Bacterial Contamination into Ready-to-Eat Foods Sold in Middle Thailand

CHIRAPORN ANANCHAIPATTANA¹*, MD. LATIFUL BARI², AND YASUHIRO INATSU²

¹Department of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, 39 Muh1, Thanyaburi Pathum Thani, 12110, Thailand
²National Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan
³Center for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh

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Bacterial contamination in ready-to-eat (RTE) foods prepared and sold by small businesses in middle Thailand was surveyed. The 135 samples were randomly purchased from open markets and supermarkets in Bangkok and Pathum Thani provinces during May 2013 to February 2015. The results indicated that the rate of contamination in cooked pork, chicken meat and cooked aquatic items were 13/39 (33%), 18/45 (40%) and 14/57 (25%), respectively and the rate of bacterial contamination of collected samples from open market and supermarket were 26/67 (39%) and 19/68 (27%), respectively. Therefore, no statistically significant difference of contamination rate between two kinds of market or among three categories of food. The most contaminated pathogenic bacteria was Staphylococcus aureus 27/135 (20%) while that of Salmonella spp. was the lowest 5/135 (4%) in each categories of collected food samples. Implementation of suitable hygienic practices in the small food businesses are thought to be required to reduce the risk of foodborne illnesses caused by the consumption of RTE foods sold in middle Thailand.

Key words: Bacterial contamination / Small businesses / RTE foods.

INTRODUCTION

Foodborne illness especially diarrheal disease is one of the important issues in the developing countries (Majowicz et. al., 2014). This illness is caused by pathogenic bacteria (such as Salmonella enterica, enterohemorrhagic Eschericia coli, Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes, virus, and parasites. Even though enough surveillance studies have been conducted, various kinds of foodborne bacteria have been isolated from Thai raw food products (Boonmar et. al., 2003; Indrawattana et. al., 2011; Minami et. al., 2010; Vindigni et. al., 2007) and several cases of outbreaks have been reported (Japatai et. al., 2010; Chanachai et. al., 2008).

In middle developing countries such as Thailand, there are two categories of food markets. One is the traditional open (wet) market under limited temperature and hygiene control and the other is the modern super market under better condition for food hygiene. These differences may cause the difference of the microbial quality of foods sold in each kind of the markets. In the recent case of Thailand, 71 raw food items (vegetables, meat, fish and sea foods) purchased from open markets exhibited statically (P<0.01) higher contamination rates of E. coli (72% vs 44%) and Salmonella (59% vs 23%) than those of 55 items purchased from supermarkets (Ananchaipattana et. al., 2012).

Increasing number of urban workers come from rural area and increasing their income have enhanced the increase of chance in the consumption of Ready-to-eat (RTE) foods produced by small businesses (such as street venders or small shops in local open markets) in middle developing countries. In Thailand, RTE foods have been very popular for urban people over for 20 years (Euromonitor, Thailand, 2008). Even though heating cooking process is included for the production
of RTE foods, the possibility of the existence of pathogenic bacteria in the final products may not be ignored because of the possibility of insufficient heating or the occurrence of cross contamination after heating. However, the information about the contamination rate of pathogenic bacteria into RTE foods sold in developing countries is limited (Cardinale et al., 2005; Manquiat and Fang, 2013). 

The objective of this study is to evaluate the contamination rate or kinds of foodborne bacteria in RTE food product (fried, boiled, grilled, roasted or steamed chicken/pork/aquatic food) sold in open markets or supermarkets in middle Thailand.

**MATERIALS AND METHODS**

**Collection of RTE food samples**

Heat cooked RTE foods such as grilled chicken or fried pork were chosen as the target of this study. One hundred and thirty five RTE foods made of pork (39 samples), chicken (45 samples) and aquatic products (51 samples) were purchased randomly from different 25 open markets and 16 supermarkets in the Bangkok and Pathum Thani provinces (in the north of Bangkok 30 km) in Thailand during May 2013 to February 2015. The collected samples put in sterile plastic bags were kept in 4-6°C during transport and subjected to the microbiological assay in the same day.

**Aerobic plate count**

A 25 g food sample was homogenized with 225 mL of sterile 0.85% sodium chloride solution. Each 0.1 mL of 10<sup>1</sup>, 10<sup>2</sup> and 10<sup>3</sup> times diluted homogenized sample were spread on plate count agar (PCA) and incubated at 35°C for 48 h. Viable cell counts were calculated from the number of developed colonies.

**Isolation and identification of Salmonella spp.**

Each of the 25 g samples was homogenized with 225 mL of sterile buffered peptone water (BPW) (Lab M. Ltd. Toplay House, UK.) and incubated at 35°C for 20 h. Each of 0.5 or 1.0 mL portions of pre-enriched BPW samples were transferred into 10 mL of Rappaport-Vassiliadis (RV) broth (Lab M. Ltd. Toplay House, UK) or Muller-Kaufmann Tetrathionate Novobiocin (MKTTn) broth (Lab M. Ltd. Toplay House, UK), respectively. The RV broth was incubated at 42°C for 20 h, and the MKTTn broth at 35°C for 20 h, respectively. Following incubation, both cultures were streaked on Xylose Lysine Deoxycholate agar (Sisco Research Laboratory Pvt. Ltd. India), and Hektone enteric agar (Sisco Research Laboratory Pvt. Ltd. India), and incubated at 35°C for 20 h. Suspected colonies were subjected to biochemical tests by using triple sugar iron agar, L-lysine decarboxylate agar and Simmon’s citrate agar (Sisco Research Laboratory Pvt. Ltd., India) after purification on the same agar plates. API 20E (BioMe’rieux, France) diagnostic kits was also used to confirm these isolates. 

Salmonella serotypes were determined by using Salmonella antisera “Seiken” (Denka-Seiken, Tokyo, Japan). The Kauffman-White serotyping scheme (Grimont and Weill, 2007) were used for classifies the genus Salmonella into serotypes.

**Isolation and identification of S. aureus**

A 25 g sample was homogenized with 225 mL of sterile BPW and diluted 10<sup>1</sup>, 10<sup>2</sup> and 10<sup>3</sup> times by BPW. A 0.1 mL of diluted ones were spread on manitol salt egg yolk (MSEY) agar (Sisco Research Laboratory Pvt. Ltd., India) or Baird Parker agar (Sisco Research Laboratory Pvt. Ltd., India) and incubated at 30°C for 20 h. Suspected colonies were isolated and identified by microscopic examination, checking the coagulase activity of isolated S. aureus by immune latex test (Eiken Chemical Co., Ltd.). And they were confirmed using an API Staph (BioMe’rieux) diagnostic kit.

**Isolation and identification of B. cereus**

A 25 g sample was homogenized in 225 mL of dilution buffer (0.1% Tryptone and 0.85% NaCl) and diluted 10<sup>2</sup> times. One mL of this solution was spread on manitol egg yolk polymyxin agar (Biomerk Labolatorys Pume Co., Ltd. India) and incubated at 30°C for 20 h. Suspected colonies were counted and isolated, identified by microscopic examination, hemolysis test on sheep blood agar and confirmed using an API 50CH with API CHB (BioMe’rieux) diagnostic kit.

**Isolation and identification of L. monocytogenes**

A 25 g food sample was homogenized with 225 mL of sterile Buffered Peptone Water (Lab M. Ltd. Toplay House, UK.) and incubated at 35°C for 24 h. After incubation, 0.1 mL of enrichment culture was spread onto Listeria selective PALCAM agar (Oxoid Ltd.) supplemented with PALCAM selective supplement (Oxoid Ltd.) and incubated at 30°C for 24 h. Typical colonies were subjected to catalase reaction test and haemolysis activity test after streaked again on tryptone soya yeast extract (TSYE) agar for purification. Suspected strains by these tests were identified by using API Listeria (BioMe’rieux) diagnostic kit.

**Isolation and identification of Escherichia coli**

The 3 tube most probable number test was used to estimate the number of coliform bacteria in food samples. Positive tube was identified by the gas production in EC medium after 24 h incubation at 42°C. Suspected colo-
nies of E. coli isolated from positive tubes were picked up from Eosin Methylene Blue agar and characterized by MR-VP test, indole test and citrate utilization test.

**RESULTS**

Contamination of four kinds of potential foodborne pathogens (Salmonella spp., B. cereus, L. monocytogenes, and S. aureus) and E. coli (as an indicator of hygiene condition) were studied by conventional methods on 135 RTE foods sold in 25 open markets and 16 supermarkets in middle Thailand during May 2013 to February 2015. All samples (39 of cooked pork, 45 of cooked chicken meat and 51 of cooked aquatic items) were heat cooked and sold in the same day. The temperature of open market (in the shop) were 28-35°C and supermarket were 22-28°C. Some samples form supermarket were put in 10-15°C.

In 56 of 135 (42%) collected food samples were found total bacteria (Aerobic plate count) over 6 log CFU/g and 45 of 135 (33%) were found at least one strain of five selected bacteria (Table1) and 37% of

**TABLE 1.** Relationship of APC and contamination of selected five bacteria.

<table>
<thead>
<tr>
<th>Order of APC (CFU/g)</th>
<th>number of positive samples</th>
<th>Ratio of positive samples (%)</th>
<th>Order of APC (CFU/g)</th>
<th>number of positive samples</th>
<th>Ratio of positive samples (%)</th>
<th>Order of APC (CFU/g)</th>
<th>number of positive samples</th>
<th>Ratio of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cooked pork (n=24)**</td>
<td>&lt;2.0 2 (0)</td>
<td>0</td>
<td>2.0-3.0 2 (0)</td>
<td>0</td>
<td>3.0-4.0 0 (0)</td>
<td>cooked chicken (n=30)**</td>
<td>&lt;2.0 2 (0)</td>
<td>0</td>
</tr>
<tr>
<td>cooked aquatic food (n=13)**</td>
<td>&lt;2.0 1 (0)</td>
<td>0</td>
<td>2.0-3.0 4 (25)</td>
<td>0</td>
<td>3.0-4.0 0 (0)</td>
<td>cooked aquatic food (n=38)</td>
<td>&lt;2.0 2 (0)</td>
<td>0</td>
</tr>
<tr>
<td>total (n=67)**</td>
<td>&lt;2.0 5 (20)</td>
<td>0</td>
<td>2.0-3.0 13 (31)</td>
<td>0</td>
<td>3.0-4.0 0 (0)</td>
<td>total (n=68)</td>
<td>&lt;2.0 5 (20)</td>
<td>0</td>
</tr>
</tbody>
</table>

*positive sample** means the samples contaminated with any of one kind of targeted five strains.

**Speaman’s rank correlation coefficient between the order of viable cells and positive rates of targeted five strains was higher than 0.8 (P<0.05).
Pathogenic E. coli were not identified in this study as same as the previous study [Ananchaipattana et al., 2012], where no pathogenic E. coli was found in 137 raw food samples from the same area.

Five serotypes of Salmonella spp. include of S. Anatum, S. Corvallis, S. Mbandaka, S. Rissen, and S. O4:i:- were identified from five positive Salmonella spp. contaminated samples (3.7%=5/135) including of grilled pork, boiled pork, grilled squid, grilled chicken, and fried sea fish. However, no L. monocytogenes was found in all collected RTE food samples. In 17 of 135 (13%) and 1 of 135 (1%) collected food samples were detected S. aureus and B. cereus over 100 CFU/g, respectively. That is not unacceptable for microbial standard parameter of Thai food.

**TABLE 2** Comparison of the contamination rate of five foodborne pathogen in RTE food between open markets and supermarkets in Bangkok and Pathum Thani province during May 2013 to February 2015.

<table>
<thead>
<tr>
<th>category of food</th>
<th>bacteria</th>
<th>(1) Open market</th>
<th>(2) Super market</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>cooked pork</td>
<td>Salmonella spp.</td>
<td>2 / 24  8.3%</td>
<td>0 / 15  0%</td>
<td>2 / 39  5.1%</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>5 / 24  20.8%</td>
<td>1 / 15  6.7%</td>
<td>6 / 39  15.4%</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>4 / 24  16.7%</td>
<td>4 / 15  26.7%</td>
<td>8 / 39  20.5%</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td>1 / 24  4.2%</td>
<td>0 / 15  0%</td>
<td>1 / 39  2.6%</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>0 / 24  0%</td>
<td>0 / 15  0%</td>
<td>0 / 39  0%</td>
</tr>
<tr>
<td>cooked chicken</td>
<td>Salmonella spp.</td>
<td>1 / 30  3.3%</td>
<td>0 / 15  0%</td>
<td>1 / 45  2.2%</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>3 / 30  10.0%</td>
<td>0 / 15  0%</td>
<td>3 / 45  6.7%</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>7 / 30  23.3%</td>
<td>5 / 15  33.3%</td>
<td>12 / 45 26.7%</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td>4 / 30  13.3%</td>
<td>2 / 15  13.3%</td>
<td>6 / 45  13.3%</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>0 / 30  0%</td>
<td>0 / 15  0%</td>
<td>0 / 45  0%</td>
</tr>
<tr>
<td>cooked aquatic food</td>
<td>Salmonella spp.</td>
<td>1 / 13  7.7%</td>
<td>1 / 38  2.6%</td>
<td>2 / 51  3.9%</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>2 / 13  15.4%</td>
<td>1 / 38  2.6%</td>
<td>3 / 51  5.9%</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>3 / 13  23.1%</td>
<td>4 / 38  10.5%</td>
<td>7 / 51  13.7%</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td>3 / 13  23.1%</td>
<td>3 / 38  7.9%</td>
<td>6 / 51  11.8%</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>0 / 13  0%</td>
<td>0 / 38  0%</td>
<td>0 / 51  0%</td>
</tr>
<tr>
<td>total</td>
<td>Salmonella spp.</td>
<td>4 / 67  6.0%</td>
<td>1 / 68  1.5%</td>
<td>5 / 135 3.7%</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>10 / 67 14.9%</td>
<td>2 / 68  2.9%*</td>
<td>12 / 135 8.9%</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>14 / 67 20.9%</td>
<td>13 / 68 19.1%</td>
<td>27 / 135 20.0%</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td>8 / 67 11.9%</td>
<td>5 / 68  7.4%</td>
<td>13 / 135 9.6%</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes</td>
<td>0 / 67  0%</td>
<td>0 / 68  0%</td>
<td>0 / 135 0%</td>
</tr>
</tbody>
</table>

**Note:** Show the significant difference of the contamination rates between (1) and (2).

Total food samples were contaminated with coliform bacteria. The results indicated that the rate of contamination in cooked pork, chicken meat and cooked aquatic items were 13/39 (33%), 18/45 (40%) and 14/57 (25%), respectively, and the rate of bacterial contamination of collected samples from open market and supermarket were 26/67 (39%) and 19/68 (27%), respectively. Therefore, no statistically significant difference of contamination rate between two kinds of market or among three categories of food. The relationship of aerobic plate count (APC) and contamination of selected five analyzed bacteria were shown in Table 1. The most contaminated pathogenic bacteria was S. aureus 27/135 (20%) while that of Salmonella spp. was the lowest 5/135 (4%) in each categories of collected food samples (Table 2). The contamination rate of E. coli in total samples sold in open markets (14.9%) was significantly higher (P<0.05) than that of supermarkets (2.9%). The comparison of the contamination rate of five foodborne pathogens in collected RTE food samples between open market and supermarket were shown in Table 2. However, pathogenic E. coli were not identified in this study as same as the previous study (Ananchaipattana et al., 2012), where that no pathogenic E. coli was found in 137 raw food samples from the same area.

Five serotypes of Salmonella spp. include of S. Anatum, S. Corvallis, S. Mbandaka, S. Rissen, and S. O4:i:- were identified from five positive Salmonella spp. contaminated samples (3.7%=5/135) including of grilled pork, boiled pork, grilled squid, grilled chicken, and fried sea fish. However, no L. monocytogenes was found in all collected RTE food samples. In 17 of 135 (13%) and 1 of 135 (1%) collected food samples were detected S. aureus and B. cereus over 100 CFU/g, respectively. That is not unacceptable for microbial standard parameter of Thai food.

**DISCUSSION**

Although there is the difference between the hygiene condition of open market and supermarket in Thailand,
the statistically significant difference of contamination rate of RTE foods was not found between two kinds of market. There was showed that the similarity of the contamination rate of raw food samples in the same market of this area (Ananchaipattana et. al., 2012). But the important data was that the several kinds of bacterial contamination in RTE foods from open market samples was more than supermarket samples (Table 2). Thus, the difference in the kind of market may effect to the kind of bacterial contaminated but not effect to the contamination rate in RTE foods samples. The most contaminated bacteria was S. aureus whereas Salmonella spp. was the lowest contamination in each categories of collected of RTE foods samples (Table 2). This study, no Salmonella spp. were found in cooked pork and chicken meat samples from supermarket. However, the previous study found that the most prevalence of pathogenic bacteria in raw food from the same area (Bangkok and Pathum Thani) were Salmonella spp., while low number of S. aureus were found (Ananchaipattana et. al., 2012). It might be due to that heating for cooking Salmonella spp., but it makes no effect on the cross contamination by hand of human or animal after cooking and the storing makes S. aureus grow well in RTE food. All five serotypes of Salmonella spp. (S. Anatum, S. Corvallis, S. Mbandaka, S. Rissen, and S. O4:i:-) detected in RTE foods of this study might be related to the most common serotypes of Salmonella spp. in raw food samples from the previous study in this area (Ananchaipattana et. al., 2014; Vindigni et. al., 2007). The most important factor on relationship of Salmonella spp. serotype between raw food and RTE food should be heating process of cooking. Insufficient heating could make some Salmonella spp. survived in food samples. Although the data showed that no L. monocytogenes were found in all collected RTE foods samples, it does not mean no L. monocytogenes in this kind of food. It might be due to loss of L. monocytogenes in isolation step by the difficulty of cultivation of this bacteria. On the other hand the previous reports (Chen et. al., 2014; Gonzalez et. al., 2013) found L. monocytogenes in RTE food samples. Thus, the isolation step of L. monocytogenes in this study should be improved.

In conclusion, the difference in the kinds of market (open market and supermarket) may effect on the kind of bacterial contamination, but may not effect on the contamination rate in RTE foods samples. In addition, S. aureus could be contaminated mainly after cooking whereas Salmonella spp. could be contaminated before cooking process. Furthermore many RTE food samples were contaminated with coliform bacteria (37%) indicating to be postheat-treatment contamination from improper sanitation. Therefore, in order to avoid the bacterial contamination in RTE foods the most important points are to select clean raw materials for cook, to provide heating to cook thoroughly, to keep food without contamination, and not to keep RTE foods over 4 h at 37-54°C (Forsythe, 2000).

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REFERENCES


