การประเมินความเสี่ยงเชิงปริมาณเชื้อ *Klebsiella pneumoniae* ที่ดื้อต่อ Ciprofloxacin จากเนื้อสุกรในเขตกรุงเทพมหานคร

นางสาวรจนา นามกระโทก

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Quantitative Microbial Risk Assessment of *Klebsiella pneumoniae* to Ciprofloxacin from Pork in Bangkok

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รจนา นามกระโทก : การประเมินความเสี่ยงเชิงปริมาณเชื้อ *Klebsiella pneumoniae* ที่ดื้อต่อ Ciprofloxacin จากเนื้อสุกรในเขตกรุงเทพมหานคร (Quantitative Microbial Risk Assessment of *Klebsiella pneumoniae* to Ciprofloxacin from Pork in Bangkok) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร.ศุภชัย เนื้อนวลสุวรรณ, 64 หน้า.

การประเมินความเสี่ยงของเชื้อจุลินทรีย์เชิงปริมาณนั้นเป็นวิธีการวิเคราะห์ข้อมูลเพื่อประเมินความเสี่ยงที่เกิดขึ้น วิธีการนี้ถูกนำมาปรับใช้ประเมินความเสี่ยงจากเชื้อแบคทีเรียเคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin ต่อสุขภาพของประชากรในกรุงเทพมหานครที่บริโภคเนื้อสุกร การศึกษานี้แบ่งออกเป็น 4 ขั้นตอน ได้แก่ การเก็บตัวอย่าง การตรวจแยกแยะและนับปริมาณเชื้อ การตรวจเชื้อดื้อยา และการประเมินความเสี่ยง จากตัวอย่างเนื้อสุกรทั้งหมด 378 ตัวอย่าง ซึ่งได้มาจาก 6 เขตของกรุงเทพมหานคร ได้แก่ เขตกรุงเทพมหานครกลาง เขตกรุงเทพมหานครตะวันออก เขตกรุงเทพมหานครเหนือ เขตกรุงเทพมหานครใต้ เขตธนบุรีเหนือ และเขตธนบุรีใต้ การศึกษานี้พบว่าค่าเฉลี่ยความชุกของเชื้อเคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin คือ 89.95% และ 7.65% ตามลำดับ เช่นเดียวกันกับความเข้มข้นของเชื้อ เคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin คือ 6.56 และ 5.89 log cfu/g ตามลำดับ พบว่าความเข้มข้นของเชื้อเคลบเซียวล่า นิวโมเนีย ในเขตธนบุรีเหนือมีปริมาณสูงกว่าเขตอื่นๆ อย่างมีนัยสำคัญ ($p < 0.05$) แต่ความเข้มข้นของเชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin นั้นไม่มีความแตกต่างกันในแต่ละเขตอย่างมีนัยสำคัญ ($p > 0.05$) อย่างไรก็ตามค่าความเสี่ยงในการบริโภคเนื้อสุกรของประชากรกรุงเทพมหานครต่อเชื้อเคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin คือ 4.94×10^{-4} และ 8.57×10^{-7} คน/วัน ตามลำดับ จะเห็นได้ว่ามีค่าความเสี่ยงต่างกันอย่างน้อย 500 เท่า ซึ่งเทียบได้กับใน 1 ปี ต่อประชากรกรุงเทพมหานคร 100,000 คน จะมีความเสี่ยงต่อเชื้อเคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin แล้วได้ป่วยจากการบริโภคเนื้อสุกร คิดเป็นจำนวน 18,067 และ 33 คน ตามลำดับ นั้นหมายถึงว่ามีความเสี่ยงน้อยต่อเชื้อเคลบเซียวล่า นิวโมเนีย ที่ดื้อต่อยา ciprofloxacin ดังนั้นสุขลักษณะและสุขาภิบาลในขั้นตอนต่างๆ ของห่วงโซ่ผลิตอาหาร จะสามารถชะลอการเจริญเติบโต และอีกทั้งยังลดการปนเปื้อนของเชื้อเคลบเซียวล่า นิวโมเนีย ที่เจือปนมากับอาหารได้ อย่างไรก็ตามการปรุงอาหารให้สุขอย่างสุขลักษณะจะช่วยลดปริมาณเชื้อจุลินทรีย์ได้จำนวนมากก่อนการบริโภค สำหรับการจะทำให้ค่าประเมินความเสี่ยงต่อเชื้อเคลบเซียวล่า นิวโมเนีย และ เชื้อเคลบเซียวล่า นิวโมเนียที่ดื้อต่อยา ciprofloxacin จะได้ผลที่ถูกต้องแม่นยำมากขึ้นเมื่อมีการพัฒนารูปแบบในการประเมินความเสี่ยงต่อเชื้อเคลบเซียวล่า นิวโมเนียโดยเฉพาะ

ภาควิชา สัตวแพทยสาธารณสุข ลายมือชื่อนิสิต .....

สาขาวิชา สัตวแพทยสาธารณสุข ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2560

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KEYWORDS: BANGKOK / CIPROFLOXACIN / KLEBSIELLA PNEUMONIAE / MARKET / RISK ASSESSMENT

RODJANA NAMKRATOK: Quantitative Microbial Risk Assessment of *Klebsiella pneumoniae* to Ciprofloxacin from Pork in Bangkok. ADVISOR: ASSOC. PROF. SUPHACHAI NUANUALSUWAN, Ph.D., 64 pp.

Risk estimates are evaluated by using quantitative microbial risk assessment (QMRA) which is a scientific tool. The methodology can be applied to model of human adverse health effect associated with *K. pneumoniae* and ciprofloxacin-resistant *K. pneumoniae* (CRKP) from pork consumption in Bangkok. This study had four phases which were 1) sample collection, 2) bacterial isolation and enumeration, 3) antimicrobial susceptibility test and 4) risk assessment. A total of 378 pork samples from fresh markets were collected from six Bangkok areas which contained Central Bangkok, Eastern Bangkok, Northern Bangkok, Southern Bangkok, Upper Thonburi and Lower Thonburi. The mean prevalence of *K. pneumonaie* and CRKP from retail pork in Bangkok was approximatedly 89.95% and 7.65%, respectively. Likewise, the mean concentration of *K. pneumonaie* and CRKP was 6.56 and 5.89 log cfu/g, respectively. The highest *K. pneumoniae* concentration was in Lower Thonburi which was significantly higher than that of any Bangkok areas (p value < 0.05). The CRKP concentrations across all Bangkok areas were not significantly different (p value > 0.05). Daily risk estimates from *K. pneumoniae* and CRKP were 4.94×10^{-4} and 8.57×10^{-7}. These were equivalent to annual risk of 18,067 and 33 cases per 100,000 Bangkok residents from *K. pneumoniae* and CRKP, respectively. Interestingly, the risk estimate from CRKP was atleat 500 times lower than that of *K. pneumoniae*. This means that the concern of AMR risk from CRKP was negligible. Hygiene and sanitary measure in the entire pork production chain can affect reduce microbial growth and contamination. Additionally, proper cook can also reduce the amount of microbial load by the point of consumption. The accurate risk estimates from *K. pneumoniae* and CRKP shall be achieved when the models of *K. pneumoniae* and CRKP are available in the further studies.

Department: Veterinary Public Health Student's Signature .....

Field of Study: Veterinary Public Health Advisor's Signature .....

Academic Year: 2017

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistant
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BMA	Bangkok Metropolitan Administration
BPW	buffered peptone water
C	concentration
°C	degree (s) Celsius
CRKP	ciprofloxacin-resistant *Klebsiella pneumoniae*
cfu	colony forming unit
DNA	Deoxy-ribonucleic acid
D	ingested dose
D_T	decimal reduction time
E.	*Escherichia*
K.	*Klebsiella*
LB	Luria-bertani broth
log	logarithm
M	dairy pork consumption
min	minute (s)
MIC	minimum inhibitory concentration
MLE	maximum likelihood estimator
ml	milliliter (s)
mm	millimeter (s)
MRA	microbial risk assessment
P	prevalence
P_E	probability of exposure
P_I	probability of illness
P_M	probability of mortality
P_S	risk estimate (s)
QMRA	quantitative microbial risk assessment
r	Spearman's rho or correlation coefficient
spp.	species

CHAPTER 1

INTRODUCTION

1.1 Importance and rationale

The *Klebsiella* spp. is a Gram-negative, rod-shape, non-motile bacterium which belongs to Family Enterobacteriaceae (Edwards, 1986). The *Klebsiella* spp. was isolated from human gastrointestinal tract (Boonyasiri et al., 2014). *K. pneumoniae* has been recognized as a foodborne pathogen (Casewell, 1978; Kiddy, 1987; Sabota et al., 1998) besides various infections of respiratory tract, gastrointestinal tract, urinary tract and septicemia (Morris and Yates, 1956; Heredia et al., 1960; Rennie et al., 1990; Guerin et al., 1998; Sabota et al., 1998; Davis et al., 2015). These infections were caused by capsule interferes with adhesion and invasiveness (Brisse et al., 2006). *K. pneumoniae* has been isolated from the intestinal tract of animals, foods and the environment (Jiwa, 1981; Singh and Kulshreshtha, 1992; Sabota et al., 1998; Viswanathan, 2000; Robertson et al., 2002; Boonyasiri et al., 2014). Then, *K. pneumoniae* was generally transmitted among humans, animals and the environment (Davis and Price, 2016). Therefore, foods e.g. retail meats and vegetables could serve as a vehicle of such transmission through contamination (Davis and Price, 2016). The foodborne disease caused by *K. pneumoniae* could be aggravated by antimicrobial resistant (AMR) strains of *K.pneumoniae* (Boonyasiri et al., 2014; Davis et al., 2015; Davis and Price, 2016). The consequences of AMR *K. pneumoniae* could be an antimicrobial treatment failure, a longer hospitalization and a higher mortality rate (Blahova et al., 1997). Moreover, the extent of antibiotic resistance of *K. pneumoniae* infection has rapidly changed among various antimicrobial classes or multidrug resistance (MDR) (Brisse et al., 2006).

Prevalence of the *Klebsiella* spp. in pork was low as 60% (Boonyasiri et al., 2014). The consumption of pork of Bangkok population was about 20.70

gram/person/day[1]. Consumption of contaminated pork with antimicrobial resistant *K. pneumoniae* could pose a serious illness. Information regarding likelihood and magnitude of adverse health effect of antimicrobial resistant *K. pneumoniae* from pork consumption is still limited. Moreover, ciprofloxacin is the antibiotic of choice in hospital for treating *K. pneumoniae* infection yet the treatment failures in some cases were reported (Phumart, 2012). Therefore, it is essential to determine the contamination level and risk estimate of both *K. pneumoniae* and ciprofloxacin-resistant *K. pneumoniae* (CRKP) acquired from pork consumption derived from fresh markets.

1.2 Objectives of this study

1.2.1 To evaluate the prevalence and concentration of *K. pneumoniae* and CRKP contamination from retail market in Bangkok

1.2.2 To determine the probability of exposure and illness of *K. pneumoniae* and CRKP from pork consumption

1.2.3 To estimate and compare the risk attributed to *K. pneumoniae* and CRKP from pork consumption

[1] National Bureau of Agricultural Commodity and Food Standards, 2005. "consumption ratio." [Online]. Available:http://consumption.acfs.go.th/index.php?content=consumption&topic=ratio. Accessed June 5, 2017.

CHAPTER II

LITERATURE REVIEW

2.1 *Klebsiella* species

The *Klebsiella* spp. is a Gram-negative, rod-shape, non-motile bacterium which belongs to the Family Enterobacteriaceae (Edwards, 1986). The *Klebsiella* spp. ferments lactose and is dome-shaped, 1.5-4.0 mm in diameter of colony after incubation for 24 hours at 30°C or 37°C. The colony is mucoid and sometimes sticky (Brisse, 2006). Genus *Klebsiella* is composed of six species, which are *K. pneumoniae* (subspecies *pneumoniae, ozaenae, rhinoscleromatis*), *K. oxytoca, K. terrigena, K. planticola, K. ornithinolytica* and *K. mobilis* (Brisse, 2006).

2.2 *Klebsiella* spp. in human and transmission route

The *Klebsiella* spp. could be isolated from the gastrointestinal tract of human with a concentration of 7 log cfu/g of feces (Finegold, 1983; Leclerc et al., 2001; Boonyasiri et al., 2014). Two major pathogenic species of the *Klebsiella* spp. are *K. pneumoniae* and *K. oxytoca*. The prevalence of *K. pneumoniae* were twice as much as those of *K. oxytoca* (0.66:0.34) (Davis and Matsen, 1974; Bauernfeind et al., 1981). *K. pneumoniae* has been recognized as a foodborne pathogen (Casewell, 1978; Kiddy, 1987; Sabota et al., 1998) as well as various infections in respiratory tract, gastrointestinal tract, septicemia and urinary tract (Morris and Yates, 1956; Heredia et al., 1960; Rennie et al., 1990; Guerin et al., 1998; Sabota et al., 1998; Davis et al., 2015). In 1990, *K. pneumoniae* was highly contaminated (6 log cfu/g) in prepared food in a restaurant and then produced a heat-labile (LT)-like enterotoxin, that caused gastroenteritis cases (Rennie et al., 1990). And in 1997, the first evidence of

K. pneumoniae as an enteroinvasive foodborne pathogen transmitted from a hamburger in USA was reported (Sabota et al., 1998).

Some studies have reported *K. pneumoniae* from intestinal tract of warm-blood animals, foods and environment e.g. water and soil (Jiwa, 1981; Singh and Kulshreshtha, 1992; Sabota et al., 1998; Viswanathan, 2000; Robertson et al., 2002; Boonyasiri et al., 2014). Therefore, foods contaminated with *K. pneumoniae* was not necessary derived from fecal contamination i.e. *K. pneumoniae* from environment could also contaminate foods. Since the main source of *K. pneumoniae* are the gastrointestinal tract of both humans and food animals. The contamination routes among human, animal and the environment were suggested (Davis and Price, 2016). At first, *K. pneumoniae* is circulating among food production chain steps since both animal and the environment were the primary sources of this foodborne pathogen. Individuals, who get involved any steps along the food production chain, are mainly responsible for the transmission of *K. pneumoniae* from food production chain to the community. The potential vehicles of such transmission could be foods such as retail meats and vegetables in figure 1 (Davis and Price, 2016).

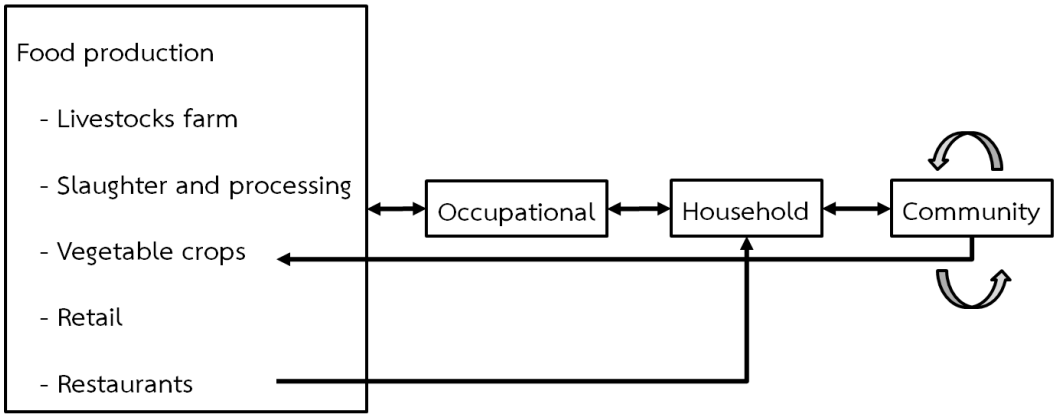

Figure 1 Transmission of *K. pneumoniae* among human, animal and the environment

The foodborne disease from *K. pneumoniae* could also be aggravated by AMR strains of *K. pneumoniae* (Boonyasiri et al., 2014; Davis et al., 2015; Davis and Price, 2016). Since the treatment of *K. pneumoniae* infections were complicated with additionally adverse health consequence of AMR such as an antimicrobial treatment failure, a longer hospitalization and a higher mortality rate (Blahova et al., 1997).

2.3 *K. pneumoniae* in foods and food products

K. pneumoniae can be isolated from various foods such as pork, chicken, turkey, beef, shrimps, seafood, vegetables, hamburger and prepared foods (Bauernfeind et al., 1981; Rennie et al., 1990; Singh and Kulshreshtha, 1992; Sabota et al., 1998; Boehme et al., 2004; Kim et al., 2005; Kilonzo-Nthenge et al., 2013; Davis et al., 2015; Noor, 2015; Davis and Price, 2016). The investigation of hospital outbreak in 2008 shown that *K. pneumoniae* was traceable back to the kitchen. *K. pneumoniae* was contaminated on the prepared foods and the kitchen's surface and was also colonized the kitchen workers (Calbo et al., 2011).

2.4 *Klebsiella* in Thailand

In 2001, prevalence of nosocomial-associated *K. pneumoniae* was between 5-14% (Indrawattana, 2015). In 2012, Phumart et al. demonstrated that *K. pneumoniae* was associated with various nosocomial infections e.g. lower respiratory infection, urinary tract infection, septicemia and surgical wounds and then resulted in the national economic impacts. Even though this study reported that the morbidity rate in terms of nosocomial infection attributable to AMR *K. pneumoniae* was only 5.67% (15,239/268,628) of total nosocomial infection in the hospitals in Thailand. However, among all *K. pneumoniae* mortality cases, the annual mortality rate attributable to AMR *K. pneumoniae* was as high as 51.55% (7,855/15,239). This result indicated that

K. pneumoniae has a low nosocomial morbidity rate but a high mortality rate (Phumart, 2012).

In addition, there was an outbreak of nosocomial infection and multidrug resistance in the Neonatal Intensive Care Unit at Buengkarn hospital as a result of the *Klebsiella* spp. and *K. pneumoniae* contamination on the environmental surface as well as nurse during nursing (Saepueng, 2015).

In 2014, Boonyasiri et. al. reported prevalence of *Klebsiella* spp. among samples from healthy animal farm workers, healthy food animals, fresh foods, cooked foods, water. The results of prevalence and corresponding antimicrobial resistant pattern (Boonyasiri et al., 2014) are summarized in Table 1.

Table 1 Prevalence of *Klebsiella* spp. and antibiotic resistant patterns from swab, food and environmental samples in Thailand

Swab, food and environmental samples	*Klebsiella* spp.	
	Prevalence (%)	Antibiotic resistance
Healthy animal farm workers	70	N/A
Healthy food animals		
- Pig	8	N/A
- Chicken	17	N/A
Fresh pork meat (slaughterhouses)	13	N/A
Fresh foods(market)		
- Chicken	55	ceftriaxone, cefoxitin, gentamicin,
- Pork	60	and/or nalidixic acid
- Beef	100	
- Fish	50	
- Shrimp	62	
- Spring onion	100	
- Parsley	91	
- Bean sprout	91	
Cooked foods		
- Chicken with rice	89	ceftriaxone, cefoxitin, gentamicin,
- Grilled chicken	0	nalidixic acid and/or ciprofloxacin
- Grilled pork	50	
- Spicy minced meat	33	
- Spicy grilled meat	50	
- Spicy green papaya	50	
Fish and shrimp pond water	24	ceftriaxone, cefoxitin, imipenem, ertapenem, gentamicin, amikacin, nalidixic acid, ciprofloxacin, or colistin
Canal water	87	N/A
Stagnant water (food animal farms)	52	ceftriaxone, cefoxitin, gentamicin, nalidixic acid, ciprofloxacin or colistin

2.5 Resistance mechanism of ciprofloxacin

Ciprofloxacin is an antibiotic which belongs to fluoroquinolone group. Mechanisms of antibacterial action of fluoroquinolones are the function of two enzymes (DNA gyrase, topoisomerase IV) and efflux pumps. The drug has small molecular weight approximately 300-400 daltons and crosses the bacterial cell wall to reach DNA gyrase and topoisomerase IV in the cytoplasm. While the mechanisms of bacteria resistance against ciprofloxacin were the chromosomal mutation which change the drug target enzymes (DNA gyrase, topoisomerase IV) and changing the drug permeation thus blocking the target enzymes access (changing drug entry and efflux). The role of DNA gyrase is to unwind superhelical twists of DNA as the initial stage for DNA replication. Likewise topoisomerase IV operates at the terminal stage of DNA replication which separates interlinked daughter chromosomes. Bacterial cell death occurred as a result of the inhibition of DNA synthesis and interruption of DNA strand generation. Whereas another bacterial resistance mechanism is the expression or over-expression of energy-dependent efflux pumps. Interestingly these efflux mechanisms are more advantageous than the former mechanism because of the lower likelihood of selecting resistant mutants (Hooper, 1999; Jacoby, 2005).

2.6 Antimicrobial resistance risk assessment

Essentially, the approach of AMR risk assessment was based on non-AMR risk assessment (CAC, 1999). However, the exposure assessment of AMR risk assessment focuses only the amount of pathogen, which is resistant to antimicrobial rather than total pathogen load. The hazard characterization needs to include the additional adverse health effect as a result of AMR conditional to the non-AMR dose-response model. The risk estimate follows non-AMR risk characterization by integrating the probability of exposure and probability of illness due to the amount of AMR pathogen contaminated in the consumed food (CAC, 2011) (Table 2).

Table 2 The comparison of non-AMR and AMR risk assessment

Risk assessment step	Variable / Model	Non-AMR risk assessment	AMR risk assessment
Exposure assessment	Prevalence & concentration	Total pathogen	Only AMR pathogen
Hazard characterization	Dose response model	Probability of illness from total pathogen	Probability of illness from only AMR pathogen
	AMR consequence model	N/A	Hospitalization, prescription, and mortality

AMR risk assessment

The AMR risk assessment is composed of four steps (Snary, 2008; CAC, 2011).

1. Hazard identification

An initial process of conducting microbial risk assessment (MRA) where the biological hazards in foods or commodity is addressed. Microbiological, epidemiological and clinical information of the pathogen of interest and its characteristics along the food-supply chain should be acquired from scientific evidences (CAC, 1999).

2. Hazard characterization

This process establishes the probability of adverse health responses as a function of exposure to non-AMR and AMR doses of pathogen in either qualitative or quantitative manner. Previously, this step was called dose-response assessment. Preferably, the dose response models should be applied to characterize the hazards.

In quantitative microbial risk assessment (QMRA) practice, the dose-response relationships were generally assumed that each bacterial cell independently causes the infection with equal, non-zero probability. Therefore, one could have been infected with single cell of hazard even with a significantly low probability. In addition, the extension of dose-response models of AMR-QMRA should cover the probability of mortality as a result of antimicrobial adverse health effect. This extension describes the conditional probability of the patients who get AMR pathogen from consuming contaminated pork after hospitalization and antimicrobial treatment failures (WHO, 2003; CAC, 2011).

3. Exposure assessment

Exposure assessment is an evaluation of total amount and frequency of population exposure to the contaminated microbial hazards during a certain period of time. The major influences on exposure estimates are the prevalence and concentration of microbial contamination including consumption data of foods. Predictive models are commonly used to describe dynamic of microbial contamination along the food supply chain via the growth and inactivation models. Furthermore, the linkage of various steps in the from-farm-to-fork approach should be emphasized to identify the risk factors influencing the risk estimates to the consumers. These risk factors are likely to form the basis of considering all appropriate risk management options (CAC, 1999).

The exposure assessment is directly related to hazard characterization. Since, the dose of bacterial concentration and consumption is used as an input of dose-response model. Thus, the growth and inactivation model of predictive microbiology were used to predict the concentration of AMR pathogens along the food chain particularly from retail to the household levels. Finally, the concentration of AMR pathogen at the point of consumption could be estimated (Snary, 2008).

4. Risk characterization

Outputs from risk characterization are expressed as risk estimates from the integration of exposure assessment and hazard characterization steps. Risk estimates could be presented as risk per serving size of foods, individual-based risk or population-based risk depending on the risk question from risk managers. In stochastic microbial risk assessment approach, Monte Carlo simulation technique is used to simulate across all possible scenarios of variables in the models (CAC, 1999).

CHAPTER III

MATERIALS AND METHODS

3.1 Sampling frame and sample collection

This study had four phases which were sample collection, bacterial isolation enumeration, and antimicrobial susceptibility test and risk assessment.

Sample size was essentially calculated from a previous report where the prevalence of *Klebsiella* spp. in pork was about 60% (Boonyasiri et al., 2014) with desire confidence level at 95% ($Z_{\alpha/2}$ = 1.96 for two side tests) and magnitude error (d) at 5% (Hajian-Tilaki, 2011). From equation (1), the pork sample size to estimate the prevalence of this pathogen was supposed to be 369.

$$n = \frac{Z^2_{\frac{\alpha}{2}} P(1-P)}{d^2} \quad (1)$$

All pork samples were collected from six Bangkok areas as officially allocated by Bangkok Metropolitan administration (BMA), which were Central, Eastern, Northern, Southern Bangkok, Upper and Lower Thonburi. Fresh markets of each individual Bangkok area were selected by convenient sampling. Total 378 pork samples from fresh markets were categorized by Bangkok areas and shown in Table 3.

Table 3 Pork samples collected from fresh markets in six Bangkok areas

Area	Location	No. of Sample	Subtotal
Central Bangkok	1. Huai Khwang Housing	36	60
	2. Ming Khwan Ban Na	4	
	3. Sisawat	6	
	4. Talad Noi	4	
	5. Leng Buai Ia	6	
	6. 22 Karakadakhom Circle	4	
Eastern Bangkok	1. Bangkapi	48	66
	2. Happyland	10	
	3. Nakorn Thai	8	
Northern Bangkok	Yingcharoen	64	64
Southern Bangkok	1. Samyan	24	68
	2. Rung Chareon	20	
	3. Klong Toei	24	
Upper Thonburi	1. Ngoen Wichit	42	60
	2. Krung Non	18	
Lower Thonburi	Bangkae	60	60
		Total	378

3.2 Microbiological isolation and enumeration

Twenty-five grams of pork sample were 10-fold serially diluted by buffered peptone water (BPW) (Difco™, Pont de Claix, France). A total of 100 microliters of the sample suspension was spread onto MacConkey agar plate (Difco™). After incubation for 24 hours at 37°C, countable plates (between 25-250 colonies/plate) were chosen for typical colonies and counted. The typical morphology of *K. pneumoniae* colony is pink (lactose positive), dome-shaped and mucoid. Biochemical test was then used for *K. pneumoniae* confirmation. The MIL medium (Difco™) was used for Indole and motility test (Podschun and Ullmann, 1998; Alves et al., 2006; Brisse, 2006). The confirmed *K. pneumoniae* colonies were counted and calculated to obtain *K. pneumoniae* concentration and prevalence. Then, all confirmed isolates were cultured in Luria-Bertani (LB) broth (Sifin diagnostics gmbh, Berlin, Germany) for 24 hours at 37°C, then mixed with glycerol and stored at -20°C for further steps (Brisse, 2006).

3.3 Antimicrobial susceptibility test

Antimicrobial susceptibility was determined by the minimum inhibitory concentration (MIC) using agar dilution method according to the version of VET01, the Clinical and Laboratory Standard Institute for bacteria isolated from animal (CLSI, 2013). The antimicrobial agent was ciprofloxacin (SIGMA-ALDRICH, St. Louis, USA) (Phumart, 2012). Veterinary-specific criteria interpretation for ciprofloxacin resistant to *K. pneumoniae* was not available, thus interpreting the result by the version of M100-S24. *Escherichia coli* ATCC 25922 was used as quality control strain (CLSI, 2014).

All pork samples contaminated with *K. pneumoniae* and CRKP were used to calculate both prevalence and concentrations of either *K. pneumoniae* or CRKP. These data were necessary to determine the hazard characterization and exposure assessment of quantitative microbial risk assessment.

Table 4 Ranges of Minimum Inhibitory Concentration (MIC) of ciprofloxacin for *K. pneumoniae*

Antimicrobial agent	Minimum Inhibitory Concentration(MIC)[a]		
	Susceptible	Intermediate	Resistance
Ciprofloxacin	≤ 1	2	≥ 4

[a]*Escherichia coli* ATCC 25922 was used as quality control strain in which ciprofloxacin concentration ranged from 0.004 to 0.015

3.4 Quantitative antimicrobial resistance risk assessment

K. pneumoniae quantitative microbial risk assessment followed the principles and guidelines for conducting microbiological risk assessment (CAC, 1999), while the CRKP risk assessment followed the guidelines for risk analysis of foodborne antimicrobial resistance (CAC, 2011).

3.4.1 Hazard Identification

K. pneumoniae belongs to family Enterobacteriaceae. *K. pneumoniae* has been considered as a foodborne pathogen and colonized various systems of human body such as respiratory tract, gastrointestinal tract, urinary tract and blood stream. Moreover, *K. pneumoniae* has been resistant to several antibiotics including ciprofloxacin (Boonyasiri et al., 2014; Davis et al., 2015; Edwards, 1986; Guerin et al., 1998; Heredia et al., 1960; Morris and Yates, 1956; Rennie et al., 1990; Sabota et al., 1998).

3.4.2 Exposure assessment

The objective of exposure assessment for *K. pneumoniae* was to evaluate the likelihood of consumers to expose to *K. pneumoniae* through pork consumption. The exposure assessment for CRKP was to determine the magnitude of exposure to CRKP in pork consumption.

The prevalence and concentration of *K. pneumoniae* obtained from pork samples were collected from fresh markets at the retail level as shown in Figure 2. The inactivation model was applied in order to predict the concentration of *K. pneumoniae* at the household level. Finally, daily pork consumption of Bangkok residents was used to multiply with *K. pneumoniae* concentrations to obtain both *K. pneumoniae* and CRKP doses ingested by Bangkok residents.

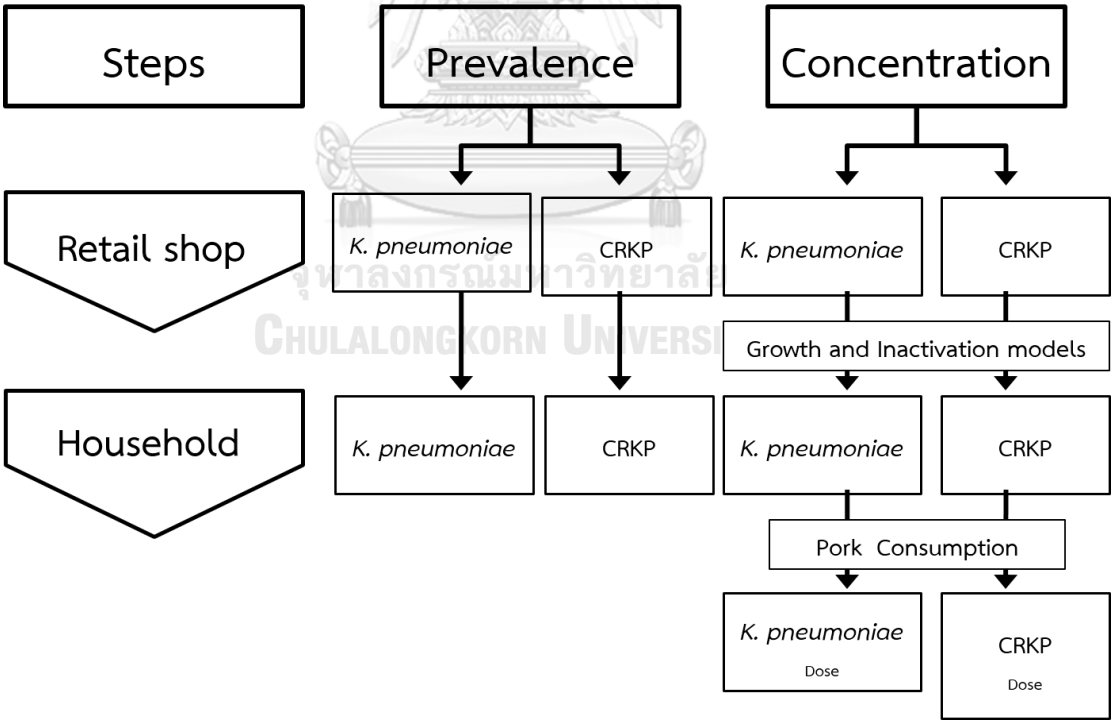

Figure 2 Pathway of pork contamination and sampling from retail to household in the exposure assessment

The following variables were used for either *K. pneumoniae* or CRKP in an exposure assessment step.

3.4.2.1 Prevalence of *K. pneumoniae* was described by Beta distribution which ranged between 0-1.0 (0-100%).

$$\mathrm{Beta}(\mathrm{s}+1, \mathrm{n}-\mathrm{s}+1) \quad (2)$$

Where n is the total number of samples tested and s is the number of samples contaminated with either *K. pneumoniae* or CRKP.

3.4.2.2 Concentration of *K. pneumoniae* was described by normal distribution to represent real-valued random variables.

$$f(x|\mu,\sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (3)$$

Where μ is the mean or expectation concentration of the distribution of *K. pneumoniae* or CRKP (and also its median and mode), **σ** is the standard deviation of *K. pneumoniae* or CRKP concentration, *e* is a constant (2.7183) and $\boldsymbol{\sigma}^2$ is the variance of *K. pneumoniae* or CRKP concentration.

3.4.2.3 Probability of exposure (P_E) is the probability that population or individual has been exposed to at least one cell of *K. pneumoniae* or CRKP from pork consumption (Cassin et al., 1998).

$$P_E = P \times (1 - e^{-D}) \quad (4)$$

$$D = C \times M \quad (5)$$

Where, for either *K. pneumoniae* or CRKP, P is prevalence, *e* is a constant (2.7183), D is ingested dose (log cfu/person/day), C is concentration (cfu/g) and M is daily pork consumption (g) by Bangkok residents per day.

3.4.2.4 Inactivation model is described by a thermal inactivation model (Smelt and Brul, 2014). Since pathogen is essentially eliminated or inactivated by heat treatment during a food processing. Then, this step was crucial to determine the exact dose or the number of pathogens contaminated in food ingested by consumers. Recommended cooking temperature and time, one centimeter depth from the meat surface should be cooked at 60°C for at least two minutes (Toyofuku, 2012). Because of the lack of decimal reduction time (D_T) for *K. pneumoniae* in food, D_T of *E. coli* is substituted for D_T of either *K. pneumoniae* or CRKP in a log-linear inactivation model (Orta-Ramirez et al., 1997).

$$\log N_t = \log N_0 - \frac{t}{D_T} \quad (6)$$

Where log N_t is the log concentration of either *K. pneumoniae* or CRKP after inactivation time *t* (min), log N_0 is the initial log concentration of either *K. pneumoniae* or CRKP at time 0 or from retail markets, t is the inactivation time (min) and D_T is decimal reduction time (min) at inactivation temperature T (°C).

The cooking temperature and time in this study were 63°C for two min. The D_{63} of *E. coli* in ground beef was 0.43 min (Orta-Ramirez et al., 1997).

3.4.3 Hazard characterization

3.4.3.1 Probability of illness of *K. pneumoniae* or CRKP

Probability of illness is calculated by means of a dose-response model. However, there was no dose-response model specifically for *Klebsiella* spp. Similar to thermal inactivation model, dose-response model of *E. coli* model substituted dose response model of *Klebsiella* spp. It was assumed that the distribution of microorganism follows exponential distribution and that one single cell of pathogen is sufficient to cause infection or illness. The exponential model takes into account the variations that exist in pathogen host interactions. The pathogen-host survival probability can be described by probability distribution. The probability of illness (P_I) can be expressed as the following (Haas et al., 2000).

$$P_I = 1 - \exp(-\frac{D}{k}) \quad (7)$$

Where, for either *K. pneumoniae* or CRKP, D was ingested dose (log cfu/person/day), *k* was constant at 1.6×10^7 (Haas et al., 2000).

3.4.3.2 Probability of mortality for CRKP

The objective of AMR risk assessment was to evaluate the additional health consequence upon the exposure of AMR hazard conditional to illness from conventional foodborne illness (CAC, 2011).

In this study, the probability of mortality (P_M) attributable to CRKP was considered as the additional health consequences from CRKP. Probability of mortality attributable to CRKP was determined by a series of independent events from

hospitalization to the death of patients due to the failure of prescribed ciprofloxacin treatment.

$$P_M = H \times P \times M \quad (8)$$

Where H is the hospitalization rate, P is the antimicrobial prescription rate and M is CRKP mortality rate conditional to the treatment by the antimicrobial of interest. Only AMR risk assessment will apply this model (CAC, 2011).

3.4.4 Risk characterization

Risk characterization is the likelihood of overall adverse health effect upon an exposure to hazard contaminated in food and then develop diseases (P_S). This risk estimate from *K. pneumoniae* was the integration of the outputs of two earlier steps of risk assessment which were probability of exposure (exposure assessment) and probability of illness (hazard characterization). The risk estimate of CRKP was the integration of probability of exposure and probability of mortality conditional to illness from CRKP.

3.4.4.1 Risk estimate from *K. pneumoniae*

The risk estimate from *K. pneumoniae* was a function of probability of exposure and illness consecutively. The model to calculate the risk estimate from *K. pneumoniae* was the following.

$$P_S = P_E \times P_I \quad (9)$$

Where P_E is probability of exposure of *K. pneumoniae*, P_I is a probability of illness from *K. pneumoniae*

3.4.4.2 Risk estimate from CRKP

The risk estimate from CRKP was a function of probability of exposure of CRKP and probability of illness from *K. pneumoniae* with the finally probability of mortality from CRKP. The model to calculate the risk estimate from CRKP was the following.

$$P_S = P_E \times P_I \times P_M \quad (10)$$

Where P_E is probability of exposure of CRKP, P_I is probability of illness from CRKP and P_M was probability of AMR consequence (CAC, 2011).

3.5 Monte Carlo simulation

Since, all models used the probability distribution to describe the total uncertainty of variables (stochastic model). Therefore, Monte Carlo simulation was used to calculate all possible scenarios. The data and models were simulated for 20,000 iterations by using the Simulación 4.0. This Monte carlo simulation (Universidad del CEMA, Buenos Aires, Argentina) was a freeware which developed in the form of VBA (Visual Basic for Applications) as Add-Ins operated on MS Excel (spreadsheet software by Microsoft corp.) by Jose Ricardo Verela.

3.6 Data analyses

This statistical analyses of this study were descriptive statistics, ANOVA (SAS System for Windows 9.0) and Tukey's multiple comparisons (SAS System for Windows 9.0). A *p* value less than 0.05 was considered statistically significant.

CHAPTER IV

RESULT

4.1 Contamination levels of *K. pneumoniae* in retail pork across six Bangkok areas

A total of 378 pork samples from fresh markets across six locations in Bangkok was collected. Prevalence and concentration of *K. pneumoniae* and CRKP were indicated.

The average prevalence of *K. pneumoniae* from retail pork in Bangkok were 89.95% (340/378). When the range of *K. pneumoniae* prevalence were between 83.3% (55/66) and 96.9% (62/64) in Eastern and Northern Bangkok, respectively. The concentration of *K. pneumoniae* were between 6.26 and 6.96 log cfu/g in Northern Bangkok and Lower Thonburi, respectively (Table 5 and Figure 3).

Table 5 Prevalence and concentration of *K. pneumoniae* in pork in Bangkok areas

Location	Prevalence and concentration of *K. pneumoniae*	
	Percentage (N)	log cfu/g*
Lower Thonburi	88.3 (53/60)	6.96[A]
Central Bangkok	96.7 (58/60)	6.57[B]
Eastern Bangkok	83.3 (55/66)	6.42[B]
Upper Thonburi	88.3 (53/60)	6.39[B]
Southern Bangkok	86.8 (59/68)	6.37[B]
Northern Bangkok	96.9 (62/64)	6.29[B]
Average	89.95 (340/378)	6.56

*Indicated the statistical difference of concentrations across Bangkok areas

The highest concentration of *K. pneumoniae* found in the Lower Thonburi was reported the highest concentration compared to other sampling locations (*p* value < 0.05). While *K. pneumoniae* concentrations of all locations were not significantly different (*p* value > 0.05). Interestingly, the correlation coefficient of prevalence and concentration of *K. pneumoniae* across six Bangkok areas was -0.092 or -9.2%. This low correlation coefficient indicated that *K. pneumoniae* prevalence and concentration were almost uncorrelated. In other word, at any *K. pneumoniae* prevalence, *K. pneumoniae* concentration could be either high or low and another way around. The negative correlation coefficient indicated that *K. pneumoniae* prevalence was inversely correlated with concentration i.e. the higher *K. pneumoniae* prevalence tended to have a lower *K. pneumoniae* concentration.

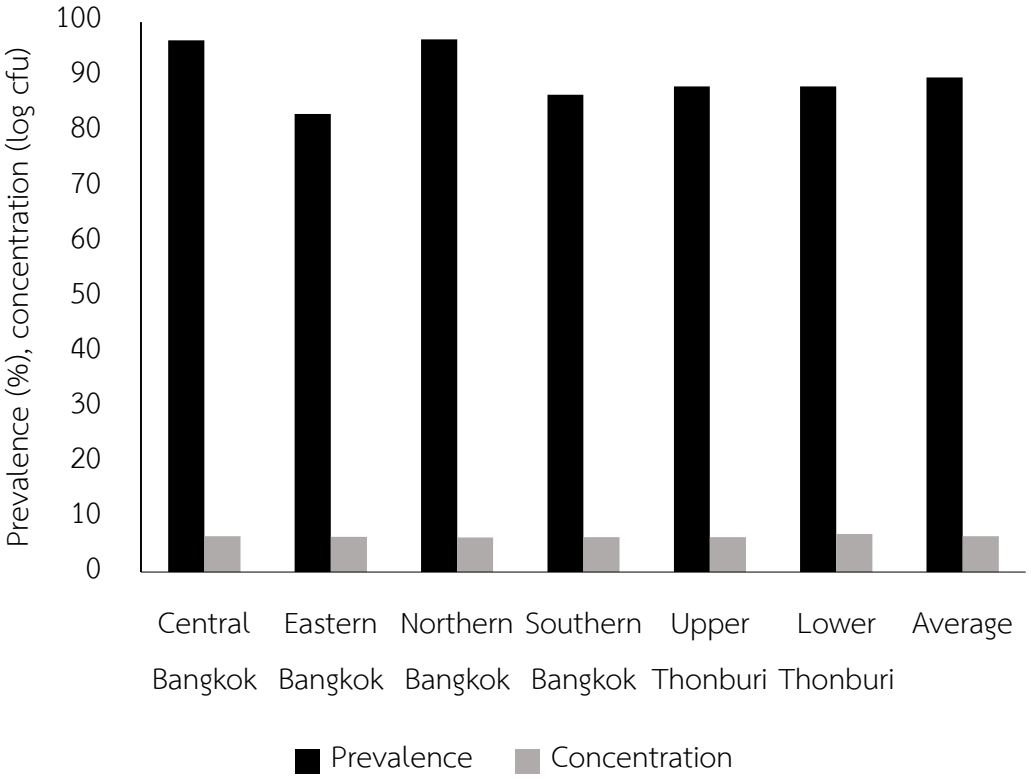

Figure 3 Prevalence and concentration of *K. pneumoniae* in retail pork across six Bangkok areas (n=378)

4.2 Contamination levels of CRKP in retail pork across six Bangkok areas

The average prevalence of CRKP in retail pork in Bangkok was 7.65% (26/340). When the range of CRKP prevalence were between 1.6% (1/62) and 11.3% (6/53) in the Northern Bangkok and the Lower Thonburi, respectively. The range of CRKP concentrations were between 5.33 and 6.25 log cfu/g in the Upper Thonburi and the Eastern Bangkok, respectively (Table 6 and Figure 4).

Table 6 Prevalence and concentration of CRKP in pork in Bangkok

Bangkok areas	CRKP	
	Percentage (N)	log cfu/g*
Eastern Bangkok	10.9 (6/55)	6.25[A]
Southern Bangkok	5.1 (3/59)	6.08[A]
Lower Thonburi	11.3 (6/53)	5.73[A]
Northern Bangkok	1.6 (1/62)	5.68[A]
Central Bangkok	6.9 (4/58)	5.45[A]
Upper Thonburi	11.3 (6/53)	5.33[A]
Average	7.65 (26/340)	5.89

*Indicated no statistical difference of concentrations across Bangkok areas

From Table 6, a narrow range was observed in CRKP concentration that causing the concentrations across were not significantly different (p value > 0.05). Like *K. pneumoniae*, the correlation coefficient of prevalence and concentration of CRKP across six locations of Bangkok was -0.024 or -2.4% which was lower than the correlation coefficient found in *K. pneumoniae*. This low correlation coefficient indicated that CRKP prevalence and concentration were nearly uncorrelated i.e. CRKP prevalence and CRKP concentration could be either high or low and another way around. However, this negative correlation coefficient of CRKP prevalence and concentrations indicated that CRKP prevalence were oppositely correlated with it concentration i.e. CRKP prevalence tended to have a lower concentration.

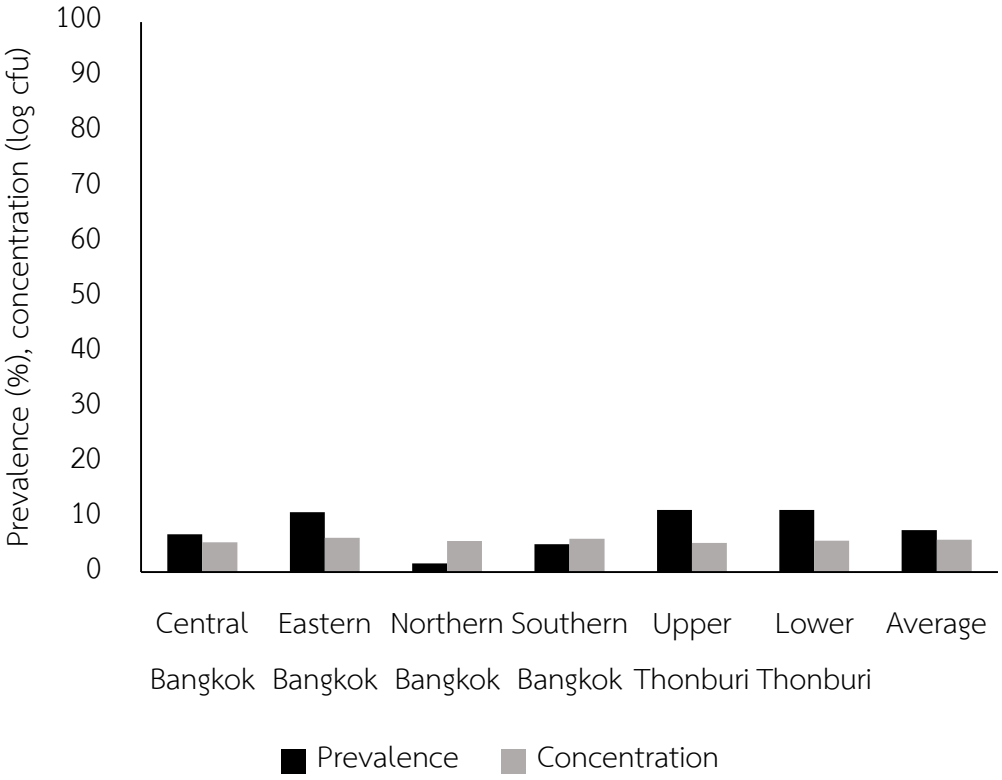

Figure 4 Prevalence and concentration of CRKP in retail pork across six Bangkok areas (n=340)

4.3 Quantitative risk assessment and antimicrobial resistance risk assessment

K. pneumoniae quantitative microbial risk assessment followed the principles and guidelines for conducting microbiological risk assessment as shown in Figure 5 (CAC, 1999). While the CRKP risk assessment follow the guidelines for risk analysis of foodborne antimicrobial resistance (CAC, 2011).

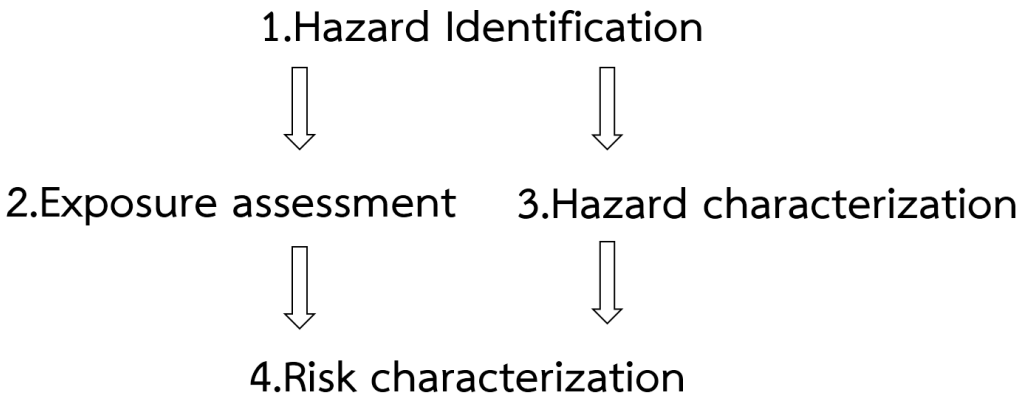

Figure 5 Risk assessment steps of both *K. pneumoniae* risk assessment and CRKP risk assessment

4.3.1 Hazard identification

The hazard identification of *K. pneumoniae* has been described earlier in some previous chapters. Essentially *K. pneumoniae* has been aware of public health concern as foodborne pathogen (Casewell, 1978; Kiddy, 1987; Sabota et al., 1998) which is also resistant to a variety of antibiotics including ciprofloxacin (Boonyasiri et al., 2014).

4.3.2 Exposure assessment

Exposure assessment is the step to evaluate the probability of exposure (P_E) from pork consumption by using prevalence and concentration of *K. pneumoniae* and CRKP through the exposure pathway from retail to consumption level (Figure 6).

The prevalence and concentration of both *K. pneumoniae* (P_{KP} and $C_{R\text{-}KP}$) and CRKP (P_{CRKP} and $C_{R\text{-}CRKP}$) were obtained from pork samples from retail shops as shown in Figure 6. Since the thermal inactivation by cooking at the household level, both $C_{R\text{-}KP}$ and $C_{R\text{-}CRKP}$ at retail level were decreased as a function of cooking temperature and time. Then, the concentrations of *K. pneumoniae* ($C_{H\text{-}KP}$) and ($C_{H\text{-}CRKP}$) were used

to multiply with daily pork consumption (M) to obtain both *K. pneumoniae* dose (D_{KP}) and ciprofloxacin resistant to *K. pneumoniae* doses (D_{CRKP}).

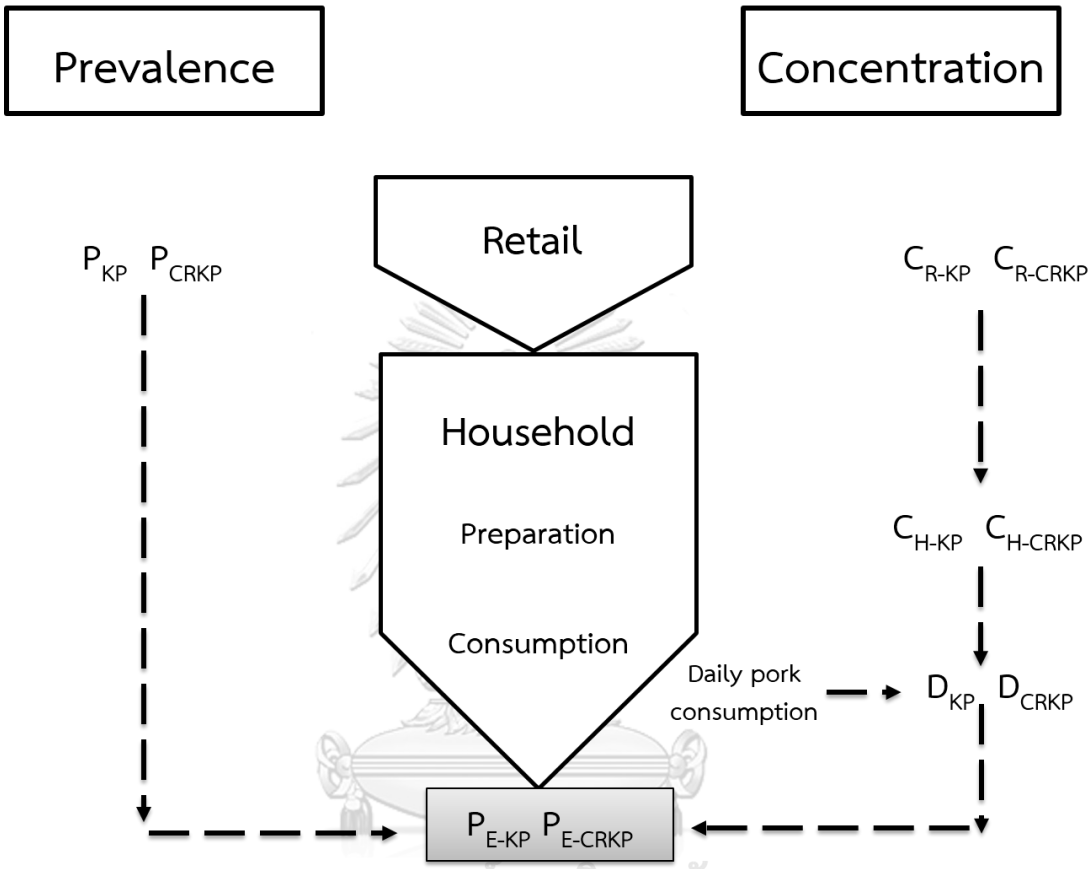

Figure 6 Parameters used to calculate probability of exposure (P_E) of *K. pneumoniae* and CRKP from retail to consumption level in the exposure pathway

K. pneumoniae prevalence (P_{KP}) and dose (D_{KP}) were used to model probability of exposure of *K. pneumoniae* (P_{E-KP}). Likewise, CRKP prevalence (P_{CRKP}) and dose (D_{CRKP}) were used to model probability of exposure of CRKP (P_{E-CRKP}).

4.3.2.1 Probabilistic prevalence of *K. pneumoniae* (P_{KP}) and CRKP (P_{CRKP}) in pork

Probabilistic prevalence of *K. pneumoniae* and CRKP in pork across six locations of Bangkok were described by Beta distribution (equation 2). After simulation, the expected value of *K. pneumoniae* prevalence in pork in all Bangkok areas were 89.2% (Table 7). The ranges prevalence of *K. pneumoniae* were from 82.4% to 95.1% from Eastern Bangkok and Northern Bangkok.

Statistical analyses by ANOVA (SAS System for Windows 9.0) indicated that the *K. pneumoniae* prevalence across six locations of Bangkok were significantly different (*p* value < 0.05). Then, the Tukey's multiple comparisons were run to elaborate the pairwise difference of *K. pneumoniae* prevalence among six locations of Bangkok. The comparisons indicated that *K. pneumoniae* prevalence of Northern Bangkok at 95.1% was significantly higher than those of the rest (*p* value < 0.05). While *K. pneumoniae* prevalence of Central Bangkok, Lower and Upper Thonburi were not statistically different (*p* value > 0.05). Additionally, *K. pneumoniae* prevalence of Southern Bangkok at 85.3% is significantly higher that of Eastern Bangkok at 82.4% (Table 7).

Table 7 Expected values and selected percentiles of *K. pneumoniae* prevalence (P_{KP}) in retail pork across six Bangkok areas

Location	*K. pneumoniae* Prevalence (%)		
	Mean*	5th percentile	95th percentile
Northern Bangkok	95.1[A]	92.5	98.9
Central Bangkok	88.2[B]	92.6	98.9
Lower Thonburi	87.3[B]	82.6	92.9
Upper Thonburi	87.3[B]	82.3	92.7
Southern Bangkok	85.3[C]	81.1	92.1
Eastern Bangkok	82.4[D]	76.7	88.9
Average	89.2	85.1	94.8

* Indicated the statistical difference of prevalence across Bangkok areas

Note: *K. pneumoniae* prevalence was performed 20,000 iterations

After simulation, the expected value of CRKP prevalence in pork in Bangkok market were 8.42% (Table 8). The range of CRKP prevalence were from 2.6% to 11.8 % from Northern Bangkok and Upper Thonburi.

Table 8 Expected values and selected percentiles of CRKP prevalence (P_{CRKP}) in retail pork across six Bangkok areas

Location	CRKP Prevalence (%)		
	Mean*	5th percentile	95th percentile
Upper Thonburi	11.8[A]	7.9	18.5
Eastern Bangkok	11.8[A]	7.4	17.8
Lower Thonburi	11.7[A]	8.6	18.9
Central Bangkok	7.9[B]	4.9	12.9
Southern Bangkok	5.9[C]	3.4	10.9
Northern Bangkok	2.6[D]	1.2	5.8
Average	8.4	4.9	14.2

* Indicated the statistical difference of prevalence across Bangkok areas

Note: CRKP prevalence was performed 20,000 iterations

ANOVA (SAS System for Windows 9.0) was used to indicate that the CRKP prevalence across six locations of Bangkok were significantly different (p value < 0.05). Tukey's multiple comparison test was used to elaborate the pairwise difference of CRKP prevalence among six Bangkok areas. The comparison result indicated that CRKP prevalence of both Upper Thonburi and Eastern Bangkok were the highest approximately 11.8% (p value < 0.05). While CRKP prevalence of Central Bangkok, Southern Bangkok and Northern Bangkok at 7.9%, 5.9% and 2.6% were statistically

different (Table 8). Collectively compared between *K. pneumoniae* and CRKP prevalence, the lowest *K. pneumoniae* prevalence was statistically higher than the CRKP prevalence (p value < 0.05). From Tables 7-8 and Figure 7. *K. pneumoniae* prevalence were approximately 10 times higher than CRKP prevalence.

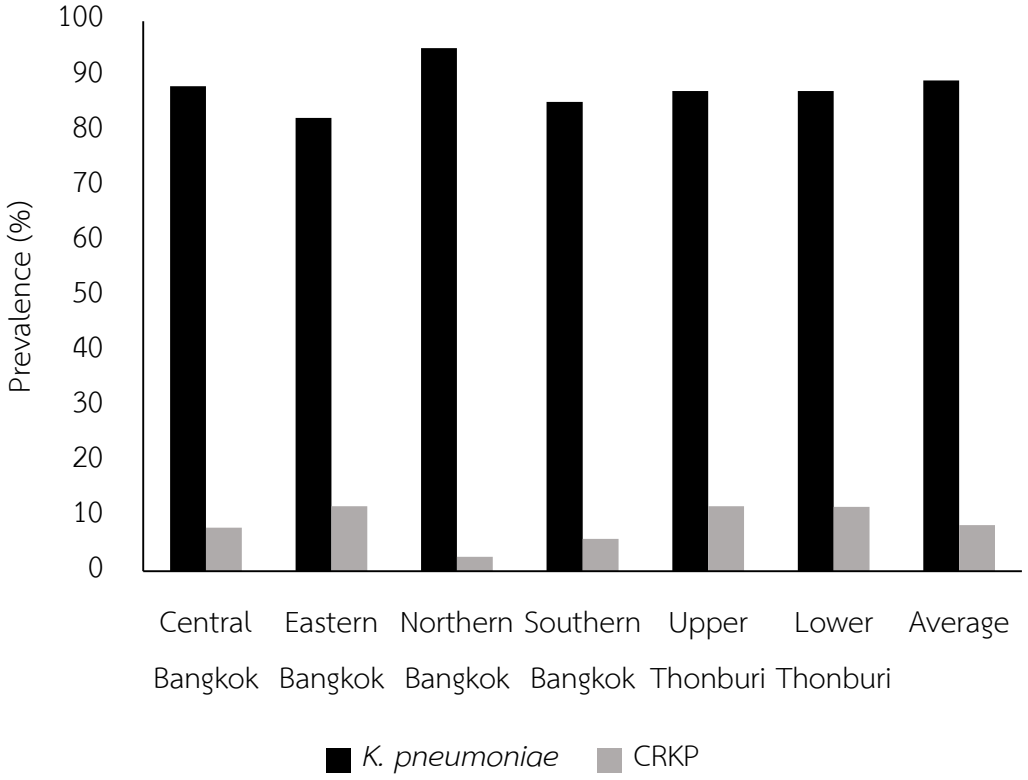

Figure 7 *K. pneumoniae* and CRKP prevalence in six Bangkok areas

4.3.2.2 Probabilistic concentration of *K. pneumoniae* (C_{KP}) and CRKP (C_{CRKP}) at retail level

The theoretical probability distribution for concentration of *K. pneumoniae* and CRKP was described by normal distribution (equation 3). *K. pneumoniae* concentration (C_{KP}) isolated from pork in six Bangkok areas were ranged between 6.29 and 6.97 log cfu/g. While the average *K. pneumoniae* concentration isolated from pork in Bangkok areas was 6.56 log cfu/g (Table 9).

Table 9 Expected values and selected percentiles of *K. pneumoniae* concentration (C_{KP}) in retail pork across six Bangkok areas

Location	*K. pneumoniae* concentration (log cfu/g)		
	Mean*	5th percentile	95th percentile
Lower Thonburi	6.97^{A}	6.43	7.73
Central BKK	6.58^{B}	6.38	7.36
Eastern BKK	6.43^{B}	6.23	7.27
Upper Thonburi	6.39^{B}	5.54	7.16
Southern BKK	6.38^{B}	5.95	7.25
Northern BKK	6.29^{B}	5.86	7.09
Average	6.56	5.34	7.43

* Indicated the statistical difference of concentrations across Bangkok areas

Note: *K. pneumoniae* concentration was performed 20,000 iterations

The *K. pneumoniae* concentration from Lower Thonburi was at the highest level at 6.97 log cfu/g. Interestingly, the narrow range of *K. pneumoniae* concentration across six Bangkok area, *K. pneumoniae* concentration from Lower Thonburi was also significantly higher than the second highest in Central Bangkok area at 6.58 log cfu/g (*p* value < 0.05). On the contrary, *K. pneumoniae* concentration from five other Bangkok areas were not statistically different (*p* value > 0.05).

The concentration of CRKP (C_{CRKP}) isolated from pork in six Bangkok areas were ranged between 5.33 and 6.24 log cfu/g. While the average CRKP concentration isolated from pork in Bangkok areas was 5.89 log cfu/g. Mean CRKP concentrations collectively for Bangkok (four areas) and Thonburi (two areas) were 6.05 and 5.58 log cfu/g, respectively (Table 10).

Table 10 Expected values and selected percentiles of CRKP concentration (C_{CRKP}) in retail pork across six Bangkok areas

Location	CRKP concentration (log cfu/g)		
	Mean*	5th percentile	95th percentile
Eastern BKK	6.24[A]	5.69	6.69
Southern BKK	6.08[A]	5.61	6.63
Northern BKK	5.68[A]	5.24	5.98
Central BKK	5.45[A]	4.92	5.86
Bangkok average	*6.05[a]*		
Lower Thonburi	5.74[A]	4.84	6.31
Upper Thonburi	5.33[A]	4.86	5.82
Thonburi average	*5.58[b]*		
Overall average	5.89	5.51	6.42

* Indicated the statistical difference of concentrations across Bangkok areas

Note: CRKP concentration was performed 20,000 iterations

Statistical analyses by ANOVA indicated that mean CRKP concentration from all six Bangkok areas were not significantly different (*p* value > 0.05). Informal the narrow range of concentrations of both *K. pneumoniae* and CRKP from six Bangkok areas were observed by ANOVA and Tukey's multiple comparisons. The statistical difference was found in *K. pneumoniae* concentration, but not in CRKP concentration. On the other hand, the mean CRKP concentration of four Bangkok areas was statistically higher than that of two Thonburi areas (*p* value < 0.05). This might indicated that two close

clusters of CRKP concentrations were not found in Bangkok and Thonburi areas by chance.

From Tables 9-10 and Figure 8, *K. pneumoniae* concentrations were higher than CRKP concentrations in all six Bangkok areas.

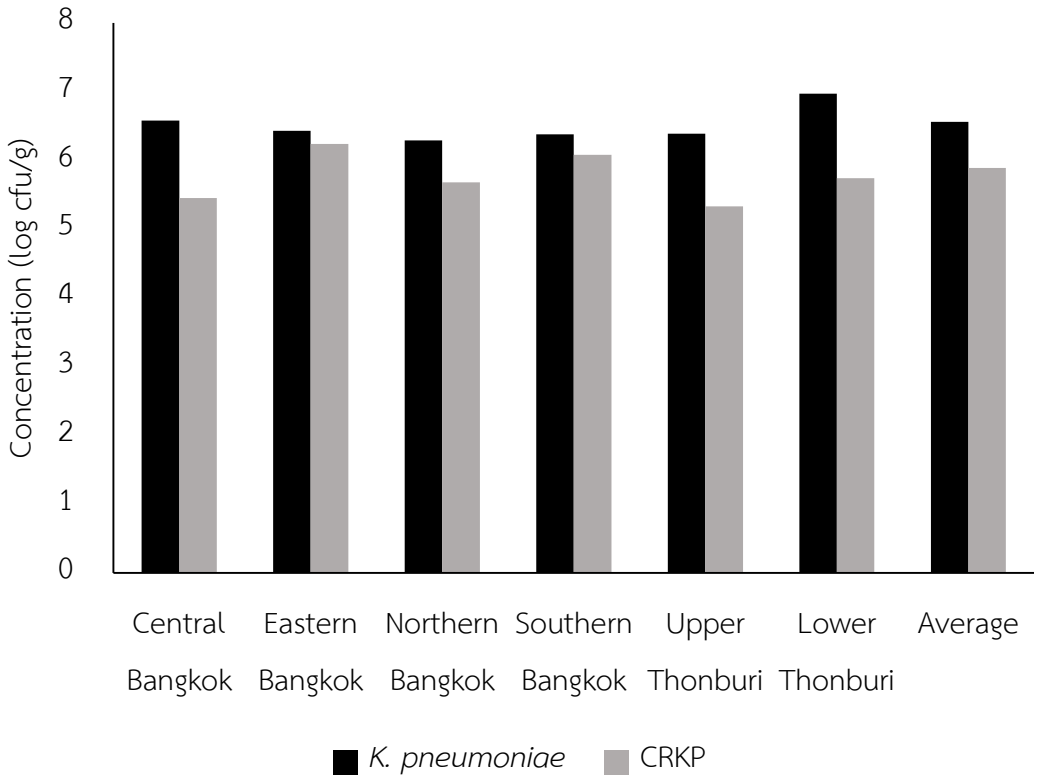

Figure 8 *K. pneumoniae* and CRKP concentration in six Bangkok areas

4.3.2.3 Concentration at the household level

The thermal inactivation by means of appropriate cooking at the household level could usually lower the concentration of pathogen contaminated in raw material. The cooking temperature and time play a vital role in determining the final concentration of *K. pneumoniae* and CRKP at the point of consumption. *K. pneumoniae* concentration ($C_{H\text{-}KP}$) and CRKP concentration ($C_{H\text{-}CRKP}$) at household level were predicted by inactivation models (equation 6) (Smelt and Brul, 2014). The inactivation temperature and time were 63°C and two min, respectively. The decimal reduction time at 63°C was 0.43 min.

After thermal inactivation, *K. pneumoniae* concentration ($C_{H\text{-}KP}$) and CRKP concentration ($C_{H\text{-}CRKP}$) at household level were 1.91 log cfu/g and 1.24 log cfu/g, respectively.

4.3.2.4 Daily pork consumption of Bangkok residents (M)

As the pork consumption data of Bangkok residents was limited, the amount of pork consumption (M) was derived from the annual pork consumption of Thai population in 2016. The consumption of pork was 0.971 ton annually from observed total Thai population 65.93 million in 2016. Daily pork consumption per person was then described by triangular distribution (equation 11).

$$Triangular(min, most\ likely, max) \quad (11)$$

After simulation of the pork consumption using triangular distribution, the minimum, mean and maximum ingested doses were 0, 20.70 and 100 grams/person/day, respectively.

4.3.2.5 Ingested dose of *K. pneumoniae* (D_{KP})

Ingested dose of *K. pneumoniae* was calculated from the equation (5) which was the product of *K. pneumoniae* concentration at the household level ($C_{H\text{-}KP}$) and daily pork consumption by each individual Bangkok resident per day (M). After simulation, the mean of ingested doses of *K. pneumoniae* (D_{KP}) of Bangkok residents was 3.95 log cfu/person/day as shown in Table 11. The range of ingested doses of *K. pneumoniae* of Bangkok residents were ranged from 3.63 to 4.21 log cfu/person/day.

Table 11 Ingested dose of *K. pneumoniae* (D_{KP}) by Bangkok residents

Location	Ingested dose (log cfu/person/day)
Lower Thonburi	4.21
Central BKK	3.96
Eastern BKK	3.84
Southern BKK	3.77
Northern BKK	3.63
Upper Thonburi	3.65
Average	3.95

Note: Ingested dose of *K. pneumoniae* was performed 20,000 iterations

4.3.2.6 Ingested dose of CRKP (D_{CRKP})

Ingested dose of CRKP was calculated from the equation (5) which was the product of CRKP concentration at the household level ($C_{H\text{-}CRKP}$) and daily pork consumption by each resident per day (M).

After simulation, the mean of ingested doses of CRKP (D_{CRKP}) of Bangkok resident was 3.05 log cfu/person/day as shown in Table 12. The range of ingested doses of CRKP of Bangkok residents were from 2.36 to 3.24 log cfu/person/day.

Table 12 Ingested dose of CRKP (D_{CRKP}) by Bangkok residents

Location	Ingested dose (log cfu/person/day)
Eastern BKK	3.24
Southern BKK	3.20
Lower Thonburi	2.86
Upper Thonburi	2.40
Central BKK	2.36
Northern BKK	2.36
Average	3.05

Note: Ingested dose of CRKP was performed 20,000 iterations

4.3.2.7 Probability of exposure of *K. pneumoniae* (P_{E-KP})

After simulation, the mean probability of exposure of *K. pneumoniae* (P_{E-KP}) of overall Bangkok residents was 0.89 or 89% (Table 13). For interpretation, each Bangkok resident consuming pork 100 times was supposed to ingest at least one cell of *K. pneumoniae* 89 times. However, the highest and lowest probabilities of exposure to *K. pneumoniae* (P_{E-KP}) was from Central and Eastern Bangkok corresponding to 0.96 and 0.83, respectively. These high probabilities of exposure of six Bangkok areas was mainly attributable to the high *K. pneumoniae* prevalence in pork.

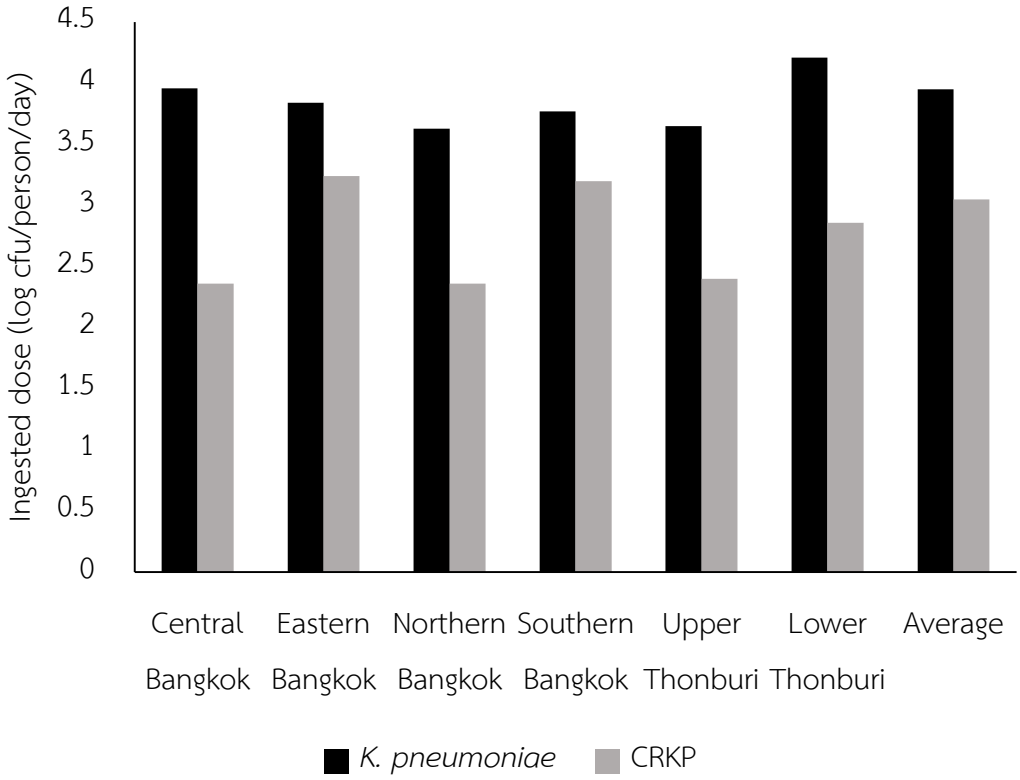

Figure 9 Ingested dose of *K. pneumoniae* and CRKP in six Bangkok areas

4.3.2.8 Probability of exposure of CRKP ($P_{E\text{-}CRKP}$)

After simulation, the mean probability of exposure of CRKP ($P_{E\text{-}CRKP}$) of overall Bangkok residents was 0.085 or 8.5% (Table 13). For interpretation, each individual who consuming pork purchased 1,000 times was supposed to ingest at least one cell of CRKP 85 times. However, the highest and lowest probabilities of exposure to *K. pneumoniae* was from Eastern and Northern Bangkok corresponding to 0.120 and 0.026, respectively. The probability of exposure of CRKP was about 10 times lower than that of *K. pneumoniae*.

Table 13 Probability of exposure of *K. pneumoniae* ($P_{E\text{-}KP}$) and CRKP ($P_{E\text{-}CRKP}$)

Location	Probability of exposure (P_E)	
	K. pneumoniae	CRKP
Central BKK	0.96	0.076
Northern BKK	0.96	0.026
Lower Thonburi	0.88	0.120
Upper Thonburi	0.87	0.120
Southern BKK	0.86	0.060
Eastern BKK	0.83	0.120
Average	0.89	0.085

Note: Probability of exposure was performed 20,000 iterations

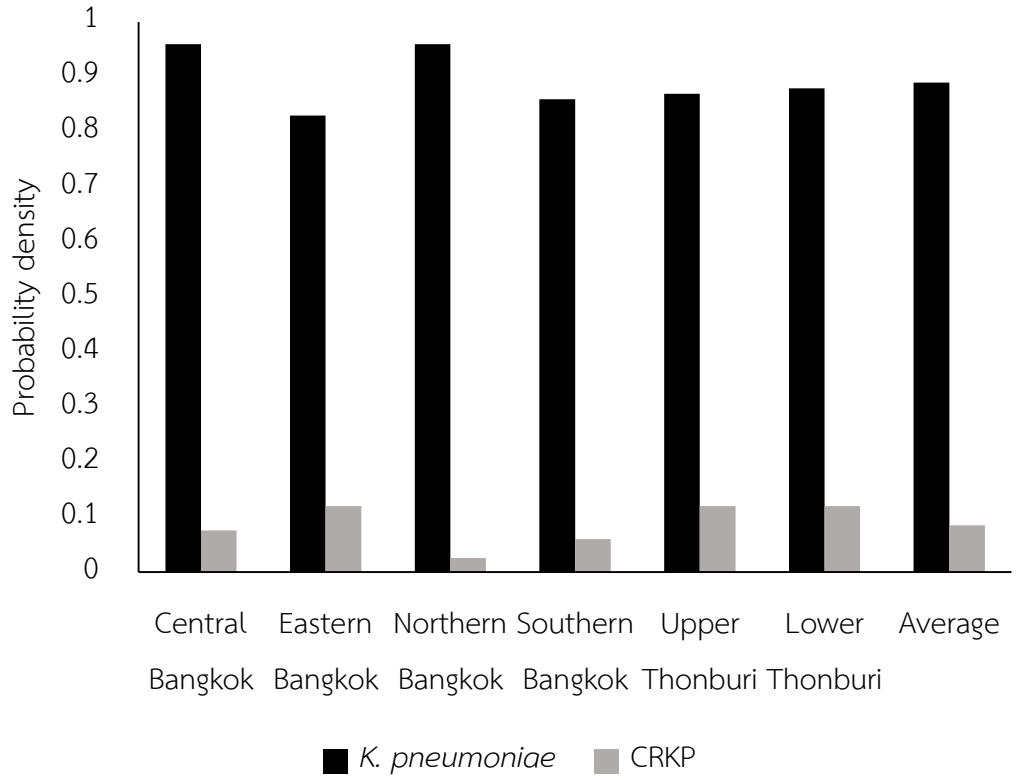

Figure 10 Probability of exposure to *K. pneumoniae* and CRKP in six Bangkok areas

4.3.3 Hazard characterization

4.3.3.1 Probability of illness from *K. pneumoniae* ($P_{I\text{-}KP}$)

After simulation, the mean probability of illness from *K. pneumoniae* ($P_{I\text{-}KP}$) of overall Bangkok residents was 5.56×10^{-4} (Table 14). In terms of individual Bangkok areas, the lowest probability of illness from *K. pneumoniae* was 2.66×10^{-4} from Northern Bangkok, while the highest probability was 1.01×10^{-3} from Lower Thonburi.

Table 14 Probability of illness from *K. pneumoniae* ($P_{I\text{-}KP}$) and CRKP ($P_{I\text{-}CRKP}$)

Location	Probability of illness (P_I)	
	K. pneumoniae	CRKP
Central BKK	5.65×10^{-4}	1.44×10^{-5}
Northern BKK	2.66×10^{-4}	1.44×10^{-5}
Eastern BKK	4.37×10^{-4}	1.08×10^{-4}
Southern BKK	3.69×10^{-4}	9.92×10^{-5}
Upper Thonburi	2.81×10^{-4}	1.57×10^{-5}
Lower Thonburi	1.01×10^{-3}	4.50×10^{-5}
Average	5.56×10^{-4}	6.96×10^{-5}

Note: Probability of illness was performed 20,000 iterations

4.3.3.2 Probability of illness from CRKP ($P_{I\text{-}CRKP}$)

After simulation, the mean probability of illness from CRKP ($P_{I\text{-}CRKP}$) of overall Bangkok residents was 6.96×10^{-5} (Table 14). In terms of individual Bangkok areas, the lowest probability of illness from CRKP was 1.44×10^{-5} from Central and Northern Bangkok, while the highest probability of illness from CRKP was 1.08×10^{-4} from Eastern Bangkok.

4.3.3.3 Probability of mortality for CRKP (P_M)

In this study, the probability of mortality attributable to CRKP (P_M) was considered as the additional health consequence from CRKP. Probability of mortality attributable to CRKP was determined by a series of independent events from hospitalization to the death due to the failure of prescribed ciprofloxacin treatment of patient (equation 8). Only AMR risk assessment applied this model (CAC, 2011).

The hospital rate for *K. pneumoniae*-infected patients (H) was 0.22%. This value was calculated from the ratio of "number of *K. pneumoniae*-infected patients" to "total patients in the hospital" (Phumart, 2012).

The prescription rate for ciprofloxacin (P) was assumed to be33.33% which was the ratio of "prescription of ciprofloxacin" to "prescription of some other antimicrobials" for *K. pneumoniae* treatment (Phumart, 2012).

Moreover, CRKP mortality rate conditional to treatment with ciprofloxacin (M) was 51.55%. This number was derived from ratio between "number of patients who came down with *K. pneumoniae* in hospital but ciprofloxacin treatment failure" and "number of patients who hospitalized with *K. pneumoniae* infection" (Phumart, 2012).

Finally, probability of mortality for CRKP (P_M) was 0.0377%.

4.3.4 Risk characterization

4.3.4.1 Risk estimate *K. pneumoniae*

The risk estimate from *K. pneumoniae* ($P_{S\text{-}KP}$) was a function of probability of exposure ($P_{E\text{-}KP}$) and illness ($P_{I\text{-}KP}$) consecutively. The model to calculate the risk estimate from *K. pneumoniae* was the following (equation 9).

After simulation, the expected value of overall risk estimate from *K. pneumoniae* of Bangkok population (P_S) was 4.95×10^{-4} (Table 15). The interpretation of Ps equal 4.95×10^{-4} was that among 10,000 of Bangkok residents at risk of *K. pneumoniae* contaminated in pork infection through the consumption of

contaminated pork approximately five Bangkok residents was supposed to hospitalize with *K. pneumoniae* daily. In order to be compatible with Annual Epidemiological and Surveillance Report (AERS) of Bureau of epidemiology (BOE), this risk estimate can be converted to be 18,067 cases per 100,000 Bangkok residents at risk of *K. pneumoniae* foodborne illness caused by pork consumption annually, respectively (Figure 11).

Table 15 Risk estimates from *K. pneumoniae* ($P_{S\text{-}KP}$) and CRKP ($P_{S\text{-}CRKP}$)

Location	Risk estimates (P_S)	
	K. pneumoniae	CRKP
Central BKK	5.47×10^{-4}	1.62×10^{-7}
Northern BKK	2.58×10^{-4}	5.21×10^{-8}
Eastern BKK	3.65×10^{-4}	1.83×10^{-6}
Southern BKK	3.21×10^{-4}	8.58×10^{-7}
Upper Thonburi	2.45×10^{-4}	2.74×10^{-7}
Lower Thonburi	8.78×10^{-4}	7.88×10^{-7}
Average	4.94×10^{-4}	8.57×10^{-7}

Note: Risk estimate was performed 20,000 iterations

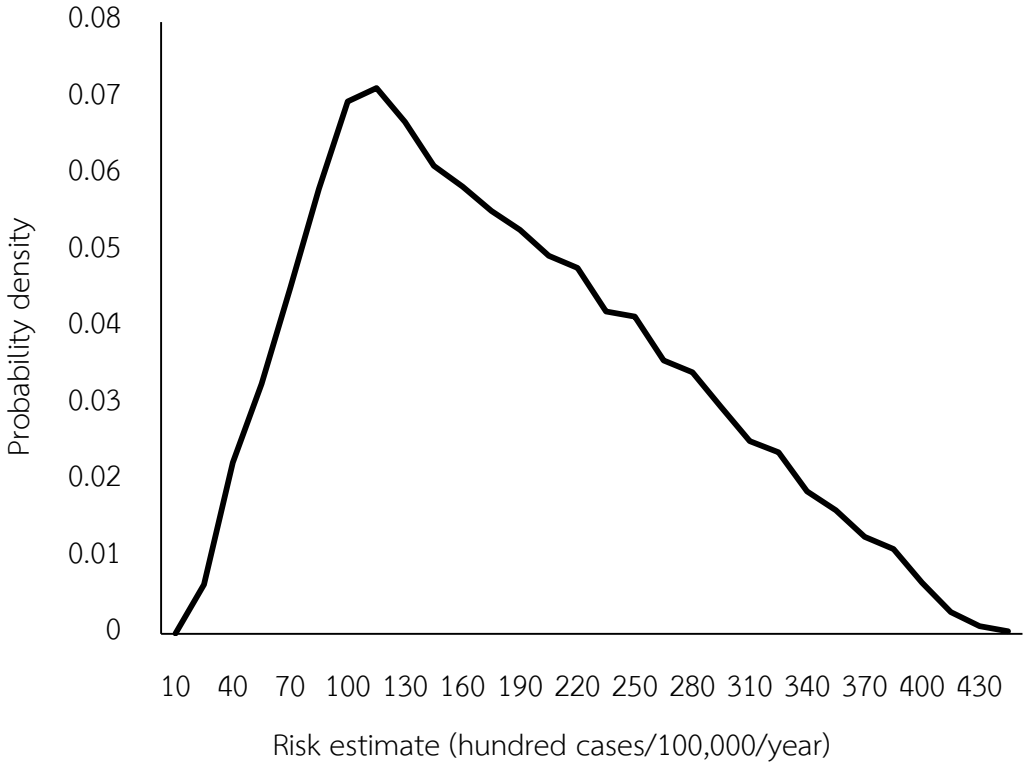

Figure 11 Risk estimate of *K. pneumoniae* from pork consumption ($P_{S\text{-}KP}$) in Bangkok in 2016-2017

4.3.4.2 Risk estimate from CRKP

The risk estimate from CRKP was a function of probability of exposure of CRKP, probability of illness from *K. pneumoniae* and finally probability of mortality and CRKP. The model to calculate the risk estimate from CRKP was the following (equation 10).

After simulation, the expected value of overall risk estimate from CRKP of Bangkok population (P_S) was 8.95×10^{-7} (Table 15). The interpretation of this value is that among 10,000,000 of Bangkok residents at risk of from CRKP contaminated in pork approximately nine persons was supposed to hospitalize with CRKP daily. In order to be compatible with Annual Epidemiological and Surveillance Report (AERS) of Bureau of epidemiology (BOE), this risk estimate can be converted to be 33 cases per 100,000 Bangkok residents at risk of CRKP foodborne illness caused by pork consumption annually, respectively (Figure 12).

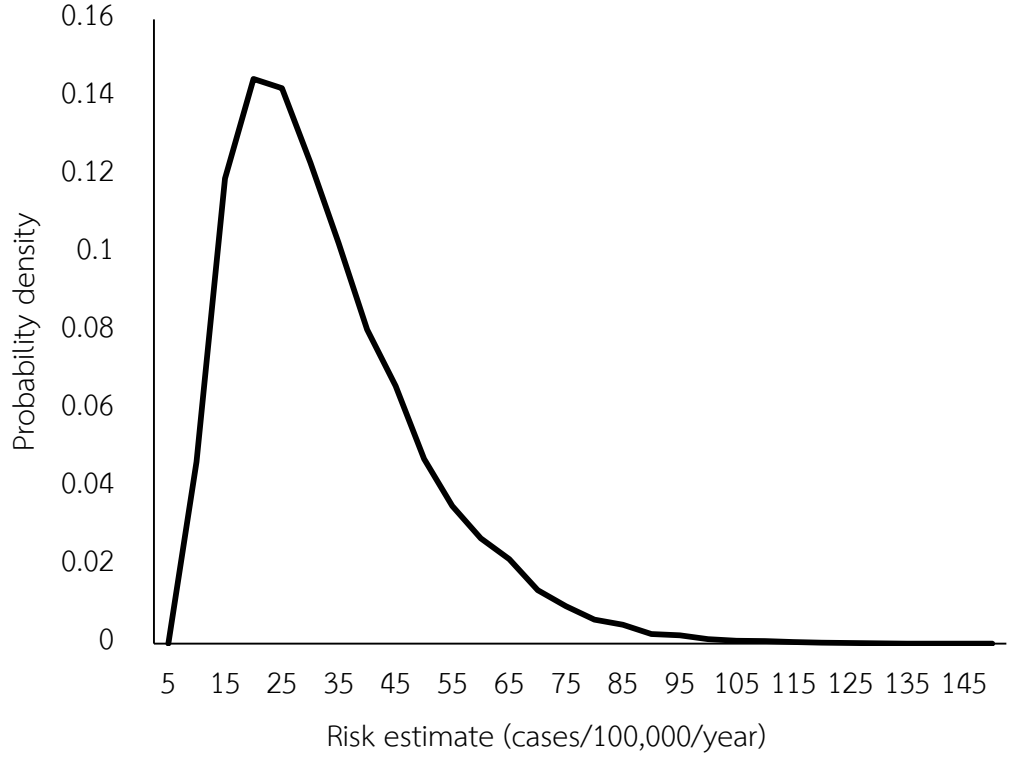

Figure 12 Risk estimate of CRKP from pork consumption ($P_{S\text{-}CRKP}$) in Bangkok in 2016-2017

4.4 Sensitivity analysis

A sensitivity analysis was product from simulation which performed to correlate model input variables with the model output variable. However, these model variables must be described by a probability distribution e.g. Normal or Uniform distribution. In the present study, some of the model input variables were *K. pneumoniae* prevalence, *K. pneumoniae* concentration or daily pork consumption. The performed using output model variable was risk estimate. The correlation was Spearman's rank correlation. The Spearman's rho or correlation coefficient (r) indicates magnitude of correlation between output and input variables i.e. the higher correlation

coefficient had higher degree of correlation between input and output variables. Additionally, the positive correlation coefficient also indicated that the input variables were correlated with output variable in the same way i.e. the increase of input variable bring about the increase of output variable as well. However, the negative correlation coefficient indicated that the input variables were correlated with output variable in the opposite manner i.e. the increase of input variable bring about the decrease of output variable as well. The rule of thumb for interpreting the magnitude of a correlation coefficient as shown in Table 16 (Hinkle et al., 2003).

Table 16 Interpretation of the magnitude of a correlation coefficient

Range of correlation coefficient	Interpretation
0.9 - 1.0	Very high correlation
0.7 -0.9	High correlation
0.50 -0.7	Moderate correlation
0.3 -0.5	Low correlation
0 - 0.3	Little if any correlation

Source: Hinkle et al., 2003

$P_{i\text{-}KP}$, D_{KP} and M have very high positive correlation coefficient approximately 0.9974 (99.74%). On the other hand, correlation coefficient of P_{KP} and $P_{E\text{-}KP}$ were low as 0.0624 (6.24%) (Table 17).

Table 17 Correlation coefficient of input variables of risk estimate from *K. pneumoniae* ($P_{S\text{-}KP}$)

Variables	Correlation coefficient
Probability of illness of *K. pneumoniae* ($P_{i\text{-}KP}$)	0.997
Ingested dose of *K. pneumoniae* (D_{KP})	0.997
Pork consumption by Bangkok resident per day (M)	0.997
Prevalence of *K. pneumoniae* (P_{KP})	0.062
Probability of exposure of *K. pneumoniae* ($P_{E\text{-}KP}$)	0.062

Moreover, $D_{CRKP,}$ M, $P_{i\text{-}CRKP}$ were highly correlated with $P_{S\text{-}CRKP}$. While $P_{E\text{-}CRKP}$was moderately correlated with $P_{S\text{-}CRKP}$ (Table 18) .

Table 18 Correlation coefficient of input variables of risk estimate from CRKP ($P_{S\text{-}CRKP}$)

Variables	Correlation coefficient
Ingested dose of CRKP (D_{CRKP})	0.824
Daily pork consumption (M)	0.824
Probability of illness of CRKP ($P_{i\text{-}CRKP}$)	0.824
Probability of exposure of CRKP ($P_{E\text{-}CRKP}$)	0.499
Prevalence of CRKP (P_{CRKP})	0.499

CHAPTER V

DISCUSSION

This study provided information on concentrations, prevalence, ingested doses, probabilities of exposure, probabilities of illness, probabilities of mortality and risk estimates of *K. pneumoniae* and CRKP from pork consumption in Bangkok.

Convenient sampling was utilized to collect pork samples in retail markets. The samples cluster in some areas such as one market of Northern Bangkok and Lower Thonburi and two markets of Upper Thonburi. Thus, the prevalence and concentrations of both *K. pneumoniae* and CRKP could be either underestimated or overestimated in some areas i.e. the result might not reflect the true prevalence and concentrations of *K. pneumoniae* and CRKP. This could be a source of a selection bias. Nonetheless, this bias has been reduced or compensated by the probability distributions of variables used in the stochastic models. Then the true values of prevalence and concentration of both *K. pneumoniae* and CRKP were supposedly covered and included by Monte Carlo simulation. Eventually, the result from this simulation has covered the true risk estimates from *K. pneumoaniae* and CRKP.

The prevalence of *K. pneumoniae* was 89.95%. Unlike *K. pneumoniae*, the prevalence of CRKP was 7.65%. The mean prevalence of *K. pneumoniae* was almost 12 times higher than that of CRKP. This finding was in line with Boonyasiri study where the prevalence of *K. pneumoniae* was much lower than that of CRKP. The prevalence of *K. pneumoniae* and CRKP were as low as 60% and 0%, respectively (Boonyasiri et al., 2014). Even though the proportion of *K. pneumoniae* and CRKP prevalence of this study was similar to that from Boonyasiri et al. ,2014 the prevalence of *K. pneumoniae* from a previous preliminary study was much lower than mean prevalence of *K. pneumoniae* from this present work. A major difference between these two studies

was the sample size. As Boonyasiri collected pork samples merely 15 samples (Boonyasiri et al., 2014) while this study collected almost 400 pork samples. Therefore sample size could play a significant role to estimate the true prevalence.

Additionally, Boonyasiri found that prevalence of *Klebsiella* spp. in pig (n=400) (fecal swab) was 7.6% and that of fresh pork meat (n=18) was 13.3%. This could be concluded that the high prevalence of *K. pneumoniae* in this study was contaminated after transportation to retail market. Thus, the suggestion should be concern and aware of sanitary in the steps of transfer pork from slaughterhouse to market.

As described in the earlier chapters, dose-response model specifically for *K. pneumoniae* was not available. Then, this study used dose-response model of *E. coli* as a substitute. Additionally, several parameters were modelled by information or data from *E. coli* in various steps of risk assessment such as thermal inactivation and probability of mortality for CRKP. After simulation for 20,000 iterations, an average prevalence and concentration of *K. pneumoniae* at retail level were as high as 89% and 6.56 log cfu/g, respectively. Mean CRKP prevalence and concentration were 8.42% and 5.89 log cfu/g, respectively. This finding was interesting, because the wide range of prevalence difference between *K. pneumoniae* and CRKP. Whereas the concentration difference between this *K. pneumoniae* and CRKP was turned out to be much narrower. This might indicated that the dissemination of CRKP in pork was limited, but CRKP could be highly adaptive and leading to be more proliferative in its niche environment in its host and down to some other steps in the food chain. Another possibility of similarly contamination level of both *K. penumoniae* and CRKP at retail level was the similar high load of manure contamination from the slaughter house steps (pig-to-pork) (Davis and Price, 2016).

The probabilities of exposure in *K. pneumoniae* and CRKP were 0.89 and 0.085, respectively. These outputs were not unexpected at all, since the probability of exposure was mainly influenced by the prevalence of *K. pneumoniae* and CRKP in the

pork at the time of consumption. The probability of exposure of this pathogen would be asymptotically approaching the prevalence of that pathogen having high ingested doses (D). On the contrary, the model of probability of exposure was not sensitive to amount of food consumption. According to results from simulation, probability of exposure was highly correlated with prevalence (r=1) yet lower correlated with pork consumption (r=0.0089). Thus, the higher prevalence, the higher probability of exposure. This model behavior was noted in a previous study of QMRA of *E. coli* O157:H7 in hamburger (Cassin et al., 1998). The beef consumptions per single meal of adult and children were 83±48 and 42±27 g, respectively. However the probability of exposure to *E. coli* O157:H7 in adult and children was almost the same approximately2.9% (Cassin et al., 1998).

According to the exponential model which is the simplest dose-response model with a single parameter k, this output from this model was mainly determined by the ingested doses of *K. pneumoniae* and CRKP which was in turn determined by concentrations in pork and pork consumption. The mean ingested doses of *K. pneumoniae* and CRKP in this study were 3.95 and 3.05 log cfu/g, respectively. After simulation, mean probabilities of illness in *K. pneumoniae* and CRKP were 5.56×10^{-4} and 6.96×10^{-5}, respectively. Therefore, a general conclusion can be made by that the lower concentration would arrive at a lower probability of illness. However, from a previous study of *E. coli* O157:H7 in hamburger using the same exponential model, the ingested dose of *E. coli* O157:H7 in hamburger was less than 1 log cfu/g, and the result of the simulation indicated that the mean probability of exposure was only 10^{-5} (Cassin et al., 1998) . Interestingly, the very low ingested dose did have similar probability of illness with very high ingested dose. Therefore, one can also assumed that the exponential model of the probability of illness has a threshold. This means that consumers would suffer from a pathogen at a very low likelihood up to a certain dose of pathogen. However, if the ingested dose of pathogen was higher than that

threshold of ingested dose then the probability of illness would abruptly increase. And regardless of high of ingested doses were, this probability of illness would converge to a certain level of probability of exposure.

The risk estimate from *K. pneumoniae* and CRKP were 4.94×10^{-4} and 8.57×10^{-7}, respectively. These values were equivalent to the annual risk of 18,067 or 33 cases per 100,000 Bangkok residents from *K. pneumoniae* and CRKP foodborne illness, respectively. While the annual risk of *E. coli* as Hemolytic Uremic Syndrome and mortality was about 135 and 7 cases per 100,000 people, respectively (Cassin et al., 1998). This means that the chance of getting illness including treatment failure with ciprofloxacin from *K. pneumoniae* appears to be higher than that of *E. coli*.

For sensitivity analysis after the simulation, ingested dose, daily pork consumption and probability of illness of both *K. pneumoniae* and CRKP were high correlated with risk estimate from *K. pneumoniae*. This could suggest some risk management measures such as appropriate cooking temperature and time. The susceptible groups particularly immunocompromise group should be more cautious to the infection by this pathogen.

This study was the first finding to alert the possibility of risk estimate of foodborne *K. pneumoniae* from pork consumption. However, the risk estimate of *K. pneumoniae* and CRKP should be more accurate and precise upon more available and specific of fit parameters and models to *K. pneumoniae* and CRKP.

CONCLUSIONS

These were annual risk of 18,067 and 33 cases per 100,000 Bangkok residents of *K. pneumoniae* and CRKP foodborne illness, respectively. In order to reduce *K. pneumoniae* and CRKP contamination in pork at retail level, fresh markets should pay attention to the sanitary and clearly separate meat and visceral organs apart. Moreover, pork was supposed to keep in a low temperatures (°C) to extend the self-life of meat. Since mesophilic bacteria especially *Klebsiella* spp. grow slower at temperature below 7-10°C. Additionally, the thermal inactivation or cooking before consuming can lower the risks from *K. pneumoniae* and CRKP. Since several models of *E. coli* were used in this study, the result might not represent the true risk estimate of *K. pneumoniae* and CRKP. So, further studies regarding specific information of *K. pneumoniae* are needed.

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APPENDIX

APPENDIX A

Culture media used for *K. pneumoniae* isolation

1. Buffered peptone water (BPW) (Difco™, Pont de Claix, France)

   Approximate Formula Per Liter

Peptone	10	g
Sodium Chloride	5	g
Disodium Phosphate	3.5	g
Monopotassium Phosphate	1.5	g

2. MacConkey agar (Difco™)

   Approximate Formula Per Liter

Pancreatic Digest of Gelatin	17	g
Peptone (meat and casein)	3	g
Lactose	10	g
Bile Salt No.3	1.5	g
Sodium Chloride	5	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	0.001	g

3. MIL medium (Difco™)

   Approximate Formula Per Liter

Peptone	10	g
Pancreatic Digest of Casein	10	g
Yeast Extract	3	g
L-Lysine HCl	10	g
Dextrose	1	g

Ferric Ammonium Citrate	0.5	g
Bromcresol Purple	0.02	g
Agar	2	g

4. Luria-Bertani (LB) broth (Sifin diagnostics gmbh, Berlin, Germany)

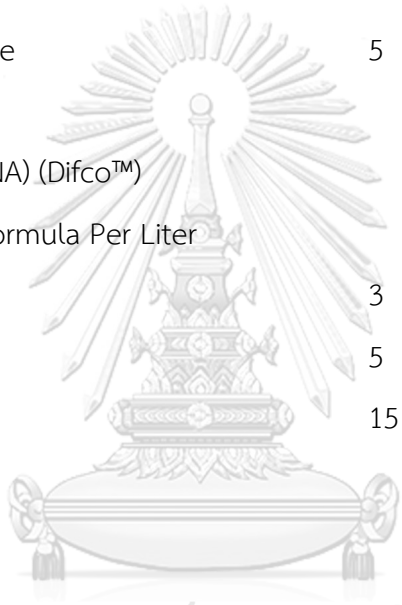
   Approximate Formula Per Liter

Tryptone	10	g
Yeast Extract	5	g
Sodium Chloride	5	g

5. Nutrient Agar (NA) (Difco™)

   Approximate Formula Per Liter

Beef Extract	3	g
Peptone	5	g
Agar	15	g



APPENDIX B

Culture media and antimicrobial agent used for antimicrobial susceptibility test

1. Muller Hilton agar (Difco™)

   Approximate Formula Per Liter

Beef Extract Powder	2	g
Acid Digest of Casein	17.5	g
Starch	1.5	g
Agar	17	g

2. Ciprofloxacin (SIGMA-ALDRICH, St. Louis, USA)

$C_{17}H_{18}FN_3O_3$	MolecularWeight	331.34 g/mol
HPLC		≥ 98.0%

APPENDIX C

Ranges of Minimum Inhibitory Concentration (MIC) of CRKP

Location	Sample	Isolate	MIC
Central Bangkok	RTA02	3.2	16
		3.3	16
	RTA32	3.2	4
	RTA43	3.7	8
	RTE11	5.1	32
Eastern Bangkok	RTB15	3.3	4
	RTB16	3.1	4
	RTB59	5.1	4
	RTB60	5.7	16
	RTB65	4.4	32
	RTB68	5.3	4
Northern Bangkok	RTC31	5.1	32
Southern Bangkok	RTC66	3.2	16
	RTC69	3.3	32
	AA01	3.1	4
Lower Thonburi	RTD11	3.1	16
	RTD13	3.4	16
		5.1	16
	RTD14	4.3	16
	RTD43	3.1	16
	RTD46	3.1	8
		3.2	16
		4.2	8
	RTE17	5.16	8
Upper Thonburi	RTD51	4.2	8
	RTD58	3.3	4
	RTD62	3.3	32
	RTD66	3.1	4
	RTD78	4.5	32
	RTD79	3.2	32

VITA

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