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

บทความ โดย ผู้รับผิดชอบบทความ
ภาควิชาสัตวแพทย์สาธารณสุข มหาวิทยาลัยขอนแก่น
ejrcruanchuen@yahoo.com

บทคัดย่อ

ความชุกของ Salmonella enterica, Escherichia coli และ Staphylococcus aureus ในเนื้อตับในร้านอาหารบริการตนเองแบบไทยในเทศบาลเมืองขอนแก่น

สรรพชูล อังกิติตระกูล 1 อรุณี พัลภัตติ 1 รุ่งฟ้า ชวนชื่น 2

เก็บตัวอย่างเนื้อดิบจำนวน 158 ตัวอย่างจากร้านอาหารไทยแบบบริจาคมเองทั้งหมดจำนวน 33 ร้านในเขตเทศบาลเมืองขอนแก่น ระหว่างเดือนกรกฎาคมถึงตุลาคม 2554 โดยเก็บตัวอย่างเนื้อสุกร เนื้อวัว และเนื้อไก่ที่เตรียมเสร็จเพื่อมากินหรือต้มด้วยตนเอง ตรวจสอบการปนเปื้อนของเชื้อก่อโรคอาหารเป็นพิษที่สำคัญ 3 ชนิด คือ Salmonella enterica, Escherichia coli และ Staphylococcus aureus ในตัวอย่างทั้งหมดตัวีขั้นมาตรฐาน โดยกระทรวงการแพทย์ S. enterica ร้อยละ 44 E. coli ร้อยละ 60 และ S. aureus ร้อยละ 25 โดยรวมตัวอย่างให้ผลบวกต่อเชื้ออย่างน้อย 1 ชนิด ในขณะที่ตัวอย่างจำนวนร้อยละ 8 มีเชื้อทั้ง 3 ชนิด ตรวจสอบ Salmonella, S. aureus และ E. coli ในเนื้อสุกร (ร้อยละ 59, 69 และ 33 ตัวอย่าง) พบ Salmonella ร้อยละ 87 S. aureus ร้อยละ 78 และ E. coli ร้อยละ 74

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 Petrifilm 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 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>
<thead>
<tr>
<th>Raw meat</th>
<th>No. (%)</th>
<th>No. (%) of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Salmonella*</td>
</tr>
<tr>
<td>Pork</td>
<td>54 (34)</td>
<td>32 (59)</td>
</tr>
<tr>
<td>Beef</td>
<td>54 (34)</td>
<td>28 (52)</td>
</tr>
<tr>
<td>Chicken</td>
<td>50 (32)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Total</td>
<td>158 (100)</td>
<td>69 (44)</td>
</tr>
</tbody>
</table>

*Number 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
In this study, however, it was not performed in this study. Probabilistic models (Gronewold and Wolpert, 2008). Analytical procedures may be made by using measurements. Comparison of samples from different microbiological standards. 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.

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.

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**References**


