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Original Research Article

Comparison between PCR and Culture Methods for Detection of *Salmonella typhimurium* from Food and Beverage

Kamil M. AL-Jobori* and Ali K. AL-Bakri

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

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In this study, Polymerase chain reaction (PCR) was compared to culture method to determine the presence of *Salmonella typhimurium* in selected food, beverage and ice cream in Baghdad, Iraq. A total of 400 different food samples were collected, including 25 sample each of Frozen meat, Minced meat, Frozen Chicken, Hamburger, Basturma, Fresh Kebab, Salad, Chickpea, Mayonnaise, Tabbouleh, Fruit Cocktail, Pomegranate juice, Melon juice, Orange juice, Raisin juice, and ice Cream from street vendors, exposed foods that are sold on the sidewalks, and in popular restaurants.were collected over a 7-month period between December 2013 and June 2014 and examined for the presence of *Salmonella typhimurium*. The results of culture method indicated that 73 samples (18.25%) out of the 400 showed positive results, and displayed that (10)40% of the examined frozen meat, (9)36% of minced meat, (16) 64% of frozen chicken, (5)20% of hamburger, (6)24% of fresh kebab, (4)16% of salad and ice cream, (3)12% of each basturma, fruit Cocktail, orange juice and raisin juice, (2)8% of mayonnaise and tabbouleh were contaminated with *Salmonella* Spp., whilst pomegranate juice and watermelon were not contaminated. The traditional method for the detection of *Salmonella* reveals *Salmonella* and bacteria-like *Salmonella*, a Serological detection was used to distinguish the *Salmonella* only. The results indicate 61 samples (83.56 %) out of the 73 were *Salmonella* spp., and 13(30.14%) samples out of 61 were *Salmonella typhimurium.* The results of conventional PCR indicated that 61 samples (15.25%) out of the 400 demonstrated positive results for the invA target gene as *Salmonella* spp. The results displayed that (8)32% of the examined frozen meat, (13)52% of frozen chicken, (6)24% of minced meat and fresh kebab, (4)16% of hamburger and salad, (3)12% of each basturma, Chickpea, fruit cocktail and raisin juice (2) 8% of each Mayonnaise, Tabbouleh, orange juice and ice cream were contaminated with *Salmonella* Spp., whilst pomegranate juice and watermelon not contaminated. On the other hand 22 samples (5.5%) out of the 400 demonstrated positive results for Mdh or fliC target gene as *salmonella typhimurium*. The results displayed that(5) 20% of the examined frozen meat, (7)28% of frozen chicken, (4)16% of minced meat (2)8% of each hamburger and fresh kebab, and (1)4% of basturma and salad were contaminated with *Salmonella typhimurium*, whilst other plant products, beverage and ice cream were not contaminated with *Salmonella typhimurium.* The results of this study revealed that the traditional method is less accurate because it detects *Salmonella* and bacteria-like *Salmonella*. Whilst PCR was a rapid and useful tool for detection of *Salmonella typhimurium* in food and beverage samples.

Keywords: *Salmonella typhimurium*, Food, Beverage, Culture method, PCR.

INTRODUCTION

Foodborne diseases represent a problem for public health because of their sanitary and economic consequences (Malorny *et al*., 2008). Microbial contamination of foods has been a major source of food borne disease in human beings. Microbial foodborne pathogens are widespread and cause thousands of cases of human illness every year in Iraq, resulting in major public health issues and substantial economic burden. *Salmonella* is regarded as one of the primary bacterial foodborne pathogens of significance to humans (Little *et al*., 2007). Poor sanitation of school's street foods (that are obtained from street vendors outside schools), exposed foods that are sold on the sidewalks, and in popular restaurants which is commonly found in Iraq, may lead to the disease that could risk human health. Therefore, *Salmonella typhimurium* is an important food hygiene indicator to access the quality of street foods. Common sources of transmitting foodborne pathogens are raw meat, including sheep, beef, buffalo, and poultry products.

Other food products such as milk, cheese, eggs, vegetables, fruits and ready-to-eat food also could be contaminated with these pathogens. Consumption of food contaminated by this pathogen has not only caused numerous infections, but also resulted in numerous foodborne outbreaks. The World Health Organization estimates that some 2.2 million deaths occur annually due to food and water-borne illnesses, and 1.9 million among them are children (Singh *et al*., 2013).

**Corresponding Author: Kamil M. AL-Jobori. Institute of Genetic Engineering and Biotechnology for Postgraduate studies, University of Baghdad-Iraq*
Email: kamilaljobori@yahoo.com

To better control microbial contaminations of food and consequently to reduce foodborne illnesses, rapid and accurate pathogen detection methods are required for effective monitoring of microbial pathogens in food supplies. Different methods have been developed to reduce the time required for the detection of this pathogen, because standard culture methods, require up to 5 d (including biochemical and serological confirmations (FDA, 2007, Chen *et al*., 2011). PCR-based methods have been found increasingly suitable for the detection of pathogens in food products because of their rapid and simple application (Olsen *et al*., 1995; Loongyai *et al*., 2010). Furthermore, PCR offers the possibility of specifically detecting the genes involved in the virulence of *Salmonella spp.* (Daum *et al*., 2002).

PCR studies have also been carried out to evaluate the specificity of *invA* primers to detect *Salmonella* by PCR technique (Kumar, 2012; das Chagas *et al*., 2013). Other studies have targeted *filC* gene for the detection of *Salmonella typhimurium* (Mirzaei *et al*., 2010, Dilmaghani *et al*., 2011). The objective of this study was to compare the sensitivity of a PCR assay to a culture method and to evaluate the 2 methods for the detection of *Salmonella typhimurium* in food, beverage and ice cream samples which were collected from street vendors, exposed foods that are sold on the sidewalks, and in popular restaurants in Baghdad, Iraq.

MATERIALS AND METHODS

Collection of samples

Through the period extending from December 2013 till June 2014, A total of 400 different food and beverage samples were collected, 25 sample of each (Frozen meat, Minced meat, Frozen Chicken, Hamburger, Basturma, Fresh Kebab, Salad, Chickpea, Mayonnaise, Tabbouleh, Fruit Cocktail, Pomegranate juice, Melon juice, Orange juice, Raisin juice, and ice Cream) from street vendors, exposed foods that are sold on the sidewalks, and in popular restaurants, Baghdad, Iraq. Samples were collected using sterile bags and transported to the Central Public Health Laboratory (CPHL) in Baghdad for detection of pathogenic bacteria (*Salmonella ser. Typhimurium*).

Preparation of Samples

Allot of 400 food and beverage samples were collected from street vendors, exposed foods that are sold on the sidewalks, and in popular restaurants, Baghdad, Iraq. samples were selected for the possibility of contamination of *Salmonella* during the handling, processing and storage of raw material of the foods and beverages. All samples that were labeled and recorded have to be analyzed as soon as possible. Samples can be refrigerated on 0-4 °C for not more than 24 h after collection. The pre-enrichment of samples was performed according to (ISO, 2002). Briefly, twenty five g of cheese sample was placed in 225 ml of nutrient Broth for the enrichment, incubated for 24 hours at 37° C.

Transfer one ml from the mix into Tetrathionate broth and Selenite Sistein broth, incubated for 24 hours at 37°C. Loop full of the enrichment broth was cultured on selective media (XLD), incubated for 24 hours at 37°C. The pre-enrichment culture was then divided into two aliquots. The first aliquot was subjected to DNA extraction by boiling method and the second aliquot was used to confirm the presence of *Salmonella* by standard cultural method, and followed by biochemical and serological confirmatory tests.

DNA Extraction

A volume of 1.5 ml of the post-enriched sample was centrifuged at 14,000g for 1 min, DNA was extracted using Presto Mini g DNA Bacteria Kit according to manufacturer's instructions(Geneaid, Korea). The extracted DNA was stored −20 ◦C until use.

Agarose Gel

After genomic DNA extraction, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA. Ten µl portion of the sample was analyzed by electrophoresis in agarose gel (2%), staining with ethidium bromide (Promega, USA), and visualized in UV light. A DNA molecular weight standard 50 bp was analyzed along with the samples (Wang *et al.,* 1997).

Specific primers

The primers listed in table 1 were selected for this study; these primers were provided in a lyophilized form, dissolved in sterile distilled water to give a final concentration of 100pmoles/ µL as recommended by provider and stored in deep freezer until used in PCR amplification.

PCR Assay

Detection of invA Gene in Salmonella spp

The polymerase chain reaction (PCR) amplification was performed in a final volume of 20µl containing 4µl of DNA template added 1µl of each primer given in table 1, and 14µl of nuclease free water, and distributed components at a rate of 16µl each sample. The samples were transported to a thermal cycle using the amplification program consisting of initial denaturation at 95°C for 5 min, 35 cycles with a denaturation at 94°C for 40s, annealing at 60°C for 1 min and extension at 72°C for 1min, followed by the final extension at 72°C for 10 min.

Detection of mdh Gene in Salmonella typhimurium

The polymerase chain reaction (PCR) amplification was performed in a final volume of 20µl containing 4µl of DNA template added to 1µl of each primer given in tables (3.10) and 14µl of nuclease free water, and distributed components at a rate of 16µl each sample. The samples were transported to a thermal cycle using the amplification program consisting of initial denaturation at 95°C for 5 min, 35 cycles with a denaturation at 94°C for 40s, annealing at 68°C for 1 min and extension at 72°C for 1min, followed by the final extension at 72°C for 10 min.

Detection of fliC Gene in Salmonella typhimurium

The polymerase chain reaction (PCR) amplification was performed in a final volume of 20µl containing 5µl of DNA template where added 0.6 µl of each primer given in tables(3.12) and 13.8 µl of nuclease free water, and distributed components at a rate of 16µl each sample, transported the samples to a thermal cycle using the amplification program consisting of initial denaturation at 94°C for 5 min, 35 cycles with a denaturation at 94°C for 40s , annealing at 57.5 °C for 40s and extension at 72°C for40s, followed by the final extension at 72°C for 7min.

Table 1. The sequences and conventional of forward and reverse primer

Species	Target gene	PCR primers' sequences (5' – 3')	Product size
Salmonella spp.	*invA gene*	invA F(5-GCTGCGCGCGAACGGCGAAG-3)	389-bp
		invA R (5-TCCCGGCAGAGTTCCCATT-3)	
Salmonella Typhimurium	*Mdh gene*	mdh F: 5′ TGCCAACGGAAGTTGAAGTG	261bp
		mdh R: 5′ CGCATTCCACCACGCCCTTC	
Salmonella Typhimurium	*fliC gene*	fliC F 5′ CGG TGT TGC CCA GGT TGG TAA T	620bp
		fliC R 5′ ACT GGT AAA GAT GGC T	

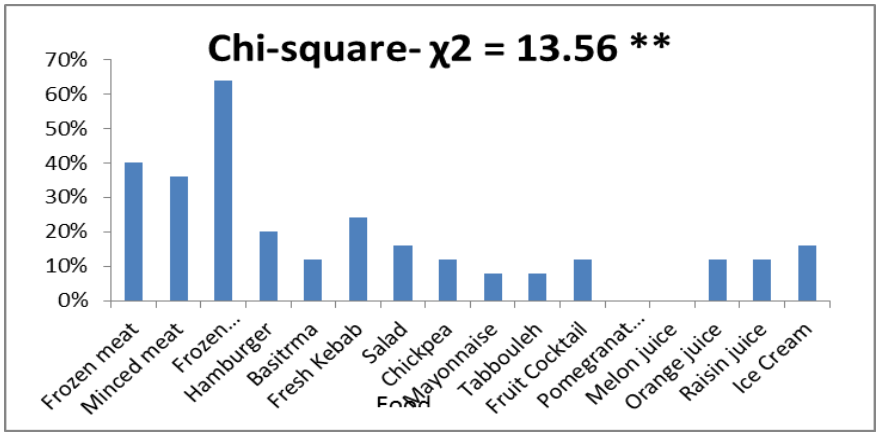

Figure 1. Percentage of *Salmonella* spp. isolated from food samples by using the traditional method.

The PCR products were subjected to electrophoresis in 2% (w/v) agarose gel, stained with ethidium bromide and photographed under UV trans-illuminator then documented with a gel documentation apparatus.

Statistical Analysis

The Statistical Analysis System- SAS (2012) was used for the evaluation of the effect of different factors in study parameters. Chi-square test was used to compare between the percentage in this study at 1% and 5% probability level.

RESULTS

Detection by Traditional Method

Through the period extending from December 2013 till June 2014, 400 different food sample were collected, 25 samples of each (Frozen meat, Minced meat, Frozen Chicken, Hamburger, Basturma, Fresh Kebab, Salad, Chickpea, Mayonnaise, Tabbouleh, Fruit Cocktail, Pomegranate juice, Melon juice, Orange juice, Raisin juice, and ice Cream) from street vendors, exposed foods that are sold on the sidewalks, and in popular restaurants. The results indicate that seventy three samples (18.25%) out of the 400 were positive results (Table 2). All kinds of food, beverage and ice cream were contaminated with *Salmonella* in varying degrees with the exception of pomegranate juice and watermelon, which were not contaminated. Frozen chicken, frozen meat, and minced meat were most polluted with *Salmonella* and differ significantly (χ^2 =13.56) from plant products. In general, meat products were the more contaminated than plant products (Table 2).

The microbiological procedure used for the detection of studied bacteria in food, beverage and ice cream were performed according to protocols of *Salmonella* organism. The results of culture method displayed that 40% of the examined frozen meat, 36% of minced meat, 64% of frozen chicken, 20% of hamburger, 24% of fresh kebab, 16% of salad and ice cream, 12% of each basturma, fruit Cocktail, orange juice and raisin juice, 8% of mayonnaise and tabbouleh were contaminated with *Salmonella* Spp., whilst pomegranate juice and watermelon not contaminated (Figure 1).

Table 2. *Salmonella* spp isolated from food samples by using the traditional method.

N. of sample / Type of food	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total	%
Frozen meat	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	10	40
Minced meat	-	+	-	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	9	36
Frozen Chicken	+	+	-	+	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	+	+	+	16	64
Hamburger	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	5	20
Basturma	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	3	12
Fresh Kebab	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	6	24
Salad	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	16
Chickpea	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	3	12
Mayonnaise	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8
Tabbouleh	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	2	8
Fruit Cocktail	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	3	12
Pomegranate juice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Melon juice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Orange juice	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	3	12
Raisin juice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	3	12
Ice Cream	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	4	16
Total																										73	18.25
Chi-square- χ^2	-------																									---	13.56 **

** (P<0.01).

Figure 2. The shape of *Salmonella* in food sample

Table 3. Serological identification of Salmonella serotype.

No.	Species	No.	Species
1	*salmonella enteritidis*	45	***salmonella Typhimurium***
2	*salmonella anatum*	46	***salmonella Typhimurium***
3	*salmonella enteritidis*	47	***salmonella Typhimurium***
4	*salmonella Dublin*	48	***salmonella Typhimurium***
5	*salmonella Dublin*	49	***salmonella Typhimurium***
6	*salmonella anatum*	50	***salmonella Typhimurium***
7	*salmonella anatum*	51	***salmonella Typhimurium***
8	*salmonella anatum*	52	***salmonella Typhimurium***
9	*salmonella anatum*	53	***Citrobacter spp***
10	*salmonella anatum*	54	***Proteus spp***
11	*Proteu sspp*	55	***Citrobacter spp***
12	*Citrobacter spp*	56	***Citrobacter spp***
13	*salmonella Typhimurium*	57	***Citrobacter spp***
14	*salmonella Typhimurium*	58	***Citrobacter spp***
15	*salmonella Typhimurium*	59	***salmonella ohio***
16	*salmonella Typhimurium*	60	***Salomnella enteritidis***
17	*salmonella Typhimurium*	61	***salmonella anatum***
18	*salmonella Dublin*	62	***salmonella anatum***
19	*salmonella Typhimurium*	63	***salmonella Typhimurium***
20	*salmonella Typhimurium*	64	***salmonella ohio***
21	*salmonella Typhimurium*	65	***salmonella braenderup***
22	*salmonella Typhimurium*	66	***salmonella braenderup***
23	*salmonella Newport*	67	***salmonella braenderup***
24	*salmonella Newport*	68	***salmonella braenderup***
25	*salmonella enteritidis*	69	***salmonella braenderup***
26	*salmonella enteritidis*	70	***salmonella braenderup***
27	*salmonella hato*	71	***salmonella anatum***
28	*salmonella hato*	72	***salmonella anatum***
29	*salmonella Typhimurium*	73	***salmonella braenderup***
30	*salmonella Typhimurium*		
31	*proteusspp*		
32	*proteusspp*		
33	*salmonella Typhimurium*		
34	*salmonella Typhimurium*		
35	*salmonella hato*		
36	*salmonella hato*		
37	*proteusspp*		
38	*proteusspp*		
39	*Salomnella ohio*		
40	*salmonella anatum*		
41	*salmonella anatum*		
42	*salmonella anatum*		
43	*salmonella anatum*		
44	*salmonella anatum*		

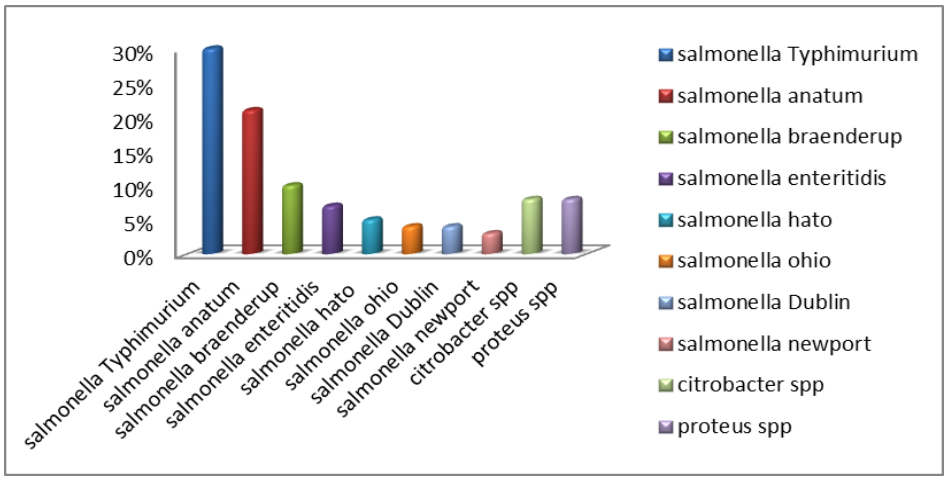

Figure 3. Percentage of microbial species detected in food samples by using the Serological method.

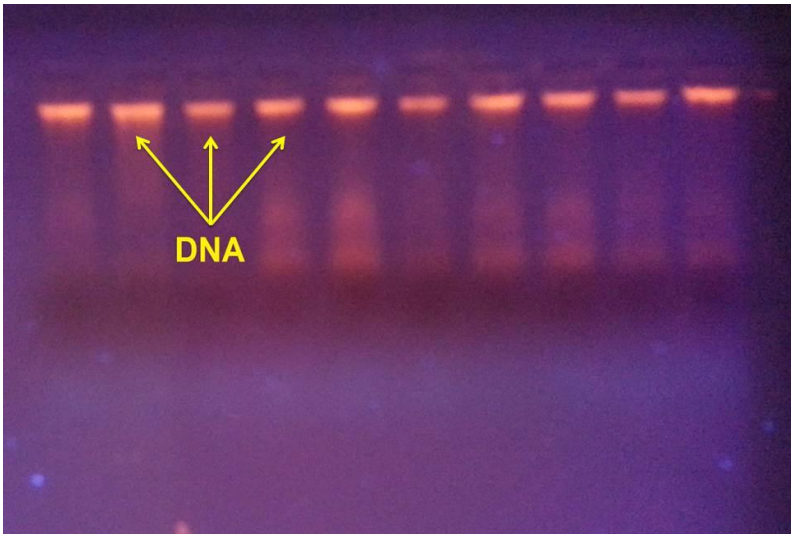

Figure 4. DNA bands extracted from food, beverage and ice cream samples using Genomic DNA extraction Kit (agarose 1%, TBE buffer (1X), 5V/Cm for 1h stained with ethidium bromide).

Depending on morphology, round pale colony with black center on XLD agar (Fig. 2), and the outcome of biochemical test clarified that the 3 isolates of *Salmonella* Spp., fermented glucose not lactose, appeared as red surface and yellow bottom of KIA with gas and H_2O formation.

The traditional method for the detection of *Salmonella* reveal *Salmonella* and bacteria-like *Salmonella*. A Serological detection was used to distinguish the *Salmonella* only. Serological identification of *Salmonella* spp. established the presence of *Salmonella* spp. in food, beverage and ice cream samples. The results indicate sixty one samples (83.56 %) out of the 73 were *Salmonella* spp., and 13 samples out of 61 were *Salmonella typhimurium* (Table 3). Serological examination showed that the highest contamination of food with bacteria was by *salmonella typhimurium* (30.14%) followed by *salmonella anatum* (20.55%) (Fig. 3).

Detection by Molecular Method

DNA Extraction from Food, Beverage and ice cream samples

The DNA was extracted from all food, beverage and ice cream samples by using a simple alternative method depended on enrichment of the samples on nutrient broth and using simple protocol genomic DNA kit. A high yield of purified DNA can be isolated, the DNA quality and integrity were estimated through remaking the DNA bands by electrophoresis on agaros 1% for 30 min. The bands appear sharp single not diffused and have no smear which may result from DNA degradation as shown in figure 4.9. The total DNA was used as templates in conventional PCR and mPCR reactions for *Salmonella* spp., and serotype *Typhimurium* research.

Table 5. *Salmonella typhimurium* isolated from food samples by using Conventional PCR method.

N. of sample / Type of food	Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total	%
Frozen meat	*invA*	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	8	32
	Mdh	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	5	20
	fliC	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	5	20
Minced meat	*invA*	-	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	6	24
	Mdh	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	4	16
	fliC	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	4	16
Frozen Chicken	*invA*	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	+	+	+	13	52
	Mdh	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	7	28
	fliC	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	7	28
Hamburger	*invA*	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	4	16
	Mdh	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	2	8
	fliC	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	2	8
Basitrma	*invA*	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	3	12
	Mdh	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4
	fliC	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4
Fresh Kebab	*invA*	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	6	24
	Mdh	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	2	8
	fliC	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	2	8
Salad	*invA*	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	16
	Mdh	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4
	fliC	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4
Chickpea	*invA*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	3	12
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Mayonnaise	*invA*	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Tabbouleh	*invA*	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	2	8
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Fruit Cocktail	*invA*	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	3	12
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Pomegranate juice	*invA*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Melon juice	*invA*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Orange juice	*invA*	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Raisin juice	*invA*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	3	12
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Ice Cream	*invA*	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	2	8
	Mdh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	fliC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
Total																											105	8.75
Chi-square- χ^2		-----																									---	11.07 **
Total	*invA*																										61	15.25%
	Mdh																										22	5.5%
	fliC																										22	5.5%
Chi-square- χ^2																											-----	8.92 **

** (P<0.01)

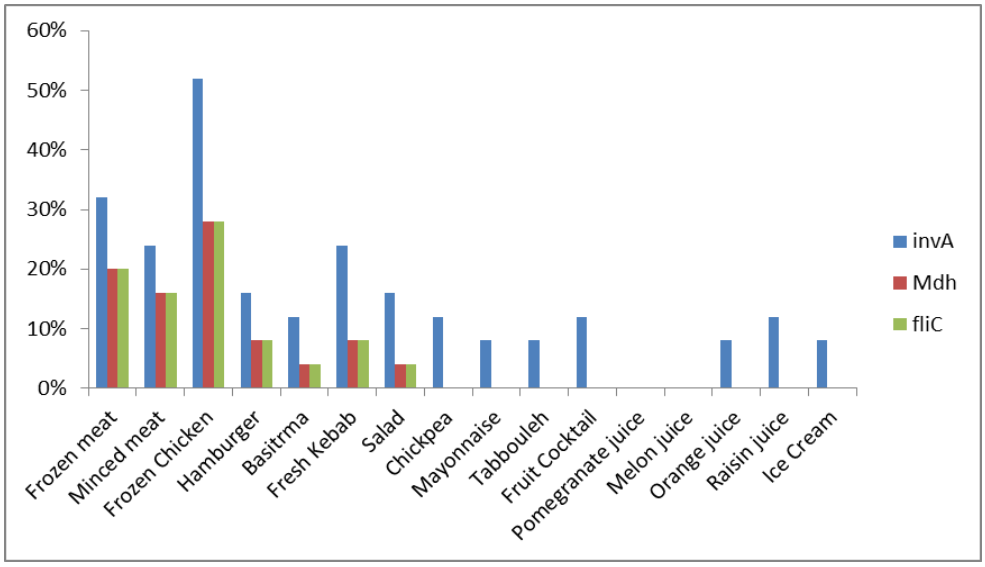

Figure 5. The percentages of *Salmonella* spp. and *Salmonella typhimurium* detected in food, beverage and ice cream.

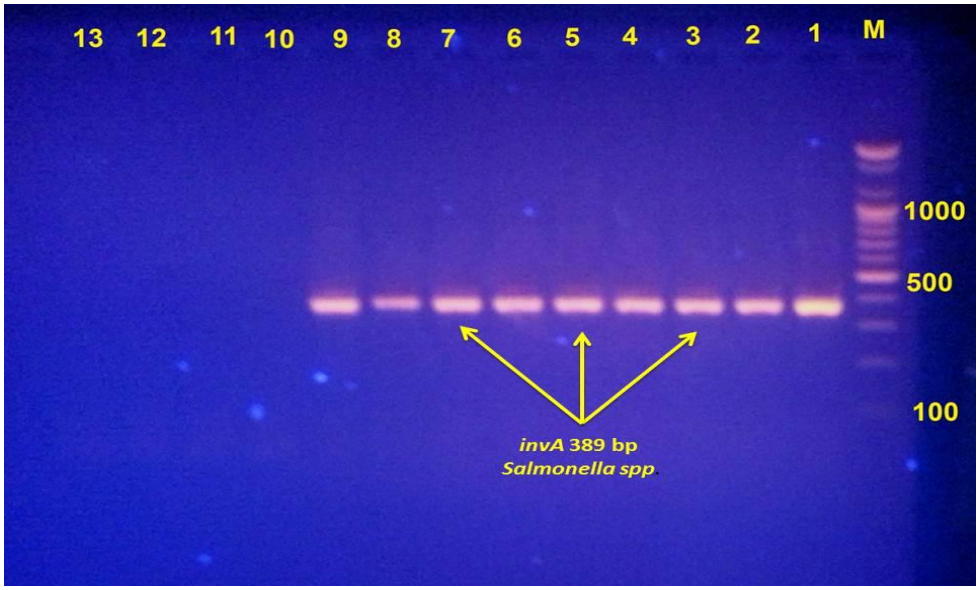

Figure 6. PCR amplification of 389bp *Salmonella* Spp., representative samples determined by PCR and detected by 2% agarose gel electrophoresis. Lane M: 50pb molecular size marker ladder. Lanes: 1-10 positive samples. Lane 11: negative control.

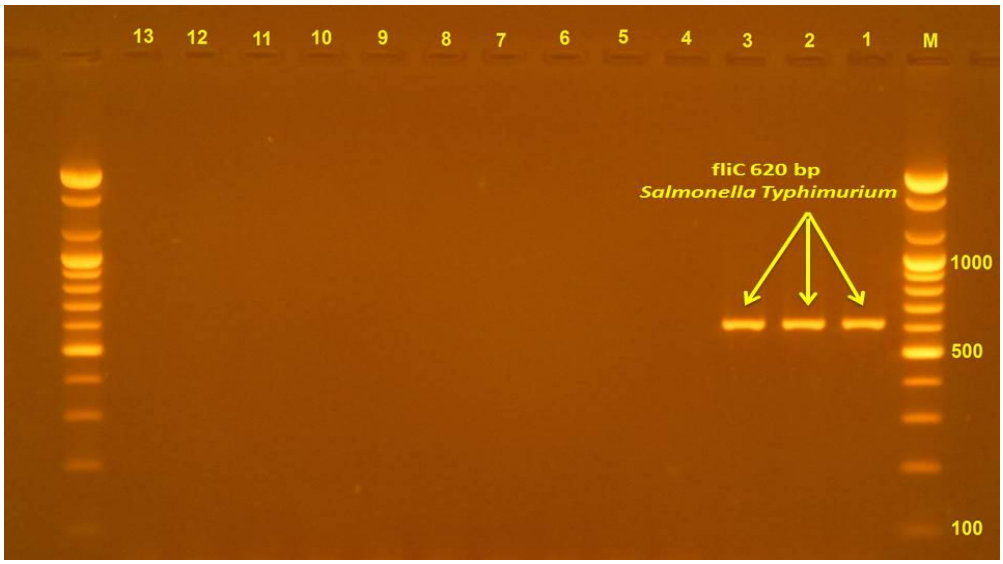

Figure 7. PCR amplification of 620 bp *Salmonella typhimurium*, representative samples determined by PCR and detected by 2% agarose gel electrophoresis. Lane M: 50pb molecular size marker ladder. Lanes: 1-10 positive samples. Lane 11: negative control.

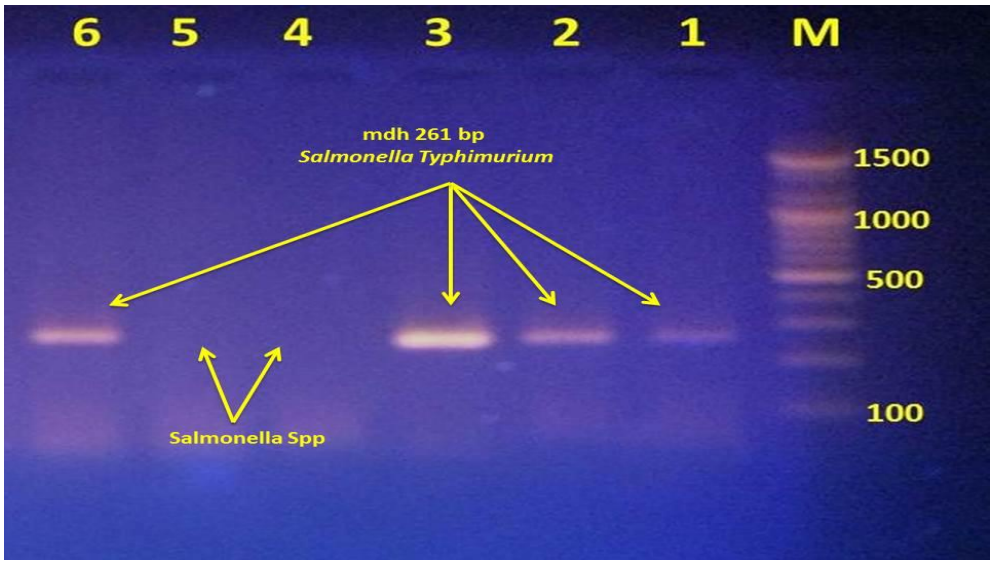

Figure 8. PCR amplification of 261bp *Salmonella typhimurium*, representative samples determined by PCR and detected by 2% agarose gel electrophoresis. Lane M: 50pb molecular size marker ladder. Lanes: 1-10 positive samples. Lane 11: negative control.

DNA Purity and Concentration

DNA concentration and purity were assessed by the use of nanodrop DNA concentrations were between 5-8ng/µl and purity ranging from 1.7-2 which indicates the presence of pure DNA.

Detection by Conventional PCR

The sixty one *Salmonella* strains were confirmed as *Salmonella* positive by the serological test. These strains, comprising 9 different *Salmonella* serotypes. The *invA*, *Mdh*, and *fliC* genes primer pairs specific for *Salmonella* were used in conventional PCR reaction on the genomic DNA isolated from samples, all demonstrated positive results for *the invA* target in conventional PCR assay as *Salmonella* spp. Testing of the *fliC* and *Mdh* serotype specific target yielded positive results for serotypes *Typhimurium* only and negative results for all other *Salmonella* serotypes tested (Table 4).

The results indicate sixty one samples (15.25%) out of the 400 demonstrated positive results for the *invA* target gene as *Salmonella* spp. (Table 5). All kinds of food and beverage were contaminated with *Salmonella* spp. in varying degrees with the exception of pomegranate juice and watermelon, which were not contaminated. Frozen chicken, frozen meat, minced meat and fresh kebab were most polluted with *Salmonella* and differ significantly **(P<0.01)** from plant products (χ^2 =11.07). In general, meat products were the more

contaminated from plant products (Table 5). The results of displayed that 32% of the examined frozen meat, 52% of frozen chicken, 24% of minced meat and fresh kebab, 16% of hamburger and salad, 12% of each basturma, Chickpea, fruit cocktail and raisin juice 8% of each Mayonnaise, Tabbouleh, orange juice and ice cream were contaminated with *Salmonella* spp., whilst pomegranate juice and watermelon were not contaminated (Figure 5).

On the other hands twenty two samples (5.5%) out of the 400 demonstrated positive results for *Mdh* or *fliC* target gene as *salmonella typhimurium* **(**Table 5). The results indicated that frozen chicken, frozen meat, meat products and salad were contaminated with *Salmonella typhimurium*. Whereas other plant products, beverage and ice cream were not contaminated with *Salmonella typhimurium*. The results displayed that 20% of the examined frozen meat, 28% of frozen chicken, 16% of minced meat 8% of each hamburger and fresh, and 4% of Basturma and salad were contaminated with *Salmonella typhimurium*., whilst other plant products, beverage and ice cream were not contaminated with *Salmonella typhimurium* (Figure 5).

PCR successfully amplified the DNA fragments corresponding in size as follows**:** *Salmonella* spp. 389 bp **(***invA* target gene**),** *Salmonella typhimurium* 620bp and 261bp **(***fliC* and *Mdh* target genes, respectively) figures (6-8).

DISCUSSION

Detection of pathogenic bacteria from 400 samples were tested by Conventional method, the results indicate seventy three samples (18.25%) out of the 400 showed positive results for more than one type as shown in table (2). All kinds of food ,beverage and ice cream were contaminated with Salmonella in varying degrees with the exception of pomegranate juice and watermelon, which were not contaminated. The presence of *Salmonella* in foods and beverages could be due to several reasons such as contamination of raw material, poor hygienic conditions, contamination of water sources and unsanitary processes of foods and beverages.

Frozen chicken, frozen meat, and minced meat were most polluted with *Salmonella* (Figure 1). The results indicated that meat products were the more contaminated than plant products. Compared to foods of animal origin, which are usually consumed once cooked, fruit and vegetables are mostly eaten raw and therefore a significant part of foodborne outbreaks due to the consumption of raw vegetables has been attributed to *Salmonella* (Cantoni & Bersani, 2010). In the current study, *S. **t**yphimurium* was detected in 64% of examined frozen chicken samples.

This result is higher than that reported by Abdellah *et al* (2009) who reported *Salmonella* contamination in chicken meat and giblets, 4 different serotypes were identified of which *S. typhimurium* (40.35%) was the most frequent, and Abd El-Aziz (2013) who detected *S. typhimurium* at rate of 44%, 40% and 48% in chicken meat, liver and heart, respectively, but not in gizzard. *Salmonella* spp. was analyzed in beef and chicken and in beef hamburgers, of the 80 hamburger samples analyzed, 22 (27.5%) were positive for *Salmonella* spp., 10 (12.5%) beef and 12 (15%) chicken and beef hamburgers (Fortuna *et al*., 2012). In a similar study Almeida Filho *et al*. (2006) analyzed 30 samples, of which 15 (30%) were contaminated with *Salmonella* spp. On the other hands other studies conducted to analyze *Salmonella* spp. in hamburgers did not reveal the presence of the pathogen in this food (Bezerra *et al*. ,2010)

The traditional method for the detection of *Salmonella* reveal *Salmonella* and bacteria-like *Salmonella*, so we need to work the serological detection to distinguish the *Salmonella* spp. Conventional techniques for the detection of bacteria involve intensive labour and time consuming cultural procedures including enrichment in selective media, agar isolation, biochemical and serological identification. Moreover, these methods may lead to false-negative results if the sample contains the target species in a high background of a mixed bacterial population (Amagliani *et al*., 2007). Traditional approaches for analysis of *Salmonella* has relied on cultural techniques and several selective differential media have used for differentiation. However, biochemical analysis for an enzyme associated with the particular pathogenic trait could be cross reactive with other enteric bacteria.

The results of serological test indicate that sixty one samples (83.56 %) out of the 73 were *Salmonella* spp. ,and 13 samples out of 61 were *Salmonella typhimurium* (Table 3). Serological examination showed that the highest contamination of food with bacteria was by *salmonella typhimurium* (30.14%) followed by *salmonella anatum* (20.55%) (Figure 3).

In the current study, detection of pathogenic bacteria from 400 samples were tested by conventional PCR, the results indicated that sixty one 15.25% out of 400 examined samples were positive results (Figure 5). This result is higher than that reported by Stock & Stolle (2001) and Molla *et al*.(2003) which his result (6.3%) and (12.1%)from minced meat samples, respectively, whereas lower than Hassanein *et al*. (2011) who reported that out of the total 75 meat samples examined, *Salmonella* was detected in 5 (20%) of minced frozen beef, 9 (36%) of frozen chicken leg and 13 (52%) of frozen chicken fillet samples. The method was evaluated with 1,293 naturally contaminated food samples and compared to the conventional cultural method, of 55 positive PCR samples, 45 were confirmed by the cultural method. (Made *et al*., 2004). Raafat *et al*. (2011) found high relationship between isolates from chicken meat and patient with food poisoning signs indicates a close genetic relationship between *Salmonella typhimurium* isolated from poultry meat and that isolates from human. Results obtained by Saeed *et al*. (2013) support this finding.

Primers targeting *invA, Mdh* and *fliC* genes were tested for rapid detection by conventional PCR. The *invA* gene was present in 61 samples, each of *Mdh* and *fliC* genes were present in 22 samples but not in all of tested samples. All three genes were specific to *Salmonella* because they were absent in other foodborne organisms tested, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* (Figures 6-8). A number of studies have targeted *invA* gene for the detection of *Salmonella* species (Upadhyay *et a*l., 2010; Kumar, 2012; das Chagas *et al*., 2013). Other studies have targeted *filC* gene for the detection of *Salmonella typhimurium* (Shanmugasundaram & Radhika ,2009; Mirzaei *et al*. ,2010, Dilmaghani *et al*. , 2011) . The *fliC* gene is responsible for the expression of a protein known as flagellin in *Salmonella* spp. (Thorns *et al*., 1996). This *invA* gene is recognized internationally as a standard for detecting the genus *Salmonella*, and its amplification has been used by many workers to detect contamination (Jeyasekaran *et al*., 2011) in chicken carcasses (Hassanein *et al*., 2011) and environmental samples (Moganedi *et al*., 2007

This result proved the specificity of PCR compared to the conventional culturing and serological method. All *Salmonella* carry the *invA* , *Mdh* and *fliC* genes, which is not carried by any other bacterial species. Therefore , if 389, 261 and 620 bp amplified product appeared in the PCR with the *invA, Mdh* and *fliC* primers it would indicate that the sample contains these

genes of *Salmonella.* In contrast to the 7 days culture method, in this study, 24 h pre-enrichment-PCR assay using *invA, Mdh* and *fliC* primers, offer a rapid and good diagnostic tool for the routine monitoring for detection of *Salmonella* in food, beverage and ice cream samples. The results of our study are generally in agreement with (Chiu & Ou, 1996). The results of this study revealed that the traditional method is less accurate because it detect *Salmonella* and bacteria-like *Salmonella*. Whilst PCR was a rapid and useful tool for detection of *Salmonella typhimurium* in food and beverage samples.

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