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Detection of *Salmonella* spp. in different food sources in Baghdad City: A Comparison between Conventional and Chromogenic Methods

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Abstract

Salmonella spp. was analyzed in different food sources using two methods conventional method and chromogenic method .The bacteria were cultured, isolated and biochemically characterized by the analytical profiling index (API 20E system)and serological test. Of the 400 food samples analyzed, 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.* were detected by the conventional method alone, The results of Chromogenic method was indicate that 61 samples (15.25%) out of the 400 were positive. 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 not contaminated .This methods detect *Salmonella* spp., Further identification of *Salmonella typhimurium* was achieved by using the serological test or API 20E test. In conclusion was that the traditional method is laborious , time consuming and less accurate because it detects *Salmonella* and bacteria-like *Salmonella*. Whilst Chromogenic method was found to accurately, sensitive, and remarkably low cost, shortening the time needed for the pathogenic agent identification

Keywords: *Salmonella* spp., *Salmonella typhimurium*, Food, Beverage, Culture method, Chromagenic method.

Introduction

Bacteria and viruses are widely found in nature and in the environment, such as in food, soil, water and the intestinal tracts of humans and animals [Ballantine *et al*., 1997]. Foodborne diseases are caused by ingestion of contaminated foods and include a broad group of illnesses [Ferreira *et al*., 2009]. The foods and food products can be contaminated at different points in the food production and preparation process. Salmonella serotypes are among the most common bacteria responsible for foodborne gastroenteritis and can be classified as a potential microorganism for bioterrorism [Khan *et al*., 2001]. The pathogen has been associated with foods such as raw milk, cheeses, ice cream, raw vegetables, raw and cooked poultry, raw meats and raw and smoked fish [Humphrey, 2006; Rapeanu *et al*., 2009; Suo *et al*., 2010]. Meat and meat products such as raw meat, ground meat and liver as well as meat products such as Sausage, Kofta, Burger and Luncheon sandwiches [Koseki *et al*., 2004; Curtis and Lee, 2005] .

The detection and characterization of *Salmonella* spp. in foods and water is very important in the control and prevention of food poisoning outbreaks. However, concerning health inspection routines, which require fast and reliable results so as to issue approval

certificates for commercialization and consumption, standard methodologies to evaluate *Salmonella* spp. in foods based on classical culture media are time-consuming and impractical. As a rule, standard microbiological techniques require between 5 and 7 days of laboratory work, especially when a large number of samples has to be processed, as observed in the food industry. These methods are also prone to producing false negative results, mainly due to the interference of other microorganisms in food samples that are direct competitors with *Salmonella* spp. bacterial cells [Fortuna *et al*., 2012]**.** Microbial contamination is an important problem in medicine, food, the pharmaceutical industry, and in biotechnology. Since food products have short shelf life, they are released before microbial results are available. Rapid detection of pathogens and spoilage microorganisms is critical to ensure food safety and quality [Tothill and Magan, 2003; Lazcka *et al*., 2007].

Over a long period of time, a large part of the innovations in this field has been linked to improvements and automation of phenotypic methods, using chromogenic or fluorogenic molecules. In parallel, powerful methods based on chemical characterization of bacteria themselves have emerged [Crunaire *et al*., 2014]. In compare with other diagnosis methods chromogenic media have more advantage and can be an appropriate alternative for conventional and routine procedure. According to Reis and Camargo [2008], chromogenic media are used to differentiate and isolate *Salmonella* spp. from other genera and also other numerous species. A chromogenic medium relies on the ability of *Salmonella* spp. to produce acid from propylene glycol, which differentiates these species from other enteric bacteria. Apart from this, the presence of the chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) affords to detect the production of beta-galactosidase D produced by another enterobacterium. *Salmonella* spp. cultures are positive only in the first reaction (acid production from propylene glycol). These colonies are brilliant red in color, while *Escherichia coli* and other coliforms are positive only for beta-galactosidase D, and form blue colonies. *Proteus* spp. are negative for both reactions and therefore its colonies are transparent. *Citrobacter* spp. form violet colonies due to the combination of colors (red and blue), since the two reactions are observed [Garrick and Smith, 1994; Pignato *et al*., 1995].

Chromogenic culture media is considered one of the most popular techniques applied for the detection of microbial pathogens nowadays for its sensitivity, accuracy and remarkably low coast, these media are very specific containing different components within its composition which acts like a substrate targeting certain enzymes produced by variety of pathogenic microorganisms to exhibit a distinguished color. In addition, Chromogenic culture media is a rapid technique of detection as it neglect the additional steps of sub-culturing and further biochemical tests thus shortening the time needed for the pathogenic agent identification [Tavakoli *et al*., 2008]. In recent years many research has been done in relation to rapid diagnosis pathogenic agent in food and water by chromogenic media has been published [El Shamy *et al*., 2008;; Salam and Tothill, 2009 ; Fortuna *et al*.,2012 Tallen *et al*. 2012; Albokari, 2013; Saeed *et al*., 2013; Crunaire *et al*., 2014; Zaghloul *et al*., 2014].

There has been little information regarding the incidence of street-food related diseases. This has raised many concerns because the conditions under which street vendors operate are usually unsuitable for the preparation and selling of food [Bryan *et al*.,1988 ; Mosupye and Von Holy , 1999; Radji *et al*., 2010]. In most cases running water is not available at vending sites and hand and dishwashing are usually done in one or more buckets or pans of water, sometimes without soap. Waste water and garbage are discarded in the streets providing food and harborage for insects and rodents. Foods are usually not effectively protected from dust and flies which may harbor food borne pathogens also safe food storage temperatures are difficult to maintain [Bryan *et al*.,1988; Ekanem,1998]. Thus, there are potential health risks associated with initial contamination of raw foods with pathogenic bacteria as well as subsequent contamination by vendors during preparation and through post-cooking handling and cross contamination [Bryan *et al*., 1988; Zaghloul *et al*., 2014]. According to Scott and Gravani [2003], temporary food service, such as mobile unit may operate on a more regular basis but unlike modern food service establishments operate under less than optimum conditions. The goal of this study was to evaluate the performance of chromogenic method for the detection of *Salmonella Spp.* in different food sources sold in streets and popular restaurants in comparison to conventional methods.

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, burger, 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.

Pre-enrichment

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.

Selective enrichment

Ten ml of the pre-enriched samples were Transferred to 100 ml of Tetrathionate (TT) and Selenite Sistein (SS) enrichment broth bottles respectively, incubated for 24 hours at 37°C.

Plating on solid selective media

Each selective enrichment broth bottle was snaked well and then a loopful from each was streaked onto plates of selective media (XLD) and Chromagenic gar *Salmonella* ,Hicrome *Salmonella* Differential Agar and Hicrome MM Agar, Modified [HiMedia Laboratories,2011a, b], incubated for 24 hours at 37°C.

Five typical colonies from each agar plate were picked and streaked on nutrient agar and incubated for 24 hours at 37°C to confirm the purity of the culture.

Samples were used to confirm the presence of *Salmonella* by standard cultural method and Chromagenic method, followed by biochemical such as Urease Test Medium [Atlas *et al*., 1995], Indole Test Medium [Collee *et al*., 1996], Simmon Citrate Test Medium [Wallace *et al*., 1998], API 20E strip, and serological confirmatory tests. A false positive result was defined as a typical colony that could not be identified as *ScdmoneUa* by biochemical and serological assay.

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 and Discussion

Detection by Traditional Method

The results indicate that 73 samples (18.25%) out of the 400 were positive results [Table 1]. 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 ($\chi 2$ =13.56) from plant products. In general, meat products were the more contaminated than plant products [Table 1].

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 64% of the examined frozen chicken, 40% of frozen meat, 36% of minced meat, 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].

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 H2O formation.

Table 1. *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).

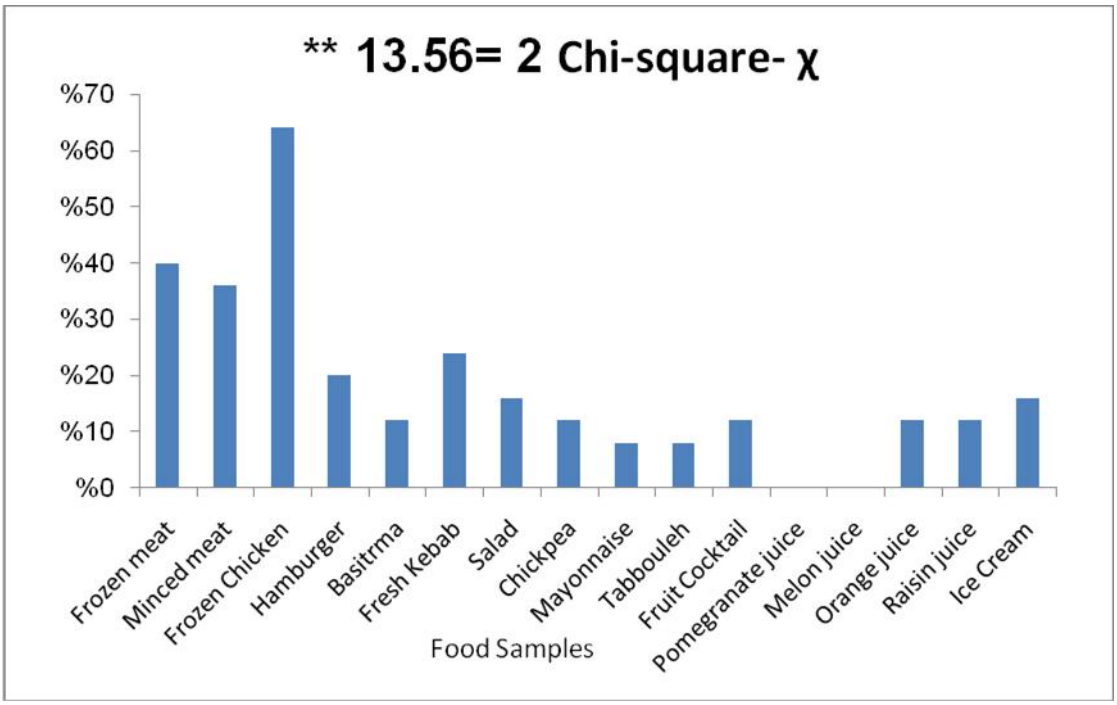

Figure 1. Percentage of *Salmonella* spp isolated from food samples by using the traditional method.

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 2]. Serological examination showed that the highest contamination of food with bacteria was by *salmonella typhimurium* (30.14%) followed by *salmonella anatum* (20.55%) [Table. 2].

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

Table 2. Serological identification of Salmonella serotype

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

Detection by Chromogenic Method

Detection of *salmonella* spp. were tested by Chromogenic methods. After culturing the enrichment broth on selective media , MM Agar showed the presence of blue green color[Fig. 3] and differential Agar showed the presence of pink color [Fig.4] suspected colonies were selected for biochemical identification of *Salmonella* spp. with API 20E strip.

Results of the API 20E Strip reading shows in table 3 Which shows that these isolates were able to give positive results for Arginine dihydrolase, Lysine decarboxylase , Ornithine decarbxylase, Citrate utilization, Hydrogen sulphide, Glucose Fermentation, Mannitol Fermentation, Inositol Fermentation, Sorbitol Fermentation , Rhamnose Fennentation, Melibiose Fennentation, and Arabinose Fermentation. Whilst they gave negative reactions for Beta- galactosidase, Urease , Tryptophane deaminase, Indole, Voges-Proskauer , Gelatin Liquefaction, Sucrose Fermentation, and Amygdalin. Fermentation. This indicated that 99.9% of isolates was *Salmonella* spp. [Fig.5].

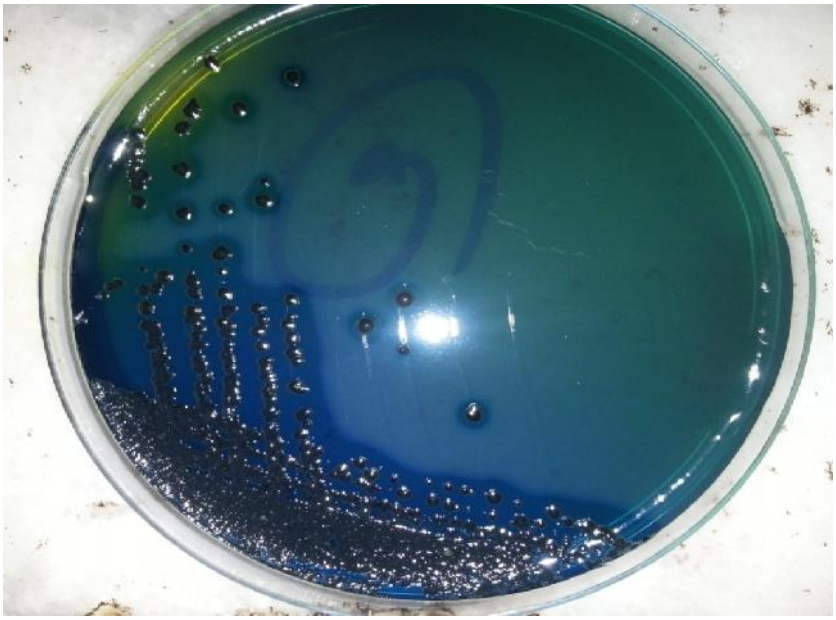

Figure 3. MM Agar Modified for identification and differentiation of *Salmonella* and non *Salmonella*.

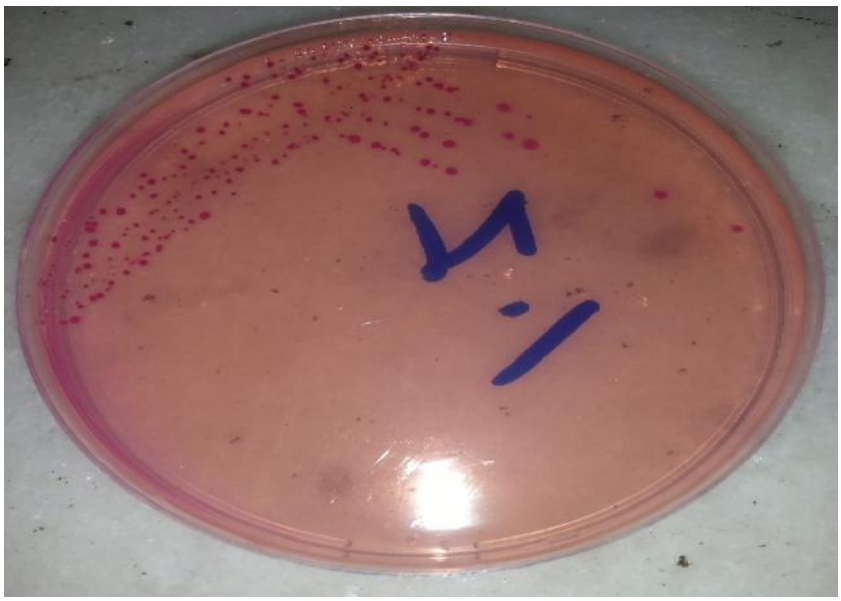

Figure 4. Differential Agar for identification and differentiation of *Salmonella* species

Table 3.Biochemical characterization by API 20E Tests grouping

API 20E tests	Results	API 20E tests	Results
ONPG	-	MEL	+
ADH	+	AMY	-
LDC	+	ARA	+
ODC	+		
CIT	+		
H_2S	+		
URE	-		
TDA	-		
IND	-		
VP	-		
GEL	-		
GLU	+		
MAN	+		
INO	+		
SOR	+		
RHA	+		
SAC	-		

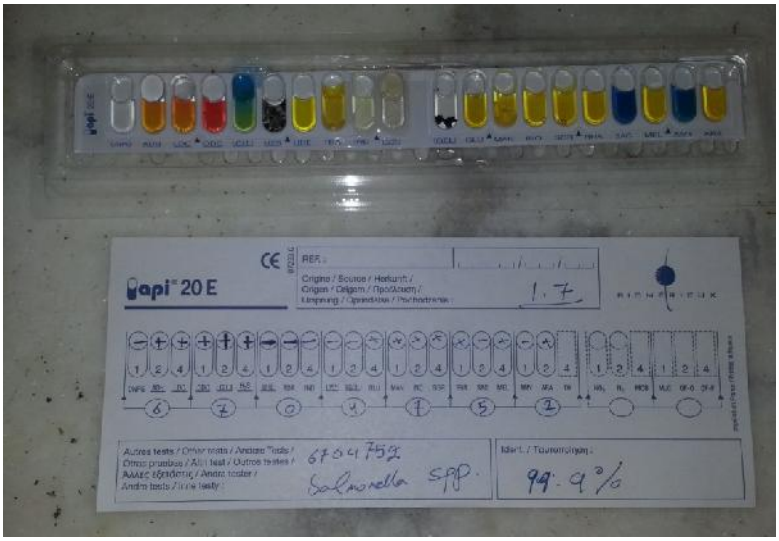

Figure 5. Api 20E test for the identification of *Salmonella* spp.

The results indicate sixty one samples (15.25%) out of the 400 were positive results is shown in table 4. 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 (χ^2 =11.07) from plant products . In general , meat products were the more contaminated from plant products[Table 4].

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 not contaminated [Fig. 6]. Chromogenic method detect *Salmonella* spp., Further identification of *Salmonella typhimurium* was achieved by using the serological test .

Table 4. *Salmonella* spp isolated from food samples by using Chromogenic Agar 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	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	8	32
Minced meat	-	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	6	24
Frozen Chicken	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	+	+	+	13	52
Hamburger	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	4	16
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	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8
Raisin juice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	3	12
Ice Cream	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	2	8
Total																										61	15.25
Chi-square- χ^2	------																									---	11.07 **

** (P<0.01).

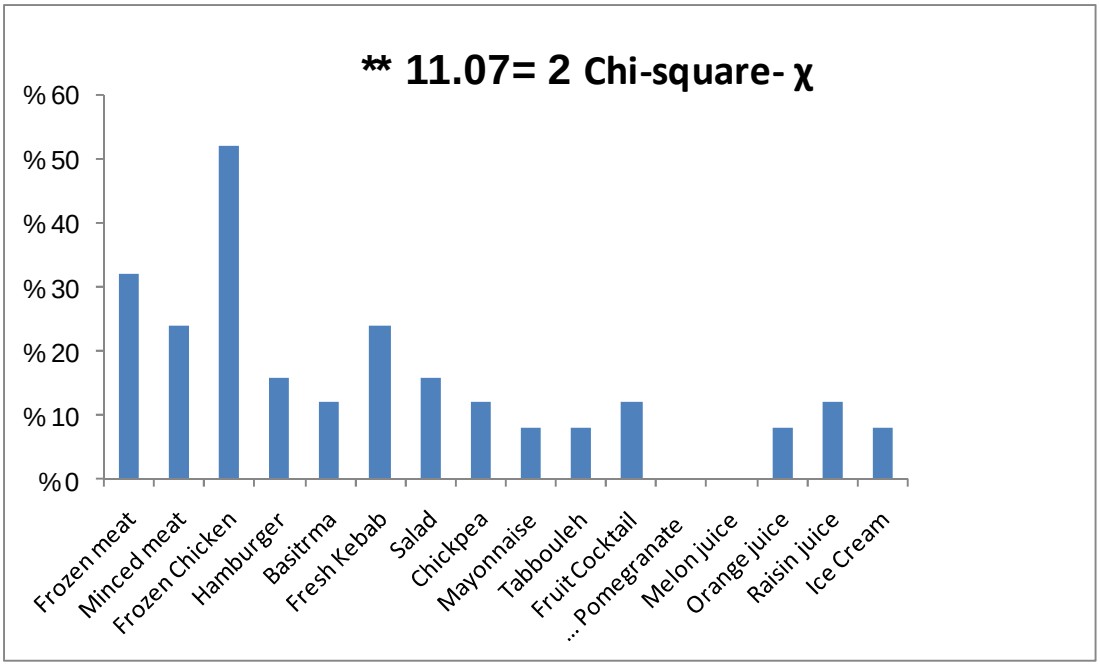

Figure 6. Percentage of *Salmonella* spp isolated from food samples by using the Chromogenic method.

Discussion

Four hundred of food sources which street-vended , and in popular restaurants, including 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 were investigated for their presence of *Salmonella* using two different microbiological examination methods including classic selective media and chromogenic media. Results of Conventional method indicate 73 samples (18.25%) out of the 400 showed positive results for more than one type as shown in table 1. 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. typhimurium* 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 that need further serological detection to distinguish the *Salmonella* spp. Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests [Ben Salem *et al*., 2010].

Traditional culture-based methods for detecting Salmonella are reliable but labor-intensive and time-consuming, demanding several days for a definitive result[Amagliani *et al*., 2007 ;Kataria *et al*., 2013]. 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 61 samples (83.56 %) out of the 73 were *Salmonella* spp. ,and 13 samples out of 61 were *Salmonella typhimurium* [Table 2]. Serological examination showed that the highest contamination of food with bacteria was by *salmonella typhimurium* (30.14%) followed by *salmonella anatum* (20.55%) [Table 2].

Detection of *salmonella* spp. were tested by Chromogenic methods. After culturing the enrichment broth on selective media , MM Agar and differential Agar suspected colonies were selected for biochemical identification of *Salmonella* spp. with API 20E strip. Depending on the results of the API 20E Strip reading shows in table 3 , sixty one samples (15.25%) out of the 400 were positive results [Table 4]. 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. The results of displayed that 32% of the examined frozen meat, our results demonstrated a considerably higher prevalence of *Salmonella* spp. 52% in frozen chicken meat samples . our results demonstrated a considerably higher prevalence of *Salmonella* in chicken meat samples[Figure 6]. These rates were comparably with Vera *et al*.[2005)] which his result (58.6%) from chicken meat , and this finding higher than Dhaher *et al*.[2011] which his results was 24.76%. *Salmonella* spp. was isolated (29/33) samples with percent (87.8 %) [Nancy *et al*., 2005]which was significantly higher what has been reached in this study, the reason of this variation due to the difference in the number of samples examined ,and health standards in the massacres.

Conventional selective media for *Salmonella* isolation have very poor specificity, and the numerous false-positive results necessitate time-consuming complementary tests [Perez *et al*., 2003].The conventional technique for the detection of the microorganism includes the following steps: preenrichment, selective enrichment, isolation and selection, biochemical characterization, serological characterization and final identification. This technique requires at least four days for a negative result and six to seven days for the identification and confirmation of positive samples [Soumet *et al*., 1999]. The presence of *Salmonella* has to be determined in at least 25g or mL of sample. Whilst CHROMagar *Salmonella* detect *salmonella* as mauve colonies at 18 to 24h of incubation, which other members of the family *Enterobacteriaceae* appearing as blue or uncolored colonies[Maddocks *et al*., 2002].

The discovery of new, chromogenic substrates incorporated into selective agars allows the differentiation of *Salmonella* colonies from background colonies by specific colony color changes [Tavakoli *et al*., 2008].Utilizing of these media can eliminate necessity of further subculture and biochemical test in identification process of bacteria [Manafi *et al*., 2005], and at the shortest period of time possible, pathogenic agent can be identified. This feature especially in disasters and military condition like maneuver and military camps have special important for preventing food and water borne outbreak [Tavakoli *et al*., 2008]. These technique based on production substrate material for specific microorganism enzyme, according to the produced color the microorganism can be identified easily [Manafi *et al*., 2005]. Chromogenic media have many advantages like rapid detection, high sensitivity, highly specific, needles to further biochemical test in microorganism identification. [Manafi *et al*., 2005;Tavakoli *et al*., 2008]. Albokari [2013] used the chromogenic successfully to detect the presence of microbial pathogens including *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and some yeast and moulds. When chromogenic medium was used for detection of the bacterium, at least one more positive sample was detected in all the examined food types indicating that chromogenic medium was more efficient [Zaghloul *et al*., 2014].In a study conducted by Saeed *et al*.[2011] showed that the total percentage of isolation *Salmonella* spp. according to the reading of API 20-E system were 25 isolates from 27 with percentage 92.5% . CHROmagar was evaluated by Merlino *et al*. [1996] on a total of 1478 isolates of *Enterobacteriaceae* including *Salmonella* , it was found that 60% of the isolates were correctly presumptively identified on CHROmagar by color and morphology alone. The authors concluded that CHROmagar allowed the easy visual detection of the target organism from either subculture of the mixed culture or when used as a primary medium in direct specimen plating, it was also found that this medium prevented the growth of *Proteus* species, *E.coli* and *Klebsiella pneumonia* [El Shamy *et al*., 2008] .

Conclusion

The obtained results indicated that these foods presented a source of infection to the consumer. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving should be taken. Cromogenic was found to accurately, rapid and useful tool for the detection of *Salmonella* in different food sources. Moreover cromogenic method need only 22-24 hours to obtain the results, while culture methods need 5-7 days duration.

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