

Incidence of Salmonella Serotypes and *E. coli* O157: H7 Microorganisms or Strains Isolated from Some Meat Products

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Abstract: The objective of this study is to detect the presence of salmonellae and *E. coli* O₁₅₇:H₇ microorganisms in meat products in order to insure the validity of these foods of animal origin that were traded in supermarkets of Kafr El-Sheikh governorate, Egypt, as well as the health awareness for producing meat product safe for human health. One hundred and fifty samples of meat products (50 each of sausage, beef burger and luncheon) were randomly collected from different supermarkets at Kafr-El-sheikh governorate for isolation of salmonellae and *E. coli* O₁₅₇: H₇ microorganisms. The results revealed that 10, 2 and 0% of examined sausage, beef burger and luncheon samples, respectively presumed to contain salmonella organisms according to biochemical identification whereas 8, 2 and 0% of examined samples were positive for salmonella organism after PCR and serological identification. Salmonella serovars identified in sausage samples were 4 strains as *S. kentucky*, one strain of both *S. tounouma* and *S. saintpaul* while the examined beef burger samples the salmonella serovars were identified as one strain of *S. liverpool*. Results showed that 2, 4 and 4% of examined sausage, beef burger and luncheon samples, respectively presumed to contain *E. coli* O₁₅₇: H₇ according to biochemical identification, respectively. The identified *E. coli* O₁₅₇: H₇ strains after serological biotyping of both beef burger and luncheon samples were 2% while the organism could not be detected in the examined sausage samples. Verotoxin however could not be detected in any of the samples examined with the aid of the ELISA technique. The results reflect cross-contamination from multiple sources during preparation of meat products and poor hygiene during meat handling and storage at supermarkets.

Key words: *Salmonella* · *E. coli* O₁₅₇:H₇, meat products

INTRODUCTION

Food is the most important vehicle for transmission of salmonellae and *Escherichia coli* (*E. coli*) to human. These pathogens have frequently been associated with meat and meat products and linked to number of human illness [1] as fresh meat is highly perishable due to their enriched nutrient composition, high pH (5.5-5.6) and water activity (0.98-0.99), which support survival and growth of almost contaminating microorganisms [2].

Food contaminated with Salmonella are unsafe and considered to be injurious to health and unfit for human consumption, where greater level of the pathogen carry an increased risk of illness due to infectious dose of the organism, which varies by strain and host susceptibility reportedly as low as 15 to 20 cells [3].

Salmonellosis can manifest in a number of disease syndromes including gastroenteritis, bacteremia, typhoid fever and focal infections [4]. The incubation period for developing gastroenteritis is 6-72 h following ingestion of contaminated food or water [5]. Abdominal pain is frequent and may cause mild to severe discomfort. In uncomplicated cases, the acute state usually resolves within 48 h, however, illness is occasionally more protracted with persistent diarrhea and low-grade fever for 10-14 days [6].

Salmonella carrier of human are of concern of the food manufacturing and the food service industries because of the perceived risk of contamination of food by infected food handlers as well as the presence of even small numbers of Salmonella microorganism in carcass meat and edible offal may lead to heavy contamination of minced meat and sausage [7].

***E. coli* O157:** H7 is one of the potentially deadly forms of *E. coli* that can cause severe illness even death in human, particularly in young children, elderly and people with weakened immune system are more susceptible to hemorrhagic colitis and show more serious symptoms [8]. *E. coli* O157: H7 produce potent toxins called shiga toxins that cause severe damage to the cells of human intestine and kidneys resulting in bloody diarrhea and kidney failure [9].

***E. coli* O157:** H7 cause acute hemorrhagic diarrhea with abdominal cramps that in its severe cases result in hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) [10].

Sporadic cases and breaks of human diseases caused by *E. coli* O157: H7 has been associated to contaminated food, particularly ground meat and meat products, person-to-person transmission and contact with animal reservoirs. Prevalence of *E. coli* O157: H7 in feedlots can reach 63-100%, particularly during summer season [11].

Salmonella and verocytotoxin-producing *E. coli* O157: H7 have been shown to survive some fermentation, maturation and drying processes of dried and fermented meats [12].

E. coli strains (*E. coli* O157:H7) and also some other *E. coli* serotypes produce verotoxins (VT1 and VT2) which have a cytotoxic effect and cause disease [13]. Infection with STEC/VTEC may produce no symptoms at all but most people may experience stomach cramps, vomiting, bloody diarrhea, mild fever. These symptoms begin 2 – 8 days after ingestion of bacteria and generally it lasts one week. People can be infectious up to 3 weeks afterwards.

Therefore, application of good manufacturing practice, good hygiene practice and Hazard Analysis Critical Control Point (HACCP) systems are important to prevent the occurrence of pathogens and microbial toxins in meat and meat products.

Our study was performed to detect the presence of Salmonella and *E. coli* O157: H7 microorganisms in meat products in order to insure the safety of meat products.

MATERIALS AND METHOD

Collection of Samples: One hundred and fifty samples of meat products (50 each of sausage, beef burger and luncheon) were randomly collected from different supermarkets at Kafr-El-Sheikh governorate, Egypt. The collected samples were transferred to the laboratory

in frozen state and immediately prepared and examined for presence of Salmonella and *E. coli* O157: H7 microorganisms.

Isolation and Identification of Salmonellae [14]:

Pre-Enrichment: 25 grams of the examined samples were weighed aseptically in sterile container and homogenized with 225 ml of sterile buffered peptone water then were incubated at 37°C for 24 h.

Selective Enrichment: One ml of incubated pre-enrichment homogenate is transferred to 10 ml muller-Kauffmann tetrathionate novobiocin broth (MKTTn) and incubated at 37°C + 1°C for 24h + 3h.

Selective Plating: A loopful from the selective enrichment broth was streaked into Harlequin™ salmonella ABC media and incubated at 37°C for 18- 24 h. Suspected colonies is that colony which appeared green in colour.

Biochemical Identification [15]: The suspected colonies were purified and identified using IMVIC pattern.

2 PCR Confirmation:

Bacterial DNA Preparation [16]: A smooth single colony was inoculated in 5ml nutrient broth and incubated at 37°C for 18 hrs, then 200 µl from bacterial culture was mixed with 800 µl of distilled water. The mixture were mixed in the vortex for good mixing then heated at 96°C for 5 minutes in heat block. The resulting solution was centrifuged at 10,000 rpm for 5 minutes and 200 µl from supernatant was used as the DNA template.

PCR Amplification: Amplification condition step was performed according to Jitrapakdee *et al.* [17]. PCR amplification was carried out using 0.5 ml PCR tube, in which 25 µl of PCR mixture were added for each sample (5 µl Taq Master Mix 5X, 1.25 µl from working solution of each primers, 5 µl of extracted DNA and 12.50 µl distilled water). The amplification was performed in a DNA thermal cycler (Peltier thermal cycler, USA) according to Jitrapakdee *et al.* [17].

The PCR Product Visualization: The amplicons of 204 bp of Salmonella species-specific fragments were visualized by running in 1% agarose gel by using horizontal gel electrophoresis (SciPlast, USA).

Table 1: The PCR amplification cycles condition for identification of Salmonella organisms

Steps	Cycle	Objective	Temperature	Time
First	1	Preheating	95 ° C	2 minutes
Second	30	Denaturation	95°C	1 minute
		Annealing	57°C	1 minute
		Extension	72 °C	2 minute
Third	1	Final extension	72°C	5 minutes
Fourth	1	Holding	4 °C	99.99 minutes

Gel Preparation: Tris Acetate Edita (TAE) buffer was prepared by mixing 20 ml of 50x conc. solution of TAE and 980 ml distilled water Agarose gel 1% was prepared by dissolving 1 gm agarose in 100 ml working solution of TAE buffer and then the mixture was heated for 15 minutes at 121 °C, then cooled to 45°C and poured in plastic tray.

Loading Step: The gel was put in TAE buffer in electrophoresis and then size marker was loaded in first well, in next wells buffer dye was loaded which thoroughly mixed with PCR product of each sample (2 µl loading buffer dye with 10 µl PCR products).

Running Step in Electrophoresis: The horizontal electrophoresis unit was connected by the power supply which adjusted at 140 Voltage for 45 minutes.

Gel Staining: Agarose gel was immersed in ethidium bromide solution (50 µl stock solution of ethidium bromide in 400 ml D. H₂O) for 30 minutes.

PCR Documentation: Loaded gel was inserted in UltraViolet Devices Inc. (UVDI) (Major Science, USA), observed bands showed by picture which were captured by the associated camera for Salmonella species specific fragment was 204 bp.

Serological Identification: The suspected Salmonella were serologically confirmed in the Ministry of Health, Cairo, ARE.

Isolation and Identification of E. coli O157: H7 [18]:

Selective Enrichment: 25 grams of the examined samples were weighed aseptically into sterile container and thoroughly homogenized with 225 ml of supplemented MTSB (Modified Tryptone-Soya broth) for 2 minutes, then incubated at 42°C for 24 hrs.

Selective Plating: A loopful from selective enrichment broth was streaked into Harlequin™ SMAC-BCIG media (Sorbitol McConkey Agar with BCIG) and incubated at 37°C for 18-24 hrs. Plates were examined for sorbitol

negative, β-glucuronide negative colonies which appear translucent.

Biochemical Identification [15]: The suspected colonies were purified and identified using IMVIC pattern.

Serological Identification: The suspected *E. coli* O157: H7 colonies were serologically confirmed using IGM antibodies to *E. coli* O157: H7 (*E. coli* O157: H7 test kit, Oxid) according to instruction of manufacturer.

Detection of verotoxin in meat products (according to manufacturer's instructions of r-Biofarm, Art. No. R5701):

The presence of verotoxin was determined by ELISA based on verotoxin test kit (r-Biofarm, Art. No. R5701). Toxin detection was carried out after culture enrichment. Samples (25g) were added into 225ml modified Tryptic Soya Broth mTSB supplemented with Novobiocin and incubated at 37°C for 6 hrs then 1ml of pre-enrichment brth to 4ml mTSB supplemented with Mitomycin was added and incubated at 37oC overnight (16-18 hrs) while shaking. The culture transferred to centrifugal vial for centrifugation for 10 min./3500g/room temperature. The assay was performed following the manufacturer's instructions to detect verotoxin in meat products. The reading was taken at 450nm wavelength using microtiter plate spectrophotometer. Results were evaluated using cut-off value which determined by adding 0.1 units to the mean absorbance of the negative control. Specimens were considered positive when the absorbance value was higher than the cut-off and considered negative when the absorbance value was equal or lower than the cut-off.

RESULTS AND DISCUSSION

Food-borne diseases and food poisoning are the widespread and great public health concerns of the modern world. Developing countries are largely affected by food-borne infections. Food-borne diseases not only affect people's health and well-being, but also have economic impacts on individuals and the countries [19].

Table 1: Incidence of salmonellae in examined samples:

Types of samples	No. of examined samples	positive samples after biochemical identification	Positive samples after PCR	Positive samples after serological identification	No. of isolates after serological identification
Sausage	50	5(10)	4(8)	4(8)	6
Beef burger	50	1(2)	1(2)	1(2)	1
Luncheon	50	0(0)	0(0)	0(0)	0

Table 2: Serotyping of Salmonellae isolated from examined samples:

Types of samples	No. of positive isolates after serological identification	Serotype	Antigenic formula
Sausage	6	(4) Salmonella keutucky	O:8, 20 H ₁ : i H ₂ : Z ₆
		(1) Salmonella tounouma	O:8, 20 H ₁ : b H ₂ : Z ₆
		(1) Salmonella saintpaul	O:1, 4 [5], 12 H ₁ :e, h H ₂ : 1,2
Beef burger	1	Salmonella Liver Pool	O: 1,3, 19 H ₁ :d H ₂ : e, n, Z ₁₅

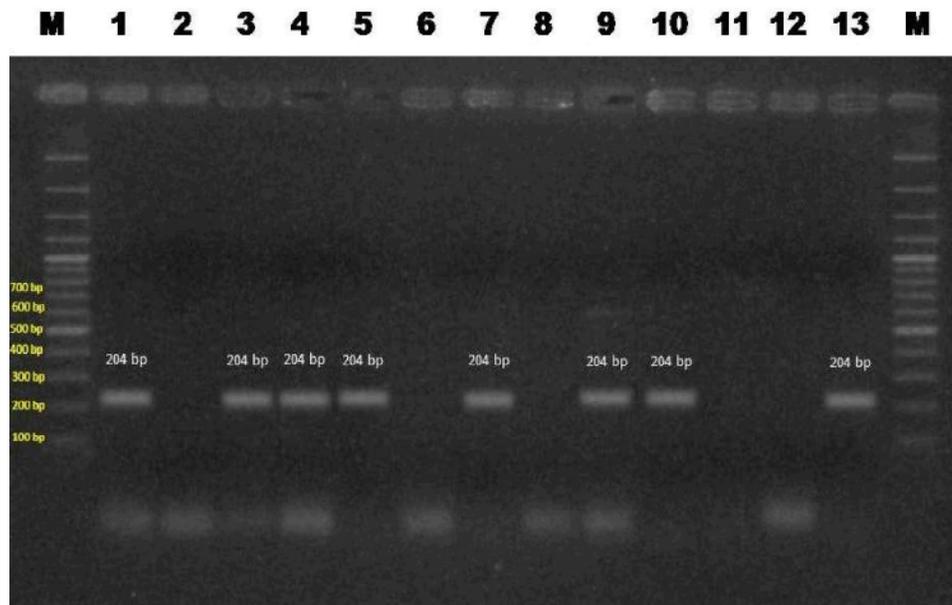


Fig. 1: Agarose gel Electrophoresis of PCR shows the results of identification of Salmonellae (M= ladder 100 bp, lane 1 control positive, lane 2 control negative, lane 3,4,5,7,9,10 the isolated Salmonella spp from sausage samples, lane 6,8 negative sausage samples, lane 11 negative luncheon sample, lane 12 negative beef burger sample, lane 13 the isolated Salmonella spp from beef burger sample.

Salmonella is an important pathogen that causes major problems of morbidity and mortality around the world. Food borne salmonellosis often follows consumption of contaminated animal products resulting from infected animals used in food production or contamination of the carcass or edible organs [20].

Table (1) revealed that 10, 2 and 0% of examined sausage, beef burger and luncheon samples, respectively presumed to contain Salmonella organisms according to biochemical identification whereas 8, 2 and 0% of examined samples were positive for salmonella organism after PCR and serological identification.

The high incidence of Salmonella in sausage may be attributed to the fact that this product is made from raw meat in addition to microorganisms gaining access into sausage from spices and other ingredients, from environment, equipment and handlers during processing affect the microbiological status of the products especially in the absence of proper hygiene [21].

In human, salmonellosis varies from self-limiting gastroenteritis to septicemia depends on host factors as well as the virulence of the strain. Gastroenteritis is characterized by nausea, vomiting, cramping abdominal pain and diarrhea. Severe dehydration can occur in infants

Table 3: Incidence of *E. coli* O157: H7 in examined samples and determine of verotoxin using ELISA technique:

Types of samples	No. of examined samples	Positive samples after biochemical identification	<i>E. coli</i> O157: H7 samples after serological identification	<i>E. coli</i> O157: H7 producing verotoxin (VT1and VT2)
Sausage	50	1(2)	0(0)	0
Beef burger	50	2(4)	1(2)	0
Luncheon	50	2(4)	1(2)	0

and the elderly. In many cases the symptoms resolve spontaneously in 1 to 7 days; deaths are rare except in very young, very old and immune-compromised persons [22]. The absence of *Salmonellae* in luncheon meat is normally due to the addition of food additives such as spices and preservatives, which have an antimicrobial activity and inhibit survival and multiplication of microorganisms and also the exposure to high temperature during processing and cooking procedures [23]

From the results recorded in Table (2) it's clear that *Salmonella* serovars identified in sausage samples were 4 strains as *S. kentucky*, 1 strain of both *S. tounouma* and *S. saintpaul*. Concerning to the examined beef burger samples *Salmonella* serovars were identified as one strain of *S. liverpool*.

The various serotypes of *Salmonella* isolated from meat products indicate the presence of cross-contamination from multiple sources and poor hygienic measures during meat cutting and handling at supermarkets.

Table (3) showed that 2, 4 and 4% of examined sausage, beef burger and luncheon samples, respectively presumed to contain *E. coli* O157: H7 according to biochemical identification, respectively. The confirmed samples for *E. coli* O157: H7 after serological identification of both beef burger and luncheon samples were 2% while the organism could not be detected in the examined sausage samples. In addition verotoxin could not be detected even after enrichment according to procedures. This means that the samples did not contain vero-toxinogenic *E. coli*. The presence of *E. coli* O157: H7 may attribute to the fecal contamination from food handlers during processing and marketing [24]. *E. coli* O157:H7 also contaminated beef trimmings during normal slaughter processes and persist throughout beef burger production from processing of beef trimmings to production and frozen storage of the formed burger. *E. coli* O157:H7 can survive well in meat stored at low temperature (-20 °C) for a long period of time with little change in the initial number of organism [25, 26].

CONCLUSION

The results reflect the possible cross-contamination from multiple sources during preparation of meat products and poor hygiene during meat cutting, handling and storage at supermarkets. Therefore, proper cooking of these meat products before consumption should be adopted to ensure the safety of these products of human consumption.

All authors conceived and designed the review, executed the experiment and analyzed the data as well as all authors interpreted the data, critically, revised the manuscript for important intellectual contents and approved the final version.

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