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FULL PAPER

Prevalence of *Salmonella* contamination in processing chain of selected chicken-based side dishes

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Abstract

This study was aimed to determine the prevalence, the level and the main contributing factors to contamination of *Salmonella* spp. in four selected chicken-based side dishes prepared for the school canteens. One hundred and seven samples were collected from four different food processing chains, i.e. fried chicken with precooking, fried chicken without precooking, breaded fried chicken, and sauced chicken. *Salmonella* contamination was determined by the most probable number (MPN) and confirmed with polymerase chain reaction. *Salmonella* spp. were detected in 8 of 21 chicken cuts samples (360-920 MPN/g) and in 4 of 30 end products samples (0.61-3 MPN/g). The fact that *Salmonella* was still found at the end product indicated that cross-contamination and/or inadequate heating process likely occurred. Besides the chicken cuts, the contributing factors to the *Salmonella* contamination were water (4 of 17 samples) and seasonings (8 of 13 samples). To ensure the safety of chicken-based side dishes prepared for the school canteen, adequate cooking process must be performed by all food handlers. The results of this study might contribute to analysing the risk of salmonellosis in Indonesia.

1. Introduction

Poultry and its products are among the most nutritious food, but also a well-known reservoir of *Salmonella* spp. (Gould *et al*., 2013; Kang *et al*., 2017). *Salmonella* spp. is a very adaptive microorganism, which can cause human salmonellosis, an important foodborne disease (Giaouris and Nychas, 2006). In Indonesia, not many *Salmonella* spp. cases have been reported. A report from Chusniati *et al*. (2009) stated that domestic chicken eggs used in a traditional herbal drink (jamu) in Sidoardjo City, East Java of Indonesia, were contaminated by *Salmonella* spp. (5.56% from 36 samples). In 2011, the microbial examination conducted on 4808 food samples from the school canteens revealed that 13 (0.27%) samples contaminated by *Salmonella* (NADFC, 2012). In 2016 NAFDC examined 106 fried chicken samples using the Most Probable Number (MPN) method and confirmed by Polymerase Chain Reaction (PCR) method, found that 45 samples were contaminated by *Salmonella* spp. with 42.0% of prevalence, and 0.36-2.30 MPN/g of concentration (NADFC, 2016a). In 2017 NADFC reported 53 foodborne outbreaks in Indonesia, where 2041 people ill and 3 people died. About 13.21% of the outbreaks confirmed as microbial related, and 15 outbreaks (28.30%) happened in school (NADFC, 2017).

Salmonella on food has to be negative (NADFC, 2016b), but diseases related to *Salmonella* are still be found although not always foodborne related. Many studies showed that poultry or its products are the sources of *Salmonella* contamination. Barua *et al*. (2014) studied the sources of non-typhoidal *Salmonella* enterica serovars in human and found that nine serovars of *Salmonella* were isolated from human stool samples, and it's commonly found on poultry. Since 1981 the outbreaks related to salmonellosis had been reported by Cobet *et al*. (1981) who isolated 158 *Salmonella* Oranienburg from 150 hospitalized patients with diarrhoea. Punjabi *et al*. (2013) reported 296 laboratory-confirmed enteric fever cases during the 7 months surveillance period in North Jakarta Indonesia, of which 221 (75%) were typhoid fever and 75 (25%) were the paratyphoid fever. Most of the cases occurred among children under five years old.

Chicken is one of the most popular food products in

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Indonesia. High level of protein and low-fat content contributed to the popularity of chicken. Furthermore, chicken is easy to prepare and widely used in restaurants, cafes, or canteens as a main meal or side dishes. Foods at the school canteens contributed to 15-20% of daily nutrition for the students. Most of the students (98.9%) who did not have breakfast will eat at the canteen. Those foods gave 31.06% of energy and 27.44% of protein every day (NADFC, 2012). Chicken based side dishes is a common food found in the school's canteen in Indonesia. This food has a risk of being contaminated by *Salmonella*. It is very important to ensure food safety at school considering the fact that the children are the future of the nation. Considering the severity and risk level of *Salmonella* and the regulation stated by NADFC (NADFC, 2012), the study of *Salmonella* contamination in chicken-based side dishes in school canteen is very important. This study aimed to identify the prevalence, the level, and the sources of *Salmonella* spp. contamination in selected chicken-based side dishes in the school's canteen.

2. Materials and methods

The chicken-based side dishes samples were collected from nine merchants of eight school canteens, namely fried chicken with precooking, fried chicken without precooking, breaded fried chicken, and sauced chicken. A total of 107 samples was collected, consisted of raw materials (chicken cuts, seasoning, egg, and water), precooked chicken, final product, swab sample from the hand of food handlers, and cutting boards.

2.1 Enumeration of Salmonella spp.

The enumeration method was conducted according to Pavic *et al*. (2009). A total of 25 g of samples were put into 225 mL of Buffer Peptone Water (BPW, Oxoid, UK) in stomacher bag and homogenized by stomacher (Seward™ Stomacher™ Model 400C Circulator Lab Blender, Fisher Scientific, USA) for 2 mins. A total of 10 mL of the initial suspension was transferred into each of 3 tubes containing 10 mL of BPW (double strength). An aliquot of the initial suspension was transferred into each of 3 tubes containing 9 mL of single strength BPW. Next, the initial suspension was diluted in BPW to provide 10^{-2}, and 1 mL was transferred to each of 3 x 9 mL of BPW. All the tubes were incubated at 37°C for 24 hrs and observed for the turbidity.

A 100 µL of the positive tubes were transferred into a petri dish containing Modified Semi-Solid Rappaport-Vassiliadis Agar (MSRVA, Oxoid, UK) and incubated at 42°C. White colonies were observed after 24 and 48 h of incubation. The white colony was then subcultured on Xylose Lysine Deoxycholate Agar (XLDA, Oxoid, UK) and incubated at 37°C for 24 hrs A single colony from specific colonies (red with or without black globule in the middle) was inoculated on Brain Heart Infusion Agar (BHIA, Oxoid, UK) medium and incubated at 37°C for 24 hrs. *Salmonella enterica* serovar Typhimurium ATCC 14028 and E. coli ATCC 25922 were used as positive and negative controls.

2.2 Confirmation of Salmonella by PCR method

PCR method was conducted according to Rahn *et al*. (1992) with modification. One loop colony from BHIA was transferred into 500 µL of sterilized 0.85% Nalco (Merck, Germany) in 1.0-1.5 mL tube, homogenized and boiled in thermomixer (Eppendorf Thermomixer C, Germany) at 100°C for 15 mins, then kept at -20°C for 2 mins as a cold shock treatment. Then centrifuged (Hettich Zentrifugen Universal 320 R, Germany) at 12000 rpm for 5 mins. The DNA template was pipetted from the supernatant and transfer into 500 µL of sterilized microcentrifuge tubes.

PCR reaction (25 µL) using Go Taq Green Master Mix (Promega, US) kit with a composition of 2.5 µL DNA template from previous step, 0.5 µL each from forward and reversed primer which was F139 5'GTGAAATTATCGCCACGTTCGGGCAA-3' and R141 5'TCATCGCACCGTCAAAGGAACC-3' (Macrogen, Singapore), 12.5 µL Go Taq Green Master Mix, and 9 µL nuclease-free water (Promega, US) were prepared. The mixture of master mix and DNA template in PCR tube was homogenized and placed into thermal cycler (PCR) machine (Blue Ray Biotech, Taiwan) according to the shown in Table 1.

Table 1. PCR protocol

Steps	Temperature (°C)	Time (Minutes)
Pre-denaturation	72	7
Denaturation	94	1
Annealing	53	1*
Elongation	72	2**
Post Elongation	72	7
Hold	4	Forever
Cycle	35	

Reference: Rahn *et al*. (1992) with modification: * from 2 modified to 1 minutes; ** from 3 modified to 2 minutes

2.3 Detection of DNA amplification by electrophoresis

Tris-Borate EDTA (TBE) (1st base, Singapore) solution was prepared using miliQ water. Agarose 2% (1st base, Singapore) was prepared and 4-6 µL per 100 mL of flourosafe were added. The agarose was poured into the block to solidify. The remaining TBE solution was poured into the electrophoresis vessel (Biometra, Germany) then added the solid agarose. About 10 µL

from each of PCR product transferred into all the wells of agarose. Some of the wells were filled with marker (DNA ladder 100 bp of nucleotides), non-template control (NCT), positive and negative control bacteria. Then the vessel connected with the electrical source (ampere meter), with 75 V for 45-60 mins. The result was observed and documented using GelDoC devices (Bio-Rad, US). The criteria for the band acceptance was 284 bp.

3. Results and discussion

This study found that 30 of 107 samples were contaminated by *Salmonella* spp., indicated by the appearance of 284 bp band in PCR product (Figure 1). Invasive A gene (invA) was used as the target gene since it is the regulator for the invasive character. This character enabled *Salmonella* to penetrate the epithelium tissues of the intestine (Galán and Curtiss, 1989). A total of 4 of 30 end product samples were contaminated, but the level of contamination was significantly lower than chicken cuts (Table 2-5).

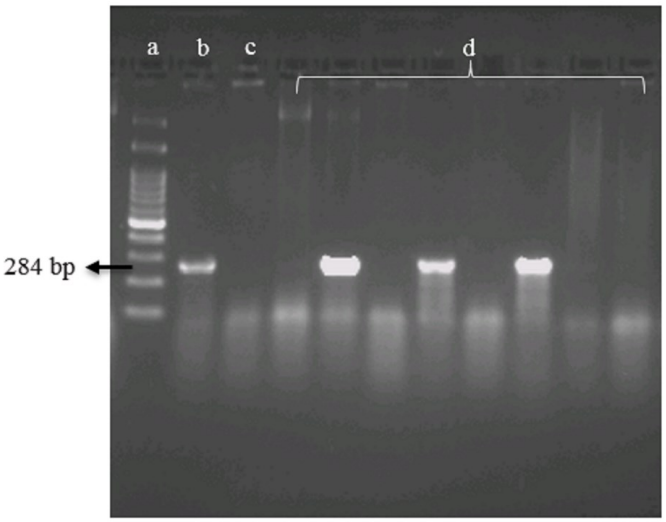

Figure 1. PCR amplification of *Salmonella* spp. in samples. a. Ladder, b. positive control (*Salmonella enterica* serovar Typhimurium ATCC 14028, c. negative control (*E. coli* ATCC 25922), d. DNA of samples

Table 2. *Salmonella* spp. contamination in the processing chain of fried chicken with precooking

Samples	Total Sample	Positive *Salmonella* spp.	Concentration
Chicken cuts	6	3	360-920 MPN/g
Seasoning	6	4	0.3 MPN/g
Water	6	0	<0.3 MPN/mL
Hand	2	0	<0.3 MPN/Hand
Cutting board	1	0	<0.3 MPN/25 cm^2
Precooked chicken	6	0	<0.3 MPN/g
Fried chicken	8	0	<0.3 MPN/g
Total	35		

$t_{boiling}$ = 30 min; $T_{meat\ core}$ = 75°C; t_{frying} = 13 min; T_{meat} core = 91°C.

The number of contaminated end products was mainly associated with contamination in 8 of 21 (38.09%) chicken cut samples used as raw materials. Chicken cut samples were contaminated by *Salmonella* spp. with a range of 300-920 MPN/g. It was lower than the report from Kholifah *et al*. (2016) in Samarinda City, Indonesia, but higher than Naik *et al*. (2015) in India and Alali *et al*. (2012) in Russia.

Hajrawati *et al*. (2016) reported that the intrinsic parameters of chicken carcasses in the Indonesian market were 0.84-0.85 for water activity and 6.00-6.37 for pH. This condition is suitable for *Salmonella* spp. which need water activity of 0.93 and pH of 4.5-9.0 to grow (Cary *et al*., 2000). El-Aziz (2013) reported that *Salmonella enterica* serovar Typhimurium was detected from 100 samples (each 25 frozen samples of chicken meat, liver, heart and gizzard) collected from Assiut city market, with a prevalence of 44% in chicken meat, 40% in liver, 48% in heart and none in the gizzard. The contamination on chicken cuts might occur during the slaughter process. In this study, chicken cuts and seasoning were brought from the traditional market. Maulita *et al*. (2017) reported that 8 of 10 samples of equipment used in the cutting process in the traditional market in Aceh, a province in Indonesia, were contaminated by *Salmonella* spp. with the highest concentration on the cutting board, 4.8×10^4 MPN/g. A similar report came from Nidaullah *et al*. (2017) that found 161 *Salmonella* spp. isolated from 182 samples (88.46%) consisting of chicken carcasses, cages, knives, cutting boards, water, defeathering machines, and aprons collected Malaysian traditional markets,

Two of four processing chain on the preparation of chicken-based side dishes in this study applied precooking process and was able to eliminate *Salmonella* spp. (Tables 2 and 5). The boiling process was conducted at 82-100°C for 15-20 mins. The occurrence of *Salmonella* spp. in the end products may be due to cross-contamination after precooking process. The possible sources of contamination were seasoning, water, cutting board and the hand of food handlers (Table 2-5).

Two of 6 end products of sauced chicken were contaminated (Table 5). The boiled chicken of sauced chicken was chopped and stir-fried with seasoning. *Salmonella* spp. was recovered from the seasoning, cutting board and hand of food handlers in this process (Tables 2 and 4). Swab samples of cutting boards and the hand of food handlers were conducted before the chopping process.

Furthermore, eight of 13 seasoning samples was contaminated. The seasonings consisted of onion, garlic, turmeric, and pepper. The level of *Salmonella* spp. was

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in a range of 0.3-0.92 MPN/g. However, the seasoning used for fried chicken without precooking was negative for *Salmonella* spp. (Table 3). A report from van Doren *et al*. (2013) stated that *Salmonella* spp. could be isolated from turmeric and pepper in Canada, Denmark, England and Wales, France, Germany, New Zealand, Norway, Serbia, and the United States.

Table 3. *Salmonella* spp. contamination in the processing chain of fried chicken without precooking

Samples	Total Sample	Positive *Salmonella* spp.	Concentration
Chicken cuts	4	1	300 MPN/g
Seasoning	2	0	<0.3 MPN/g
Water	4	1	0.3 MPN/mL
Hand	2	0	<0.3 MPN/Hand
Fried chicken	8	1	3.0 MPN/g
Total	20		

$t_{boiling}$ = 30 min; $T_{meat\ core}$ = 78.22°C

Table 4. *Salmonella* spp. contamination in the processing chain of breaded fried chicken

Samples	Total Sample	Positive *Salmonella* spp.	Concentration
Chicken cuts	8	2	300-740 MPN/g
Seasoning	4	2	0.3 MPN/mL
Water	2	2	0.92 MPN/g
Hand	2	1	0.3 MPN/g
Breaded Fried chicken	10	1	0.61 MPN/g
Total	26		

$t_{boiling}$ = 6 min; $T_{meat\ core}$ = 86.38°C

Table 5. *Salmonella* spp. contamination in the processing chain of sauced chicken

Samples	Total Sample	Positive *Salmonella* spp.	Concentration
Chicken cuts	3	2	360-720 MPN/g
Water	3	1	0.3 MPN/mL
Boiled chicken	3	0	<0.3 MPN/g
Chopped chicken	2	1	0.3 MPN/g
Cutting board	3	1	0.3 MPN/25 cm^2
Hand	3	2	0.3 MPN/Hand
Seasoning	3	3	0.8-0.92 MPN/g
Sauced chicken	6	2	0.74-1.1 MPN/g
Total	18		

$t_{boiling}$ = 25 min; $T_{meat\ core}$ = 78.5°C; t_{frying} = 8 min; T_{meat} core = 96°C.

Groundwater and municipal water were used in the cooking process, but only groundwater was contaminated by *Salmonella* spp. The prevalence of *Salmonella* contamination in water was 23.5% (4 of 17 samples) and higher than the report from Momtaz *et al*. (2013) which was 0.89% in Iran. Groundwater was well known as a source of *Salmonella* Enteritidis (Kovacic *et al*., 2017) and also the carrier of typhoidal *Salmonella* serovar (Levantesi *et al*., 2012).

Another material used in chicken side dishes processing was eggs. Eggs were used in breaded fried chicken processing and contaminated by 0.92 MPN/g of *Salmonella* spp. Contamination in egg in Indonesia was reported by Chusniati *et al*. (2009) who found *Salmonella* spp. in 5.56% of 36 egg samples. It was higher than reported by Singh *et al*. (2010). The results showed that contamination of *Salmonella* spp. in eggs was 4.82%, with lower incidence in farms samples. Furthermore, they obtained 27 isolates that included *S*. Typhimurium (55.5%), *S*. Lagos, *S*. Africana, and *Salmonella* II. Kouam *et al*. (2018) studied *Salmonella* spp. contamination of 140 eggs from 19 farms. All farms sample contain at least one type serovar of *Salmonella* spp.

Another possible source of *Salmonella* spp. in sauced chicken processing was cutting board and hand of food handlers. Two of seven swab samples of hands and one of 4 swab samples of the cutting board was contaminated by *Salmonella* spp. The level of contamination by *Salmonella* spp. was 0.3 MPN/hand and 0.3 MPN/25 cm^2 respectively. Gorman *et al*. (2002) reported that *Salmonella* could be isolated from the counter's surface where food was processed and from aprons used by food handlers with a prevalence of 16.6% from total samples. *Salmonella* spp. can survive in a dry environment for a long time (Humphrey *et al*., 1994). *Salmonella* Enteritidis PT4 has also been reported showing the ability to form biofilms on the stainless-steel surface. These bacteria could grow in the presence of invisible leftover on the surface of the utensils as a result of inadequate washing process as reported by Giaouris and Nychas (2006).

In this study, all food handlers used frying method where only half of the chicken submerged in the oil. Although the temperature reached a high point, the possibility of uneven heat over the meat make *Salmonella* spp. could be recovered. Roccato *et al*. (2014) studied the resistance of *Salmonella* Typhimurium DT 104 in chicken-based foods with 3 cooking method, namely frying, grilling and baking. The results showed that 26 of 78 samples were contaminated and the highest prevalence came from frying method (12 positive samples), followed by grilling and baking. The average temperature of chicken meat during the frying process was 59-74°C. He *et al*. (2011) reported that *Salmonella* spp. could be eliminated when the internal temperature of food reached more than 72°C during the processing. It could reduce *Salmonella* spp. into 2 logs,

but the temperature of 90°C could reduce the number into 5.5-7.1 log. Evans *et al*. (1996) reported that *Salmonella* Enteritidis PT 4 in beef rissoles was not eliminated during the frying process on 142-157°C for 5-7 minutes. This bacterium came from eggs that were one of the ingredients. Beef rissoles were assumed as cooked when floating in the oil. It turns out the inside temperature was only 48-60°C while the external temperature was 91-95°C.

Heat resistance of *Salmonella* is different in each serovar, influenced by genetic factors, environmental conditions, and adaptability. Pre-exposure to thermal treatment on precooking could increase survival ability in thermal treatment such as frying. Chen *et al*. (2013) found *Salmonella* Senftenberg could survive in the chicken litter for 24 hrs at 80°C. A similar report from de Jong *et al*. (2012), chicken breast fillet inoculated by *Salmonella* Typhimurium was boiled, and after one minute the temperature of the chicken became 85°C. Extreme reduction on *Salmonella* Typhimurium has seen in 2.20 min, but even after 10 mins of boiling the bacteria could still be recovered. In this study, the decreasing of *Salmonella* was detected in all food processing chains. However, *Salmonella* spp. was still recovered in some of the end products, except in fried chicken with precooking.

4. Conclusion

The pre-cooking step in the selected processing chain of chicken-based side dishes reduced *Salmonella* spp. to an undetectable level. The occurrence of *Salmonella* spp. in 4 of 30 end product samples likely associated with cross-contamination. Inadequate deep-frying practices by all food handlers probably also contributed to the survival of *Salmonella*. This study suggested the need for education of the food handlers on proper cooking to improve the safety of the food.

Conflict of Interest

The authors declare no conflict of interest.

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