Animal Health Research Institute,
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# INHIBITORY EFFECT OF SOME SPICE EXTRACTS ON LISTERIA MONOCYTOGENES IN MINCED MEAT

(With 2 Tables and 3 Figures)

By
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## التأثير المثبط لمستخلصات بعض التوابل على ميكروب الليستيريا مونوسيتوجينيس فى اللحم المفروم

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ميكروب الليستيريا مونوستيوجينيس من الميكروبات الخطيرة التى تؤثر على صحة الإنسان ويزيد من خطورتها قدرتها على البقاء والنمو فى درجة الحرارة المنخفضة لأنها من الميكروبات المحبة للبرودة ولذلك أجريت هذه الدراسة لإختبار تأثير ثلاث مستخلصات من بعض التوابل الشائع إضافتهم فى صناعة الأغذية (مثل الزعتر و السماق و الفلفل الأسود) على نمو ميكروب الليستيريا مونوسيتوجينيس. و قد أتبعت طريقتان لدراسة هذا التأثير لهذه المستخلصات، طريقة agar cup method بإستخدام ٣ تركيزات ٢,٥ ، ٥ و١٠% من مستخلصات التوابل السالفة الذكر و قد وجد أن أقل تركيز فى تأثيره المثبط أعطى منطقة مثبطة (١٥مم) هو مستخلص السماق و الزعتر تركيز ٢,٥% على نمو ميكروب الليستيريا مونوسيتوجينيس. الطريقة الثانية لدراسة هذا التأثير food model قد أجريت على اللحم المفروم بإستخدام نفس التركيزات السابقة و تم حفظ العينات عند درجة ٣ م لمدة سبعة أيام وقد أظهرت النتائج أن أقل تركيز أعطى تأثير مثبط على نمو ميكروب الليستيريا مونوسيتوجينيس هو مستخلص السماق ٥ % حيث أدى إلى تناقص عدد هذا الميكروب بعد ٧ أيام من الحفظ بنسبة تثبيط ١,٩log .

## SUMMARY

*Listeria monocytogenes* is of great concern to the food industry, especially in foods stored under refrigerated conditions where, unlike most food-borne pathogens, *L. monocytogenes* is able to multiply so this investigation was conducted to study the inhibitory effect of some spice extracts namely thyme, sumac and black pepper commonly used in food

industry on the growth of this pathogen. Three different concentrations (2.5, 5 and 10%) of the spice extracts were used. Two different procedures were carried out to evaluate the inhibitory effect of these spice extracts, agar cup method and food model. In, agar cup method, the obtained results showed that the lowest concentration which produced inhibitory effect on the growth of this pathogen with inhibition zone of 15mm were of sumac and thyme 2.5%. In the second procedure on food model (minced meat) stored at 3 °C for 7 days, the results revealed that the lowest concentration which exhibited a great decline in counts of *L. monocytogenes* after 7 days of storage by log 1.9 cfu/g was sumac 5% (in comparison to control).

***Key words***: *listeria monocytogenes, inhibitory effect, thyme, sumac and black pepper*

## INTRODUCTION

*Listeria monocytogenes* is a Gram-positive asporogenous coccobacillus which gained increasing attention as a pathogen of public health importance owing to large numbers of food-borne outbreaks of listeriosis and of great concern to the food industry, especially in foods stored under refrigerated conditions where, unlike most food-borne pathogens, *L. monocytogenes* is able to multiply (Juntilla *et al.*, 1988). Consequently, refrigeration should not be relied upon as the sole method for the control of *L. monocytogenes* but should be incorporated with another means of preservation. One possible option is the use of plant extracts. Because of negative consumer perception of chemical preservatives, attention is shifting towards natural alternatives. Particular interest has been focused on the potential application of plant essential oils (EOs) and other extracts (Rasooli *et al.*, 2006).

Spices and herbs have been long used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the last 19$^{th}$ century have documented the antimicrobial properties of some spices, herbs and their components (Shelf, 1983 and Zaika, 1988).

Selected spices and their EOs have been studied with the aim of inhibiting the growth of *L. monocytogenes* in foods. Cloves, cumin, garlic powder, thyme, paprika, red and black pepper, rosemary, mace, marjoram and pimento have given good results in terms of their

capability of reducing the number of these organisms (Aureli *et al.*, 1992; Ting and Deibel, 1992; Hefnawy *et al.*, 1993; Pandit and Shelef, 1994 and Hao *et al.*, 1998).

Sumac (Rhus coriaria L., family Anacardiaceae) grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and the Southeastern Anatolian Region of Turkey. The name derived from "sumâqâ," meaning red in Syriac. The spice, produced by grinding the dried fruit with salt, is used as condiment and sprinkled over kebabs and grilled meat as well as over salads that often accompany these dishes. It has a sour taste (pH 2.5) that is derived from the citric and malic acids found in its juice. In folk medicine, it is used for treatment of indigestion, anorexia, diarrhea, hemorrhagia and hyperglycemia (Wetherilt and Pala, 1994). Sumac is commonly used as spice in the Mediterranean region especially in meat and fish dishes.

Among the aromatic plants belonging to the Lamiaceae family, the genus Thymus is noteworthy for the numerous species and varieties of wild-growing plants. Many of these are typical of the Mediterranean area. The plants are extensively used (fresh and dried) as a culinary herb. The EO is utilized as flavour ingredients in a wide variety of food, as well as in perfumery. Because of its antiseptic, antispasmodic and antimicrobial properties is also used for medicinal purposes (Van Den Brouke and Lemli, 1981 and Panizzi *et al.*, 1993).

It is well documented that for most spices and plant materials the most active constituent against microorganisms was found to be the essential oil fraction (Aktuğ and Karapinar, 1986 and Zaika, 1988). As sumac contains very low quantities of essential oil, i.e., 0.02-0.03% (Brunk *et al.*, 1993), as well as thyme and pepper contain very low quantities of essential oil, which is difficult to collect separately and because essential oils are generally alcohol soluble, studies with alcohol extracts were conducted.

Therefore, this study was conducted to study the inhibitory effect of some spice extracts namely thyme, sumac and black pepper commonly used in food industry on the growth of *L. monocytogenes* in minced meat stored under refrigerated conditions.

## MATERIALS and METHODS

**Bacterial strain:**

*L. monocytogenes* strain was obtained from Institute für Milchhygiene ünd Milchtechnologie, Vet. Med. Univ., Vienna, Austria.

A fresh culture was prepared by inoculating 10 ml of tryptic soy broth (TSB) with 0.6 yeast extract (TSBYE) with a loopful of the stock culture and incubating the inoculated tube at 32° C for 18-20h (Thongson *et al.*, 2005).

**Spices used:** sumac powder, thyme powder and black pepper powder, which purchased from a local market in Assiut city.

**Extraction procedures:**

Extraction of active constituents of sumac powder, thyme powder and black pepper powder using maceration technique (Abd El-Mawla, 1996): 10 gm of each spice were soaked in 50 ml alcohol 70%, left for complete extraction then filtration in air until complete evaporation then diluted in Tween 80 to obtain 10, 5 and 2.5% concentrations.

**The Cup Method:**

The method described by Zaika, (1988) was applied. Fifty milliliter Nutrient Agar (NA) cooled to 50 °C after autoclaving at 121 °C for 15 min, were inoculated well with 0.5 ml of an overnight (12-18h) *L. monocytogenes* culture, mixed well and poured into standard Petri plates. After setting of medium after about 1 h, cups of 1cm diameter were prepared. The base of each cup was sealed with 50ul of sterilized molten NA. The cups were filled by adding 300 ul of spice extracts (2.5, 5 and 10%) concentrations while Tween 80 was added in one cup as a control. The plates having cups were incubated for 48h at 37 °C. After incubation the growth inhibition zones around every cup (including cup) were measured with a caliper and recorded.

**Food model** (Ceylan *et al.*, 1998)**:**

1000 g of fresh meat was purchased from a local butcher. The samples were minced and divided into groups; the first group was divided into three equal portions each of 100 g in sterile plastic bags. The 1st, 2nd and the 3rd bags received 2.5, 5 and 10% thyme extracts. Also, the second group was divided into three equal portions each of 100 g in sterile plastic bags, then 2.5, 5 and 10% sumac extracts were added into the 1st, 2nd and the 3rd bags Moreover, the third group was divided into three equal portions, the 1st, 2nd and the 3rd bags received 2.5, 5 and 10% black pepper extracts, respectively, whereas, the fourth group was considered as control sample which had no spice. *L. monocytogenes* then was added to these mixtures to obtain 1X $10^7$ cfu/g initial inoculum level. Both the bacterial inoculua and spice extracts were distributed in the minced meat by stomacher for 2 min. All plastic bags were

refrigerated at 3 °C and examined at the 0, 2nd, 5th and 7th days to evaluate the viable cell counts of *L. monocytogenes*.

**Evaluation of *L. monocytogenes* in the inoculated samples:**

Ten gm of the inoculated ground beef sample were transferred into sterile 250-stomacher bag together with 90 ml of sterile 0.1-peptone water. The sample was thoroughly homogenized by using a stomacher for 2 min. Serial dilutions of the homogenate were prepared by using 0.1-peptone water as diluents. 0.1 ml portions of three consecutive dilutions were spread-plated on tryptic soy agar (TSA) with 0.6 yeast extract (TSAYE) (Thongson *et al.*, 2005). The plates were incubated at 37° C for 24h.

## RESULTS

Results were demonstrated in Tables 1&2 and Figures 1-3.

**Table 1:** Growth inhibition zones (mm) by different conc. of thyme, sumac and black pepper extracts on the growth of *Listeria monocytogenes*.

| Type of extracts | Control | Conc. of thyme extracts | | | Conc. of sumac extracts | | | Conc. of black pepper extracts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2.5% | 5% | 10% | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| Inhibition Zones (mm) | 10 | 15 | 17.1 | 26.2 | 15 | 17.9 | 28 | 10 | 10.5 | 13.7 |

**Table 2:** Inhibitory effect of different conc. of thyme, sumac and black pepper extracts on the growth of *Listeria monocytogenes (log cfu/g)*.

| Days | Control | Thyme | | | Sumac | | | Black pepper | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2.5% | 5% | 10% | 2.5% | 5% | 10% | 2.5% | 5% | 10% |
| 0 time | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
| After 2 days | 7.3 | 6.4 | 6.3 | 4.2 | 6.6 | 6.3 | 4.2 | 7.2 | 6.5 | 6.2 |
| After 5 days | 7.8 | 6.3 | 6.2 | 5.6 | 6.3 | 6.2 | 5.3 | 6.3 | 6.3 | 6.3 |
| After 7 days | 8.9 | 6.3 | 6.2 | 5.3 | 5.7 | 5.3 | 6.3 | 6.3 | 6.3 | 6.3 |

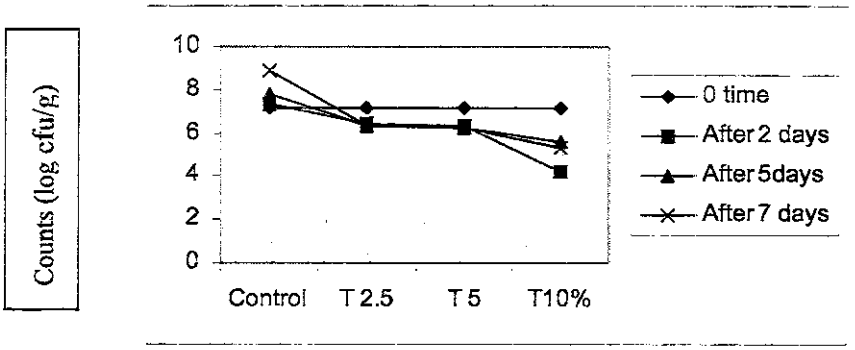


**Fig. 1:** Effect of thyme extracts (T) on counts of *L. monocytogenes*

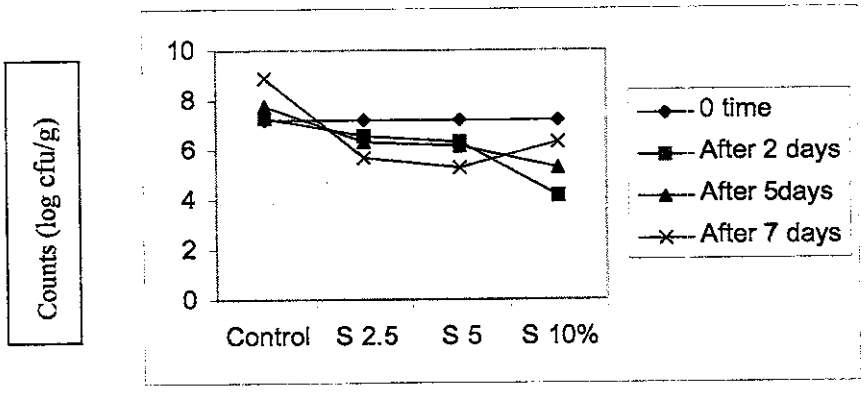


**Fig. 2:** Effect of sumac extracts (S) on counts of *L. monocytogenes*

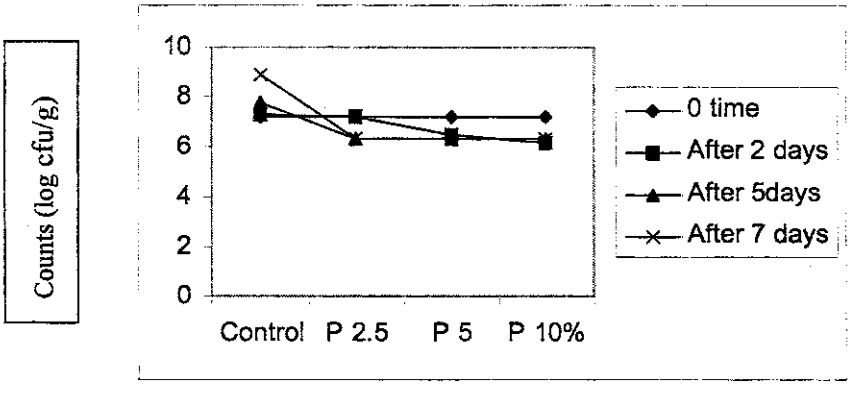


**Fig. 3:** Effect of black pepper extracts (P) on counts of *L. monocytogenes*

# DISCUSSION

*L. monocytogenes* has been recognized as one of the major food-borne pathogen due to its ability to survive in adverse conditions.

The current interest in the use of compounds derived from spices as antimicrobial agents was sparked in the 1980s by changes in consumer attitudes toward the use of preservative agents such as nitrates and NaCl in foods (Shelef, 1983).

The inhibitory effect of different concentrations of thyme, sumac and black pepper extracts on the growth of *L. monocytogenes* tested by agar cup method is represented in Table 1. The obtained results showed that the most inhibitory extract which gave the greatest zone of inhibition (28mm) on the growth of *L. monocytogenes* was sumac 10% followed by 26.2mm by thyme 10%, whereas, the lowest concentration (2.5%) of sumac and thyme caused a zone of inhibition of 15mm. On the other hand, different concentrations of black pepper extract had no inhibitory effect on the growth of *L. monocytogenes* (Table 1).

From the results outlined in Table 2 and Figures 1, 2 and 3 it could be observed that thyme and sumac extracts (10%) had the highest inhibitory effect on the growth of *L. monocytogenes* in minced meat stored at 3 °C at the 2$^{nd}$ day of storage with reduction of 3 log cfu/g, whereas, the lowest concentrations which gave the most inhibitory action on the growth of this pathogen by log 1.9 cfu/g was sumac 5% after 7 days of storage. These obtained results were in agreement with those previously published by Aureli *et al.*, (1992); Nasar-Abbas and halkman, (2004); Abu-Shnab *et al.*, (2005) and Rasooli *et al.*, (2006).

Aureli *et al.* (1992) found that minced meat with thyme oil reduced *L. monocytogenes* population over the first week of storage.

Alcohol extract of sumac was found to be effective against all the tested organisms (6 Gram-positives and 6 Gram-negatives). Among the Gram-positives, Bacillus species (*B. cereus*, *B. megaterium*, *B. subtilis* and *B. thuringiensis*) were found to be the most sensitive, surviving up to only 500 mg/L of the spice, followed by *Staphylococcus aureus* (1000 mg/L), and then by *L. monocytogenes* (1500mg/L). Of the Gram-negative bacteria, *Salmonella enteritidis* and *Escherichia coli* type 1 were found to be more resistant, surviving up to 3000 mg/L of the spice (Nasar-Abbas and halkman, 2004).

Sumac is of Semitic origin and appears to derive ultimately from an Aramaic adjective summaq "dark red" (Semitic root SMQ or ŚMQ "to be red"); compare Modern Hebrew sumak. The name was

transported to European languages via Arabic summaq [سماق] "sumac" (Wetherilt and Pala, 1994).

Sumac is a very popular condiment in Turkey and Iran, where the ground fruits are liberally sprinkled over rice. Mixed with freshly cut onions, it is frequently eaten as an appetizer. The well-known Turkish fast food specialty döner kebap is sometimes flavoured with sumac powder (Nasar-Abbas and halkman, 2004).

In Palastine, Sumac (Rhus coriaria L.) is a well known spice, popular and has been utilized extensively in many different meals, such as in Zatar (dukka) which is a blend of sumac, thyme and citric acid with seasame seeds; almusakhan which is composed from fragmented chicken, small fragments of onions and sumac, as well as in salads and others (Abu-Shnab *et al.*, 2005).

Regarding black pepper as shown in Table 2 and Figure 3, it was found that black pepper at maximum conc. (10%) produced weak or very small effect in populations of *L. monocytogenes* by log 0.9 cfu/g after 7 days of storage at 3°C. This result was in agreement with those obtained by Ting and Deibel (1992) who emphasized that black pepper of concentrations up to 3% had no effect on *L. monocytogenes*. Also, Hefnawy *et al.* (1993) tested two strains of *L. monocytogenes* (strain Scott A and strain V7) for their response to spices (including black pepper) in a liquid medium (TSB) held at 4°C for 7 days, and they found that black pepper had no effect on *L. monocytogenes* strain V7, whereas, it reduced but did not completely inactivate the population of strain Scott A.

While numerous in vitro studies have demonstrated the effectiveness of spices, herbs, or plant extracts and their active ingredients against pathogens, few studies have addressed the use of plant-derived antimicrobial to inhibit pathogenic or spoilage organisms associated with meat (Cutter, 2000).

Many factors in foods could be responsible for the reduction of antimicrobial activity of spices and spice extracts while applied on different types of food. This observation was recorded by many investigators such as Ismaiel and Pierson, (1990) who reported that antimicrobial activity of spices and oils diminished in food as a result of solubilization of the antimicrobial agents into the food's lipid fraction.

The results of the present study indicate the existence of the antimicrobial activity in the extracts of sumac and thyme.

It can be concluded that ethanolic extracts such as sumac and thyme extracts, which inhibited the growth of *L. monocytogenes* at low

concentrations, could be considered as preservative materials for some kinds of foods; they could find an application as additives to foodstuffs in storage to protect them from listerial contamination.

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# A STUDY ON THE OCCURRENCE OF *ESCHERICHIA COLI* IN SOME BEEF PRODUCTS WITH SPECIAL REFERENCE TO *ESCHERICHIA COLI* O157:H7.

(With 4 Tables)

By

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## دراسة عن وجود ميكروب الإشيرشيا كولاى فى بعض منتجات اللحوم مع الإشارة إلى عترة O157 :H7

***زينب ابراهيم سليمان ، عزه على حسين التابعى***

تعتـبر منـتجات اللحوم المصنعة من أهم المنتجات التي يقبل عليها المستهلك. وقد تتعرض لحوم الذبائح للتلوث بالميكروب القولونى أثناء الذبح وتختلط هذه البكتريا جيدا باللحم عند فرم وتجهيز اللحم لإعداد الأنواع المختلفة من منتجات اللحوم كالبرجر والسجق إلى غير ذلك من منتجات اللحوم المصنعة. ومع أن معظم عترات ميكروب الإشيرشيا كولاى غير ضارة إلا أن هـناك بعـض العـترات قد تشكل خطرا على صحة الإنسان. فعلى سبيل المثال العترة O157:H 7 تذكـر دائما مرتبطة بحالات شديدة من التسمم الغذائي وخصوصا في منتجات اللحـوم الغـير مطهية جيدا وقد تؤدى العدوى إلى حدوث فشل كلوي وخاصة في الأطفال. لذلك تهدف الدراسة الحالية إلى عزل وتقييم انتشار الميكروب القولوني العترة O157 :H 7 إلى جانب العترات الأخرى في بعض منتجات اللحوم المصنعة التي تباع في السوبر ماركت. تـم تجميع خمسة وعشرون عينة من كل من البرجر ، الفرانكفورتر، الكفتة، اللحم المفروم، والسجق. وقد أسفرت التحاليل عن وجود ميكروب الإشيرشيا كولاى بنسب ٥٦ % ، ٤٠% ، ٩٢% ، ٦٨% ، ٧٢% فـي عيـنات الـبرجر ، الفرانكفورتر، الكفتة، اللحم المفروم، والسـجق علـى التوالي. كذلك تم عزل وتصنيف العترة O157 H:7 من ٦ (٤,٨ %) من اجمـالى عـدد العينات التى تم فحصها. كما تم تصنيف عترات أخرى من العينات الإيجابية وهـى O68 O119, O113, O111, O55، O126. هـذا وقـد تم مناقشة خطورة وجود العـترة O157 :H7 إلى جانب العترات الأخرى على الصحة العامة وكذلك أهم التوصيات بالنسـبة لمستهلكي منتجات اللحوم المفرومة المصنعة والتى تتركز فى الطهى الجيد وضمان وصـول الحـرارة إلـى كافة الأجزاء الداخلية وليس السطح الخارجي وذلك للقضاء على الميكروب.

## SUMMARY

A total of one hundred and twenty five samples, twenty five of each beef burger, frankfurter, kofta, minced meat and sausage were collected from Port -Said markets. Samples were examined to isolate and evaluate the prevalence rate of *E. coli* O157:H7 and other *E. coli* serotypes. *E. coli* was detected in burger, frankfurter, kofta, minced meat and sausage samples at a rate of 56, 40, 92 68 and 72%, respectively. Six (4.8%) out of all 125 tested meat products samples were found to be contaminated with *E. coli* O157:H7, ten isolates of *E. coli* O157:H7 could be recovered. A total of 50 *E. coli* isolates recovered from positive samples were identified to serogroups, O55 (30%), O111 (22%), O113 (22%), O119 (16%), O68 (6%) and O126 (4%). The majority of *E.coli* serotypes recovered from the examined samples showed hemolytic activity. The public health significant of the isolated serogroups and consumer's safety were discussed.


***Key words:*** *Escherichia coli, beef products, E.coli 0157:H7.*

## INTRODUCTION

Many people enjoy beef burgers, sausages and other meat products, especially during the summer months . However, raw and improperly handled or cooked sausages and beef burgers can harbour harmful bacteria including *Escherichia coli*. The bacteria constituting the species *E. coli* are bacteria that normally live in the intestines of humans and animals. Although most strains are harmless, several are known to produce toxins that can cause diarrhea. The pathogenic groups includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC). Of these, only the first 4 groups have been implicated in food or water borne illness(Levine, 1987 and Nataro and Kaper. 1998).

In recent years, it has become apparent that one can contract a rather serious bacterial gastro-enteritis by consuming undercooked ground beef. Scientists have identified a rare but dangerous type of *Escherichia coli*, *E. coli* O157:H7 that is responsible for this foodborne illness. Scientists believed that *E. coli* O157:H7 is a mutant strain that was created when a virus infected benign *E.coli* and gave it a string of DNA from *Shigella,* a bacterium that causes severe bloody diarrhea. In both *Shigella* and *E. coli* O157:H7, as few as 10 germs can cause illness;

by comparison, it takes about a billion *Salmonella* bacteria to make sick (Wong *et al.*, 2000). *E. coli* O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to under cooked burgers served from a fast food chains (Riley *et al.*, 1983). Since 1983, an increasing in number of *E. coli* O157:H7 have been reported in association with consumption of improperly cooked ground beef (Cohen and Giannella, 1991 *and* Siegler *et al., 1993*).

The organism colonizes in the large intestine and produces one or more of the potent cytotoxins referred to as Shiga-like toxins (SLTs) (O'Brien and Kaper 1998). Although more than 60 *E. coli* serotypes produce SLTs, serotype O157:H7 is the predominant pathogen in the EHEC group and the one associated most frequently with human infections worldwide (Karmali, 1989). These toxins are responsible for severe hemorrhagic colitis in humans. In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail, about 2%-7% of infections lead to this complication (Doyle, 1991).

In a view of the importance of *E. coli* O157:H7 from a food safety stand point, this study was planned to investigate the presence of this agent and other pathogenic *E. coli* serotypes among some selected meat products. The public health significant and consumer's safety were discussed.

## MATERIALS and METHODS

One hundred and twenty five samples, twenty-five of each beef burger, frankfurter, minced meat, kofta and sausage were collected from Port- Said markets.

The frozen samples were thawed in their original containers in a refrigerator at 2-5°C. Twenty-five grams of each sample were homogenized with 225 ml of tryptone phosphate broth as a pre-enrichment fluid then incubated for 4-6 hours at 37°C. (Mehlman and Lovett, 1984). Two Mossel[18] enteric enrichment broth tubes (10 ml) were inoculated each by 1 ml from the pre-enrichment medium. One tube was incubated at 44°C for 24 hours to permit the growth of pathogenic *E.coli*, other than serovar O157. The other tube was incubated at 37°C for 24 hours to permit the growth of *E. coli* O157:H7 as well as other serovars unable to grow at high (44°C) temperature (Mehlman and Romero, 1982). Dilutions of culture in tryptone phosphate broth with

peptone water (0.1%) to $10^{-6}$ were prepared. About 0.1 ml obtained from appropriate dilution were inoculated in MacConkey Sorbitol agar (MACS) and Eosin methylene blue agar (EMB) as double parallel by using spread –plating . The plates were incubated at 37°C for 24 hours. Randomly selected white and colorless sorbitol negative colonies were picked from MACS and streaked separately onto MACS supplemented with cefixime- tellurite (CT, Difco) (CT- MACS) and onto EMB to purify the colonies. The plates were incubated at 37°C for 24 hours (FDA, 2002). Morphological and biochemical tests were applied to colorless or neutral /gray with smoky center and 1-2 mm diameter sorbitol negative colonies on CT- MACS and to metallic green colored, smooth sided colonies on EMB according to Quinn *et al.* (2002). The isolates were identified serologically by the slide agglutination test using diagnostic polyvalent and monovalent *E.coli* O antisera and H 7 antisera (*Escherichia coli* antisera, Denka Seiken Co., Ltd, Tokyo, Japan) ,following the manufacturer's specification.

Hemolysin production (Beutin *et al.*, 1989)

*E. coli* isolates were inoculated onto blood agar plates containing sheep blood (5%) and incubated at 37°C for 24 hours. The plates were examined for the presence of haemolysis.

## RESULTS

**Table 1:** Prevalence rate of *Escherichia coli* in the examined meat products samples (n=25 of each).

| Meat products | Positive samples | % of Positive samples |
|---|---|---|
| Beef burger | 14 | 56 |
| Frankfurter | 10 | 40 |
| Kofta | 23 | 92 |
| Minced meat | 17 | 68 |
| Sausage | 18 | 72 |

**Table 2:** Prevalence of *E. coli O157*: H7 among the examined meat products (n=25 of each).

| Meat products | Positive samples for serovar O157:H7 | |
|---|---|---|
| | No. | % |
| Beef burger | 2 | 8 |
| Frankfurter | 0 | 0 |
| Kofta | 1 | 4 |
| Minced meat | 3 | 12 |
| Sausage | 0 | 0 |
| Total | 6 | 4.8 |

**Table 3:** Serovars of *E. coli* isolates recovered from the examined meat products samples.

| Serovar / source | O55 | | O111 | | O113 | | O119 | | O68 | | O126 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % | No | % |
| Beef burger | 4 | 8 | 3 | 6 | 4 | 8 | 3 | 6 | 1 | 2 | 2 | 4 |
| Frankfurter | 1 | 2 | 2 | 4 | 1 | 2 | 0 | 0 | 1 | 2 | 0 | 0 |
| Kofta | 5 | 10 | 4 | 8 | 3 | 6 | 3 | 6 | 0 | 0 | 0 | 0 |
| Minced meat | 3 | 6 | 2 | 4 | 1 | 2 | 1 | 2 | 1 | 2 | 0 | 0 |
| Sausage | 2 | 4 | 0 | 0 | 2 | 4 | 1 | 2 | 0 | 0 | 0 | 0 |
| Total | 15 | 30 | 11 | 22 | 11 | 22 | 8 | 16 | 3 | 6 | 2 | 4 |

NB: Percentage was calculated according to the total number of the isolates (50)

**Table 4:** Hemolytic activity of *E. coli* isolates recovered from the examined meat products samples.

| *E. coli* serovars | No. of isolates | Hemolytic activity | |
|---|---|---|---|
| | | No. | % |
| O157 : H7 | 10 | 10 | 100 |
| O55 | 15 | 15 | 100 |
| O111 | 11 | 9 | 81.8 |
| O113 | 11 | 7 | 63.6 |
| O119 | 8 | 8 | 100 |
| O68 | 3 | 0 | 0 |
| O126 | 2 | 0 | 0 |
| Total | 60 | 49 | 81.7 |

## DISCUSSION

Most enteropathogenic *E. coli* outbreaks have been blamed on ground beef and other meat products such as beef burger, and hot dog (Desmarchelier and Grau, 1997). The present investigation was carried out to evaluate the prevalence of *E. coli* O157: H7 and other *E. coli* serotypes among selected types of meat products.

The overall incidence of *E. coli* in different samples was recorded in Table (1), *E. coli* were recovered from burger, Frankfurter, kofta, minced meat and sausage samples at a rate of 56, 40, 92, 68 and 72%, respectively. In this concern, prevalence of *E. coli* from meat and meat products ranging from 30% to76% have been reported by Doyle and Schoeni, (1987), Gallas *et al.*, (2002) and Gad El-Said *et al.*, (2005). This contamination rate of the present samples indicates unhygienic

practices prevailed in slaughter. Cattle are a major reservoir of these groups of bacteria and ground beef have been the major vehicle of *E. coli* transmission. During slaughter process, meat may become contaminated by fecal contamination during evisceration and through skin or hide during dressing (Desmarchelier, 1997 and Rice *et al.*, 1997). When the meat is ground, fecal organisms on the outside of the meat are mixed throughout the ground beef. Also contamination of meat probably occurs during processing. In this respect, Read *et al.* (1990) reported that ground beef meat- processing plants were heavily contaminated with verocytotoxin *E.coli*. In addition, *E.coli* is an indicator of food safety for dehydrated, frozen and refrigerated food, as *E. coli* does not survive well under such condition (Mossel *et al.*,1979). Therefore, its presence might indicate poor temperature control.

*Escherichia coli* O157: H7, predominantly originated from beef, is a significant pathogen to the public health and thus, need to be vigorously surveyed in meat products. Lake of sorbitol fermentation within 24 hours has been considered a stable phenotypic character of *E. coli* O157: H7 therefore; MACS was used for differentiation of *E. coli* O157: H7 from other enteric bacteria (March and Ratnam, 1986).

Results of biochemical and serological identification of sorbitol negative *E. coli* isolates revealed that six (4.8%) out of all 125 meat products examined were found to be contaminated with *E. coli* O157:H7 (Table 2), three (8%) out of 25 minced meat samples, two (8%) out of 25 burger samples and one (4%) out of 25 kofta samples. The exact contamination rate may be higher than stated here due to the low isolation rate of the culture methods compared to other immunological and genetical methods. Considerably higher isolation rates of *E. coli* O157:H7 than in this study have been reported elsewhere. In South Africa, it was isolated from a total of 74.5% and in Malaysia from 36% of beef samples (Vorster *et al.*, 1994 and Radu *et al.*, 1998). On the other hand, in some studies beef and beef samples have found to be free (Junghannss *et al.*, 1996, Simmons, 1997 and Uhitil *et al.*, 2001). In another studies it was isolated at low contaminated rate, Pai *et al.*, (1984) reported the presence of *E. coli* O157:H7 in 5 out of 17 beef samples. In USA, *E. coli* O157:H7 was isolated from six (3.7%) out of 164 beef samples (Doyle and Schoeni, 1987), in India, Dutta and Deb, (2000) isolated *E. coli* O157:H7 from two (9%) out of 22 minced beef samples. Also in turkey, Baran and Gulmez, (2005) isolated *E. coli* O157:H7 from three (6%) of ground beef samples. Positive isolation of *E. coli* O157:H7 from beef samples in Egypt was reported by Tanios *et*

*al.*, (2002) from two (6.7%) of minced meat samples and by Gad El-Said *et al.*, (2005) with a rate of 3.95% from meat samples.

Enterohemorrahagic *Escherichia coli* O157: H7 is an important foodborne pathogen, its presence even at low rate (4.8%) in the present study may constitutes dangerous beef products. The ability of *E. coli* O157:H7 to withstand the acidic conditions encountered in various foods have generally suggested that passage through the stomach would be insufficient to inactivate the pathogen. (Naim *et al.*, 2003). In addition, the organism always enters the digestive system within a food matrix, Waterman and Small (1998) postulated that high protein content in food (such as ground beef and boiled egg white) might protect enteric bacteria against the killing effect of gastric acids. The data from epidemiological investigations indicated that as few as 10 to 100 cells of *E. coli* O157:H7 per g of raw ground beef were sufficient to cause illness (Abdul-Raouf *et al.*, 1993). Moreover, Wong *et al.* (2000) believed that treatment with antibiotics is contraindicated for *E. coli* O157 poisoning, since it is when the bacteria die, they release the toxins which produce hemolytic uremic syndrome (HUS), for which there is no cure.

Symptoms of *E. coli* O157 infection include bloody and nonbloody diarrhea, vomiting, and abdominal cramps. Illness resolves typically within 7-10 days .A subset of patients, particularly the young and the elderly, will develop HUS, characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure (Russell *et al.*, 2000). In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and *E. coli* O157: H7 causes most cases of hemolytic uremic syndrome (Besser *et al.*, 1999). An estimated 73,480 people a year are infected with *E. coli* O157:H7 and about 600 of those cases are fatal, according to the federal Centers for Disease Control and Prevention (Wong *et al.*, 2000). These illnesses and deaths were factors that began changing policy towards foodborne disease. The Food Safety and Inspection Service, declared that raw ground beef contaminated with *E. coli* O157 is adulterated and must be further processed to kill the pathogen or be destroyed (FDA. , 2000) .

While *E. coli* O157: H7 is the most renowned Shiga toxin-producing *E. coli* (STEC) , over 200 different types of STEC have been documented in meat and animals, at least 60 of which have been linked with human disease. A number of studies have suggested that non-O157 STEC are associated with clinical disease, and non-O157 STEC are present in the food supply (Acheson, 2000).

Regarding to other serogroups, as shown in Table (3), O55, O111, O113 and O119 were the most prevalent serotypes recovered from the examined samples with an incidence of 30, 22, 22, and 16% respectively, followed by O68 (6%) ,O126 (4%). Most of the isolated serotypes are usually associated with many cases of foodborne outbreaks and multiple sporadic cases in different part of the world. In this concern, Anathan and Subramaniam, (1995) isolated *E. coli* belonging to serotypes O111 from cases of persistent diarrhea in young children. Enteropathogenic *E. coli* belonging to serotypes O111, O103 and O55 were isolated from patients suffering from bloody diarrhea, which may be accompanied by HUS (Desmarchelier, 1997). Non-O157 STEC, such as O111 has caused large outbreaks and HUS in the United States and other countries. (Acheson, 2000). Moreover, Hussein and Omaye, (2003) found that the serogroups belonging to O26, O113, O111, O119 and O166 have caused approximately 30% of the hemolytic uremic syndrome (HUS) in US.

Blood haemolysis is one of character of virulent *E. coli* (Stephen *et al.*, 1985).

Ten isolates identified serologically as *E. coli* O157: H7 were tested for hemolysis production using sheep blood. All tested isolates were haemolytic. Moreover, the majority of *E. coli* isolates other than O157: H7 isolated from the examined samples showed haemolytic activity (Table 4). In this respect, Adesiyun *et al.*, (1997) reported that from 94 *E. coli* isolates tested for haemolysis 13.8 % were haemolytic. Meanwhile, Gad El-Said *et al.*, (2005) stated that 81.58 % of *E. coli* isolates recovered from meat samples showed haemolytic activity.

The productions of haemolysin have a potential role in virulence of hemolytic *E. coli*. Therefore, contamination of meat products with *E. coli* O157:H7 and other *E. coli* serotypes may results in problems for consumers. There is a close association between enterohaemolysin production and SLT production (Beutin *et al.*, 1998). Moreover, the genes involved in enterohaemolysin production were carried on the EHEC plasmid (Scotland *et al.*, 1990).

The risk of contamination of raw meat products with *E. coli* O157:H7 and other pathogens constitute a major problem for human. The low infective dose *E. coli* O157:H7 present a major threat. Hemolytic uremic syndrome, a disease caused mostly by *E. coli* O157:H7 may cause sever kidney diseases and/or failure among children. The main means of combating this organism are good food hygiene covering activities on farm, in abattoir and minced beef

industries. However, until *E. coli* can be eliminated from meat processing systems, consumers should protect themselves by using safe food practices and advice for those who eat ground beef. Frozen ground beef should be thawed in the refrigerator rather than at room temperature. While thawing and preparing ground beef, raw meat must be separated from ready-to-eat foods. It is not enough to merely brown the outside of a burger; and other meat products .Ground beef should be cooked thoroughly to an internal temperature of at least 160° F (71° C), food safety experts recommends that consumers use a meat thermometer to cook ground beef to ensure that internal temperatures are high enough to kill bacteria. To reduce the risk for cross-contamination, consumers should use soap and hot water to wash hands, utensils, and other surfaces that might have been exposed to raw or undercooked ground beef and other meat products. In addition, consumers should be aware from under cooked burgers and other meat products served from fast food restaurants.

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