MINISTRY OF FOOD AND DRUG SAFETY
National Institute
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Risk assessment of *Staphylococcus aureus* and *Clostridium perfringens* in processed meat products

Introduction

Processed meat products include material extracts and meat extracts and those produced by adding foodstuffs and food additives such as those that can be classified into primary processed products solely containing soup (e.g., Seolleongtang and Yukgaejang), mixed processed products combining soup and solid ingredients (e.g., Gamjatang, Samgyetang), and processed meat products (Pyeonyuk). Although processed meat products are not considered susceptible to microbial contamination as these are heated during their preparation to prevent *Staphylococcus aureus*, which secrete heat-stable toxins, and *Clostridium perfringens* to proliferate in this particular medium. Therefore, the present study intended to assess the risks of *S. aureus* and *C. perfringens* in processed meat products that are widely consumed in Korea, to apply the results in the re-establishment of the standard criteria in screening for possible microbial contaminants, and to contribute to the reduction of incidence of food poisoning.

Statement of purpose

The purpose of the risk assessment was to present the scientific basis for the establishment of quantitative standard criteria in detecting *S. aureus* and *C. perfringens* in processed meat products, whose consumption is rapidly increasing due to recent changes in dietary patterns and social trends, and to develop microbiological risk management measures to prevent food-related incidents, including food poisoning.

Hazard identification

S. aureus

S. aureus is a non-mobile, gram-positive (0.5–1.5 μm), facultative anaerobic and asporogenous coccus that exists on the skin, nasal mucosa, and other soft tissues of most animals and humans. It usually grows well in aerobic conditions but can also survive in anaerobic conditions, while its salt tolerance enables it to rapidly proliferate in a medium containing 7.5% NaCl. It is resistant to dryness, heat (50℃, 30 min), and sodium (3%–7%). It rarely causes food poisoning via food materials, while it is the main source of food poisoning through contaminated cooked food, where it rapidly proliferates, and in turn, generates enterotoxins. SEs are secreted by S. *aureus*; these are heat-stable and water-soluble proteins with low molecular weight of 26–30 kDa and an isoelectric point of 5.7–8.6. As enterotoxins generated by *S. aureus* have extremely high heat resistance, these are not completely destroyed even after heating at 120℃ for 20 min. To reduce the risk of food poisoning, it is essential to block the generation of enterotoxins in advance. *S. aureus* exists in various food items, including dairy food (e.g., milk, cream, and cheese), processed meat products (e.g., ham and meat), and other protein food items (e.g., fish meat products) in the U.S. and Europe. In Korea, it has also been detected in a wide variety of food items. Symptoms of food poisoning by *S. aureus* include nausea, diarrhea, and severe abdominal pains due to acute gastroenteritis. The latent period is 2 to 6 h, and clinical symptoms differ according to the amount of enterotoxins that have entered the body and level of individual sensitivity. Its fatality rate is relatively low, and most patients recover within 24–28 h. The number of its outbreaks and patients that experienced food poisoning by *S. aureus* in Korea from 2011 to 2015 has decreased. Nevertheless, it is constantly reported as a key cause of food poisoning, with an average of 9.2 outbreaks (161.4 patients per outbreak) along with enteropathogenic *Escherichia coli*, Norovirus, and *Salmonella*.

C. perfringens

Clostridium perfringens is an anaerobic, gram-positive, and spore-forming bacillus. It is widely distributed in natural environments, including soil, streams, sewage, intestinal tracts, feces of human beings and animals (major mammal species), as well as food items, while its spores often remain viable for long periods of time. The bacillus produces toxins that cause food poisoning, and during this process, nursing cells also form spores. The toxins are divided into five types, namely A, B, C, D, and E, of which the A and C types have been mainly associated with food poisoning in humans. The optimal temperature range for their growth is 43°C–47℃, while these nearly stop growing at temperatures below 15℃ or above 52℃. The optimal temperature for the formation of spores and toxins is 37℃. After the generation of enterotoxins during the proliferation of *C. perfringens* and binding with specific receptors in small intestine, morphological changes, diarrhea, and abdominal pains may occur. The latent period is around 8–12 h, and patients usually recover after light symptoms of diarrhea and abdominal pain. The major symptoms of food poisoning disappear within 24 h, but minor ones may remain for another one to two weeks. For treatment, sufficient intake of fluid and electrolytes is recommended, and intravenous injection may also be utilized in severe cases. The administration of antibiotics is often not encouraged. According to the food poisoning statistics of the MFDS, the number of outbreaks and patients gradually increased from 2002 to 2014; the number of *C. perfringens* food poisoning outbreaks and patients was 28 and 1,689, respectively, in 2014 alone. On the other hand, the number of annual food poisoning outbreaks caused by *C. perfringens* was less than 10 until 2011 and has recently increased.

Exposure assessments

Exposure assessments of *S. aureus* and *C. perfringens* in processed meat products were conducted by developing a scenario that was divided into three stages, namely manufacturing stage, sale and purchase stage, and consumption stage.

Exposure assessment for *S. aureus* in processed meat products

S. aureus was detected in 3 out of 1,695 processed meat products sold in Korea. Estimation of the contamination level using the beta distribution revealed that the average level of contamination was -4.08 log CFU/g. Growth prediction models were developed for *Seolleongtang* (ox bone soup), *Galbitang* (beef short rib soup), and *Pyeonyuk* (boiled meat slices) as processed meat products using the Baranyi model as the primary model. The secondary model (square root model) was then developed through the application of the lag phase and growth rate, which were obtained from the primary model. The differences in the bacterial counts from the initial contamination level (average: -4.08, maximum: -3.38 log CFU/g) were predicted through simulation. The contamination level of *S. aureus* slightly increased during the distribution stage (average: -4.04, maximum: -3.34 Log CFU/g), whereas that during storage at home was similar to that during the purchase stage (average: -4.03, maximum: -3.33 Log CFU/g). Food intake level was derived from the Survey on the Consumption Amounts and Patterns of 50 Major Livestock Products (MFDS, 2015) conducted in conjunction with Gallup Korea, and consumption of *Seolleongtang* (603 ± 120 g), which showed the greatest proliferation rate in the growth prediction model, was applied.

Exposure assessment for *C. perfringens* in processed meat products

C. perfringens was not detected in any of the 1,695 processed meat products examined. Estimation of the contamination level using the beta distribution revealed an average contamination level of -4.44 log CFU/g. The growth prediction models were developed for *Seolleongtang*, *Galbitang*, *Gomtang* (beef bone soup), and *Pyeonyuk* chosen as processed meat products, and the growth patterns of *C. perfringens* were verified in all processed meat products. The initial contamination level of *C. perfringens* in processed food products was extremely low (average: -4.44, maximum: -3.49 log CFU/g) through simulation, whereas it slightly increased (average: -4.42, maximum: -3.47 Log CFU/g) during the distribution stage. The estimated contamination level at the time of intake after storage at home (average: -4.42, maximum: -3.47 Log CFU/g) was similar to that in the purchase stage. According to the Estimation of Amount and Frequency of Consumption of Livestock Products (1,500 persons), around 2 out of 1,000 Koreans eat 603 ± 120 g of processed meat products per serving each day.

Hazard characterization

S. aureus

The minimum concentration level of enterotoxins of *S. aureus* that are harmful to human health is 100 mg, and various studies have detected enterotoxins at a concentration level of 4–8 log CFU/g. However, most of the studies have shown that the concentration of 5 log CFU/g is determined as the appropriate minimum concentration level of *S. aureus* to threaten human health.

C. perfringens

According to a meta-analysis of minimum infective dose of bacteria for food poisoning that was conducted by Park (2014), the minimum infective dose of *C. perfringens* is 10^2 cells/g at the minimum value, 10^8 cells/g at the maximum value, 10^7 cells/g at the median, 10^2 cells/g at the 5^{th} percentile, 10^6–10^8 cells/g at the 25^{th} to 75^{th} percentiles, and 10^8 cells/g at the 95^{th} percentile. In the present study, the minimum infective dose was 10^7 cells/g, which had the highest relative frequency for the minimum infective dose, and is similar to the result of 10^5–10^8 cells/g reported by Leggett et al. (2012). The study used the exponential model (Golden et al., 2009), which is the only dose-response model for *C. perfringens*.

Risk characterization

***S. aureus* in processed meat products**

S. aureus intake through processed meat products was predicted based on the contamination level of the bacterium at the consumption stage, food intake, and intake frequency, and the final results were drawn through more than 10,000 iterations using @RISK. The average daily personal intake level of *S. aureus* per serving of processed meat product was extremely low at 0.000118 CFU/g, and the maximum intake of 0.118 CFU/g was very low at about 0.0001% of the minimum concentration to threaten human health (105 CFU/g). Estimation through simulation of the risk level of food poisoning by *S. aureus* in processed meat products showed that the probability of food poisoning was at the maximum of $1.10 \times 10^{-8} \pm 2.11 \times 10^{-10}$, which is very low. This result would be gained by daily consumption of the products.

C. perfringens in processed meat products

C. perfringens intake through processed meat products was predicted based on the contamination level of the bacterium at the consumption stage, food intake, intake frequency, etc., and the final results were derived through more than 10,000 iterations using @RISK. Daily personal intake level of *C. perfringens* per serving of processed meat product was low at 0.00569 CFU/g on average, and the maximum intake of 2.3221 CFU/g was about 0.00002% of the minimum concentration to threaten human health (10^7 CFU/g). Estimation through simulation of the risk level by *C. perfringens* in processed meat products showed that the probability of food poisoning was at the maximum of $1.42 \times 10^{-12} \pm 2.92 \times 10^{-14}$, which was very low. This result would be gained by daily consumption of the products.

Impact of establishing microbial standards in the Korean Food Code

For the establishment of standards, changes in *S. aureus* and *C. perfringens* were predicted according to a scenario of extreme conditions. With the assumption that their initial contamination levels were 1, 2, and 3 Log CFU/g, estimation of the contamination level at the consumption stage in the current distribution condition showed that when the initial contamination level was 3 Log CFU/g, the contamination levels of *S. aureus* and *C. perfringens* at the consumption stage were predicted to reach the maximum levels of 3.30 and 5.9 Log CFU/g, respectively. These levels were far lower than the minimum concentration to threaten human health with 5 and 8 Log CFU/g, respectively. Therefore, the probability of food poisoning by *S. aureus* and *C. perfringens* would be low as long as the current distribution condition is maintained. Based on the results of the present study, the standards on *S. aureus* and *C. perfringens* in processed food products were re-established from zero-tolerance to the quantitative standards that were relaxed to n = 5, c = 1, m = 10, and M = 100 (Notification No. 2016-154, December 29, 2016).

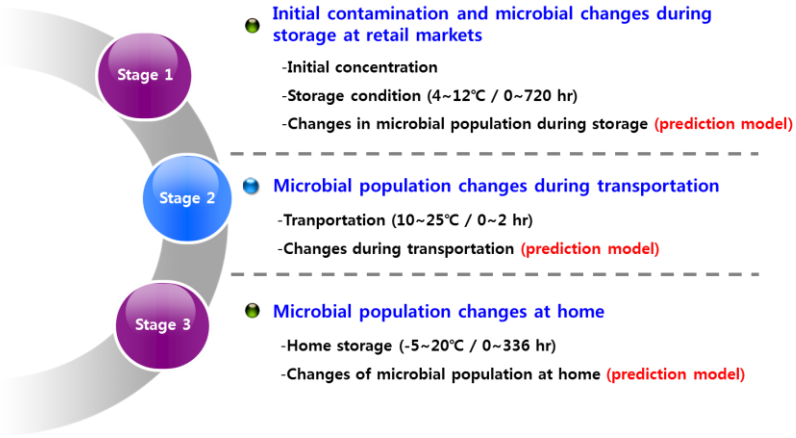

Figure 1. Flow diagram of exposure assessment in processed meat products.

Key words: *Staphylococcus aureus*, *Clostridium perfringens*, Risk Assessment, Processed meat product, Meat extract