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Modeling Transfer of *Staphylococcus aureus* and *Escherichia coli* during Raw Pork Slicing

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Nham is a popular Thai traditional fermented pork sausage and usually consumed raw. It is commonly produced in small-scale industries and has been found to be contaminated with pathogenic bacteria due to lack of personal hygiene and improper practices among the food handlers leading to cross-contamination. The objective of this research was to determine the bacterial transfer coefficients between a contaminated stainless steel knife and raw pork during slicing step in the processing of Nham. The pathogens tested were *Staphylococcus aureus* TISTR 1466 (Gram positive) and *Escherichia coli* ATCC 25922 (Gram negative) at different inoculum levels on the blades (8, 6, and 4 log CFU/blade). Raw pork pieces were sliced 15 times. The results showed that both pathogens were able to transfer to all 15 slices of pork examined at inoculum levels of 8 and 6 log CFU/blade. However, it was unable to detect both pathogens on the sliced pork at inoculum level of 4 log CFU/blade. The results also showed that even though the number of bacterial transferred for each successive pork slice decreased logarithmically for both bacteria, but their viable counts remained on the last slice. Transfer coefficients at inoculum level of 8 and 6 CFU/blade were significantly affected ($p< 0.05$) by the type of pathogen. Transfer coefficients were also influenced ($p< 0.05$) by the inoculum level for *E. coli* but not *S. aureus*. Mathematical model was fitted to transfer data for both pathogens and obtaining a good fit ($R^2 > 0.80$).

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Introduction

Recent trends in global food production, processing, distribution and preparation are creating an increasing demand for food safety research in order to ensure a safer global food supply. The World Health Organization (WHO) has been worked closely with the Food and Agricultural Organization of the United Nations (FAO) to address food safety issue along the entire food chain (from production to consumption) using new method of risk analysis (FAO and WHO, 2006). It is important to note that microbiological and chemical hazards result in the most significant sources of foodborne illness.

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) are resident and transient bacteria, respectively, on hands and are associated with poor hygiene practices (Hundy and Cameron, 2002; Adams and Moss, 2008). Toxin-producing strains of *S. aureus* are the leading cause of gastroenteritis following handling of food by persons who carry the microorganism in their noses and skin (Portocarrero et al., 2002; Le Loir et al., 2003). These bacteria are present in about 60% of a given human population and can also survive on hands, knives, chopping boards and dish cloths for hours to days after initial contamination (Scott and Bloomfield, 1990; Kusumaningrum et al., 2002; Barro et al., 2006). *E. coli* is transmitted via the fecal-oral route and is used as indicator of recent fecal contamination. The organism is naturally found in the human intestine and although most strains are harmless, some such as serotype O157:H7 can cause serious illness. The detection of *E. coli* in any sample signals the potential presence of pathogenic microorganisms originating from the intestinal tract of humans and warm-blooded animals (Brown et al., 2002; Moore and Griffith, 2002). *E. coli* has a relatively limited ability to survive on hands and in the environment but can survive on hand surfaces in sufficient numbers to allow transmission to other surfaces (Dawson et al., 2006).

Nham or fermented coarsely-ground pork sausage is a popular Thai traditional meat product. The major ingredients in Nham are raw ground lean pork, cooked shredded pork rind, garlic, cooked rice, 2-3% salt and 100-125 ppm sodium nitrite, while sugar and chili are occasionally added to enhance the flavor. The mixture is wrapped with banana leaves or fills in plastic bags, and left 3-4 days for natural fermentation (Visessanguan et al., 2006). The fermentation process involved successive growth of different microorganisms dominated by Lactobacilli and Pediococci (Valyasevi et al., 2001). Nham is commonly produced in a small-scale industries, thus its safety is questionable. Moreover it is usually served raw thus increasing the risk of consumer being

exposed to contaminated pathogenic microorganisms. Nham sold in retail markets has been found to be contaminated with pathogenic bacteria including *Salmonella* spp., *S. aureus* and *Listeria monocytogenes* (Paukatong and Kunawasen, 2001). Chomvarin et al., (2006) found that several samples of Nham in Thailand's municipality of Khon Kaen province were contaminated with *Salmonella* spp. and *S. aureus* in the amount exceeding 5 log CFU/g. Chokesajjawatee et al., (2009) reported that 39.35% of Nham samples (155 samples in total) collected from retails market in Bangkok and its environs were positive with *S. aureus*. However no Staphylococcal enterotoxin (SE) was detected. This may be due to the fact that the amount of *S. aureus* was no high enough to allow toxin production, and Nham sold in Bangkok came from medium or large factories where hygiene control was much better than in rural area.

Lack of personal hygiene among food handlers is one of the source of the most commonly reported practices contributing to food-borne illness and poor hand and surface hygiene is also a significant contributory factor (Olsen et al., 2000; Collins, 2001; Cogan et al., 2002). Previous studies conducted in food industries have shown that microorganisms are transferred to the hands in the process of handling food and through poor personal hygiene after visiting the lavatory, resulting in the hands being heavily contaminated with fecal related pathogens (Taylor et al., 2000; Barza, 2004). In many situations, the hands are major vehicles that contaminate and disseminate fecal-oral bacteria (Burt et al., 2003; Moore et al., 2003). On the skin surface, hands harbor a resident and/or transient flora (Larson, 2001; Aycicek et al., 2004). Guzewich and Ross (1999) found that 89% of outbreaks caused by food contamination, pathogens were transferred to food by workers' hands.

Food processing equipment has been shown to be a source of contamination in many studies (Lawrence and Gilmour, 1994; Pritchard et al., 1995; Autio et al., 1999; Miettinen et al., 1999; Sumelis and Metaxopoulos, 1999; Aguado et al., 2001; Fonnesbech-Vogel et al., 2001; Lunden et al., 2002; Suihko et al., 2002; Lunden et al., 2003). Utensils for food preparation, such as wooden chopping boards, stainless steel knives have also been found to harbor pathogenic microorganisms, allowing transfer to food (Miller et al., 1996; Bloomfield and Scott, 1997; Kusumaningrum et al., 2002; Moore et al., 2003). Transfer of pathogens to utensils may take place either by direct contact with contaminated objects or indirectly through airborne particle (Baker et al., 2003; Cogan et al., 2002; Kusumaningrum et al., 2002). Previous studies have quantified bacterial survival and cross-contamination between hands and from utensils to food (Scott and Bloomfield, 1990; Zhao et al., 1998; Chen et al., 2001; Kusumaningrum et al., 2003). Hygiene in equipment such as tongs, hammer and cutter are caused when microorganisms become attached to the surfaces and survive, and later become detached from the surfaces contaminating the products (Aarnisalo et al., 2006). The transfer coefficient is the proportion of cells that is transferred between surfaces (food, equipment, tools, hands, etc.) under on or more operations. Surface food-borne pathogen transfer during slicing is one of the important factors impacting the food safety in preparing sliced meat products such as ham, salami, bologna and other restructured meat. A slicer is commonly used and most likely to be the last preparation step before packaging and wrapping of the sliced foods. The slicing equipment if not properly cleaned and sanitized can cause microbial contamination (Sheen and Hwang, 2010).

In the present work, we determined the transfer coefficients at different inoculum levels of 2 types of pathogenic microorganisms: a Gram positive *S. aureus* and a Gram negative *E. coli*, between a contaminated knife blade and a raw meat for quantitative microbial risk assessment (QMRA) of Nham.

Materials and Methods

Inoculum preparation

S. aureus (TISTR 1466) and *E. coli* (ATCC 25922) were provided by Faculty of Pharmacy, Chulalongkorn University in Bangkok, Thailand. Both strains were transferred and maintained on nutrient agar (NA) slants and kept in refrigerator at 4°C. One day before the experiment each strain was transferred to NA slant and incubated at 37°C for 24 h. Three mL of 0.85% normal saline was pipetted onto the slant and mixed well. The culture was scratched by a loop to obtain the cell suspension. Two mL of the cell suspension was pipetted to a 250 mL aseptic flask containing 20 mL of 0.85% normal saline. The concentration of the cell suspension was adjusted to obtain the stock culture with OD 0.151 and 0.104 which were equivalent to 8 log CFU/mL for *S. aureus* and *E. coli* respectively. The stock culture of each microorganism was diluted with 0.85% normal saline to obtain 6 and 4 log CFU/mL inoculums.

Transferring of *S. aureus* and *E. coli* to knife blade

Prior to each experiment, a stainless steel knife was cleaned by detergent (linear alkylbenzene sulfonate and sodium lauryl ether sulfate) and then sanitized by soaking in 95% (v/v) ethanol for 5 min. To inoculate a knife blade, a cotton swab (3M Thailand, Bangkok, Thailand) was dipped into a 1 mL of cell suspension and smeared on a blade surface focusing on the area where it will contact with a meat sample. The cell suspension was inoculated at different concentration (8, 6 and 4 log CFU/mL). The inoculated blade was allowed to dry in a laminar flow cabinet for 15 min.

Transferring of *S. aureus* and *E. coli* from contaminated knife blade at different inoculum level to raw meat during cutting

The pork shoulder used to produce Nham was trimmed by sterilized knifes to obtain the desired size and shape of sample. Each piece of pork was cut to be uniform in shape in order to make all slices similar in area and weight, and also had a uniform surface for slicing. A pork piece was sliced up to 15 times to yield slices (approximately 10 g each) by inoculated knife. Each slice was picked up by a pair of sterilized pincers and placed in a sterile stomacher bags to test for the presence of microorganism. Three repetitions for each inoculum level were performed. The pressure applied to the pork piece was assumed to be equal to that occurring on typical household slicing process.

Microbiological analysis

Prior to each experiment 2 sample of pork (10 g) were placed in a sterile stomacher bags containing 1% peptone buffer and was mixed in a stomacher (Stomacher Lab-Blender 400, model BA 7201, Seward Medical, UK) for 30 s at the speed of 230 rpm to avoid the breakage of meat and only released the cell on the pork surface. Amount of *S. aureus* and *E. coli* was determined using the methods described below. These 2 samples were used as a control sample.

Firstly, estimate the number of viable cells transferred during the cutting process (up to 15 slices). Slice of meat was place independently in a sterile stomacher bag containing 90 mL of 1% peptone buffer and mixed for 30 s at 230 rpm. Ten-fold dilutions were made with 1% peptone buffer. Then 1 mL of each sample at appropriate dilution level was plated in duplicate on selected media. *E. coli* was cultured on 3M Petrifilm *E. coli*/coliform count plate (E-C plate) (3M Company, Bangkok, Thailand) and inoculated at 37°C for 24 h (Nordic Committee on Food Analysis, 1993). Blue colonies with entrapped gas bubbles were enumerated as *E. coli*. For detection of S. aureus, 1 mL of each serial dilution was spread onto *Staphylococcus* Express Petrifilm (3M Company, Bangkok, Thailand) and inoculated at 37°C for 24 h (Nordic Committee on Food Analysis, 1993). Colonies showing pink color were identified and enumerated as *S. aureus*.

Data analysis

The number of bacteria transferred from contaminated knife blade to meat slices were expressed as per square centimeter of slice and logarithmically transformed (log CFU/cm^2). Total transfer coefficient is the logarithm of the summation of the proportion (%) of bacterial cells transferred from the blade to all pork slices (15 slices) (per cm^2) as shown in Equation 1, while transfer coefficient is calculated for each slice as shown in Equation 2.

$$Tr_T\ (\%) = \log\left[\left(C_{slice}\ \text{transferred in 15 slices}/\ C_{blade}\right) \times 100\right] \quad \text{(Eq. 1)}$$

$$Tr\ (\%) = \log\left[\left(C_{slice} / C_{blade}\right) \times 100\right] \quad \text{(Eq. 2)}$$

Tr_T (%) is the total transfer coefficient, Tr (%) is the transfer coefficient, C_{slice} (CFU/cm^2) is the number of bacteria on each meat slice, and C_{blade} (CFU/blade) is the initial number of bacteria on the knife blade. The transfer coefficients were tested by analysis of variance (ANOVA) and Duncan's multiple-range test using SPSS 20 software (SPSS Inc., NC, USA) for the effect of the microorganism type and inoculum level on the knife blade.

Mathematical modeling

Mathematical models were fitted to the data to describe the bacterial concentration on the pork slice as a function of the number of the slice taken (N_{slice} = 1, 2, 3, …, 15). In our experiment log-linear model (semi-logarithmic model) and Weibull model described in Equation 3 or 4 were used to determine the bacterial concentration on the successive slices.

$$\log\left(C_{slice}\right) = \log\left(C_0\right) - k \cdot N_{slice} / \ln(10) \quad \text{(Eq. 3)}$$

$$\log\left(C_{slice}\right) = \log\left(C_0\right) - \left(N_{slice} / a\right)^b \quad \text{(Eq. 4)}$$

For log-linear model in Equation 3: C_{slice} (CFU/cm^2) is the amount of bacteria on each pork slice, N_{slice} is the number of pork slice taken, C_0 is a regression parameter (the y-axis intercept), and k/ln(10) is a regression parameter related to the slope through calculation. For Weibull model in Equation 4: a is reaction rate constant and b is behavior index.

Table 1. Transfer data of *S. aureus* and *E. coli* from a contaminated knife blade to pork slices at inoculum level of 8, 6 and 4 log CFU/blade

Inoculum level (log CFU/blade)	Total number of bacteria transferred (log CFU/cm^2)		Total transfer coefficient (%)		Transfer coefficient (%)	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
8	7.11±0.31^{a,A}	5.30±0.31^{a,B}	1.11±0.31^{a,A}	0.70±0.31^{a,B}	- 0.55±0.74^{a,A}	- 2.31±0.66^{a,B}
6	4.85±0.27^{b,A}	3.92±0.09^{b,A}	0.85±0.27^{a,A}	0.08±0.09^{b,B}	- 0.68±0.61^{a,A}	- 1.50±0.49^{b,B}
4	-	-	-	-	-	-

Mean ± standard deviation; Different lowercase letters in the same column indicate significant differences (P< 0.05); Different uppercase letters in the same row indicate significant differences (P< 0.05).

Results and Discussion

Transfer coefficient

The experimental data are summarized in Table 1. The transfer values expressed as *Tr* (%) ranges from -0.55 and -2.31 which corresponding to *S. aureus* and *E. coli* at 8 log CFU/blade respectively. The magnitude of transfer data was much lower than the levels found by other previous studies. However our data were in agreement with those reported by Perez-Rodriguez et al., (2007) following the experiment in which cooked meat was cut

by inoculated blade of slicing machine. This may be explained by a different of hand cutting process from the static contact experiment in other studies such as transfer of bacteria from hand to utensil or to food. Transfer of bacteria during slicing involves horizontal movement between surfaces when meat surface runs over the blade (Perez-Rodriguez et al., 2007).

Table 2. Regression parameters and goodness-of-fit indices for log-linear and Weibull models obtained from transfer data of *S. aureus* and *E. coli* at inoculum level of 8, 6 and 4 log CFU/blade

Type of microorganism	Inoculum level (log CFU/blade)	log-linear model			Weibull model			
		log (C_0)	*k*	R^2	log (C_0)	*a*	*b*	R^2
S. aureus	8	6.8644	0.3749	0.9545	6.9150	5.2826	0.9495	0.9548
	6	4.3535	0.2551	0.8662	4.2980	11.9660	1.0960	0.8670
E. coli	8	4.9867	0.3507	0.9429	4.9400	7.7580	1.0570	0.9432
	6	3.1767	0.1605	0.8013	3.8620	1.6892	0.3787	0.8434

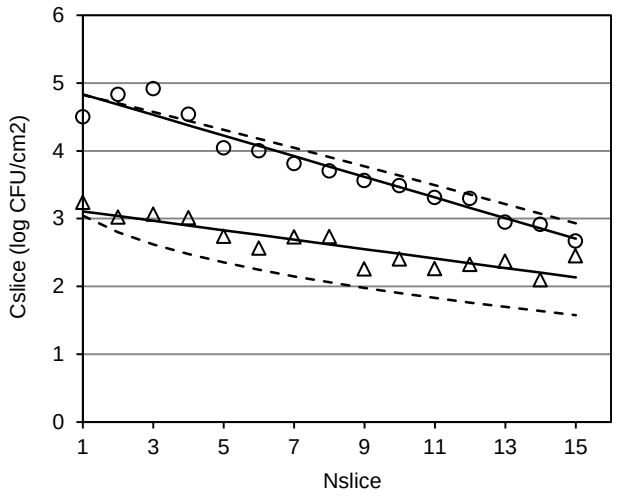

Fig. **1.** Transfer of *S. aureus* from an inoculated knife blade with 8 (○) and 6 (Δ) log CFU/blade. The log-linear models (solid lines) and Weibull models (dashed line) are fitted to transfer data

Total amount of *S. aureus* transferred at 8 and 6 log CFU/blade were 7.11 and 4.85 log CFU/cm^2 respectively, meaning that a small fraction of microorganism were actually transferred (e.g. approximately 34 million cells from 100 million cells per blade). This was in consistent with the low total transfer values expressed as Tr_T (%) of 1.11 and 0.85% respectively. Considerable amount of *S. aureus* were found on the last pork slice at both inoculum levels (4.68 and 2.48 log CFU/cm^2, respectively). Hence transfer of cells is unlikely to cease at 15 slices used in our experiment and more slices could be further contaminated. However at inoculum level of 4 log CFU/blade, no bacterial counts could be obtained in any of the slices. Moreover, amount of the *E. coli* transferred for entire pork slices taken were also low (5.30 and 3.92 log CFU/cm^2 at 8 and 6 log CFU/blade respectively) and lower than *S. aureus*. At both inoculum levels, *E. coli* were still detected on the last slice (2.65 and 2.23 log CFU/cm^2, respectively), thus further contamination could possibly occur beyond 15 slices. Although from the experiment data the possibility may be lower than that of *S. aureus*. In case of the lowest inoculum level of 4 log CFU/blade, the cell transfer did not occur.

The analysis of variance of total transfer coefficients (Tr_T) in 15 slices at 8 and 6 log CFU/blade inoculum levels revealed significant differences for the microorganism types ($P < 0.05$). The analysis of variance of transfer coefficients (*Tr*) at inoculum level of 8 and 6 log CFU/blade was conducted and the results also showed significant differences between the microorganism types ($P < 0.05$). The different in susceptibility to environmental stress between *S. aureus* and *E. coli* may explain this behavior. Since higher transfer coefficient was observed for *S. aureus*, it pointed out that *S. aureus* had higher ability to withstand the stressful environmental conditions than *E. coli*. With regard to inoculum level, analysis of variance indicated significant differences ($P < 0.05$) in total transfer coefficients for *E. coli* but not for *S. aureus*. Total transfer coefficient of *E. coli* decreased as inoculum level decreased from 8 and 6 log CFU/blade. This same observation can be observed for transfer coefficients of *E. coli*. Thus it was clear that inoculum level (8 to 6 log CFU/blade) did not influence the ability of *S. aureus* to transfer from knife blade to pork slice but significantly affected the transfer capability of *E. coli*.

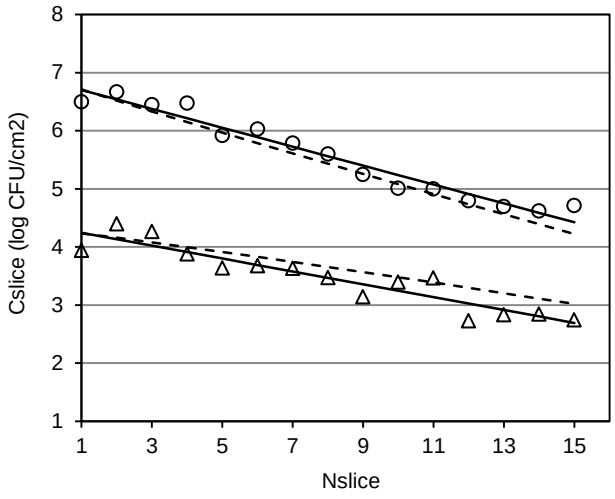

Fig. **2.** Transfer of *E. coli* from an inoculated knife blade with 8 (○) and 6 (Δ) log CFU/blade. The log-linear models (solid lines) and Weibull models (dashed line) are fitted to transfer data

The transfer data for *S. aureus* and *E. coli* expressed as bacterial concentration (log CFU/cm^2) on each pork slice are plotted against number of pork slice (1-15 slices) on a semi-log scale (Fig. 1 and 2 respectively). Both microorganisms exhibited a decreasing trend in ability to

transfer from knife blade to each pork slice when the number of slice increased. The results showed that at inoculum level of 8 and 6 log CFU/blade amount of *S. aureus* transferred per slice decreased logarithmically from the first slice taken to the last slice (1.65 and 1.39 log CFU/cm^2, respectively). Although this effect was less pronounces for *E. coli*, amount of cells transferred per slice decreased logarithmically as well (1.51 and 0.93 log CFU/cm^2, respectively). It is important to note that the amount of cells transferred per pork slice was not always highest for the first slice. This can be clearly seen in Fig. 1 and 2. For example at 8 log CFU/blade of *E. coli*, the first slice had 4.17 log CFU/cm^2 while second, third and fourth slice had 4.63, 4.68 and 4.45 log CFU/cm^2 respectively (data not shown). This same behavior also occurred at 8 and 6 log CFU/blade of *S. aureus*.

Modeling of transfer data for the QMRA

In previous studies a log-linear model has been used to describe attachment strength of bacteria on surfaces (ease of removal) assuming first-order kinetics phenomena. Such a model was fitted to experimental data consisting of the number of colonies transferred and revealed the inverted relation between slope and microorganism attachment strength (Perez-Rodriguez et al., 2007). A Weibull model is a non-linear model and its underlying principles allows modeling of the processes occurring during bacterial transfer between surfaces. Weibull distributions are usually applied to objects with large number of links and each of which has a certain probability of breaking (Perez-Rodriguez et al., 2007), and are often used to model the time interval between successive, random, independent events that occur at a variable rate (Cullen and Frey, 1999; Vose, 2000). Weibull models have been applied in predictive microbiology to represent the logarithmic process of bacterial inactivation (Peleg and Cole, 1998; Fernandez et al., 2002). In cross-contamination events, the initial bacterial population on a surface includes a complex net of interactions between the bacteria and the contact substrate through attachment, in which each component has a certain probability of breaking. To enable bacterial transfer between surfaces it is necessary that these interactions fail (attachment structures), and the bacteria can travel from one surface to another (Dickinson, 1990). Therefore, based on this hypothesis, contamination or recontamination between surfaces by contact could be described by means of a probability curve of breaking probabilities such as Weibull distributions (Perez-Rodriguez et al., 2007).

The log-linear and Weibull models could be applied in quantitative microbial risk assessment (QMRA) to describe cross-contamination scenarios in Nham manufacturing. To establish the model, the amount of bacteria transferred onto the pork slice (log(C), CFU/cm^2) was preferred to either transfer coefficient or total transfer coefficient since this could be easily interpreted and used by modelers in QMRA. Transfer data for each microorganism type at the inoculum levels 6 and 8 log CFU/blade were fitted to the 2 mathematical models described in Equation 3 and 4.

The fitting lines for the log-linear and Weibull model are plotted in Fig. 2 and 3 respectively. Model parameters and R^2 are summarized in Table 2. *S. aureus* and *E. coli* presented a logarithmic trend at 8 and 6 log CFU/blade (R^2 = 0.8013 to 0.9545) according to the log-linear model, though *S. aureus* showed better fit as shown by the values of R^2. At inoculum level of 8 and 6 log CFU/blade, log-linear model revealed lower slopes for *E. coli* (k/ln(10) = 0.1523 and 0.0697, respectively) than *S. aureus* (k/ln(10) = 0.1628 and 0.1108, respectively). Thus, it indicated that at 6 log CFU/blade the logarithmic decrease of bacterial concentration on each pork slice was more rapid for *S. aureus* when bacterial contamination was described using log-linear model. These results appeared to contradict those reported by Perez-Rodriguez et al., (2007) which revealed that *E. coli* O157:H7 decreased logarithmically at a slightly faster rate than *S. aureus* when cooked meat was cut using contaminated slicing machine at inoculum level of 6 log CFU/blade. The Weibull model presented the same goodness-of-fit as log-linear model for both microorganisms as shown by R^2 except for *E. coli* at 6 log CFU/blade which presented slightly better fit (R^2 = 0.8434 and 0.8013 for Weibull and log-linear model, respectively). The Weibull model was almost identical to the log-linear model in the case of *E. coli* at 8 log CFU/blade since the value of parameter b was nearly equal to 1 (b = 1.0570) and both curves are overlap (Fig. 3). With a slight differences in R^2 between log-linear and Weibull models, we suggested that log-linear model appropriately described the transfer data. It was supported by the fact that bacterial concentration on each pork slice decreased continuously throughout the experiment (1 to 15 slices). Though Weibull model could be chosen in the experiment where the slices at the start of cutting showed much higher bacterial concentration than the rest of the slices.

Conclusions

The results showed that all the pork slices could be contaminated by both microorganisms at inoculum level of 8 and 6 log CFU/blade, but it couldn't occur at 4 log CFU/blade. The transfer coefficients were low for both microorganisms. The first slices had the highest contamination levels and the level decreased logarithmically as number of slices increased. Hence, the specific time during slicing (for each slice taken) when contamination occurred had a significant impact on the risk level of the pork slice. The microorganism type did affect total transfer coefficient and transfer coefficient. The inoculum level on knife blade did not significantly influence the transfer of *S. aureus* but not *E. coli*. It was

not clear which mathematical model was the most suitable for estimation of transfer data to be used in QRMA. Both log-linear and Weibull models fitted the data with sufficient goodness-of-fit. Future research should consider the use of microbiological analysis method with higher sensitivity (lower detection limit) to better imitate the scenario in manufacturing environment. Other factors like the medium used to inoculate the knife blade should be investigated as there too should affect the transfer ability of the microorganisms.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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