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# Prevalence and level of *Salmonella* spp. Contamination on selected pathways of preparation and cooking of fried chicken at the household level

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#### **Abstract**

Homemade foods have been reported as an important contributor to some foodborne outbreaks. This study determined the prevalence and number of *Salmonella* spp. on selected pathways of fried chicken preparation and cooking at the household level and investigated their antimicrobial resistance. *Salmonella* serovar was confirmed by polymerase chain reaction (PCR) and partial sequencing using primer 785F and 907R. Samples consisted of chicken meat (raw, pre-cooked and fried), seasoning, water, mortar, and the hands of the food handler. The results showed that *Salmonella* spp. was found in 22.1% of 104 samples. The level of *Salmonella* found was in the range of 0.3 MPN/g (in fried chicken and water) to 920 MPN/g (in marinated raw chicken). Six *Salmonella* serovars were identified, namely *S.* Typhimurium, *S.* Bergen, *S.* Enteritidis strain FORC\_052, *S.* Enteritidis strain GD1011, *S.* Typhi strain 541, and *S.* Typhi strain 3N4. Three were resistant to nalidixic acid, while one was resistant to streptomycin.

Keywords: antimicrobial resistance; fried chicken; household; Salmonella.

Practical Application: Salmonella spp. prevalence in fried chicken and its antimicrobial resistance properties.

#### 1 Introduction

Salmonellosis due to *Salmonella* spp. in poultry has been reported in America, Asia, and Europe (Kovačić et al., 2017). Fifty-five percent of chicken carcass samples (n= 40) from seven traditional markets and eight supermarkets in Bogor were contaminated by *Salmonella* spp. (Sylviana & Kusumaningrum, 2008). The prevalence of *Salmonella* spp. has also been reported to be 46.6% (n= 87) in chicken meat samples collected from ten cities in Indonesia (Syarifah & Novarieta, 2015). In addition, the presence of *Salmonella* spp. in fried chicken has also been found to be 42.0% of 106 fried chicken samples in Jakarta, with the level between 0.36 and 2.30 MPN/g (Indonesia National Agency of Drug and Food Control, 2016).

A study conducted by Rosniawati et al. (2018) in 2018 showed that 32.5% of a sample of 40 housewives had a low understanding of how to implement safety practices in handling chicken carcasses. Therefore, chicken processing practices at the household level need to be improved in order to control the contamination of *Salmonella* spp. in the finished product. Fried chicken is a favorite side dish in South East Asian countries, including Indonesia. One of the common ways to cook chicken is to fry without flour (unbreaded) using seasoning, with or without a pre-cooking process. The precooking process means that chicken meat is cooked by boiling prior to frying. This study aimed to determine the prevalence and the number of *Salmonella* spp. on preparation and cooking of fried chicken, and to identify the isolated serovars and their antimicrobial resistance/susceptibility.

#### 2 Materials and methods

#### 2.1 Materials

10 household's food processor in Jakarta were selected to be the respondents in this study. The preparation of unbreaded fried chicken in households may vary. However, it commonly consists of five steps, starting from receiving raw materials (chicken, water, seasonings), cleaning raw chicken, preparing seasonings, boiling/marinating, and frying. 104 samples were collected, composed of raw chicken meat (18 samples), seasonings (14 samples), water (20 samples), marinated raw chicken meat (2 samples), pre-cooked chicken meat (16 samples), pre-cooked chicken meat stored for 3 hours at room temperature (4 samples), fried chicken (20 samples), the hand swabs of food handlers (7 samples), and the swabs of mortars (3 samples). The pre-cooked process was conducted by heating the chicken meat with seasoning (salt, onion, ginger, turmeric and galangal) until the meat is boiled, with a total heating time of approximately 19 to 46 minutes.

# 2.2 Sample preparation

All samples, except water, swabs of food handlers and mortars, were taken with the same weight at a different spot to collect approximately 200g and put into containers aseptically. For water, samples were collected from the tap, well, and refilled drinking water (originating from depots). The hands of food handlers and mortars were swabbed prior to preparing fried chicken.

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# 3 Sample analysis

# 3.1 Salmonella isolation by the most probable number method

*Salmonella* spp. was isolated using the MPN method (Indonesia National Agency of Drug and Food Control, 2016). Food samples (25 g), except water, swabs from food handlers and mortars, were put in sterilized stomacher bags aseptically, and subsequently homogenized in 225 mL of Buffered Peptone Water (BPW, Oxoid). All samples were diluted serially to  $10^{-1}$  and  $10^{-2}$ . All tubes were incubated at 37 °C for 24 h.

After incubation, 100  $\mu$ L of each sample was inoculated into selective enrichment Modified Semi-Solid Rappaport Vassiliadis (MSRV, Oxoid), then incubated at 42 °C for 24 h to 48 h. The growth of *Salmonella* spp. was indicated by the presence of white zones in the MSRV medium. The white zone area was streaked on the selective medium Xylose Lysine Deoxycholate Agar (XLDA, Oxoid) and incubated at 37 °C for 24 h.

A loop from a specific colony (pink colored colony with or without black circles) and a nonspecific colony were streaked on Brain Heart Infusion Agar (BHIA, Oxoid) medium, then incubated at 37 °C for 24 h. Two loops of isolates from BHIA were transferred into 50  $\mu$ L NaCl and subsequently homogenized.

# 3.2 Salmonella confirmation by the polymerase chain reaction technique

Salmonella spp. was detected using an optimized PCR technique (Indonesia National Agency of Drug and Food Control, 2016). The positive control bacterium was Salmonella Typhimurium ATCC 14028 while the negative control bacterium was E. coli ATCC 25922. Bacterial sample suspension was extracted to collect DNA using the boiling technique at 100 °C for 15 min. The boiled tube was treated with a cold shock at -20 °C for 2 min, then centrifuged (Hettich, Germany) at 12.000 rpm for 5 min. The supernatant was taken using a pipette and transferred into sterilized 500 μL microcentrifuge tubes. The obtained supernatant was used as a DNA template and the absorbance was measured using NanoDrop spectrophotometry (BioDrop, US) at wavelengths of 260 and 280 nm for the determination of the DNA concentration (ng/μL) and purity level (absorbance ratio of 260 nm/280 nm).

DNA was then amplified using the PCR technique. The master mix for PCR reactions was made to 25 µL by using a Kit Go Taq Green Master Mix (Promega, USA), consisting of 2.5 µL template DNA, 0.5 μL Forward and Reverse Primers, 12.5 μL Master Mix Go Taq Green, and 9 µL Nuclease Free Water. To detect invA genes of Salmonella spp. a specific primer was used, i.e. Forward SalinvA 139 primer (primer sequence (5'-3'): GTG-AAA-TTA-TCG-GCA-CGT-TCG-GGC-AA) and Reverse SalinvA 141 primer (primer sequence (5'-3'): TCA-TCG-CAC-CGT-CAA-AGG-AAC-C). Target/template DNA (DNA samples, positive control bacterial DNA of S. Typhimurium ATCC 1402 and negative control bacterial DNA of E. coli ATCC 25922) were amplified by PCR (Turbo Cycler, Biometra) as follows: pre-denaturation at 95 °C for 1 min; denaturation at 95 °C for 30 s; annealing at 60 °C for 30 s; elongation at 72 °C for 30 s; and final extension at 72 °C for 4 min. The PCR cycle was performed 35 times. Amplification of bacterial DNA was detected using 2% agarose solution for 45-60 min, depending on the distance of agarose. The electrophoresis (Biometra, Germany) occurred from the negative pole to the positive pole at 75 voltages. The electrophoresis process was stopped after the yellow color almost reached the lower limit. The presence of DNA bands was observed in the position of 284 base pairs and documented by using GelDoc (Biorad, USA).

#### 3.3 Salmonella serovar analysis by sequencing

DNA of *Salmonella* spp. was extracted and amplified using 27F (5' (AGA GTT TGA TCM TGG CTC AG) 3') and 1492R (5' (TAC GGY TAC CTT GTT ACG ACT T) 3') primers. Amplicon was sequenced using 785F (5' (GGA TTA GAT ACC CTG GTA) 3') and 907R (5' (CCG TCA ATT CMT TTR AGT TT) 3') primer, ABI PRISM 3730 XL Analyzer (96 capillary type) sequencer and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The result of sequencing was analyzed and base sequence obtained, then submitted to the Blast process at the NCBI (National Center for Biotechnology Information, 2017) database.

#### 3.4 Antibiotic resistance testing

Salmonella spp. isolates obtained from fried chicken processing chains were tested for their resistance according to the Clinical Laboratory Standards Institute using the disk diffusion technique (Clinical & Laboratory Standards Institute, 2013). Five antibiotics were tested, namely ampicillin (10  $\mu g$ ), streptomycin (10  $\mu g$ ), tetracycline (30  $\mu g$ ), nalidixic acid (30  $\mu g$ ), and trimethoprimsulfamethoxazole (25  $\mu g$ ). The bacterial isolates were grouped as susceptible (S), intermediate (I), and resistant (R) (Clinical & Laboratory Standards Institute, 2013).

# 4 Results and discussion

# 4.1 Results

A total of 23 of 104 samples (22.1%) were contaminated by *Salmonella* spp. confirmed by PCR. Samples containing *Salmonella* spp. were indicated by the formation of bands in agarose gel at 284 bp (Figure 1). The highest prevalence of *Salmonella* spp. was found in raw chicken meat (50.0%, 9 of 18 samples) in comparison with other samples (Figure 2). PCR step: pre-denaturation at 95 °C for 1 min; denaturation at 95 °C for 30 s; annealing at 60 °C for 30 s; elongation at 72 °C for 30 s; and final extension at 72 °C for 4 min. The PCR cycle was carried out 35 times. *Salmonella* positive samples were shown by a DNA band on 284 bp by gel electrophoresis

Salmonella spp. was also confirmed in pre-cooked chicken meat (1 of 16 samples) and fried chicken (1 of 20 samples). There was no Salmonella spp. detected in the prepared seasoning samples from the market and tap water. Tables 1 and 2 suggest that the number of Salmonella spp. in raw chicken meat increased after marinating, reaching 920 MPN/g. Further processing, boiling and frying, reduced the number, although two samples (1 of 16 pre-cooked samples; and 1 of 20 fried chicken samples) still contained Salmonella spp. of 0.4 and 0.3 MPN/g, respectively. Three of seven food handler's hands were contaminated by Salmonella spp.: two of them did not wash their hands whereas one of them washed their hands only using well water.

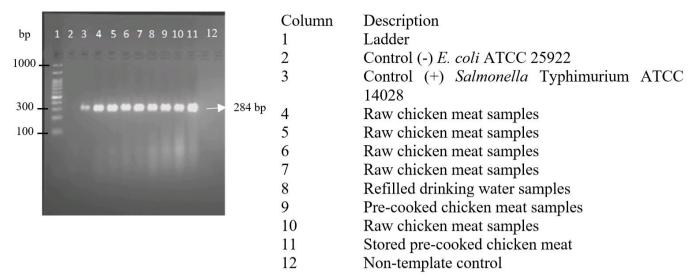
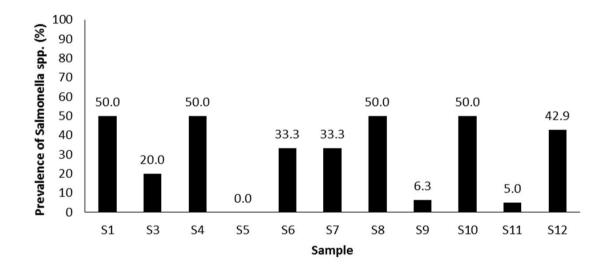


Figure 1. Detection of Salmonella spp. using the PCR technique from different isolates.



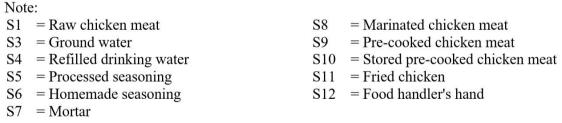


Figure 2. Prevalence of Salmonella spp. from different sources throughout the fried chicken processing chain.

Six *Salmonella* serovars were found as presented in Table 3. Five *Salmonella* serovars were found on raw chicken meat and two serovars on pre-cooked chicken, while one serovar was found in both samples. In addition, one serovar was found in refilled drinking water as well as in pre-cooked chicken and raw chicken meat. Almost all isolates were resistant to at least one antibiotic, except *S*. Bergen which was susceptible to all antibiotics (Table 4). This study also found that *Salmonella* Typhimurium from raw chicken meat showed resistance to both nalidixic

acid and tetracycline. In addition, all isolates were susceptible to trimethoprim-sulfamethoxazole and ampicillin.

# 5 Discussion

Raw chicken meat was the predominant source of *Salmonella* spp. contamination in the finished product. Ground water, refilled drinking water, homemade seasoning, cooking utensils (mortars), and food handler's hands were also confirmed as contributors to *Salmonella* spp. contamination

**Table 1**. Positive results of *Salmonella* spp. in fried chicken processing chain.

No	Sample	Complete and the control	Positive results		
		Sample number (n)	Sample number (n)	MPN value (MPN/g)	
1	Raw chicken meat	18	9	1.2-55.6	
2	Marinated raw chicken meat	2	1	920.0	
3	Pre-cooked chicken meat	16	1	0.4	
4	Stored pre-cooked chicken meat	4	2	0.5	
5	Fried chicken	20	1	0.3	

Table 2. Number of Salmonella spp. in the cooking environment of the fried chicken processing chain.

No	Sample	Cl	Positive results				
		Sample number (n)	Sample number (n)	Unit	MPN value		
1	Ground water	10	2	MPN/mL	67.0		
2	Refilled drinking water	4	2	MPN/mL	0.3-2.0		
3	Homemade seasoning	3	1	MPN/g	2.8		
4	Mortar	3	1	MPN/utensil	6.1		
5	Food handler's hand	7	3	MPN/hand	6.1-121.5		

Table 3. Salmonella serovars on selected pathways of fried chicken preparation and cooking.

Isolates	Sources	Serovar	*% Conformity
R11CH	Raw chicken meat	Salmonella enterica strain FORC_030	99
R21CH	Raw chicken meat	Salmonella enterica subsp. enterica serovar Bergen str. ST350	99
R61BH	Raw chicken meat	Salmonella enterica subsp. enterica serovar Enteritidis strain FORC_052	99
R72AMH	Raw chicken meat	Salmonella enterica subsp. enterica serovar Enteritidis strain GD1011	99
R81BH	Raw chicken meat	Salmonella enterica subsp. enterica serovar Typhi strain 541	99
R32AH	Pre-cooked chicken meat	Salmonella enterica subsp. enterica serovar Typhi strain 3N4	99
R51BH	Stored pre-cooked chicken meat	Salmonella enterica subsp. enterica serovar Enteritidis strain FORC_052	99
R42AH	Refilled drinking water	Salmonella enterica subsp. enterica serovar Enteritidis strain FORC_052	99

<sup>\*</sup>the conformity level was based on sequencing using 907 R primers.

**Table 4**. Resistance of *Salmonella* serovars from various sources to antibiotics.

Isolate	Serovar	Contamination sources	Trimethoprim- sulfame thoxazole	Streptomycin	Ampicillin	Nalidixic acid	Tetracycline
R11CH	S. Typhimurium strain FORC_030	Raw chicken meat	S	Ι	S	R	R
R21CH	S. Bergen str. ST350	Raw chicken meat	S	S	S	S	S
R61BH	S. Enteritidis strain FORC_052	Raw chicken meat	S	I	S	R	S
R72AMH	S. Enteritidis strain GD1011	Raw chicken meat	S	I	S	R	S
R81BH	S. Typhi strain 541	Raw chicken meat	S	R	S	S	S
R32AH	S. Typhi strain 3N4	Pre-cooked chicken meat	S	I	S	S	S
R51BH	S. Enteritidis strain FORC_052	Stored pre-cooked chicken meat	S	I	S	S	S
R42AH	S. Enteritidis strain FORC_052	Refilled drinking water	S	I	S	R	S

Note: S = Susceptible, I = Intermediate, R = Resistant.

in the finished product. The fact that *Salmonella* spp. was also found in pre-cooked chicken meat and fried chicken indicated inadequate heat processing during cooking or recontamination after the heating process.

Salmonella spp. contamination in raw chicken meat found in this study (9 of 18 samples, 50%) supports a previous study indicating that poultry was one of the major reservoirs of

*Salmonella* spp. (Levin, 2009). *Salmonella* was also detected in one of three samples of homemade seasoning (Figure 2). The traditional mortar which was used for seasoning preparation likely contributed to the contamination, since *Salmonella* spp. was also found in one of the three mortars sampled. As a traditional mortar is usually made from stone, if it was not washed properly, it can retain *Salmonella* spp. originating from, for example, water

used for cleaning. Another study showed that *Salmonella* spp. was found in cutlery that was washed using tap water in 24.49% of restaurants in Banda Aceh (Marissa & Arifin, 2014).

After pre-cooking, the prevalence and number of *Salmonella* spp. were reduced. However, *Salmonella* spp. was still found in one of sixteen samples (0.4 MPN/g). Cooking at an internal meat temperature of 65 °C for 6 min can reduce *Salmonella* spp. by more than 7  $\log_{10}$  (Food Standard Australia New Zealand, 2017). During storage, the number of *Salmonella* spp. slightly increased, probably due to the ability of *Salmonella* spp. to grow at room temperature. *Salmonella* spp. is reported to have the ability to grow in chicken breast meat (skinless) stored at 13 °C and 21 °C for 3 h, as well at 30 °C for 4 h (Ingham et al., 2007).

This study revealed that there are many factors such as water, seasoning, cooking equipment and food handler's hands enabling Salmonella spp. to contaminate chicken meat during the preparation and cooking. Ground water may be one of the sources of Salmonella spp. contamination, with a prevalence of 20.0% (2 of 10 samples) (Figure 2). A study of an outbreak of gastroenteritis in Croatia found that Salmonella enterica subsp. enterica serovar Enteritidis was present in well water used by patients (Kovačić et al., 2017). In addition, refilled drinking water used by households was also probable as a source of contamination with a prevalence of 50.0% (2 of 4 samples) (Figure 2). A recent study showed that refilled drinking water in Jember University Indonesia was detected containing Salmonella sp. (Nurhayati et al., 2018). In addition, during the preparation of chicken meat, 60% of housewives did not use separate utensils for cooked and uncooked food (Rosniawati et al., 2018). Salmonella spp. has the ability to form a biofilm on the surface of cooking equipment, leading to bacterial contamination on the product prepared (Myszka & Czaczyk, 2011). Therefore, proper washing practices of cooking wares are required. This study showed that unwashed food handler's hands and the use of well water for hand washing are potentially becoming the source of Salmonella spp. contamination (Figure 2). A previous survey showed that 20% of housewives were not aware of the importance of washing their hands before preparing fried chicken (Rosniawati et al., 2018). Hand sanitation is one of the important factors associated with Salmonella spp. contamination (Trimoulinard et al., 2017). In general, hand washing enables microorganisms on hands to be eliminated. Hand washing with soap for 20 seconds can reduce *E. aerogenes* artificially added to hands by a total of 1.7  $\pm$  0.8 log CFU/mL. This treatment was more effective in comparison to hand washing without soap, with a total reduction of 1.0  $\pm$  0.4 log CFU/mL (Jensen et al., 2015).

This study revealed that although *Salmonella* spp. was not found in prepared seasoning from the market, housewives should be aware of the presence of other microbial hazards. Other study showed that ground chili in a traditional market in Bogor contained total microorganism content of 7.9 x  $10^4$  – 1.9 x  $10^7$  CFU/g (Rosaria & Rahayu, 2008).

Among 23 samples which were found positive for *Salmonella* using PCR, only 8 samples or 8 isolates had thick DNA bands. These samples were then sequenced. The sequencing results using 907R primer showed that *Salmonella* spp. isolate of raw chicken

meat demonstrated 99% conformity to the sequence genome of four different serovars of *Salmonella enterica* including *S.* Bergen, *S.* Typhimurium, *S.* Enteritidis and *S.* Typhi. Strain FORC\_030 has a characteristic relationship with *Salmonella enterica* serovar Typhimurium (Lee et al., 2018). Various *Salmonella* serovars have been found on raw chicken meat. In Colombia, *Salmonella* serovar on raw chicken carcasses were identified including *Salmonella* Paratyphi B dt +, Heidelberg, Enteritidis, Typhimurium and Anatum (Donado-Godoy et al., 2014). In Vietnam, however, *Salmonella* serovar on chicken carcasses from two cities and two provinces including Albany, Agona dan Dabou were identified (Ta et al., 2012).

Salmonella serovar on chicken processed chicken meat has 99% conformity to the sequence genome of two different serovars of Salmonella enterica including serovar S. Typhi and serovar S. Enteritidis. Salmonella spp. isolates on refilled drinking water meanwhile have 99% conformity to the sequence genome of S. Enteritidis. These serovars were also found in groundwater samples of gastroenteritis outbreak that occurred in Sibenik, Croatia (Kovačić et al., 2017).

This study showed that Salmonella serovars (S. Typhimurium and S. Enteritidis) from raw chicken meat were resistant to nalidixic acid, streptomycin, and tetracycline (Table 4). This result was in accordance with previous studies. Salmonella spp. isolates derived from chicken production chains in slaughterhouses and retail markets exhibited high resistance to tetracycline (77%) and nalidixic acid (41%) (Li et al., 2013). Another study reported that Salmonella isolates derived from broiler chicken (44.4%) (Aprillian et al., 2015) and poultry origin (77.8%) (Myšková & Karpíšková, 2017) also showed resistance to nalidixic acid. This study revealed that all Salmonella serovars derived from raw chicken meat, pre-cooked chicken and refilled drinking water (S. Typhimurium, S. Bergen, S. Enteritidis, and S. Typhi) were considered "susceptible" to trimethoprim-sulfamethoxazole (100.0%), ampicillin (100.0%) and tetracycline (87.5%) (Table 4), which contrasts with previous studies. Salmonella isolates from chicken production chains (slaughterhouses and retail markets) displayed the greatest resistance to sulfamethoxazole/ trimethoprim (43%) (Li et al., 2013), while the other study found that Salmonella isolates from poultry and environmental samples were resistant to ampicillin (89.5%) (Chuah et al., 2018). This study showed that S. Typhimurium strain FORC\_030 can be classified as multidrug-resistant.

# **6 Conclusions**

The presence of *Salmonella* on raw chicken meat indicated that initial contamination plays an important role as one of the main sources of *Salmonella* in the preparation and cooking of fried chicken. Other factors, i.e. water, seasoning, cooking ware, and food handler's hands, were found to also contribute considerably to the presence of *Salmonella* in fried chicken. Since *Salmonella* serovars found on the preparation and cooking of fried chicken showed resistance to a particular antibiotic, the consumer should be aware that they should apply good handling and cooking practices during the preparation of fried chicken.

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