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Detection of *Salmonella typhimurium* in retail chicken meat and chicken giblets

Doaa M Abd El-Aziz*

Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

PEER REVIEW

Peer reviewer
Dr. Moemen A. Mohamed, Poultry Disease Department, College of Veterinary Medicine, Assiut University, Assiut-Egypt.
Tel: 0020882334699 internal line:171
0020882349991
E-mail: moemenassiut2002@yahoo.com

Comments
The study is up-to-dated and a valuable research work in which authors have demonstrated the presence of *S. typhimurium* in poultry meat and poultry as a vehicle for pathogen transmission to human consumers.
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ABSTRACT

Objective: To detect *Salmonella typhimurium* (*S. typhimurium*), one of the most frequently isolated serovars from food borne outbreaks throughout the world, in retail raw chicken meat and giblets.
Methods: One hundred samples of retail raw chicken meat and giblets (Liver, heart and gizzard) which were collected from Assiut city markets for detection of the organism and by using Duplex PCR amplification of DNA using *rfbJ* and *fliC* genes.
Results: *S. typhimurium* was detected at rate of 44%, 40% and 48% in chicken meat, liver and heart, respectively, but not detected in gizzard.
Conclusions: The results showed high incidence of *S. typhimurium* in the examined samples and greater emphasis should be applied on prevention and control of contamination during processing for reducing food-borne risks to consumers.

KEYWORDS
Salmonella typhimurium, Chicken meat, Duplex PCR

1. Introduction

Poultry meat is the combination of muscle tissue, attached skin, connective tissue, and edible organs of avian species commonly used for food. Chicken meats comprise about two-thirds of the total production in the world[1].

Poultry meat is one of the most popular food products worldwide. Several nutritional factors such as high level of protein and low fat content and favorable content of unsaturated fatty acids contribute to the popularity of poultry meat, of which sensory, dietary and economic factors are important. Poultry meat is easy to prepare at home and widely used in restaurants and fast-food establishments. There is no primary religious restriction on the consumption of poultry meat[2].

Poultry products have always topped the incidence of salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe[3]. Contamination with *Salmonella* in poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation[4]. The Center for Disease Control and Prevention has estimated that as of May 7, 2013, a total of 146 persons infected with the outbreak strain of *Salmonella*

*Corresponding author: Doaa Mohammed Abd El-Aziz, PhD, Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.
Tel: +2 088 241 1640
E-mail: doaassiut@yahoo.com
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typhimurium (*S. typhimurium*) have been reported from 26 states[5]. *Salmonella enterica* serotypes enteritidis and typhimurium are the most predominant isolated organisms in most *S. typhimurium* cases associated with the consumption of contaminated poultry, pork and beef products[6]. The Panel on Biological Hazards was asked to assess the public health risk posed by "*S. typhimurium*-like" strains, as *S. typhimurium* isolates are commonly found to be antimicrobial resistant, and to evaluate the analytical methods currently used for identifying these emerging strains, in particular to advise whether the public health risk, when detecting these strains in animals or food, should be considered similar, more or less important than (other) *S. typhimurium* strains[7]. PCR based on oligonucleotide primers called m-PCR has been developed which is more quickly and sensitive than bacterial culture[8].

Therefore the objective of this study is to detect the prevalence of *S. typhimurium* in frozen raw chicken meat and giblets to take care during cooking and consumption of these products and confirmation of isolates using Duplex PCR.

2. Materials and methods

2.1. Collection and preparation of samples

One hundred samples of frozen raw chicken meat, liver, heart and gizzard (25 each) were collected from Assiut city markets. Samples were transported to the laboratory immediately after collection in an ice chest and samples were kept frozen until analysis. Thawing of samples occurred during overnight incubation in refrigerator[9].

2.2. Isolation and identification of salmonella

Twenty-five grams of each sample were put into a stomacher bag containing 225 mL buffered peptone water and homogenized using a stomacher incubated at 37 °C for 16 to 20 h. One mL was transferred to 10 mL selenite cystine broth and incubated for 20-24 h at 37 °C. Plating carried on XLD agar and incubated at 37 °C for 24 h. The plates examined for typical colonies of *salmonella* (red with black center). Presumptive *Salmonella* colonies were confirmed by biochemical test (Indole, Methyl Red, Voges-Proskauer, Citrate and urease and glucose (TSI)).

2.3. DNA extraction

A single colony of each isolate on agar plate was picked and suspended in 200 μL of distilled water. After vortexing, the suspension was boiled for 5 min, and 50 μL of the supernatant was collected after centrifuging for 10 min at 14000 r/min[8].

2.4. Primers

For D-PCR assay, two primer sets were selected. *RfbJ* and *FliC* specific for the *rfbJ*, *fliC* genes of *S. typhimurium* to produce amplicon sizes 663-bp and 183-bp respectively[10]. Oligonucleotides primers were synthesized by Roche, Germany, The primers sequences and their corresponding genes are shown in Table 1.

Table 1
Primers sequences and their corresponding genes.

Primer	Target gene	Amplicone
Rfbj-s: 5´-CCA GCA CCA GTT CCA ACT TGA TAC-3´		
Rfbj-as: 5´-GGC TTC CGG CTT TAT TGG TAA GCA-3´	*rfbJ*	663
Flic-s: 5´-ATA GCC ATC TTT ACC AGT TCC CCC-3´		
Flic-as: 5´-GCT GCA ACT GTT ACA GGA TAT GCC-3´	*fliC*	183

2.5. Duplex PCR

PCR was performed with 5 μL of DNA sample, 25 μL of Go Taq Green Master Mix, 1 μM of each primer, 15 μL of DNase/RNase free water in a final volume of 50 μL. The reactions were performed in a DNA thermo cycler (Techne, cyclogene, UK). The m-PCR protocol consisted of the following steps: the initial denaturation step of 5 min at 95 °C; 35 cycles, with considering of 1 min at 95 °C, 1 min at 60 °C and 30 seconds at 72 °C; and a final extension step of 10 min at 72 °C. The PCR products were subjected to electrophoresis in 1% (w/v) agarose gel, stained with ethidium bromide and photographed under UV trans-illuminator then documented with a gel documentation apparatus.

3. Result

One hundred samples of retail raw chicken meat and giblets (Liver, heart and gizzard) which were collected from Assiut city markets for detection of *S. typhimurium*. Table 2 shows that *S. typhimurium* was detected at rate of 44%, 40% and 48% in chicken meat, liver and heart, respectively, but not detected in gizzard, by confirmation by duplex PCR (Figure 1A, 1B).

Table 2
Incidence of *S. typhimurium* in the examined samples.

Samples	Incidence of *S. typhimurium*		Total
	Number	%	
Raw chicken meat	11	44%	11(44%)
Liver	10	40%	22(29%)
Heart	12	48%	
Gizzard	ND		
Total			33%

ND: not detected.

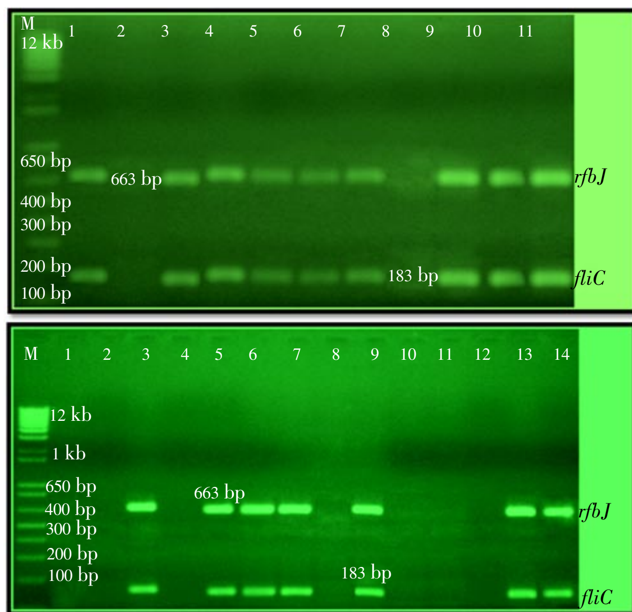

Figure 1. A and B: Gel showing duplex PCR amplification of DNA extracted from *Salmonella* strains for detection of *rfbJ* and *fliC* genes in *S. typhimurium*. Lanes M, 100-bp DNA size marker; Figure 1A showing *S. typhimurium* in lanes 1, 3-7 and 9-11; Figure 1B showing *S. typhimurium* in lanes 3, 5-7, 9 and13-14.

4. Discussion

The isolation of invasive *Salmonella* serotypes such as *S. typhimurium* and other pathogenic salmonellas in our study indicate the public health significance of these serovars as contaminated chicken meat and meat products may pose health hazards. This risk may further be higher if chicken meat or giblets are consumed undercooked or cross contamination in the kitchen with *Salmonella* during meal preparation[11].

In this study the incidence of *S. typhimurium* in raw chicken meat, liver, and heart were 44%, 40% and 48% respectively, but not detected in gizzard. Lower results reported by Dhaher, *et al.*[12] who isolated *Salmonella* sp. at rate of 24.76% and Alali, *et al.*[13], reported *Salmonella* prevalence of 27% in broiler chicken meat in Russia Federation. Another study conducted by Todd[14] in Ethiopia showed the incidence of *Salmonella* sp. contamination in retail chicken to be 13.3%. Also Molla and Mesfin reported *Salmonella* in chicken meat (15.4%), liver (34.5%), and heart (23.7%), identified of which *S. braenderup* which was the most frequent followed by *S. typhimurium*[15]. Abdellah *et al.* reported *Salmonella* contamination in chicken meat and giblets, 4 different serotypes were identified of which *S. typhimurium* (40.35%) was the most frequent[16]. *Salmonella* isolates found at level of 13.88%, 11.11% and 6.25% in chicken gizzard, liver, and breast, respectively. Higher findings were reported by Jerngklinchan *et al.* and Boniphace who isolated *Salmonella* at an incidence rate of 86% (190/221) and 42% (24/57) in chicken giblets, respectively[17,18].

The results showed that *Salmonella* isolated at higher rate from chicken meat than giblet which may interpret due to that the defeathering process may spread microorganisms between carcasses or from the defeathering equipment contributing to an increase in the numbers of psychrotrophs and aerobe mesophiles on the carcasses. The evisceration process provides an opportunity for cross contamination from human, equipments and worker's hands[19]. *Salmonella* and other microorganisms have the ability to survive and multiply in internal organs, especially the liver and heart as these areas provide sites where bacterial multiplication can occur without exposure to host defense mechanisms[20]. This probably explains the high incidence of *Salmonella* found in livers and hearts.

In conclusion contamination of chicken meat and giblets with *Salmonella* indicate bad microbiological quality of retail chicken which may due to contamination occur during processing or distribution.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This study deals with important food-borne pathogen *Salmonella enterica* var *typhimurium* that represent an important public health problem and an economic burden in many parts of the world. As well as the use of robust technique in identification of *Salmonella* which is D-PCR.

Research frontiers

From my viewpoint, this considered a good study for investigation the presence of one of important foodborne pathogen (*Salmonella*) in the poultry meat and giblets in Assiut province-Upper Egypt, Egypt. Furthermore this study uses D-PCR for rapid identification of *S. typhiumurium*.

Related reports

Number of researcher studied the prevalence of foodborne pathogens. Dookeran *et al.* 2012 reported 51-77% *Salmonella*'s presence in poultry meat in Trinidad and Tobago.

Innovations and breakthroughs

The results of this study revealed the presence of *S. typhimurium*'s in the poultry meat. Cross contamination and infection from foodborne *Salmonella* can lead to prolong illness and death in some cases.

Applications

The data in the article is helpful for the effective planning and implementation of food safety systems for the preservation and control of food related pathogenic bacteria. The article shows that poultry meat is a vehicle of bacterial transfer and plays an important role in diseases prevalence. This study support and suggest the use of D-PCR for rapid identification *S. typhimurium* to avoid the shortage and drawbacks of antisera usage that face us commonly.

Peer review

The study is up-to-dated and a valuable research work in which authors have demonstrated the presence of *S. typhimurium* in poultry meat and demonstration of poultry as a vehicle for pathogen transmission to human consumers.

References

[1] Ruban SW, Thiyageeswaran M, Sharadha R. Isolation and identification of *Salmonella* spp. from retail chicken meat by polymerase chain reaction. *Int J Microbiol Res* 2010; **1**(3): 106-109.

[2] Mulder RWAW. Hygiene during transport, slaughter and processing. In: RI Richardson, GC Mead editors. *Poultry meat science: Poultry science symposium series*. Massachusetts: CABI Publishing 1999; **25**: 277-283.

[3] Yang B, Xi M, Wang X, Cui S, Yue T, Hao H, et al. Prevalence of *Salmonella* on raw poultry at retail markets in China. *J Food Prot* 2011; **74**(10): 1724-1728.

[4] Dookeran MM, Baccus-Taylor GS, Akingbala JO, Tameru B, Lammerding AM. Transmission of *Salmonella* on broiler chickens and carcasses from production to retail in Trinidad and Tobago. *J Agric Biodivers Res* 2012; **1**(5): 78-84.

[5] Center for Disease Control and Prevention. Multistate outbreak of human *Salmonella typhimurium* infections linked to live poultry. Atlanta: Center for Disease Control and Prevention; 2013. [Online] Available from: http://www.cdc.gov/*salmonella*/typhimurium-live-poultry-04-13/index.html. [Accessed on 27 April, 2013].

[6] Vose D, Koupeev T, Mintiens K. A quantitative microbiological risk assessment of *Salmonella* spp. in broiler (*Gallus gallus*) meat production. Technical report submitted to EFSA. 2010. [Online] Available from: http://www.efsa.europa.eu/en/supporting/pub/183e.htm. [Accessed on 27 April, 2013].

[7] EFSA Panel on Biological Hazards. Scientific opinion on monitoring and assessment of the public health risk of "*Salmonella typhimurium*-like" strains. *EFSA J* 2010; **8**(10): 1826.

[8] Salehi TZ, Gharagozlou MJ, Shams N, Madadgar O, Fasaei BN, Yahyaraeyat R. Molecular characterization of a *Salmonella typhimurium* isolate from Caspian pony. *Iranian J Biotechnol* 2012; **10**: 49-53.

[9] Roberts D, Greenwood M. *Practical food microbiology*. 3rd ed. Oxford: Blackwell publishing Ltd; 2003, p. 58-65.

[10] Lim YH, Hirose K, Izumiya H, Arakawa E, Takahashi H, Terajima J, et al. Multiplex polymerase chain reaction assay for selective detection of *Salmonella enterica* serovar *Typhimurium*. *Jpn J Infect Dis* 2003; **56**: 151-155.

[11] FSIS/USDA. Compliance guideline for controlling *Salmonella* and *Campylobacter* in poultry. 3rd ed. United States: U.S. Department of Agriculture, Food Safety and Inspection Service; 2010. [Online] Available from: http://www.fsis.usda.gov/PDF/Compliance_Guide_Controling_*Salmonella*_Campylobacter_Poultry_0510.pdf. [Accessed on 27 April, 2013].

[12] Dhaher FH, Awni MN, Mahmood MM, Jamil HS. Isolation and diagnosis of *Salmonella* in animal origin food, import feed in Baghdad local markets and local poultry farms. *Iraq Acad Sci J* 2011; **5**: 1-19.

[13] Alali WQ, Gaydashov R, Petrova E, Panin A, Tugarinov O, Kulikovskii A, et al. Prevalence of *Salmonella* on retail chicken meat in Russian Federation. *J Food Prot* 2012; **75**(8): 1469-1473.

[14] Todd CD. Food borne and waterborne diseases in developing countries- Africa and the Middle East. *Dairy, Food Environ Sanitation* 1999; **21**: 110-122.

[15] Molla B, Mesfin A. A survey of *Salmonella* contamination in chicken carcass and giblets. *Revue Med Vet* 2003; **154**: 267-270.

[16] Abdellah C, Fouzia RF, Abdelkader C, Rachida SB, Mouloud Z. Prevalence and anti-microbial susceptibility of *Salmonella* isolates from chicken carcasses and giblets in Meknes, Morocco. *Afr J Microbiol Res* 2009; **3**: 215-219.

[17] Jerngklinchan J, Koowatananukul C, Daengprom K, Saitanu K. Occurrence of *Salmonella*e in raw broilers and their products in Thailand. *J Food Prot* 1994; **57**: 808-810.

[18] Boniphace JT. Cross-sectional study of *Salmonella* in chicken meat and offals in Addis Ababa, Ethiopia. Ethiopia: Addis Ababa University and Freie Universität Berlin; 2001.

[19] Jackson TC, Marshall DL, Acuff GR, Dickson JS. Meat, poultry and seafood. In: MP Doyle, LR Beuchat, TJ Montville, editors. *Food microbiology, fundamentals and frontiers*. 2nd ed. Washington DC: ASM. Press; 2001, p. 91-109.

[20] Gast R. *Salmonella* infections. 11th ed. *Diseases in poultry*. United States: Wiley; 2003, p. 587-597.