MICROBIAL RISK ASSESSMENT OF *STAPHYLOCOCCUS AUREUS* IN RETAIL PORK DISTRIBUTED IN CITY OF BANGKOK

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Abstract – *Staphylococcus aureus* (SA) has been among top-three food poisoning etiology in Thailand. Microbiological risk assessment (MRA) has been used as a scientific means to enhance consumer protection and supported by Codex Alimentarius Commission. MRA is a structural approach to estimate risk from foodborne illness. Important variables to estimate risk were contamination of SA in pork in terms of prevalence and concentration including pork consumption. The probability distributions were used to describe uncertainty and variability of these variables. Parametric distributions, Beta and Lognormal, were used to describe prevalence, concentration and consumption, respectively. The range of prevalence of SA contamination were between 8-68%. Whereas that of concentration of SA were between 0-4.73 log cfu/g. Majority of fresh markets (11/16) were at the borderline of this criterion while 3 markets were deemed acceptable. This study indicated that risk levels of SA foodborne illness in city of Bangkok were slightly pass the acceptable level of risk. Additional risk management measures are recommended to be implemented.

Key Words – Pork, Risk assessment, *Staphylococcus aureus*

I. INTRODUCTION

Staphylococcus aureus (SA) has been among top-three food poisoning etiology in Thailand. According to the most recent report from Bureau of Epidemiology, Department of Disease control, Ministry of Public Health, 22.22% of food poisoning has been attributed to SA, which is equivalent to about 46 cases/100,000 population at risk [1]. The implicated food was associated with foods contaminated with SA and stored at the optimum temperature range for toxin production 35-40^{o}C. These storage temperatures are corresponding to the ambient or storage temperatures of retail pork sold in fresh market nationwide. Since the SA enterotoxin is heat resistant, the consumption of thoroughly cooked pork still have a likelihood of developing acute food poisoning.

Microbiological risk assessment (MRA) has been used as a scientific means to enhance the consumer protection and also support the international trades. Codex Alimentarius commission (CAC) has recommended a structural approach to conduct MRA in the estimation of risk; [1.]Hazard identification, [2.]Hazard characterization, [3.]Exposure assessment, and [4.]Risk characterization (Fig. 1).

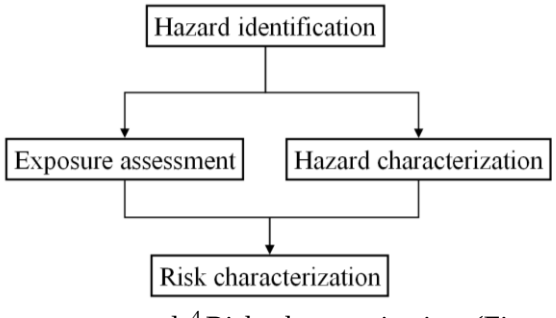

Figure 1. Steps in microbiological risk assessment

MRA is the process of describing an agent resulting in some undesired consequence to the consumers and also contaminated in a food of interest is defined as "Hazard identification." The next step by the sequential event time line is to determine the likelihood of taking pathogen and also the amount of pathogen that is taken from

consuming the implicated food. This step is called "exposure assessment." After exposing to a certain amount of pathogen so called "dose", the likelihood of having adverse health effect (e.g. infection or illness) will be determined by means of the relationship between dose of pathogens and response in terms of dose response assessment model. This step is called "Hazard characterization" or historically "dose response assessment." The final step is to integrate the later two steps to determine the likelihood of undergoing the adverse health effect (dose response assessment) as a result of consuming the food contaminated with pathogen (exposure assessment). Therefore the objective of this study is to quantify risk in terms of illness caused by consumption of pork in fresh market contaminated with SA by using approach recommended by CAC [1].

II. MATERIALS AND METHODS

Sample collection

City of Bangkok is consisted of 4 Bangkok areas; Central, East, North, and South, and 2 Thonburi areas; Upper and Lower (Fig. 2).

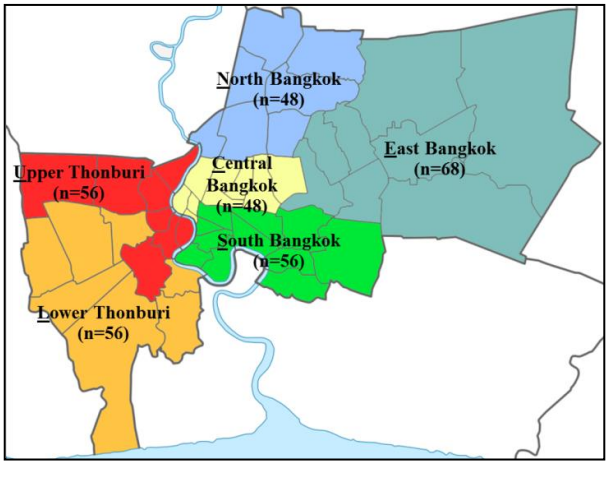

Figure 2. Pork samples collected from 6 areas of Bangkok.

A total number of pork samples was 325 whereas sample sizes of each individual areas were shown in Fig. 2. After collected from fresh markets, 200 g. sampling unit of pork samples were immediately kept on ice and transported to the laboratory for SA enumeration.

SA isolation and enumeration

The isolation and enumeration technique was modified from US. FDA. Bacteriological Analytical Manual (BAM), Chapter 12 (2001). Firstly, 25 g analytical unit of pork samples was mixed with 225 ml buffered peptone water (BPW) to obtain a dilution of 10^{-1}. Then 10-fold serial dilution by BPW was performed to achieve a dilution of 10^{-8} to cover a high level of SA contamination. Aseptically transfer 0.1 ml sample suspension to Baird-Parker (BP) agar. Spread inoculum over surface of BP plate by sterile bent glass streaking rod and leave for 10 min before incubating 45-48 h at 35-37°C. Typical colonies of SA on BP plate are gray or black, with a size of 2-3 mm diameter. Colonies appear to be round, smooth, wet surrounded by opaque zone and have buttery to gummy consistency when touched with needle. Count typical SA colonies BP plate between 25-250 colonies per plate. Typical colonies of SA which also Coagulase positive are counted as SA [3].

Conceptual model for MRA

The central idea of MRA is firstly to determine probability of exposure to SA (P_E) as a function of prevalence, concentration and dose. Probability of illness (P_I) is then described by hazard characterization (dose-response) model. Finally the risk estimate is the integration of exposure and illness in terms of P_E and P_I, respectively.

Exposure assessment model

In order to account for the exposure assessment, the extent and its frequency of

contamination of SA in pork at the point of consumption should be considered. These two variables were interpreted as the concentration and prevalence of SA contaminated in pork, respectively. However the dose of exposure was calculated by the product of concentration of SA and consumption of pork. The likelihood of these three variables (concentration, prevalence, and consumption) was described by the probability distribution using the first-order model.

Concentration variable (C)

SA concentrations at the point of consumption were assumed to be log-normally distributed. Therefore its log is normally distributed. The sample mean and sample standard deviation of concentration were calculated and used as parameter of normal distribution.

Prevalence variable (P)

Since the range of prevalence is between zero (0%) and one (100%) inclusive which is also applicable to the range of Beta distribution. The Beta distribution is characterized by 2 parameters which are alpha and beta as shown in (1).

$$\text{Prevalence} = \text{Beta}(s + 1, n - s + 1) \qquad (1)$$

Consumption variable (M)

The consumption was reported as mean and 97.5 percentile were 20.70 and 90.00 g/day, respectively. Lognormal distribution was used for consumption variable.

Dose (D)

The dose of hazard was determined by the product of concentration (C) and consumption (M)

Probability of exposure (P_E)

Probability of exposure is the likelihood of experiencing SA from eating pork. Therefore the input variables to model probability of exposure are concentration & prevalence of SA and pork consumption as shown in (2) [4].

$$P_E = P(1-e^{-D}) \qquad (2)$$

Hazard characterization models

The objective of this step of MRA is to determine the dose-response relationship quantitatively. The dose is derived from the product of concentration of SA and consumption from exposure assessment. The dose-response model for illness caused by SA was exponential model [5] as shown in (3)

$$P_D = (1-e^{-rD}) \qquad (3)$$

P_D : probability of illness from a dose of SA

r : parameter of exponential model = 7.64×10^{-12}

D : dose of SA/day

Risk characterization model

Finally the risk estimate is calculated by the product of P_E and P_D as two independent and chronological events.

III. RESULTS AND DISCUSSION

The prevalences and mean concentrations of SA in pork in 4 Bangkok (BKK) and 2 Thonburi (TBR) areas were shown in Tables 1-2, respectively.

Table 1 SA Prevalence in fresh markets

Area	Prevalence (%)		
	Market 1	Market 2	Market 3
Central BKK	27	59	N/A*
East BKK	21	33	30
North BKK	40	67	47
South BKK	43	68	N/A
Upper TBR	38	15	19
Lower TBR	58	8	30

* N/A not sampled

The frequencies of SA contamination in terms of prevalence in BKK areas were slightly more ubiquitous (21-68%) than those in TBR area (8-58%) with the similar range of

prevalence. Whereas the extents of SA contamination of both BKK and TBR areas were less varied in the range of 3-4 log cfu/g expect market 1 in East BKK having much higher concentration.

Table 2 SA concentration in fresh markets

Area	log cfu/g		
	Market 1	Market 2	Market 3
Central BKK	3.80	4.00	N/A*
East BKK	5.39	3.00	3.70
North BKK	3.67	3.38	3.67
South BKK	3.42	4.73	N/A
Upper TBR	4.41	4.15	3.42
Lower TBR	3.50	0	4.19

* N/A not sampled

When considering the relationship of prevalence an concentration, the correlation of coefficient is as low as 0.31. This mean that the detection of SA was not a good representative of SA enumeration.

The risk estimate/day of SA in pork from 4 Bangkok areas; Central, East, North, and South, and 2 Thonburi areas were shown in Table 3.

Table 3 Mean risk estimate of SA in fresh markets

Area	Mean daily risk		
	Market 1	Market 2	Market 3
Central BKK	1.2×10^{-6}	4.0×10^{-6}	N/A*
East BKK	3.6×10^{-5}	2.3×10^{-7}	1.0×10^{-6}
North BKK	1.3×10^{-6}	1.1×10^{-6}	1.5×10^{-6}
South BKK	7.7×10^{-7}	2.5×10^{-5}	N/A
Upper TBR	6.6×10^{-6}	1.5×10^{-6}	3.4×10^{-7}
Lower TBR	1.3×10^{-6}	$< 1 \times 10^{-6}$	3.2×10^{-6}

* N/A not sampled

Generally the acceptable risk level for food borne disease is less than 10^{-6}. Majority of fresh markets (11/16) were at the borderline of this criterion while 3 markets were deemed acceptable.

IV. CONCLUSION

This study indicated that the risk levels of SA foodborne illness in city of Bangkok were slightly pass the acceptable level of risk. Additional risk management measures are recommended to be implemented.

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