of *S. aureus* ATCC13565 enterotoxin in *kimbab* at 37°C (pH 5.5, 0.22% NaCl)

Incubation time (hr)	Cell number (CFU/g)	Presence of toxin
0	1.4×10^5	-
1	1.4×10^5	-
2	2.1×10^5	-
4	7.8×10^6	-
6	$\mathbf{2.0\times10^7}$	+
8	9.7×10^7	+
10	1.1×10^8	+

or packaging. Based on the physicochemical condition of specific food matrix, the microbial growth can be predicted to assess the exposure of a specific pathogen.

The growth pattern of *S. aureus* in *kimbab* was predicted using FMM. The physicochemical conditions of *kimbab* used for the prevalence tests were pH 5.5, Aw 0.999, and NaCl 0.22%. The temperature range permissible for the prediction in FMM was within 10 to 30°C, therefore the study was carried out using temperatures within this range and the results are presented in Fig. 1. The growth of *S. aureus* accelerated proportionate to the holding temperatures and the maximum growth was predicted at 30°C. Food-borne disease caused by *S. aureus* is due to the production of enterotoxins produced by this bacterium. Figure 1 can be used to predict the time taken for the production of enterotoxin by taking into consideration the temperature at which *kimbab* is placed. Several studies have shown that the concentration of *S. aureus* needed for detectable toxin production in a variety of foods varies between 1×10^6 and 4×10^7 CFU/g (18-20). The numbers of *S. aureus* ATCC13565 required for producing enterotoxin in *kimbab* at 37°C (pH 5.5, 0.22% NaCl) is presented in Table 1. It was observed for this strain that a cell number of 2.0×10^7 CFU/g was sufficient to produce toxin. The average summer temperature reaches as high as 30°C in Seoul, Korea during the month of August (21). However, the worst case holding temperature of *kimbab* can also be as high as 37°C during summer season in South Korea and at this temperature the time required for toxin production would be 6 hr.

In our earlier study carried on *kimbab* samples from different sales channels, it was observed that the overall prevalence of *S. aureus* was higher (3.1 log CFU/g) in summer and lower (2.0 log CFU/g) in winter (15). The data on the growth kinetics of *S. aureus* at pH 5.5 and Aw 0.999 under different storage temperatures predicted using FMM is given in Table 2. Based on the growth rates of *S. aureus* at different storage temperatures (Fig. 1), it can be interpreted that the time required for the cell number to become 10^6 CFU/g is 6.3 hr at 30°C. While as the growth rate is 0.671/hr, the cell number after elapse of 240 hr becomes 8.3 log CFU/g. The predicted time for cell number to be 10^6 gradually increased at lower temperatures. It was assumed that 6.3 hr could be the critical keeping time for the production of enterotoxin at 30°C. Accordingly, it can be inferred that *kimbab* at pH 5.5 and Aw 0.999 should not be kept for any longer than 6 hr when held at 30°C to avoid the risk of enterotoxin due to *S. aureus*. Similarly, the probable critical times for the production of enterotoxin (cell numbers of 10^6 CFU/g) at 15 and 20°C were determined as 58.5 and 22.6 hr, respectively.

Predictive models can be readily used to evaluate the effects of a wide range of factors upon the growth of the microorganisms of interest (17,22). Modeling the growth and survival of pathogenic and spoilage microorganisms is the basic tool to predict for toxin production and microbial deterioration in food products (23). Predictions anticipate the events of bacterial growth helping the decision-making process (12).

Table 2. Growth kinetics of *S. aureus* at pH 5.5 and Aw 0.999 under different temperatures predicted using Food MicroModel®

Storage temperature (°C)	Growth rate (1/hr)	Time (hr) for the cell count to be		Cell count after 240 hr (log CFU/g)
		Double	10^6	
10	0.023	13.1	187.2	6.8
15	0.075	4.0	59.0	8.3
20	0.195	1.6	23.0	8.3
25	0.404	0.7	11.0	8.3
30	0.671	0.5	6.3	8.3

Table 3. Growth kinetics of *S. aureus* in *kimbab* obtained from the predictive models

Temperature (°C)	Growth kinetics		
	Exponential growth rate (log CFU/mL)	Generation time (hr)	Lag phase duration (hr)
10	0.021	14.5	40.9
25	0.371	0.8	1.9

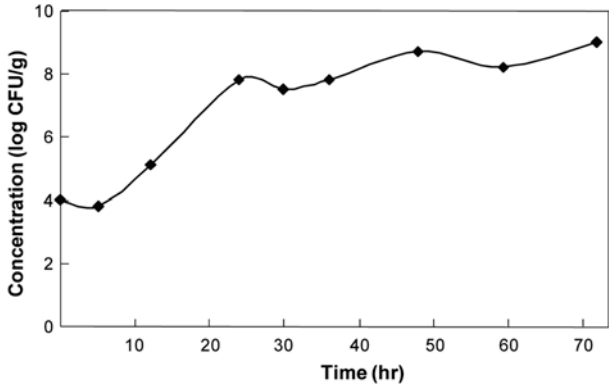

Fig. 2. Growth curves of *S. aureus* at pH 5.4, Aw 0.997, and temperature 28ºC predicted using ComBase model.

Lag phase duration and generation time A lag phase corresponds to a transition period during which microbial cells adjust to their new environment before exponential growth (24), whereas the generation time is the time needed for doubling the initial bacterial population during the exponential growth phase (25). From the prediction results obtained by using FMM, the lag phase and generation times were obtained as presented in Table 3. At a storage temperature of 10ºC, lag phase and generation times were 40.9 and 14.5 hr, respectively which were approximately 22 and 18 times greater than those at 25ºC. Similarly, the resulting exponential growth rates were 0.021 and 0.371 log CFU/g at 10 and 25ºC, respectively. It can be inferred that the cell growth at 25ºC was approximately 18 times faster than that at 10ºC. These results demonstrated that a storage temperature less than 10°C will greatly reduce the chances of *S. aureus* growth and the associated risk of staphylococcal toxins in *kimbab*.

Comparison with ComBase The prediction results obtained using FMM were compared with those of another prediction model, ComBase. ComBase is a database of predictive microbiology developed by USDA and it contains thousands of data sets that describe the growth, survival and inactivation of bacteria under diverse environments similar to food processing conditions (26). It is reported to be a large and effective database for getting microbial responses to food environments and has attracted

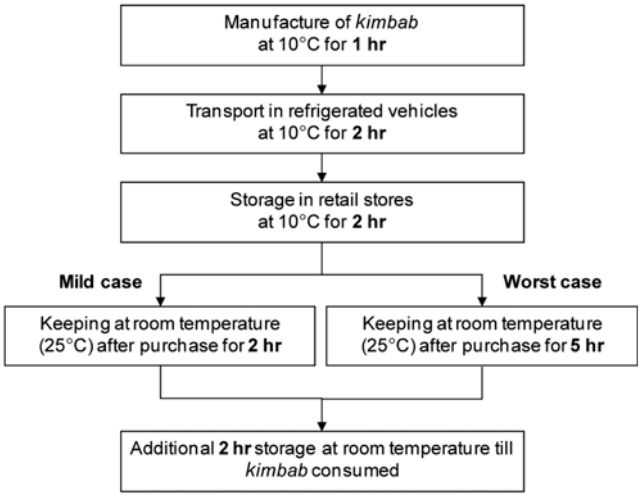

Fig. 3. A proposed conceptual scenarios of *kimbab* consumption according to the mild case and the worst case.

the attention of many researchers and food processors (27). However we observed that possible conditions of the variables in ComBase, were not as sophisticated as in FMM. Figure 2 represents the growth curve of *S. aureus* predicted using ComBase model and the variables used as input were pH 5.4, Aw 0.997, and temperature 28ºC. The food type selected in this model was "other, mixed, uncategorized or unknown type of food including sandwich filling", which was the closest class for *kimbab*. The time for the cell number becoming 10^6 was predicted as 15-20 hr. Based on ComBase model *kimbab* seems to be safe for 15 hr while held at 28ºC. This predicted holding time is much higher than the one predicted using FMM. Hence, it is essential that various models should be tried for complicated food matrix in order to find out best prediction for the growth of pathogens.

Scenario analysis The mitigations evaluated in the scenario analysis have long been recognized as important in controlling the spread of enteric pathogens in the retail food sector (28). The scenario analysis for exposure of *S. aureus* in *kimbab* was conducted in order to estimate the suitable time for consuming *kimbab*. On the basis of the surveillance of manufacturing process, *kimbab* was considered to be safe until delivery to the market and the scenario analysis was carried out for the subsequent events before final consumption. Two different types of scenarios, the mild case and the worst case, were proposed during distribution and consumption as presented in Fig. 3. The mild scenario included 1 hr for manufacturing at 10ºC; 2 hr for distribution at 10ºC in chilled vehicle, and 2 hr storage at 10ºC at the retail store. Once purchased by consumer, *kimbab* can be eaten either right away or otherwise the consumption can be delayed, therefore an extra 4 hr

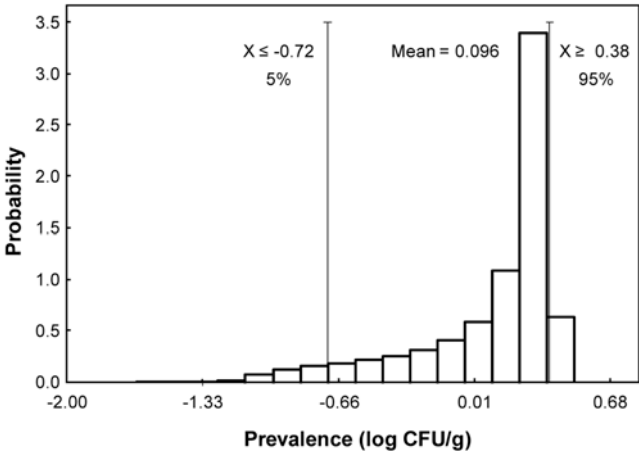

Fig. 4. Prevalence probability estimation using Monte Carlo simulation.

storage at room temperature was considered. The initial count of *S. aureus* was considered 623 CFU/g and final count in mild case, considered for a serving size of 171 g, was predicted as 1.1×10^5 CFU/g. This cell count was considered insufficient to produce staphylococcal enterotoxin, hence *kimbab* is regarded as safe for human consumption. Likewise, a worst-case scenario was also proposed as summarized in Fig. 3. It was assumed that *kimbab* was prepared, distributed, and stored at 10°C, however consumer purchased *kimbab* at 7 am, left it at room temperature (25°C) for 7 hr and consumed at 2 pm. Therefore an extra 7 hr keeping time at 25°C should be considered in this case. The cell density was predicted to reach 6.8×10^6 CFU/g. It was still lower than 7.8×10^6 CFU/g, the cell density acquired by incubating *S. aureus* in *kimbab* at 37°C and considered critical for producing enterotoxins.

Risk assessment for populations using Monte Carlo simulation Quantitative risk assessment (QRA) is a technique, which is used to estimate the likelihood and severity of an adverse event (29). When performed in conjunction with Monte Carlo simulation, QRA offers precise explanation of the uncertainty and variability associated with the risk (30). Monte Carlo simulation is an effective technique for estimating the probability of pathogenic contamination of foods by using the data of parameters as inputs that may have effects on contamination (31). The results of qualitative and quantitative analyses on 214 *kimbab* samples, presented in an earlier study (15), were used for estimating staphylococcal food poisoning incidence and prevalence probability of *S. aureus*. The prevalence level might not be the exact one for the whole population, but can be regarded as the best possible estimation. By using prevalence levels, temperatures and pH conditions determined in the previous contamination study, 'what-if' scenario was tried. The Monte Carlo simulation was run with 20,000 iterations using @Risk. Once all possibilities for given conditions of *kimbab* were explored, the prevalence probability was determined by Monte Carlo simulation for generalizing the risk as presented in Fig. 4. The distribution can be interpreted as a less risk, because the distribution has smaller range and less spread out. Log values of *S. aureus* contamination in *kimbab* has an upper limit of 0.38 log CFU/g, a lower limit of –0.72 log CFU/g, and a mean value of 0.096 log CFU/g in the β distribution application with confidence intervals of 95, 5, and 50%, respectively. These log values can be converted to 2.4, 0.2, and 1.2 CFU/g for the contamination level of *kimbab* in South Korea and probabilities are 3.4 (95%), 0.2 (5%), and 0.6 (50%), respectively. The data showed that the prevalence in the current conditions in *kimbab* has the low probability and the overall risk for food-borne outbreak is small and the enterotoxin is not produced at this simulation.

The credibility of a microbial risk assessment is based on its ability to take into account the variability and uncertainty of each parameter implied in the estimation of the final risk (32). The uncertainty factor determined in our study was temperature, since other conditions, such as pH, were relatively fixed. Diverse range of temperature can give the sensitivity of probability estimation. However if the prevalence data depending on smaller range of temperature is acquired, the sensitivity analysis can be tried to correlate temperature with prevalence.

In conclusion, the prediction modeling on the growth of *S. aureus* and enterotoxin production in *kimbab* having pH 5.5 and Aw 0.999 was performed using the Food MicroModel® program and the storage temperature varied from 10 to 30°C. Considering 2.0×10^7 CFU/g as the minimum cell number for the production of the enterotoxins, it was predicted that 6.3 hr could be the critical time for enterotoxin production at 30°C. Thus *kimbab* with above mentioned pH and Aw should not be kept for more than 6 hr while at 30°C to avoid the exposure of enterotoxin risk. Moreover a storage temperature of 10°C or even lower will greatly reduce the chance of *S. aureus* growth in *kimbab*. In scenario analysis, the mild and worst case was assumed including extra keeping times of 4 and 7 hr, respectively at 25°C. In both cases the predicted *S. aureus* counts were lower than the critical 7.8×10^6 CFU/g and hence not expected to produce staphylococcal enterotoxin. Estimation of the prevalence probability of *S. aureus* in *kimbab* was also made using @Risk program. Log value of *S. aureus* contamination in *kimbab* has an upper limit of 0.38 log CFU/g and a lower limit of –0.72 log CFU/g which have probabilities of 0.2 (95%) and 3.4 (5%), respectively. These results reflect a lower risk probability for food poisoning as a result of common handling of *kimbab* in Korea.

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