MICROBIOLOGICAL ASSESSMENT OF CHICKEN MEAT SOLD AT CHICKEN RICE STALLS IN SINGAPORE

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Abstract. Prior surveillance of food prepared at food stalls in Singapore suggested that chicken meat, particularly boiled chicken meat, may be undercooked and of unsatisfactory microbiological quality. Therefore, we conducted three studies of chicken meat, between 2010 and 2013 at chicken rice stalls using convenience sampling method in 5 areas of Singapore to evaluate chicken meat safety in order to guide food safety program. The first study compared the microbiological quality of the boiled, deep-fried and soy-sauced chicken meat by evaluating it using standard plate count (SPC) and detecting *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* counts. This was performed on 30 boiled, 30 deep-fried and 10 soy-sauced whole chicken meat samples obtained from 30 chicken rice stalls. Ninety percent of the samples had a SPC <5.0 log colony forming units per gram of meat, which is the Singapore regulatory limit. *E. coli* was detected in 10% (3/30) of the boiled chicken samples, 10% (1/10) of the soy sauced chicken samples and none of the deep-fried chicken samples. The boiled chicken samples (3/30) exceeded Singapore's regulatory limit. The first study revealed unsatisfactory levels of bacterial contamination, especially in boiled chicken meat. The second study assessed the microbiological safety of boiled chicken meat by examining for the presence of *Salmonella* or *Campylobacter* species among samples taken from the tail of the chickens near where the cloaca is found. We examined 136 samples obtained from 61 stalls, with a maximum of three samples per stall. *Salmonella* species were found in 1.5% (2/136) of samples and *Campylobacter* was not found in any of the tested samples. This study revealed a relatively low percentage of contaminated samples, but there is still room for improvement given the pathogenicity of *Salmonella*. The third study evaluated the presence of bacterial growth among chicken meat samples prepared by boiling and deep-frying. However, with this study, we took culture samples for SPC and *E. coli* counts from both groups every 2 hours for a total of 6 hours after food preparation was finished. A total of 29 boiled chicken meat samples and 29 deep-fried chicken meat samples were examined. The median SPC among boiled chicken meat samples at 0, 2, 4, and 6 hours were 2.9, 3.4, 3.4 and 3.3 log CFU/g. The number of bacteria present did not

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increase significantly between 0 and 2 hours ($p>0.05$), but did increase significantly from 0 to 4 hours ($p<0.05$) and between 0 and 6 hours ($p<0.01$). The median SPC among the deep-fried chicken meat samples at 0, 2, 4 and 6 hours were 1.6, 2.5, 2.6 and 2.5 log CFU/g, respectively. The numbers of bacteria increased significantly between 0 and 2 hours ($p<0.05$), between 0 and 4 hours ($p<0.01$) and between 0 and 6 hours ($p<0.05$), respectively. These findings suggest boiled and deep-fried chicken meat should be sold for consumption only within the first 2 hours after preparation, but not afterward. Our 3 studies of chicken meat show there is room for improvement in hygiene, especially boiled chicken. It also suggests the meat should be sold for consumption no more than 2 hours, if displayed under ambient conditions, after preparation.

Keywords: microbiological quality, chickens, shelf life, retail food, *Salmonella*

INTRODUCTION

Food stalls are frequently patronized by the public in Singapore. Microbiological contamination of the food sold at these stalls can negatively impact public health. Commercial food preparation practices are assessed and reviewed regularly by public health authorities in Singapore to ensure food hygiene and safety.

Chicken rice is a popular dish sold by food stalls in Singapore. The common types of chicken meat served in this dish are boiled chicken, deep-fried chicken, and soy-sauced chicken. Though commonly referred to as steamed and roasted chicken in Singapore, the chicken meat is actually boiled ("steamed") or deep-fried ("roasted"). Boiled chicken meat is typically cooked by cooking the whole chicken in boiling water at the stalls. Batches of 3 to 8 chickens are immersed at a time and cooked over a low fire for approximately 45 minutes. The usual way of determining the doneness of meat is through visual inspection of meat color and texture, rather than actual temperature measurements. After cooking over a low fire, chickens are immersed in iced tap water to stop the cooking process and retain the tenderness of the meat. Soy-sauced chicken meat is prepared in a manner similar to boiled chicken except the chickens are soaked in soy-sauce broth immediately after cooking to enhance the flavor. "Roasted" chickens are deep-fried to impart a crispy outer texture. The cooked chicken meat is then hung in display cabinets under ambient conditions until sold. The period the chicken meat is maintained at ambient temperature varies by stall, operating hours and number of customers. Interviews with selected food stall vendors reveal the time the chicken meat is kept at ambient temperature is usually 4-6 hours, but may be as long as 10 hours. Upon purchase, the chicken meat is chopped into bite-sized pieces and served with rice (Fig 1). Prior observations and surveillance data (unpublished) suggested that boiled chicken meat may be undercooked and microbiologically unsatisfactory.

We conducted three different studies between 2010 and 2013 to determine the microbiological safety of chicken meat sold with chicken rice obtained from chicken rice stalls in Singapore. The first study evaluated the microbiological safety of the boiled, deep-fried and soy-sauced chicken meat. The second study evaluated for the presence of *Salmonella* and *Campylobacter* species present in the tail parts of the chicken samples prepared by

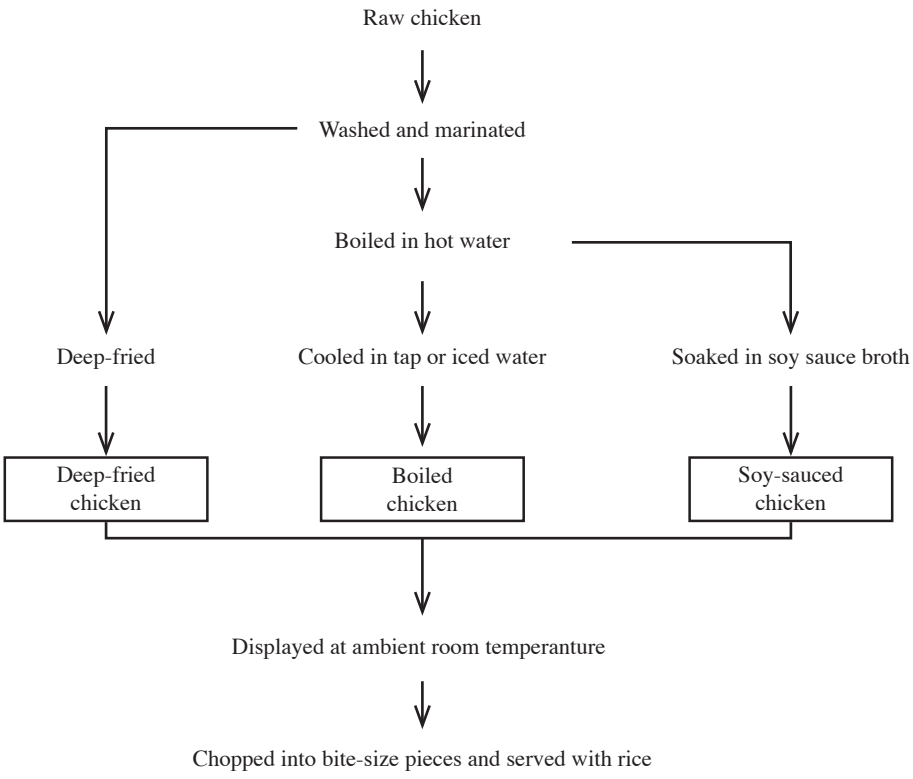

Fig 1 - Chicken meat preparation at studied sites.

boiling. The final study evaluated for the presence of bacterial growth immediately, and 2, 4 and 6 hours after cooking chicken meat by boiling compare to chicken meat prepared by deep frying. These 3 studies were conducted to determine the hygiene level of chicken meat preparation and the ideal time after preparation that the meat can be safely served.

MATERIALS AND METHODS

The three studies discussed in this paper were conducted between 2010 and 2013. For all 3 studies, chicken meat samples were obtained from five areas in Singapore to try to get a representation of the various parts of Singapore. Each chicken meat sample was placed in a sterile bag and kept on ice until transported to the laboratory where they were stored at 4°C until examined, which was done on the same day.

Study 1

Thirty chicken rice stalls were sampled using convenience sampling method where we obtained 30 whole boiled chickens, 30 whole deep-fried chickens and 10 whole soy-sauced chickens. We were unable to find 30 stalls selling soy-sauced chicken meat, so we sampled the 10 stalls we found selling the chicken meat.

Each whole chicken was aseptically dissected into 7 parts [2 wings, 2 breasts, 2 drumsticks and 1 tail (parson's nose)] in the laboratory to determine if the different

parts had different bacterial contaminations or bacteria. Standard plate counts (SPC) were recorded for each part and a median SPC was calculated for the whole chicken. Each sample was also examined for the presence of *Escherichia coli*, *Staphylococcus aureus* or *Bacillus cereus*. If any part had one of these bacteria, the entire bird was considered to have it.

Ten grams of each sample was placed in 90 ml Universal Pre-Enrichment Broth (UPB) (Acumedia Manufacfurer, Lansing, MI) and thoroughly mixed using a laboratory homogeniser (Seward Stomacher® 400 Circulator) at 230 rpm for 30 seconds. Serial 10-fold dilutions were prepared using 9 ml Butterfield's buffer (3M Center, St Paul, MN) and each dilution was cultured to determine SPC, and to detect the presence of *E. coli*, *S. aureus* or *B. cereus*, using previously published methods (Aung *et al*, 2016). The least effective cooking method was designated as the one with the highest median SPC. An unsatisfactory SPC was considered to be a SPC>5.0 log CFU/g, and/or the method with the highest positivity rates for *E. coli*, *S. aureus* and/or *B. cereus*.

The SPC was evaluated as follows: one milliliter of 10- to 1,000-fold diluted sample was inoculated onto a Petrifilm Aerobic Plate count (3M) and distributed evenly using a spreader. The Petrifilm was then incubated at 37°C for 48 hours before quantification. One milliliter of the 10-fold diluted sample was inoculated onto a Petrifilm *E. coli*/Coliform Count Plate (3M), and incubated at 37°C for 48 hours. Presumptive *E. coli* colonies (dark blue color) were counted, streaked onto Levine Eosin Methylene Blue agar (LEMB) (Acumedia) and incubated at 37°C for 24 hours for further confirmation testing. Presumptive *E. coli* colonies (dark blue color on LEMB) were then subjected to indole testing using Kovacs' reagent (Remel, Lenexa, KS) for *E. coli* confirmation.

One milliliter of the 10-fold diluted sample was equally distributed between two plates of Baird-Parker agar (Oxoid, Hamshire, UK) before incubation at 37°C for 48 hours. Presumptive *S. aureus* colonies (grey-black colonies with a narrow white margin surrounded by a zone of clearing) were counted and confirmed with a catalase reaction using 3% hydrogen peroxide (ICM Pharma, Singapore) and coagulase rabbit plasma (Remel).

A hundred microliters of 10-fold diluted sample were spread onto Mannitol-Egg yolk-Polymyxin agar (MYP) (Oxoid) and incubated at 30°C for 24 hours. Presumptive *B. cereus* colonies (bright pink color, surrounded by a zone of precipitation) were counted and confirmed using API® 50 CHB (bioMérieux, Marcy l'Etoile, France).

Study 2

Sixty-one chicken rice stalls were sampled using convenience sampling method to obtain 136 tail samples. The number of chicken meat samples dissected for testing and analysis: a total of 61 chicken rice stalls were convenience sampled to obtain 136 boiled chicken meat tail samples, with a maximum of 3 samples per stall. The minimum number of sampled stalls was increased to 60 with this study to improve sensitivity. The chicken meat tail portion was chosen because of its proximity to the cloaca where it is presumed there could be a greater chance of detecting *Salmonella* or *Campylobacter.* (Berndtson *et al*, 1996; Heyndrickx *et al*, 2002; Arsenault *et al*, 2007).

A ten-gram piece from each collected sample was placed in 90 ml Universal Pre-Enrichment Broth (UPB) (Acumedia) and thoroughly mixed using a labora-

tory homogeniser (Seward Stomacher® 400 Circulator, Seward, Worthing, West Sussex, UK) at 230 rpm for 30 seconds. The resulting solution was then cultured to detect *Salmonella* and *Campylobacter* species using the previously described methods (Chau *et al*, 2017). Each sample was incubated for 18-24 hours at 37°C, 10 µl of this solution was then streaked onto Xylose Lysine Desoxycholate (XLD) agar (Oxoid) which was then incubated at 37°C for 24 hours. Presumptive *Salmonella* colonies (red colonies with black centers) were confirmed to be *Salmonella* using API® 20E (bioMérieux) and a serological latex agglutination test (Oxoid). Another ten-gram piece of each sample was suspended in Bolton broth (Oxoid) and incubated at 42°C for 48 hours under microaerophilic condition using Campygen sachets (Oxoid) in a sealed jar. A 10 µl loopful of the solution was then streaked onto modified Charcoal-Cefoperazone-Desoxycholate agar (mCCDA) (Oxoid) and incubated at 42°C for 48 hours under microaerophilic conditions. Presumptive colonies were then confirmed to be *Campylobacter* using serological latex agglutination kit (Remel).

Study 3

Twenty-nine chicken rice stalls were convenience sampled, since we were unable to find 30 stalls at the time of specimen collection. One whole boiled chicken and one deep-fried chicken were obtained from each studied stall at baseline, 2, 4 and 6 hours after preparation. Only boiled and deep-fried chickens were selected for this part of the study since they represented the least effective (boiling) and most effective (deep-fried) cooking methods. The temperature in each of the food display cabinets where the prepared chicken meat was displayed for sale was measured using an infrared thermometer (Optex).

Each whole chicken meat sample was aseptically dissected into 3 parts: (breast, drumsticks and tail. These parts were chosen because they were thicker and most likely to be undercooked or in the case of the tail part, due to its proximity to the cloaca. Ten grams of each part of each sample were placed in 90 ml Universal Pre-Enrichment Broth (UPB) (Acumedia) and thoroughly mixed using a laboratory homogeniser (Seward Stomacher® 400 Circulator) at 230 rpm for 30 seconds. Serial 10-fold dilutions were prepared using 9 ml Butterfield's buffer (3M). The SPC and *E. coli* positivity rates were recorded.

Statistical analysis

Statistical differences in SPC for each study were evaluated using the Kruskal-Wallis and Mann-Whitney tests using the Statistical Package for Statistics Software, Version 22.0 (IBM, Armonk, NY). Statistical differences in the numbers of chicken samples with a SPC>5.0 log CFU/g, and for samples where *E. coli* was detected at the different collection times for study 3 were evaluated using the Z-score test (Social Science Statistics).

Ethical considerations

The 3 studies were approved by the Environmental Health Institute's Management Committee (Project TS103), National Environmental Agency.

RESULTS

Study 1

Ninety-four percent (66/70) of the chicken meat samples collected in study 1 met Singapore's SPC regulatory limit for ready-to-eat food (<5.0 log CFU/g) (Agri-food and Veterinary Authority Singapore, 2005). Ten percents (3/30) of the boiled chicken meat samples tested exceeded the SPC limit, 3% (1/30) of the

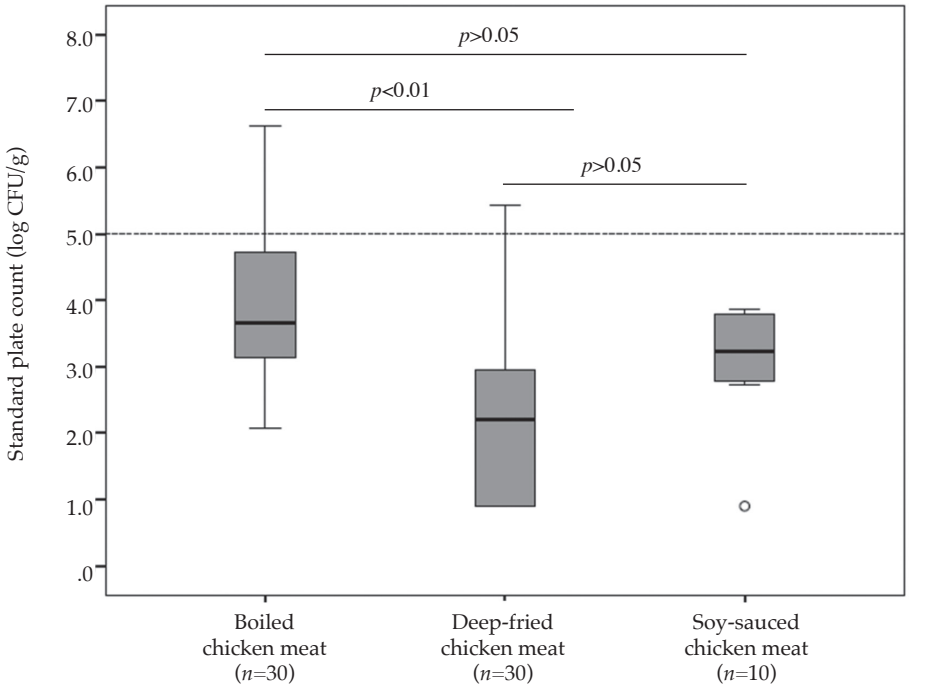

Fig 2 - Standard plate counts for chicken meat prepared by different cooking methods. The dotted line at 5.0 log CFU/g is the cut-off regulatory level specified for cooked retail food in Singapore. The boxes are drawn (vertical lines) from the 25th to 75th percentiles. The whiskers indicate the minimum or maximum values. The horizontal lines within the box indicate the median values. Circles represent outliers.

Table 1
Chicken meat samples with specific bacteria.

Bacteria type	Boiled (*N*=30) chicken meat *n* (%)	Deep-fried (*N*=30) chicken meat *n* (%)	Soy-sauced (*N*=10) chicken meat *n* (%)
E. coli	3 (10)	0 (0)	1 (10)
B. cereus	1 (3)	3 (10)	0 (0)
S. aureus	0 (0)	0 (0)	0 (0)

deep-fried chicken meat samples tested exceeded the SPC limit and none (0/10) of the soy-sauced chicken meat samples tested exceeded the SPC limit. The median SPC among the boiled chicken meat samples tested (3.7 log CFU/g) was significantly higher ($p<0.01$) than the median SPC among the deep-fried chicken meat samples (2.2 log CFU/g). However, there was no significant difference between the median SPC for the boiled chicken meat samples and the SPC for the soy-sauced chicken meat samples (3.2 log CFU/g) ($p>0.05$) (Fig 2). *E. coli* was detected in

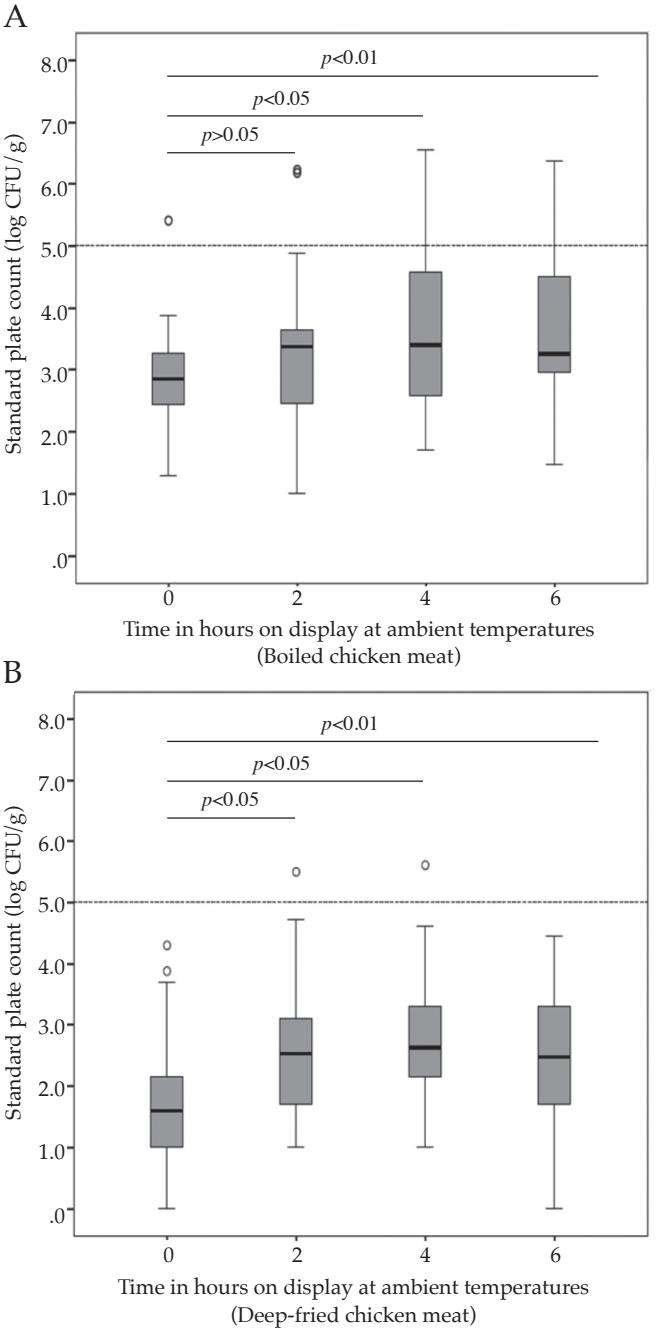

Fig 3 - Standard plate counts for boiled (A) and deep-fried (B) chicken meat. The dotted line at 5.0 log CFU/g is the cut-off regulatory level specified for cooked retail food in Singapore. The boxes are drawn (vertical lines) from the 25th to 75th percentiles. The whiskers indicate the minimum or maximum values. The horizontal lines within the box indicate the median values. Circles represent outliers.

10% of the boiled (3/30) chicken meat sample and 10% of the soy-sauced (1/10) chicken meat samples and none of the deep-fried (0/30) whole chicken meat samples. *B. cereus* was detected in 3% (1/30) of the boiled chicken meat samples and 10% (3/30) of the deep-fried chicken meat samples tested. *S. aureus* was not detected in any of the chicken meat samples tested (Table 1).

Study 2

Salmonella species were detected in 1.5% (2/136) of the chicken tail meat samples obtained from boiled chicken meat of these, 1 was determined to be serogroup D (*S.* Enteritidis), and the other was determined to be *Salmonella* serogroup C. No *Campylobacter* species were detected in this study.

Study 3

The median temperature of the display cabinets for the cooked chicken meat at the chicken rice stalls tested was 31.8°C, a temperature where most bacteria multiply rapidly (NSW Food Authority, 2011). The median SPC for the boiled chicken meat samples tested when first placed in the display cabinet immediately after the meat preparation was 2.9 log CFU/g. The median SPC did not change significantly ($p>0.05$) by 2 hours post-preparation (3.4 log CFU/g) but did increase significantly ($p<0.05$) by 4 hours post-preparation and again significantly increased ($p<0.01$) by 6 hours post-preparation (Fig 3A).

Table 2
Chicken meat samples with *E. coli* detected by time post-preparation by preparation methods (*N*=29)

Preparation method	0 hours *n* (%)	2 hours *n* (%)	4 hours *n* (%)	6 hours *n* (%)
Boiled	0 (0)	2 (7)	2 (7)	4 (14)
Deep-fried	0 (0)	0 (0)	0 (0)	0 (0)

Immediately post-preparation, 3% (1/29) of the boiled chicken meat samples exceeded the Singapore's regulatory SPC limit (<5.0 log CFU/g); by 2 hours post-preparation, this was 7% (2/29), by 4 hours this was 14% (4/29) and by 6 hours this was 17% (5/29); these were not significantly different from each other ($p>0.05$). The percentage of specimen culture positive for *E. coli* immediately post-preparation was 0.0% (0/29), by 2 hours was 7% (2/29), by 4 hours was 7% (2/29) and by 6 hours was 14% (4/29); these were significantly different between 0 and 6 hours ($p<0.05$) (Table 2). The median SPC for the deep-fried chicken meat samples was 1.6 log CFU/g immediately post-preparation; was 2.5 log CFU/g at 2 hours, 2.6 log CFU/g at 4 hours and 2.5 log CFU/g at 6 hours. The values at 2, 4 and 6 hours were significantly greater than immediately after preparation ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) (Fig 3B). *E. coli* was not detected in any of the deep-fried chicken meat samples at any time during study 3.

DISCUSSION

In our study, all three cooking methods were relatively effective for cooking chicken meat. However, boiling was the least effective method. This could be due to undercooking and/or post-cooking process. Cooling the boiled chicken in tap water or iced tap water could have resulted in re-contamination of the cooked chicken meat. The cooling process may also have removed the residual heat that could have continued to kill bacteria in the chicken meat. Undercooking also resulted in detection of *Salmonella* species in 1.5% of boiled chicken tail meat samples.

The microbiological quality of the boiled chicken meat was also compromised by display at room temperatures. The longer the meat was displayed the higher the SPC especially by 2 hours or longer after preparation.

Despite the finding of *Salmonella* species in our study, to our knowledge, there have been no outbreaks of salmonellosis reported linked to consumption of chicken rice in Singapore. Poultry is a commonly reported food associated with salmonellosis (White *et al*, 1997; Corry *et al*, 2002). The role of chicken rice dishes in the epidemiology of salmonellosis needs further investigation.

Although the deep-fried chicken meat was displayed at room temperature the same as the boiled chicken meat, the SPC for the deep-fried chicken meat was lower than the boiled chicken meat at all time-points. This may be due to the lower initial bacterial count after cooking, achieved by the higher cooking temperature of deep-frying than boiling and the omission of the tap water cooling down step with deep frying. However, the SPC did increase in

both boiled and deep-fried chicken meat by two hours of display. Therefore, it is advisable for food stalls to limit selling cooked chicken meat to only within 2 hours post-preparation.

The absence of *S. aureus* in our study suggests minimal bare-hand contact with the cooked chicken meat studied, as this bacteria is part of human skin flora (Food and Drug Administration, 2012b). This is probably due to regulation requiring using gloves when handling ready-to-eat food in retail food establishments in Singapore. The occasional presence of *E. coli* in boiled and soy-sauced chicken meat is probably due to inadequate cooking and/or post-cooking contamination.

The occasional finding of *B. cereus* in the cooked chicken meat samples in our study is not surprising since *B. cereus* is ubiquitous and can produce heat resistant spores that can survive conventional cooking processes (Health Protection Agency, 2009; Sudershan *et al*, 2012). The contamination levels with *B. cereus* observed in our study (2.0 to 2.3 log CFU/g) were well below the infectious dose of 5.0-8.0 log CFU/g and thus unlikely to cause illness if consumed (Food and Drug Administration, 2012a).

Our findings provide valuable data about the risk of inadequately boiling chicken meat as demonstrated by the relatively higher proportion of boiled chicken samples exceeding Singapore's regulatory SPC limit. Undercooking can lead to a food safety problem, as seen by finding *Salmonella* species in boiled chicken meat samples in this study. The optimum time in which chicken meat should be sold post preparation is no more than 2 hours. Although the number of food stalls sampled in this study was relatively small, the stalls were selected from various parts of Singapore to improve the likelihood the results can be applied to a variety of locations in Singapore. These data can inform food safety programs instructing food handlers working at chicken rice stalls in Singapore.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this study.

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