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Original Article

Isolation and Identification of *Salmonella* Typhimurium in Retail Meat Sources by Multiplex Polymerase Chain Reaction

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Abstract

Background: *Salmonella enterica* serovar Typhimurium is considered one of the most important emerging food-borne pathogens in public health worldwide. Meat is commonly known as the food sources responsible for the salmonellosis outbreak.

Methods: Overall, 141 different meat samples were randomly collected from local markets. The conventional culture method was performed to isolate *Salmonella* spp., and then, using two pairs of primers, a multiplex polymerase chain reaction (PCR) assay was employed to confirm the identification of isolated colonies as *Salmonella* spp. and determine serovars as Typhimurium.

Results: Out of 141 samples, 48 (34%) ones were presumptively isolated as *Salmonella* on the *Salmonella* agar medium and distributed as 24%, 23%, and 42% among veal, lamb, and chicken meat, respectively. However, the results of multiplex PCR showed that 4.9% of chicken meat was merely identified as *S.* Typhimurium. In general, *S.* Typhimurium isolates were found only in chicken meat.

Conclusion: *Salmonella* Typhimurium isolates were only observed in the chicken meat. Multiplex PCR was found to be a specific and rapid alternative method for the identification of various types of *Salmonella*.

Keywords: *Salmonella* Typhimurium, Meat, SA medium, Multiplex PCR

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Introduction

Typhoid is a serious problem in areas without clean water sources and proper sanitation. It affects around six million people worldwide and is estimated to cause 600 000 deaths a year. The disease is not common in developed countries, and most infections come from abroad or from migrants (1). Approximately 80% of infections and deaths occur in Asia, and the remaining cases belong to Africa and Latin America (2). *Salmonella* is a bacterium associated with animals. Animal skin and fur infections occur through the transmission of bacteria through the blood from the intestines of infected animals (3). This bacterium causes enteritis in poultry which is associated with a high mortality rate (4). It also causes death in newborn calves (5). These bacteria are not classified as the microbiota of the poultry intestinal flora, but they are introduced from the environment through insects, rodents, and feed. Infected adult animals show no outward signs, and infection can be spread by stable brushes and milking tools (6). *Salmonella* can also be transmitted between animals or from an infected animal to the veterinarian through the veterinary instruments used for detection (7). *Salmonella* can be isolated from the liver, spleen, and gallbladder of many species, including mammals (goats, sheep, and horses), birds (pigeons and ducks), Amphibians (frogs), and some fish species such as catfish (8). In 2009, more than 40 000 cases of salmonellosis (13.6 cases per 100 000 people) were tested by public health laboratories in the United States; this represents a decrease of about 15% from the previous year, but an increase of 4.2% from 1996 (9). Overall, the prevalence of *Salmonella* has not significantly changed since 1996 in the United States (10,11).

Non-typhoid *Salmonella* is the second most common cause of foodborne illness and causes 35% and 28% of hospital admissions and deaths in the USA, respectively (12). Among the many types of *Salmonella*, *Salmonella* Typhimurium and *Salmonella enteritidis* are among the most isolated types during foodborne outbreaks in the world (13). *S. enteritidis* is considered the leading cause of foodborne diseases (14); during recent decades, an increasing number of these bacteria has been isolated around the world (13,15).

Studies using mice play a key role in all areas of medical research. Furthermore, systemic infection patterns in mice have been widely used for more than 20 years. Mice are especially employed in infectious studies because of their anatomical similarity to humans. Mouse salmonellosis is an experimental pattern commonly used to mimic acute

infections in humans because the invasive disease caused by *S.* Typhimurium in mice is similar to the acute phase of human typhoid fever caused by *S. typhi* (16).

Diseases caused by *Salmonella* are exhausted to humans and cause the prolonged absence from work. Although mortality is relatively low, the cost of treatment is high, especially as these bacteria are starting to be resistant to many antimicrobial therapy regimens (17,18). It is known that *typhi* (and *paratyphi)* and Typhimurium are the only two types that cause the disease in humans as a result of eating infected meat or drinking contaminated water. Recently, the resistance of these bacteria to antibiotics has increased in our country. Therefore, the current work aimed to isolate and type *S.* Typhimurium from some Syrian veal, lamb, and chicken meat samples collected from different regions using conventional microbiology detection compared to the ones detected using the multiplex-polymerase chain reaction (PCR) technique. It was attempted to conduct subsequent studies on mice to determine the effectiveness of antibiotics and some alternative therapies against these bacteria.

Materials and Methods

Collection of Samples

One hundred forty-one different meat samples were collected, including 35, 81, and 25 samples of lamb, chicken, and veal meat from different markets in Damascus, respectively.

Isolation of Salmonella

Twenty-five grams of each meat sample were homogenized using a stomacher (Stomacher® 80 Biomaster Bags; Seward Ltd, UK) into 225 mL of buffered peptone water and incubated at 37˚C for 16-20 hours. Then, 1 mL was transferred to 10-mL selenite cysteine broth and incubated for 20-24 hours at 37˚C (19). The cultivation was performed on the medium of lysine iron agar; it is a differential medium for intestinal bacteria and consists of several materials per liter, including a pancreatic digest of gelatin (5 g), yeast extract (3 g), dextrose (1 g), L-lysine (10 g), ferric ammonium citrate (0.5 g), sodium thiosulfate (0.04 g), bromocresol purple (0.02 g), and agar (13.5 g). The selected isolates were then cultivated on *Salmonella* agar medium, which is a differential medium for the *Salmonella* genus. All plates were incubated at 37˚C for 24 hours.

Extraction of DNA

The cetyltrimethylammonium bromide method was used to isolate the DNA (20). Briefly, 2 µL of DNA extraction in TE buffer was read using a nanodrop machine. To detect the DNA concentration, TE buffer was employed as blank. Then, the concentration of 100 ng/mL in each sample was made and stored at -20˚C.

Multiplex Polymerase Chain Reaction

The multiplex PCR was applied to identify the specific *S.* Typhimurium isolates. Multiplex PCR amplification was performed using two sets of primer pairs. The primer pair, ST11 (5'-GCCAACCATTGCTAAATTGGCGCA-3') and ST15 (5'-GGTAGAAATTCCCAGCGGGTACTGG-3'), was specific to *Salmonella* spp. and targeted a randomly selected sequence of unknown function of 429 bp (21,22). The primer pair, Fli15 (5'-CGGTGTTGCCCAGGTTGGTAAT-3') and Tym (5' ACTCTTGCTGGCGGTGCGACTT-3'), was specific to the filiC gene of *S.* Typhimurium of 559 bp (19). Multiplex PCR was performed in a final volume of 25 µL containing 200 ng bacterial DNA, 25 pmol of each forward and reversed set primers, 0.2 mM of dNTP Mix, 1.5 mM $MgCl_2$, 1X PCR buffer, and 1U Taq DNA polymerase. The cycling conditions included one cycle at 95˚C for 5 minutes, 35 cycles (at 94˚C for 60 seconds, at 55˚C for 60 seconds, and at 72˚C for 60 seconds), then one cycle at 72˚C for 10 minutes. The results of multiplex PCR were observed under UV light after electrophoresis in agarose gel pre-stained with ethidium bromide.

Results

Isolation of Salmonella

On lysine iron agar medium, fifty-four positive isolates (38.3%) were obtained, which were distributed as 24%, 25.7%, and 48% among veal, lamb, and chicken meat, respectively, where the medium maintained its violet color, and the colonies appeared transparent with or without dark centers (Figure 1). However, using *Salmonella* agar medium, forty-eight positive isolates (34%) were obtained, which were distributed as 24%, 22.9%, and 42% among veal, lamb, and chicken meat, respectively; where the medium turned yellow around the isolates, taking a transparent color with or without dark centers (Figure 2). The distributions of *Salmonella* among different meat sources are shown in Table 1.

Molecular Typing Using Multiplex PCR

The multiplex PCR was performed on all samples, showing positive results on *Salmonella* agar medium in 6, 8, and 34 samples of veal, lamb, and chicken meat, respectively. The results in Table 1 were obtained after transferring it onto an agarose gel. As a result, only four isolates of *S.* Typhimurium were obtained in 81 samples of chicken meat (4.9%). However, no isolate of *S.* Typhimurium was found in veal and lamb meat samples. Table 1 presents the results of cultures and PCR, and Figure 3 shows the data of PCR on the agarose gel.

Discussion

In recent decades, *Salmonella*, especially Typhimurium, has rapidly developed resistance against antibiotics such as ampicillin, chloramphenicol, co-trimoxazole, and even against ciprofloxacin (23,24). In addition, multidrug-resistant enteric fever is a major problem worldwide (25,26).

Animals, particularly poultry, and their products (e.g.,

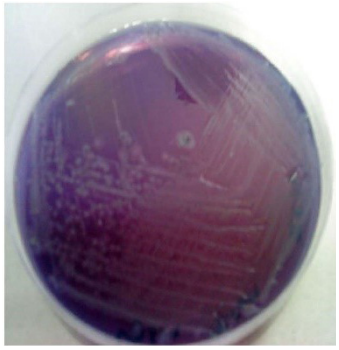

Figure 1. Appearance of *Salmonella* Colonies on Lysine Iron Agar Medium. *Note*. They were locked as transparent colonies with black centers

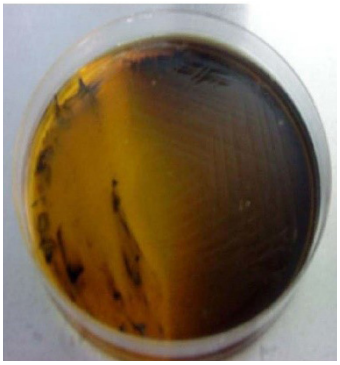

Figure 2. Appearance of *Salmonella* Colonies on *Salmonella* Agar Medium. *Note*. In this medium, transparent colonies with black spots are observed, and the color of the medium changes to yellow

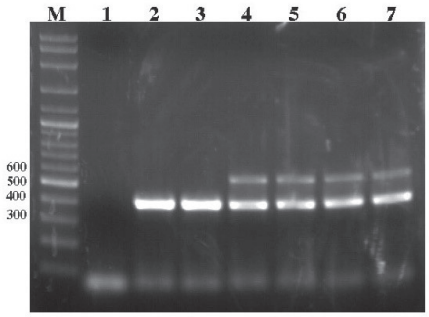

Figure 3. Agarose Gel Electrophoresis Results by Multiplex PCR. *Note*. PCR: Polymerase chain reaction; Lane M: GeneRuler DNA ladder (Thermo); Lane 1: Negative control (no template DNA was added); Lanes 2 and 3: Amplification of 429 bp fragment of *Salmonella* spp.; Lanes 4, 5, 6, and 7: Amplification of 429 and 559 bp fragments of *Salmonella* Typhimurium.

eggs and meat) are the main source of human infections caused by this pathogen (27). The epidemiological nature and severe pathogenicity of these pathogens are considered essential agents in food-related diseases. Further, diseases caused by these pathogens are of great importance to public health (28,29).

Almost all types of enteric *Salmonella*, especially Typhimurium, which infect humans and animals, can infect many types of animals and cause systemic diseases in many animals (30). However, traditional serotyping methods are highly time-consuming and laboratory intensive and require high-quality serological antibodies and extremely well-trained human laboratory staff (31-33).

The typing of *Salmonella* is essential for monitoring and controlling foodborne diseases. This allows rapid detection, identification of infection sources, and control of pandemics in addition to discovering new serotypes and new transmission routes of infection (34). Because of the great importance of *S.* Typhimurium as an animal pathogen, researchers have found it more important to reveal its presence than the other bacteria of the *Enterobacter* genus (35).

Phenotyping methods play an important role in determining the genus of *Salmonella*. Phenotyping using the Kauffman-White scale is considered in the primary phenotyping of *Salmonella* and is based on the detection of the presence of somatic and flagellated antigens on the surface of the bacterial cell, while phage profiling is employed to identify subtypes (36,37). However, the presence of some defects associated with serotyping, including cross-sections between serotypes, has encouraged researchers to discover faster and simpler diagnostic methods based on molecular techniques.

To eliminate serotyping-associated problems, PCR and other nucleotide-based methods allowed the acceleration of the detection of serotypes by single gene detection or gene amplification (32).

The economic losses caused by *S.* Typhimurium in the poultry industry cannot be ignored since this organism plays an important role in foodborne diseases.

Several studies have focused on the isolating and typing of *Salmonella* spp. and *S.* Typhimurium. It was found that most cases of *Salmonella* infection occur through eating

Table 1. Distributions of *Salmonella* Among Different Meat Sources: Bacterial Isolation and Multiplex PCR Reaction Results

Meat	Samples	LIA medium		SA Medium		Multiplex PCR			
						Salmonella spp.		*S. typhimurium*	
		No	%*	No	%*	No	%**	No	%**
Veal	25	6	24	6	24	2	8	-	-
Lamb	35	9	25.7	8	22.9	3	8.6	-	-
Chicken	81	39	48	34	42	29	35.8	4	4.9
Total	141	54	38.3	48	34	34	24.1	4	2.9

Note. PCR: Polymerase chain reaction; LIA: Lysine iron agar; SA: *Salmonella* agar. * % compared with total samples; ** % compared with suspected isolation in the SA medium.

contaminated food, especially food of animal origin (38,39).

Salmonella Typhimurium detection has been reported from poultry by several researchers. In a study of 200 samples of field meat from chicken, turkey, and pig, White et al (40) revealed the presence of 8 isolates of *S.* Typhimurium, four of which were from chicken and four from pigs. While Nagappa et al (41), who used PCR assay, found one *S.* Typhimurium isolate only in 100 chicken meat samples. In another study, 3% of *S.* Typhimurium belonged to chicken skin and feathers (42) using multiplex PCR. In contrast, Abd El-Aziz (43) showed that a proportion of 44% of Egyptian chicken meat contains *S.* Typhimurium.

In our study, only four isolates of *S.* Typhimurium were observed in 81 chicken meat samples (about 5% of the isolates). This percentage is highly consistent with most studies conducted on chicken meat. However, we observed no *S.* Typhimurium isolates in the meat of other animals used in our study. This is probably, in one way or another, due to the nature of different feeding of animal species and the contamination of the tools employed in the establishment and breeding of each type of animal.

Conclusions

Salmonella Typhimurium isolates were only detected in chicken meat. Multiplex PCR was found to be a specific, simple, and rapid alternative method for the identification of *Salmonella* spp. and allowed the observation of specific serovar (Typhimurium) contamination in the field conditions.

Authors' Contribution

Conceptualization: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
Methodology: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri, Samah Qasem, Laila Al Hallab.
Validation: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
Formal Analysis: Mazen Safi.
Investigation: Mazen Safi, Bassam Al Balaa.
Resources: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
Data Curation: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
Writing—Original Draft Preparation: Mazen Safi.
Writing—Review and Editing: Mazen Safi.
Visualization: Mazen Safi, Samah Qasem.
Supervision: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
Project Administration: Mazen Safi, Bassam Al Balaa, Ayman Al-Mariri.
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Competing Interests

None to be declared.

Ethical Approval

This article contains no studies with human participants or live animals performed by any of the authors.

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