

Prevalence of *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* in Raw Meat in Thai Self-Service Style Restaurants in Khon Kaen Municipality

Sunpetch Angkititrakul¹ Arunee Polpakdee¹ Rungtip Chuanchuen ^{2*}

Abstract

One hundred and fifty eight raw meat samples were collected from 33 Thai self-service style restaurants in Khon Kaen municipality during July-October 2011. The fresh meat included pork, beef and chicken and was provided for self-grilling or boiling. All the samples were examined for contamination of three major foodborne pathogens including *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* using standard protocol. High prevalence of *S. enterica* (44%), *E. coli* (60%) and *S. aureus* (25%) was observed. Seventy-seven percent of the samples were positive for at least one of the three pathogens, while only 8% were found to carry all three pathogens. The prevalence of *Salmonella*, *S. aureus* and *E. coli* was observed in different rates in pork (59%, 69% and 33%, respectively), beef (52%, 56% and 28%, respectively) and chicken (18%, 56% and 12%, respectively). When consider meat type, the highest contamination frequency was found in pork (87%), followed by beef (78%) and chicken (64%). The results indicate the requirement of quality and hygiene control policy for raw meat served in self-service restaurants and that public awareness of food poisoning caused by consumption of undercooked meat should be raised.

Keywords: *Escherichia coli*, Khon Kaen, raw meat, *Salmonella enterica*, *Staphylococcus aureus*

¹ Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand.

² Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, Thailand

*Correspondence author E-mail: rchuanchuen@yahoo.com

บทคัดย่อ

ความชุกของ *Salmonella enterica*, *Escherichia coli* และ *Staphylococcus aureus* ในเนื้อดิบในร้านอาหารบริการตนเองแบบไทยในเทศบาลเมืองขอนแก่น

สรรพชัย อังกิตระภู ¹ อรุณี พลวัสดุ ¹ รุ่งทิพย์ ชวนชื่น ^{2*}

เก็บตัวอย่างเนื้อดิบจำนวน 158 ตัวอย่างจากร้านอาหารไทยแบบปูรุ่งด้วยตนเองจำนวน 33 ร้านในเขตเทศบาลเมืองขอนแก่น ระหว่างเดือนกรกฎาคมถึงตุลาคม 2554 โดยเก็บตัวอย่างเนื้อสุกร เนื้อวัว และเนื้อไก่ที่พร้อมเสิร์ฟเพื่อให้ย่างหรือต้มด้วยตนเอง ตรวจหาการปนเปื้อนของเชื้อก่อโรคอาหารเป็นพิษที่สำคัญ 3 ชนิด คือ *Salmonella enterica*, *Escherichia coli* และ *Staphylococcus aureus* ในตัวอย่างทั้งหมดด้วยวิธีมาตรฐาน โดยตรวจพบการปนเปื้อน *S. enterica* ร้อยละ 44 *E. coli* ร้อยละ 60 และ *S. aureus* ร้อยละ 25 โดยร้อยละ 77 ของตัวอย่างให้ผลบวกต่อเชื้อย่างน้อย 1 ชนิด ในขณะที่ตัวอย่างจำนวนร้อยละ 8 มีเชื้อทั้ง 3 ชนิด ตรวจพบ *Salmonella*, *S. aureus* และ *E. coli* ในเนื้อสุกร (ร้อยละ 59, 69 และ 33 ตามลำดับ) เนื้อวัว (ร้อยละ 52, 56 และ 28 ตามลำดับ) และเนื้อไก่ (ร้อยละ 18, 56 และ 12 ตามลำดับ) ในอัตราที่แตกต่างกัน เมื่อพิจารณาชนิดของเนื้อสัตว์พบว่าเนื้อสุกร (ร้อยละ 87) มีอัตราการปนเปื้อนสูงสุดตามด้วยเนื้อวัว (ร้อยละ 78) และเนื้อไก่ (ร้อยละ 64) ผลการวิจัยชี้ให้เห็นถึงความจำเป็นของการควบคุมคุณภาพและความปลอดภัยของเนื้อดิบที่จำหน่ายในร้านอาหารไทยแบบปูรุ่งด้วยตนเอง รวมทั้งการกระตุ้นให้มีความระมัดระวังเกี่ยวกับโรคอาหารเป็นพิษที่มีสาเหตุมาจากการบริโภคเนื้อสัตว์ที่ปูรุ่งไม่สุก

คำสำคัญ: เอสเซอร์วิช คอสต์ ขอนแก่น เนื้อดิบ ชั้นโน้นเนลดา เอนเทอริกา สเตาฟ์ฟิล์โคคัส ออร์บีส

¹ ภาควิชาสัตวแพทยศาสตร์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อ.เมือง ขอนแก่น 40000

² ภาควิชาสัตวแพทยศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

*ผู้รับผิดชอบบทความ E-mail: rchuanchuen@yahoo.com

Introduction

Meat flesh is food of animal origin, of which consumption varies, depending on cultural or religious preferences. As seen in most parts of the world, meat particularly pork, beef and chicken is a major part of daily diet for Thai people. Meat is an excellent source of protein, various vitamins (e.g. vitamin B12, niacin and vitamin B6) and minerals (e.g. iron, zinc and phosphorus) (Romans, et al., 1994). Due to its high nutrient content, meat is perishable food that requires proper package, storage, handle and cooking to guarantee safety for consumers. Public health concern is that meat is a major source of foodborne pathogens, for example, *Salmonella enterica*, *Campylobacter spp.*, *Escherichia coli* and *Staphylococcus aureus* (Nyachuba, 2010). In general, foods at high risk of contamination with these pathogens are those made by hand and require cold storage. Therefore, most cases of foodborne illness arise from improper food handling, preparation and storage. *Salmonella*, *E. coli* and *S. aureus* are also indicators for microbiological quality of raw meat (DMSC, 2010). Based on the current microbiological standard for raw meat described in the microbiological quality of food and relevant-food containers issued by Department of Medical Science, Ministry of Public Health in 2010, *Salmonella* must be absent in 25 g raw meat. The presence of *E. coli* must not exceed 100 MPN/g and

that of *S. aureus* must not exceed 100 colonies/g (DMSC, 2010).

Self-grilling (Moo-Ka-Ta) and -boiling (Moo-Joom) Thai style restaurants have gained popularity among Thai people, especially teenagers and working people due to inexpensive price and various choices of meat. A variety of raw meat flesh is served for grilling on non-stick hot pan or boiling in soup by customers; however, such raw meat may be handled with poor hygiene in certain circumstances. Undercooked meat may be occasionally eaten by consumers due to personal preference, quick-cooking and/or poor cooking pan quality. Cross-contamination between raw meat and cooked meat may also occur. These could promote microbial spread and result in an increased risk of becoming ill from contaminated foodborne pathogens. The aim of this study was to determine the presence of *Salmonella*, *E. coli* and *S. aureus* in raw meat available in Thai self-service style restaurants in Khon Kaen municipality where this particular type of Thai restaurant is very common.

Materials and Methods

Sample collection: one hundred and fifty eight raw meat samples including pork (n = 54), beef (n = 54)

and chicken ($n = 50$) were obtained from 33 Thai self-service style restaurants in Khon Kaen municipality during July–October 2011. Each restaurant was visited for sample collection on one or two separate occasions, depending on permission. The samples were randomly collected from dishes served on tables or buffet bars. Each sample was placed in individual plastic bag, stored on ice and immediately delivered to the laboratory of Department of Veterinary Public Health, Faculty of Veterinary Science, Khon Kaen University for bacterial isolation and identification.

Bacterial isolation and identification: All the raw meat samples were examined for the presence of *Salmonella* by using ISO6579:2002(E) (ISO, 2002). Contamination of *E. coli* and *S. aureus* was determined on relevant PetrifilmTM count plate (3M, 3M Microbiology, St. Paul, MN, USA) as described in AOAC Official Method 998.08 (AOAC, 2000) and 2003.11 (AOAC, 2000), respectively.

Briefly, 25 g of each sample was pre-enriched in Buffer Peptone Water (BPW, Merck, Darmstadt, Germany) at 37°C overnight for *Salmonella* isolation. The suspension was spotted on Modified Semisolid Rappaport medium (Merck) and incubated at 42°C for 24 hours. Then, *Salmonella* was isolated on Xylose-Lysine-Desocholate agar (Merck) and Hektoen Enteric agar (Merck) at 37°C for 24 hours. Five typical colonies of *Salmonella* from each positive sample were biochemically confirmed using Triple Sugar Iron agar (TSI, Merck) and Motility Indole-Lysine agar (Merck).

For *E. coli* and *S. aureus*, 50 g sample was placed in BPW and the suspension was serially diluted in BPW to 10⁻³. One-ml was plated onto PetrifilmTM E. coli/ Coliform count plate (3M) and PetrifilmTM Staph Express Count plate (3M) for determination of *E. coli* and *S. aureus*, respectively. The inoculated petrifilms were incubated at 37°C for 24 hours. The samples were considered positive when there was the presence of *Salmonella* in 25 g raw meat and/or *E. coli* and *S. aureus* counts exceeded 100 colonies/g.

Results and Discussion

One of the major findings of this study was the high prevalence of three major foodborne pathogens, *Salmonella*, *E. coli* and *S. aureus* in raw meat

served in Thai self-service style restaurants (Table 1) and none of the restaurants carried raw meat negative for all three pathogens. Of all the raw meat samples tested, 77% were positive for at least one of the three pathogens. Only 8% were found to carry all three pathogens tested (Table 2). Combination of *Salmonella* and *E. coli* was most commonly found (22%), followed by the presence of *E. coli* only (21%). These observations confirm the potential role of raw meat served in Thai self-service style restaurants as vehicle for human foodborne diseases.

When consider meat type, the highest contamination frequency was found in pork (87%), followed by beef (78%) and chicken (64%). It is not surprising to observe such high prevalence of contamination. This could be explained by inadequate sanitation in fresh meat production. Pork and beef were originated from local slaughterhouses and sold in community fresh markets. In contrast, the restaurant owners claimed that most raw chicken was frozen-flesh meat produced by poultry-production companies supplied by local distributors. Even though the contamination rate in chicken was lower than that in the others, it is still considered high for frozen chicken produced by standard poultry producers. This indicates that cross-contamination during raw meat preparation occurred and certain restaurants still purchased raw chicken that was home slaughtered and made available at community markets.

Most samples in this study carried *E. coli* exceeding 100 CFU/g (60%), predominantly in all types of meat i.e. 69% of the pork, 56% of the beef and 56% of the chicken. While most pork (30%) and beef (26%) carried both *Salmonella* and *E. coli* simultaneously, most chicken (36%) were positive for *E. coli* only. *E. coli* is normal flora in gastrointestinal tract and serves as a common indicator for fecal contamination. Therefore, the presence of *E. coli* in food suggests poor handling, poor hygiene practices and/or poor temperature control for storage. *Salmonella* is very common in farm animals and food contamination with this pathogen could happen throughout food chain. Regardless, such existence of *Salmonella* and *E. coli* confirmed the risk of infection through ingestion of raw meat served in Thai self-service style restaurants.

Table 1 Prevalence of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* in raw meat ($n = 158$)

Raw meat	No. (%)	No. (%) of positive samples		
		<i>Salmonella</i> ^a	<i>E. coli</i> ^b	<i>S. aureus</i> ^c
Pork	54 (34)	32 (59)	37 (69)	18 (33)
Beef	54 (34)	28 (52)	30 (56)	15 (28)
Chicken	50 (32)	9 (18)	28 (56)	6 (12)
Total	158 (100)	69 (44)	95 (60.13)	39 (25)

^aNumber of samples with the presence of *Salmonella* in 25 g

^bNumber of samples with *E. coli* counts exceeding 100 colonies/g

^cNumber of samples with *S. aureus* counts exceeding 100 colonies/g

Table 2 Distribution of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* in raw meat (n = 158)

Raw meat	No. (%)	No. (%) of positive samples							
		Sa only	Sa+E	Sa+St	E+St	E only	St only	All	None
Pork	54(34)	5(9)	16(30)	4(7)	7(13)	7(13)	1(2)	7(13)	7(13)
Beef	54(34)	5(9)	14(26)	5(9)	4(7)	8(15)	2(4)	4(7)	12(22)
Chicken	50(32)	3(6)	5(10)	0	4(8)	18(36)	1(2)	1(2)	18(36)
Total	158(100)	13(8)	35(22)	9(6)	15(9)	33(21)	4(3)	12(8)	37(23)

Sa: *Salmonella*, E: *Escherichia coli*, St: *Staphylococcus aureus*

It should be noted that *E. coli* detection in this study derived from colony enumeration while the MPN-based method is described in the current microbiological standard. This may limit data comparison due to discrepancy between these two measurements. Comparison of samples from different analytical procedures may be made by using probabilistic models (Gronewold and Wolpert, 2008). However, it was not performed in this study.

In this study, *Salmonella* was present in 18% of the chicken, much less than that in pork (59%) and beef (52%). This is inconsistent with previous studies reporting the high occurrence of *Salmonella* in chicken (75%), pork (65%) and beef (67%) sold in local fresh market (75%) (Angkititrakul et al., 2005). Based on the claim that raw chicken used in the restaurants were supplied by frozen-chicken manufacturers, this flesh should be *Salmonella* free. Once again, the presence of *Salmonella* in raw chicken confirmed cross-contamination during meat preparation process in the restaurant kitchens and/or unhygienic chicken meat supplies.

When compared to other bacteria tested, *S. aureus* was found with the least frequency in all kinds of meat. The presence of *S. aureus* is an indicator of poor hygiene and sanitation of food handlers. Additionally, contamination of raw meat may occur during slaughtering process as shown in a previous report of the presence of *S. aureus* on pig skin at pig slaughterhouses and pork at fresh markets (Theerapong and Angkititrakul, 2011). *S. aureus* is not always pathogenic and commonly found on skin and in respiratory tract of healthy people. Its large presence in food may indicate hygiene and sanitation deficit among food handlers. However, it is not sufficient evidence to incriminate food as the cause of food poisoning because the pathogen causes disease by producing different dreadful toxins that are heat stable and not destroyed by cooking (Murray, 2005). Therefore, enterotoxins must always be tested to trace the cause of illness associated with *S. aureus*.

In conclusion, the results indicate that raw meat served in Thai self-service style restaurants is an important source of foodborne pathogens and necessitates the basic food safety education for food handlers and other relevant workers. Good hygiene practices must be carried out before, during, and after food preparation. Relevant governmental authorities should routinely monitor food to ensure their quality and safety. Importantly, consumers should be aware of the risks associated with consumption of undercooked meat and educated in safe food handling, particularly when eating outdoor.

Acknowledgements

We thank Khon Kaen municipal officers for sample collection. We also thank D. Sithigo, J. Phakam, C. Chuntaraoat, S. Liangchaisiri, P. Khumphui, S. Boonpluk, P. Chompukhow, C. Tessrimuang, C. Jaturakomol and P. Laobannuue, Faculty of Veterinary Science, Khon Kaen University for sample collection and laboratory assistance. This work was partially supported by Thailand Research Fund MRG5180154.

References

- Angkititrakul S, Chomvarin C, Chaita T, Kanistanon K and Waethewutajarn S 2005. Epidemiology of antimicrobial resistance in *Salmonella* isolated from pork, chicken meat and humans in Thailand. Southeast Asian J Trop Med Public Health. 36(6): 1510-1515.
- AOAC 2000. 3MTM PetrifilmTM Staph Express Count Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Types of Meat, Seafood, and Poultry. AOAC Official Method 2003.11: AOAC International, MD, USA. 2 pp.
- AOAC 2000. Escherichia coli Counts in Poultry, Meats, and Seafood, Dry Rehydratable Film Method (Petrifilm EC Plate Method). AOAC Official Method 998.08: AOAC International, MD, USA.
- DMSC 2010. Microbiological quality of food and food containers. Department of Medical Science, Ministry of Public Health, Nonthaburi. volume 2. 6 pp.
- Gronewold AD and Wolpert RL 2008. Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. Water. Res. 42(13): 3327-3334.
- ISO 2002. Microbiology of food and animal feeding stuffs-Horizontal method for the detection of *Salmonella* spp: ISO6579. 1-27 pp.
- Murray RJ 2005. Recognition and management of *Staphylococcus aureus* toxin-mediated disease. Intern Med J. 35 (Suppl 2): S106-119.
- Nyachuba DG 2010. Foodborne illness: Is it on the rise? Nutr Rev. 68(5): 257-269.
- Romans J, Costello WJ, Carlson CW, Greaser ML and Jones KW 1994. The Meat We Eat: Interstate Pub Inc, Damville, IL, USA. 1193 pp.
- Theerapong J and Angkititrakul S 2011. Contamination of *Staphylococcus aureus* in pork and palms of butchers at fresh-food markets in Khon Kaen municipality. KKU Vet J. 21(2): 147-153.