Transmission of *Listeria monocytogenes* from raw chicken meat to cooked chicken meat through cutting boards

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**A B S T R A C T**

*Listeria monocytogenes* (L. monocytogenes) is a food-borne pathogen contaminating poultry products. Ready-to-eat (RTE) cooked chicken meat can easily be contaminated with *L. monocytogenes* in post-processing activities. This study aimed to determine transmission of *L. monocytogenes* from raw chicken meat to hot and cooled chicken meat through polyethylene and wooden cutting boards. Raw chicken breast samples were purchased from retail markets and were artificially contaminated with *L. monocytogenes* at concentration of 7.35 ± 0.22 log CFU/ml. Contaminated raw samples were placed on polyethylene and wooden cutting boards to simulate bacterial transfer to cutting boards. Cooked chicken samples (hot and cooled) were then placed on the same cutting boards to simulate transfer of bacteria from cutting boards to cooked meat. *L. monocytogenes* successfully attached to polyethylene and wooden cutting boards and recovered after holding time up to 1 h. Transmissions of *L. monocytogenes* to cooled cooked samples from both types of cutting boards were relatively higher than hot cooked samples. Moreover, transfer rates of *L. monocytogenes* from wooden cutting boards at holding time of 1 h to both cooled and hot cooked samples were lower than those from polyethylene cutting board. It is recommended to use different cutting boards for raw and cooked materials and apply detergents and hot water for cleaning procedure to eliminate *L. monocytogenes* attached to the cutting boards and prevent cross-contamination of final products.

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1. Introduction

*Listeria monocytogenes* (L. monocytogenes) is a food pathogen contaminating variety of food products such as vegetables, milk, dairy products, poultry and meat products (Ryser & Marth, 2000). Ready-to-eat (RTE) cooked chicken meat can easily be contaminated with *L. monocytogenes* in post-processing activities (Beresford, Andrew, & Shama, 2001). Consumption of contaminated RTE cooked chicken meat results in severe health problems, including listeriosis with high mortality rate (Low & Donachie, 1997; Siegman-Igra et al., 2002). Listeriosis is a rare food-borne disease comparing to salmonellosis and campylobacteriosis. However, like any other zoonotic disease, its significance is not fully dependent on its occurrence (EFSA, 2010). The fatality rate and severity of the disease depends on other factors. USDA set a zero tolerance policy for *L. monocytogenes* in RTE foods (USDA-FSIS, 2004).

*L. monocytogenes* can be easily inactivated by heat treatments, such as cooking (Cygarnowicz-Provost, Whiting, & Craig, 1994). Muriana, Quimby, Davidson, and Grooms (2002) showed that post-package pasteurization by submersion heating can reduce *L. monocytogenes* in RTE deli meats. Liquid smoke, potassium/sodium lactate, quaternary ammonium compounds and bacteriocins are used to reduce *L. monocytogenes* in RTE poultry and meat products (Mereghetti, Quentin, Marquet-Van Der Mee, & Audurier, 2000; Ryser & Marth, 2000). Fabrizio and Cutter (2005) showed that application of electrolyzed oxidizing water on RTE meat can reduce number of *L. monocytogenes*.

Presence of *L. monocytogenes* in cooked meat is due to cross-contamination. Cross-contamination is referred to transmission of
bacteria (direct or indirect) from contaminated source to a non-
contaminated source. Food-related infections are attributed to
cross-contamination of food products after processing or during
storage and handling. Moreover, pathogens can be spread from one
contaminated location to a clean location (Mylius, Nauta, &
Havelaar, 2007). Cross contamination is well known as a route of
transmission of bacteria from naturally contaminated sources to
finished products (Kusumaningrum, van Asselt, Beumer, &
Zwietering, 2004). Cross-contamination of cooked meat during
food preparation in kitchen, results in an unsafe food (de Boer &
Hahné, 1990).

Cutting and chopping boards are common utensils in kitchens
and food processing units. Different types of cutting boards can be
used such as polyethylene, wood and bamboo boards. Wooden
cutting boards are considered as potential source of food pathogens
as deep cracks on the surface of the board provides a suitable
route for bacterial growth (Aitken & Gardner, 1992). These cutting
boards are considered as potential source of food pathogens
when they come in contact with raw material (Kusumaningrum,
van Asselt, Beumer, & Zwietering, 2004). Polyethylene and rubber
wood cutting boards were purchased in 1970s to replace traditional
wooden cutting board. Cutting boards used for food preparation in
domestic kitchens, had always been recognized as possible vehicles for cross-contamination (Cliver, 2006). Different cutting boards
must be used for cutting raw materials and cooked food as pathogens on the surface of cutting boards can easily be transmitted to cooked food. Re-use of the same cutting board for raw and RTE food without washing is a potential
source of L. monocytogenes transmission (Jevnický, Hoyer, & Rasper,
2008). It is recommended to wash cutting boards with detergents
and warm water between using the cutting board for raw material
and RTE or cooked food (Anonymous, 2008). Several researches
were conducted to find a material that can reduce the risk of cross-
contamination while using cutting boards. Triclosan is a compound
with antimicrobial properties which is allowed to be used in plastic
food-contact materials in European countries (European
Commission, 2010). Triclosan showed antibacterial activity over a
wide range of food pathogens (Møretrø, Haiby-Pettersen,
Habimana, Heir, & Langsrud, 2011). In another research, Møretrø,
Haiby-Pettersen, Halvorsen, and Langsrud (2012) showed that
cutting boards containing silver have antibacterial activity against
some important food pathogens.

L. monocytogenes is able to grow at ambient temperature or
below. Remnant of raw meat left on cutting board is considered a
potential transmission vehicle for bacterial transfer to RTE food (Ak,
Cliver, & Kaspar, 1994a). There is limited research on transmission
of L. monocytogenes through cutting boards. Thus, this study was
directed to determine transmission of L. monocytogenes from raw
chicken meat to cooked meat via cutting boards.

2. Materials and methods

2.1. Preparation of L. monocytogenes inoculum

Pure culture of L. monocytogenes ATCC 19112 was inoculated on
PALCAM agar (Merck), and incubated for 48 h at 30 °C. To prepare
the reference suspension, single colony of L. monocytogenes
was inoculated into 10 ml of Tryptic Soy Broth (TSB) (Merck) and
incubated for 1 day at 37 °C (Wong et al., 2011). The harvested broth
culture was serially diluted (10⁻¹–10⁻⁸) in normal saline (0.85%
sodium chloride) to determine the density of the bacterium by
spread plate method using PALCAM agar. The mean concentration
of the inoculums was approximately 7.35 ± 0.22 log CFU/ml.

2.2. Cutting boards

Polyethylene and rubber wood cutting boards were purchased
from supermarkets and cut into approximately 3.5 × 3.5 cm. Every
single cutting board was sterilized under UV light for 1 h in laminar
flow hood before experiment.

2.3. Chicken samples

Sample preparation was carried out according to Tang et al.
(2011). Chicken breasts were purchased from wet markets located
at Serdang, Selangor, Malaysia. Chicken breasts were cut into
approximately 10 ± 0.5 g pieces. Cooked chicken samples were
prepared by boiling the samples in sterile distilled water for 30 min.
Cooled chicken samples were prepared by storing the cooked
chicken meat in sterile beaker at room temperature (28 ± 0.5 °C)
for 1 h. Hot chicken samples were used immediately after boiling
(100 ± 0.5 °C).

2.4. Cross-contamination experiment

All utensils were autoclaved before use. Four groups of raw
chicken meat and 2 groups of cooked (cooled and hot) chicken
meat were used in this experiment. All chicken samples were
inoculated with 0.2 ml microbial load of 7.35 ± 0.22 log10 CFU/ml of
L. monocytogenes. First group of the samples were used as control
samples to determine the number of L. monocytogenes attached to
chicken meat. The mean value was determined as mean concen-
tration of L. monocytogenes attached to chicken meat. The second
group samples were spread over the surface of cutting boards for
5 s. The surface of cutting board was enumerated for L. monocytogenes
immediately. The mean value was determined as the mean number of bacteria transferred from chicken samples to
cutting boards. The third group samples were also spread over the
cutting boards for 5 s, and then cooled cooked chicken meats were
put on the same area immediately. Cooled cooked chicken meat
was then enumerated for L. monocytogenes. The mean value was
determined as the mean number of bacteria transferred from cutting
tools to cooled cooked meat samples. The fourth group’s
samples were treated as the third group, but hot cooked chicken
meat was used instead of cooled cooked meat. The mean value was
determined as the mean number of bacteria transferred from cutting
tool to hot cooked meat. All experiments were done in
triplicate.

The whole experiment was repeated twice for two holding time,
which were 30 min and 1 h after attachment of contaminated
chicken on cutting board. Inoculated chicken samples were spread
over the cutting board’s surface for 5 s, and then the cutting boards
were left at ambient temperature for either 30 min or 1 h holding
time. Cooked chicken samples (cooled and hot) were put on the
same area after the holding time.

2.5. Enumeration of L. monocytogenes

Control samples, hot and cooled cooked chicken meat samples
were mixed with 90 ml of normal saline. Serial dilutions were
prepared in same diluents and plated in PALCAM agar using spread
plate technique. Plates were incubated for 48 h at 30 °C and the
typical Listeria spp. colonies grown on PALCAM agar were consid-
ered as L. monocytogenes. The experiment was conducted twice
using triplicate samples each time.

2.6. Recovery method

According to our knowledge, there is no standard method for
recovery of bacteria from cutting boards’ surfaces. The method used
in this study was rinsing method proposed by Tang et al. (2011). The
surface of cutting board was rinsed using 100 ml of normal saline.
Successive dilutions were prepared in same diluents and plated in
PALCAM agar using spread plate technique and plates were incubated at 30 °C for 48 h.

2.7. Data analysis

Quantitative data were expressed as mean ± standard deviation log$_{10}$ CFU/g for chicken meat sample and log$_{10}$ CFU/ml for contaminated cutting board. Percentage of _L. monocytogenes_ (LMPs), (%) were reported as percentage of _L. monocytogenes_ on the recipient in comparison with the number of _L. monocytogenes_ found in the donor calculating as follows:

\[
\text{LMP \( (\%) \) = (CFU}_{\text{recipient}} / \text{CFU}_{\text{donor}} \times 100\%
\]

For statistical analysis, Minitab (v. 14) statistical package (Minitab Inc., PA, USA) (Mann–Whitney Test) was used to determine the significant (P ≤ 0.05) difference between transfer rates from different type of cutting board to chicken samples.

3. Results

Results of transmission of _L. monocytogenes_ from artificially contaminated raw chicken samples to both polyethylene and wooden cutting boards are presented in Table 1. Number of bacteria transmitted to cooked chicken samples (cooled and hot) were expressed as log$_{10}$ CFU/g chicken.

Both types of cutting board were free of _L. monocytogenes_ at the beginning of the experiment. Inoculated samples were donor in transmission of bacterium from raw sample to cutting boards (polyethylene and wooden). The initial concentration of 7.35 ± 0.22 log$_{10}$ CFU/ml was used for all inoculated samples.

No significant difference (P > 0.05) was observed for bacterial attachment to polyethylene cutting boards for time intervals of 0 s (immediately), 30 min and 1 h. About 1 h after removal of contaminated chicken sample from wooden cutting boards, transmission of bacteria was significantly (P ≤ 0.05) lower than 0 s (immediately), 30 min and those on polyethylene cutting boards (Table 1).

The LMP of bacteria to cooled samples from wooden cutting board (1 h after contamination of cutting board) was significantly (P ≤ 0.05) lower than all other bacterial transmission to cooled samples. Transmission of bacteria from polyethylene cutting board after 1 h to hot sample was lower (P ≤ 0.05) than polyethylene cutting board [0 s (immediately)] and wooden cutting board [0 s (immediately)]. Besides, _L. monocytogenes_ could not be detected from hot samples attached to wooden cutting boards at 1 h after contamination of cutting boards.

At 0 s (immediately), there were no statistical significant (P > 0.05) differences between the rates of _L. monocytogenes_ transmission from both polyethylene and wooden cutting boards to cooled and hot samples. However, at 30 min, transmission of bacteria from both polyethylene and wooden cutting boards to cooled samples were higher (P ≤ 0.05) than transmission of bacteria to hot samples on wooden cutting boards. Furthermore, at 1 h, the transfer rate of bacteria to cooled samples from wooden cutting board were lower (P ≤ 0.05) than polyethylene cutting boards. _L. monocytogenes_ was undetectable after 1 h of inoculation on wooden cutting board.

4. Discussion

There are several ways of exposure to disease including consumption of contaminated food or cooked food that has been cross-contaminated during preparation. Cross-contamination is a well-known route for transmission of bacteria from naturally contaminated sources to the finished products (Kusumaningrum et al., 2004). Presence of bacteria on surfaces of cutting boards can lead to transmission of bacterium to food and cause food-borne diseases. _L. monocytogenes_, is recognized as cross-contaminant on kitchen cutting boards as it is able to be transmitted via food. As mentioned before, remnant of raw meat left on cutting board is a risk for transmission of bacteria to RTE food (such as raw vegetable or cooked meat) (Ak et al., 1994a). This study simulated the condition of using un-washed cutting boards for cooked meat, after removal of raw meat from the cutting boards. In this study, both polyethylene and wooden cutting boards were initially free of _L. monocytogenes_. Cooked meat was chosen to be used in this experiment as a vehicle for transmission of bacteria to consumers as it is assumed that all _Listeria_ spp. were inactivated during heat treatment. The study aimed to simulate cross-contamination on cooked meat when using the cutting boards after 0 s (immediately), 30 min and 1 h of exposure to raw chicken meat. Generally, cross-contamination occurs if the food processing surfaces (such as cutting boards) are not cleaned properly. According to Ak et al. (1994a) recovery of bacteria from cutting board within 3 min after inoculation, does not influence by the age of the cutting boards (whether it was new or used cutting board).

### Table 1

Transmission of _L. monocytogenes_ from artificially contaminated raw chicken samples to different types of cutting board and cross contamination of cooled and hot chicken through plastic and wooden cutting boards.

<table>
<thead>
<tr>
<th></th>
<th>0 s (immediately)</th>
<th>30 min</th>
<th>1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log$_{10}$ CFU/ml</td>
<td>LMP (%)</td>
<td>Log$_{10}$ CFU/ml</td>
</tr>
<tr>
<td><strong>Transfer to cutting board</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic cutting board</td>
<td>5.2 ± 0.5</td>
<td>71.2 ± 8.3ab</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Wooden cutting board</td>
<td>5.5 ± 0.6</td>
<td>74.1 ± 7.8a</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td><strong>Transfer to cooled chicken meat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From plastic cutting board</td>
<td>4.7 ± 0.6</td>
<td>88.5 ± 6.4Aab</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>From wooden cutting board</td>
<td>4.8 ± 0.3</td>
<td>87.9 ± 6.9Aa</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td><strong>Transfer to hot chicken meat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From plastic cutting board</td>
<td>3.9 ± 1.6</td>
<td>73.2 ± 28.5Aa</td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td>From wooden cutting board</td>
<td>3.8 ± 2.2</td>
<td>65.6 ± 37.9Aa</td>
<td>0.8 ± 1.6</td>
</tr>
</tbody>
</table>

The results were expressed as mean ± SD log$_{10}$ CFU/g. Each value in the table represents mean ± SD of three measurements from triplicate experiments (Log$_{10}$ CFU/g for chicken samples and log$_{10}$ CFU/ml for washes from cutting boards).

LMP (%) were percentage of _L. monocytogenes_ on the recipient in comparison with the number of _L. monocytogenes_ found in the donor. LMP (%) = (CFU$_{\text{recipient}}$/CFU$_{\text{donor}}$) × 100%.

* Within columns, means ± SD followed by same capital letter are not significant (P ≤ 0.05) different.

* Within rows, means ± SD followed by same small letter are not significant (P ≤ 0.05) different.
It was found that *L. monocytogenes* can be transferred from raw chicken samples to polyethylene cutting boards and was recovered after 1 h of holding time and subsequently contaminate cooked meat samples. Wooden cutting boards showed lower (*P* ≤ 0.05) bacteria count after 1 h of using raw meat. This might be due to the topography of different kind of cutting board. Inoculated bacteria were readily recovered from polyethylene surfaces. Conversely, inoculums were readily absorbed into wooden cutting board. This finding was in agreement with previous findings by Ak, Cliver, and Kaspar (1994b).

However, the temperature at which the samples were held had an influence on transmission of *L. monocytogenes* from cutting boards to cooked samples. Transfer rate of *L. monocytogenes* to hot cooked samples at 30 min and 1 h was lower (*P* ≤ 0.05) comparing to cooled samples. The finding is in agreement with the finding of Tang et al. (2011), who reported that transfer rate of *Campylobacter jejuni* from breast fillet to cooked sample maintained at room temperature and boiling temperature (100 °C) were 57.6% and 25.3% respectively.

Wooden cutting boards showed lower transmission of bacteria to cooked samples (hot and cooled) at 1 h holding time compared to polyethylene cutting boards. This might be due to the nature and topography of wooden cutting boards which allowed absorption of bacteria into the holes and cracks on the cutting board.

Washing of cutting boards with hot water and detergents after use is an efficient method to eliminate the bacteria attached to the surface of cutting boards. Unfortunately, some food-processing equipment cleaners are not aware of the health risks associated with *L. monocytogenes*. Improper cleaning procedures might concomitantly displace bacteria from contaminated equipments to other area within the processing environment. Moreover, some bacterial cells can be transmitted to sterile surface and subsequently contaminate final food product (Taormina & Beuchat, 2002).

According to Wong et al. (2011), *L. monocytogenes* was able to be transferred to food through direct contact with contaminated area or during food handling. In their study, samples were cooked before the experiment to destroy vegetative pathogens. As confirmed by other researchers, cross-contamination of cooked samples can be occurred during handling and distribution (Walls, 2006). Several factors (such as density of the bacteria) affect cross contamination process. In this study, samples were inoculated with high level of *L. monocytogenes* to demonstrate the worst case at which raw chicken samples were highly contaminated with *L. monocytogenes* before being brought into kitchen. The inoculum used in this experiment was approximately 10⁵ CFU/ml of *L. monocytogenes*. Transfer rate in reality might even be higher than the transfer rates determined in this study.

As mentioned before, re-use of the same cutting board for raw and RTE cooked food without washing is a critical factor in bacterial transmission. In study of Worsfold and Griffith (1997), 60% of the respondents used a single cutting board to perform all cuttings in kitchen. While Altekruse, Street, Fein, and Levy (1996) stated that only 67% of the respondents cleaned the cutting boards after being used directly for raw meat. For safety purposes, different cutting boards must be used to cut raw material and cooked food as pathogens on the surface of cutting boards can easily be transmitted to cooked food. It is also recommended to conduct proper washing process and minimize the contact time of food and cutting board.

5. Conclusions

As shown in this study, cutting boards are potential vehicles for cross contamination of *L. monocytogenes* from raw meat to RTE meat, irrespective of material of the cutting boards. Generally, *L. monocytogenes* can be transferred from naturally contaminated raw chicken meat to cooked chicken meat and can even be spread to other utensils and surfaces around kitchen. So, hands and utensils involved in the preparation of cooked meat must be cleaned and properly washed. Different utensils such as cutting boards must be used for raw and cooked meat. Transfer rates of *L. monocytogenes* from raw meat to cooked meat were influenced by the material of the cutting boards. In order to study the survival of *L. monocytogenes* on different materials, further study on the cleaning and drying methods for the cutting boards is recommended.

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