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Identification and probability of illness of *S. aureus* contaminated food for school children

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Abstract

Food for school children holds an important role in the fulfillment nutritional intake of children at the school age, therefore it has to be safe for consumption. The research that had been conducted in Bogor to thirty five samples from twenty two food sellers in eight elementary schools in two different time periods. The analysis results showed that none of the samples was contaminated by *L. monocytogenes, Salmonella* spp or *Vibrio* spp. Two samples (otak-otak and siomai) were contaminated by *S. aureus*. The contamination source tracing showed that the hands, equipment, and environment around the selling place were contaminated by *S. aureus*. The likelihood of illness by *S. aureus* after consuming food and snack for school children was 0.00103 or one case for every 972 servings.

Introduction

Food for school children holds an important role in the fulfillment of energy and nutrient intake for children at school age. Generally, school children buy food from the canteens in the school and food vendors around the school. The microbiological, chemical, and physical hazard quite possibly contaminate food sold in the school canteens or by food vendors because of poor food safety practices and polluted environment.

Food for school children that is not handled properly is susceptible to microbial contamination. Pathogenic bacteria that may cause contamination are *Listeria monocytogenes, Salmonella* spp., *Vibrio* spp. and *S. aureus*. The use of raw materials contaminated by pathogenic microbes that is followed by the imperfect processing can produce unsafe food. To produce safe food for school children, handling can be done by controlling a critical control point that is the stage to prevent or reduce the hazard to the safe level.

S. aureus is a normal flora on the skin and in the respiratory organs and generally found in 20-50% of healthy population. *S. aureus* can survive on unclean hands and kitchen utensils (Teixeira, 2007). *S. aureus* contamination in food usually occurs after the food has been cooked. *S. aureus* contamination in food can cause intoxication with symptoms that typically occur, i.e. nausea and vomiting, accompanied by abdominal cramps, sometimes followed by diarrhea. In severe cases, a person can suffer headache, muscle cramps and dehydration (Lawley, 2008).

Characterization of the hazards which is one of the stages in the risk assessment can be carried out, among others by knowing the dose-response relationship between the hazard and its arised consequences. Dose-response relationship can be observed by using the exponential model, beta poisson, beta-binomial and Weibull-gamma (Vose, 2008). The result of illness likelihood calculation caused by eating food contaminated by pathogenic bacteria can be a reference to estimate the magnitude of the hazard risk of pathogenic bacteria in food. The magnitude of these risks can be the basis to determine contaminated food for school children handling policy.

Considering the importance of food for school children development, it should be safe which is shown by a low risk of hazard. Therefore, it is necessary to identify hazards and to calculate the likelihood of illness due to pathogenic bacteria in the food for school children and identifying the source of pathogenic bacteria contamination, so that critical control point of food for school children can be determined.

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Materials and Methods

Materials

Food for school children samples were taken from eight elementary schools in Bogor city. The materials which were used to analyze the pathogenic bacteria for example, i.e.: Chromocult® *Listeria* selective agar acc. Ottaviani and Agosti (ALOA) (Merck, Germany), buffered peptone water (Merck, Germany), Singlepath L'Mono (Merck, Germany), Brain Hearth-Broth (Oxoid), Rappaport-Vassiliadis (RV) (Oxoid), Tetrathionate Broth (TTB) (Oxoid), Hektoen Enteric (HE) agar (Oxoid), Xylose Lysine Deoxycholate (XLD) agar (Oxoid) and Bismuth Sulfite Agar (BSA) (Merck), Triple Sugar Iron (TSI) medium (Merck, Germany), Lysine Iron Agar (LIA) (Merck, Germany), Alkaline Peptone Water (APW) (Scharlau), TCBS, and Baird Parker Agar (BPA) (Oxoid). The research consists of five stages, those are sampling, identification of hazards, identification of contamination source, identification of critical control point, and calculation of illnes likelihood due to pathogenic bacteria in food.

Sampling

Food samples were taken from eight elementary school of six sub-districts in Bogor. Elementary school number of sampling sites for each district was determined proportionally based on Ministry of Education and Culture reference data (2014). Sampling locations were determined by random selection. Observations of sampling sites were done based on location of the school, hygiene conditions, the number of schools in one location, the type and number of food sellers. The samples consisted of 35 foods, those were nineteen fish-based samples (fish meatballs, otak-otak, pempek and siomai), four meat-based samples (beef burger, nuggets, and sausages), two dairy-based samples (milk pasteurization) and ten fruit and vegetable-based samples (melon, pineapple, watermelon, cucumber, and lettuce).

Hazard identification of pathogenic bacteria in food for school children

Food for school children samples analysis was conducted on the presence or amount of pathogenic bacteria in the following methods: analysis of *L. monocytogenes* with BAM method (Hitchins *et al*., 2011) and ISO 11290-2004, *Salmonella* spp. with SNI method 01-2332.2-2006 (NSA, 2006), *Vibrio* spp. with method BAM (Kaysner *et al.*, 2004), and *S. aureus* with SNI 2332.9: 2011 method (NSA, 2011).

Identification of the source of pathogenic bacteria contamination in food for school children

Identification of the pathogenic bacteria contamination source in the food for school children which included the raw materials, equipment, hands, and air around the outlet was performed on food for school children samples containing pathogens. Analyses were performed according to the method developed by Carpentier and Barre (2012). Analysis of raw materials was performed the same way as analyzing the content of pathogenic bacteria in food. Microbial analysis on the knife was done by taking the microbes from the surface of the knife with swab system which then was dipped in 10 mL of 0.85% NaCl diluent. Furthermore it was analyzed with the same way like analysis for food. Microbial test on the food seller's hands was done by contact method, i.e. by placing fingers of the right and left hand on the testing media for two seconds. Whereas microbial testing of the air was carried out by the air contact with the bacterial growth media for 30 minutes.

Identification of critical control points in food for school children

Identification of critical control points (CCP) was performed toward the microbiological hazards in the process stages in food sale locations. Determination of critical control points was done with decision tree of critical control point processing determination as the aid (Figure 1).

Calculation of ill probability by pathogenic bacteria in food for school children

Assessment of hazard characterization of pathogenic bacteria was done by the literature study to obtain dose-response models that can be used to predict the risk of disease caused by consuming food for school children. Dose-response models that were examined were four models as expressed by Vose (2008). The selected dose-response model was the model that was appropriate with the type of pathogenic bacteria found in food for school children.

The average value of pathogenic bacteria contamination and weight of food per serving were calculated using Monte-Carlo simulation with @ RISK software® as the aid. Monte-Carlo simulation results provided an objective and mathematical calculation with various scenarios. Iteration which was used in this study was 10,000 times so it could show the average value of pathogenic bacteria contamination and weight of food per serving based on 10,000 scenarios that might occur.

Data processing to calculate the likelihood of illness was done with Monte-Carlo simulation with

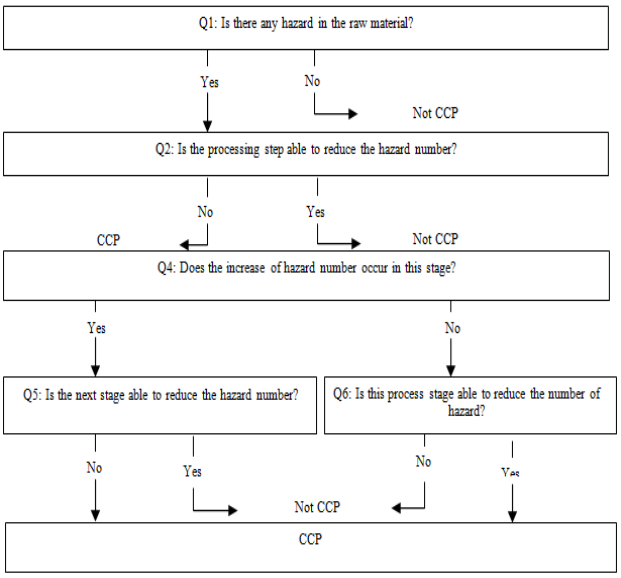

Figure 1. Decision tree for CCP determination (Schorhorst, 2004)

@RISK software® toward pathogenic bacteria, based on dose per serving of food. Ill probability obtained re-simulated using @RISK software® in order to obtain objective calculation results of the 10,000 possible scenarios.

Results and Discussions

Identification of hazard and pathogenic bacteria contamination sources in food for school children

The results of 35 samples analysis obtained from 22 food sellers showed that there was no food contaminated by microbes *L. monocytogenes, Salmonella* spp., and *Vibrio* spp. Food samples which were contaminated by *S. aureus* exceeded the maximum limit (1.0×10^2 colonies/g) set by NADFC (2009) were otak-otak and siomai. Table 1 shows that *S. aureus* contamination was only in fish-based food and not in the other foods. The tracing result of *S. aureus* contamination sources in otak-otak and siomai indicated the source of the contamination came from the hands of sellers, air, knife, and raw product (Table 2).

S. aureus contamination source from food seller's hands in this study was supported by Kadariya *et al.* (2014) which showed the investigation results in many cases of outbreaks of food poisoning due to *S. aureus*. They were successfully traced that food sellers were the source of contamination. Strains of *S. aureus* on the food sellers were exactly the same with the strains of *S. aureus* in the food. Similarly Tan *et al.* (2013) conducted an analysis of 85 food sellers' hands of 38 elementary schools in Selangor showed that 61 (71.76%) of food sellers' hands containing *S. aureus* contamination. Ifeadike *et al.* (2012) also conducted an analysis on the fingernails of 168 food sellers in the capital city of Nigeria and the resulted in 7.1% handlers' fingernails containing *S. aureus*.

If the hands of sellers and equipment used were contaminated by *S. aureus* when processing the food, they would contaminate the food. The process of cooking (boiling/ steaming/ frying) actually is able to kill *S. aureus* because it has D65.5 12-120 seconds heat resistance (Forsythe, 2002), but fish-based food can be contaminated with *S. aureus* after cooking. Recontamination to product that has been cooked can be occured from the food processor, equipment and environment.

Tracing results of *S. aureus* contamination sources on fish-based food for school children also showed food stall was contaminated by *S. aureus*. Street vendors have no appropriate space provision, i.e. occupying the road that overly crowded, hence blocking the motorists' traffic. The number of parents who waited outside the school and the number of people and vehicles passing on the narrow road led the food for school children being vulnerable to contamination of not only the observed bacteria.

Critical control point of fish-based food for school children processing at the vendor's location

Raw material of fish-based food which was sold in the school environment was a semi-finished material. Otak-otak and siomai were produced by boiling or steaming the dough. But before consumption, these products were usually displayed or stored before being sold. Storage could be done in the room temperature (28-32°C), cold temperature or solidification temperature. The usual processing of otak-otak before being sold were refrying or resteaming.

S. aureus bacteria were identified in the raw materials, sellers' hand, knife and air around food for school children sellers. Based on the hazard of *S. aureus* in the otak-otak and siomai, the critical control point (CCP) had been identified. Table 3 shows the results of determination fish-based food for school children CCP on the sale area based on the hazard of *S. aureus*.

Initially, raw material already contained *S. aureus*. However, the subsequent preparation that incorporates thermal processing, i.e. steaming, is able to kill *S. aureus*. Therefore, the raw material reception stage is not a CCP. On the other hand, a CCP is established for steaming, since the removal of the bacteria relied on the adequacy of the thermal processing. This stage is able to reduce microbiological hazard to the acceptable level. Processing steps of raw materials into finished products which were ready

Table 1. Food for school children which were contaminated by *S. aureus* based on food type

Food Type	Sample number	Contaminated Sample Exceeding the Limit (Contamination Number (CFU/g))	Contaminated Sample Exceeding the Limit
Fish-based food	19	2 (8.9×10^2, 2.2×10^3)	*Otak-otak, Siomai*
Meat-based food	4	0	-
Vegetable and fruit-based food	10	0	-
Milk-based food	2	0	-

Table 2. Tracing results of *S. aureus* contamination sources in food for school children

No	Food	Seller's hands (+ and -)	Air (1000 cm^2 for 30 minutes)	Knife (CFU/cm^2)	Raw product/ Before cooking (CFU/g)
1	*Otak-otak*	+	53	7.0×10^2	2.9×10^5
2	*Siomai*	+	53	7.0×10^2	1.2×10^4

for school children consumption were steaming, cutting and packaging. Cutting and packaging are also determined as CCP because after the process of cutting and packaging there was no other stage that could eliminate the hazard of *S. aureus*.

Characterization of pathogen bacteria in food for school children

The use of dose-response relationship with the exponential model, beta poisson, beta-binomial and Weibull-gamma had been studied in this research. The results showed that the dose-response model that could be used to determine the ill probability properly due to *S. aureus* was exponential model. The model determines the hazard dose-response relationship of *S. aureus* had been done by Tamrakar (2013). The suggestion of this model was based on the analysis conducted by Rose and Haas (1999) that recalculated the research that had been done by Singh *et al.* (1971). Singh *et al.* (1971) inoculated *S. aureus* from the skin of the volunteers' forearm with a certain initial dose. After that, the rate of infection and bacteria growth kinetic were observed for 6 days. Based on these data, Rose and Haas (1999) stated that there had been an increase on *S. aureus* density significantly after inoculation. Tamrakar (2013) recommended exponential model to obtain the probability of illness as reproduced in Formula 1.

$$Pi = 1 - e^{-kN} \text{ and } k=7.64E-08 \quad (1)$$

Where : Pi = the probability of illness for exposured participant at a determined
k = the probability of a cell causes illness
N = number of swallowed microbes

S. aureus can cause illness if the population is exceeding 10^6 microbes in food. This is caused by *S. aureus* is capable to produce enterotoxin if the amount has exceeded 10^6 CFU/g microbes (Bennett and Amos, 2006). In this study, ill probability due to *S. aureus* per serving of food for school children was calculated based on the results of the analysis of 35 samples using the Tamrakar (2013) formula. In Monte-carlo scenario of *S. aureus* contaminated food, @Risk software® simulated 0-2.2 x 10^3 CFU/g of *S. aureus* population for fish-based food per serving, given 145 g as the serving size. Based on these data, the probability of illness due to *S. aureus* per serving of food which was calculated using the exponential model was equal to 0.00103 or one case per 972 servings. The probability of illness value was determined by the initial contamination, serving size and the constant value (k). The value of k is constant value of interaction probability between host and pathogen which shows amount of the pathogenic bacteria which is ingested and able to survive, then start the occurrence of illness (Fretz, 2006). The greater the value of k, then the pathogenic bacteria have greater probability to cause illness compared to bacteria that have smaller value of k.

Conclusion

The results of the hazard identification in the food for school children showed one sample of otak-otak and one sample of siomai containing *S. aureus* exceeded the allowed limit. Whereas the source of contamination in the food for school children was the raw materials, sellers' hands, air and knife which was used to prepare the food in school. Ill probability as the consequence of food that had been contaminated by *S. aureus* consumption calculated by exponential model was one case per 972 servings. Ill probability per serving due to consumption of pathogenic bacteria contaminated food was not only influenced by the

Table 3. CPP determination of fish-based food for school children (otak-otak and siomai) at sale location

Processing stage/ raw materials	Microbiology hazard	Microbiology hazard source	CCP	Explanation
Raw product	*S. aureus* identified	Possibility from: raw material, food processor, environment, equipment	No	In fish-based food for school children raw products were identified *S. aureus* contamination. Sources of contamination could be derived from the raw materials, food processing, environment and equipment used in the manufacturing process. This process is not a CCP because after this process there is cooking process that can control *S. aureus* number.
Cooking (Frying/ Steaming)	*S. aureus* identified raw product	Raw product	Yes	Cooking process should be done well to control *S. aureus*. This process is a CCP because it is specifically designed to reduce hazard to an acceptable level.
Cutting	*S. aureus* contamination possibility	Knife, seller's hand, air	Yes	In the cutting process, cross contamination can occur from the knife, seller's hand, and air. This process is a CCP because after this process there is no stage which is able to control number of *S. aureus*.
Packaging	*S. aureus* contamination possibility	Knife, seller's hand, air	Yes	In the packaging process, cross-contamination can occur from seller's hand and the air. This process is a CCP because after this process there is no stage which is able to control the number of *S. aureus*.

type of pathogenic bacteria and the food types, but also by the amount and frequency of consumption. So it is suggested to conduct an exposure study to estimate the risk characterization of pathogenic bacteria contaminated food with better way.

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