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INCIDENCE OF *SALMONELLA* AND *E.COLI* IN PACKED MEAT PRODUCTS SOLD IN ASSIUT CITY

(With 5 Tables)

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مدى تواجد ميكروبي السالمونيلا والقولوني (الأيشيريشيا كولاي) في منتجات اللحوم المعبأة والمباعة في مدينة أسيوط

شوكت فتحي ، عبدالراضي ثابت

تم جمع ١٠٠ عينة عشوائية من منتجات اللحوم من أسواق مدينة أسيوط بواقع ٣٠ عينة من كل من البيف برجر والسجق، ٤٠ عينة من اللحم المفروم وذلك لدراسة مدى تواجد ميكروبي السالمونيلا والأيشيريشياكولاي في هذه المنتجات. وقد تم عزل ميكروب السالمونيلا في ٦عينات حيث تم تصنيف العترات المعزولة إلى سالمونيلا تنفيموريم (٢) وسالمونيلا تيفويد (٣) تم عزلهم من عينات البيف برجر وعترة واحدة من السالمونيلا باراتيفي تم عزلها من عينات السجق. كما تم عزل ميكروب الأيشيريشياكولاي من ١٤ عينة بواقع ٢ من البيف برجر بنسبة ٦,٦٧% ، ٥ عينات من السجق بنسبة ١٦,٦٧% ، ٧ عينات من اللحم المفروم بنسبة ١٧,٥% ، وقد وجد أن ثماني عترات من ميكروب الأيشيريشياكولاي قد صنفت تحت نوع I ، أربع عترات تحت نوع II وأثنين لم يصنفا. وقد وجد أن نسبة ميكروب الأيشيرشياكولاي المرضي المعزولة من عينات البيف برجر ، السجق واللحم المفروم كانت ٥٠ ، ٨٠ ، ١٠٠% على التوالي . هذا وقد تمت مناقشة الإشتراطات الصحية الواجب توافرها أثناء تجهيز هذه المنتجات لرفع مستوى الحالة الصحية لمنتجات اللحوم حتى لاتشكل خطرا على الصحة العامة لمستهلكي هذه المنتجات.

SUMMARY

Recovery of *Salmonellae* and *Escherichia coli* from a total number of 100 random samples of different types of packed meat products were evaluated. The collected samples were 30 from each beefburger and sausage and 40 from minced meat samples. Out of the analysed 100 samples, *Salmonellae* were detected only in 6(6%), 5 isolates from

beefburger and only one strain from sausage samples, while they could not be recovered from the examined minced meat samples. The isolated *Salmonella* serotype were two strains of *S. typhimurium* and three strains of *S. typhi* which were detected in the examined beefburger samples, while the only *S. paratyphi* A strain was recovered from sausage samples. Regarding the isolation of *E.coli*, they were detected in only 14 samples, 2 strains from beefburger, 5 from sausage and 7 from the examined minced meat samples, where their incidence was 6.67%, 16.67% and 17.50% in the examined samples, respectively. The isolated *E.coli* strains were identified serologically into eight strains as *E.coli* biovar I and 4 strains as biovar II. The serotyped strains revealed 6 different serovars. Sources of contamination, precautions during preparation and manufacturing of such meat products, as well as the public health hazards of the presence of *Salmonella* and *E.coli* in meat products were discussed.

Key words: *Salmonella, E.coli, meat products.*

INTRODUCTION

Meat and meat products are considered as a major vehicle of most reported outbreaks of foodborne diseases. Epidemiologic data have identified improperly handled meat products as important vehicles for infection (ICMSF, 1980).

Role of *Salmonella* as an agent of foodborne disease has received increasing attention all over the world. *Salmonellae* are found chiefly on meat and in meat products. In Egypt, many investigators like Karim (1976); Ibrahim (1981), Tolba (1986), Abdel-Aziz (1987) and Khalafalla (1988) failed to isolate *Salmonella* from hamburger, while, the organism could be detected in 5, 6 and 6% of the examined beefburger samples by Darwish et al. (1986), Ahmed and Abdel-Aziz (1988) and El-Mossalami et al. (1989), respectively. *Salmonella* could be recovered also from sausage samples in variable incidence by Sadek (1963)(4%), Pains and Manzini (1966) (10%), Kool and Bes (1973) (12%), Tolba (1986) (10%), Abdel-Aziz (1987) (10%), Khalafalla (1988) (8%), Nabbut (1988) (11.2%) and El-Mossalami et al. (1989) (8%). Furthermore, very high incidence of *Salmonella* in sausage was recorded by Weissman and Carpenta (1969) (38%), Roberts et al. (1975) (29.65%) and Abdel-Aziz (1979) (28%). Regarding minced meat, many researchers such as

Roushdy (1971), El-Mossalami and Roushdy (1973), Foster et al. (1977) and Hefnawy (1980) failed to isolate *Salmonella*. In contrast, other authors could recover the organism from minced meat samples in variable percentages as El-Agroudi and Sadek (1963) (7.3%), Edel et al. (1978) (24.7%), Vassiliadis et al. (1978) (18.5%), Deseo and Engeli (1979) (0.5%), Darwish et al. (1986) (5%), Ahmed and Abdel-Aziz (1988) (6%), Khalafalla (1988) (8%) and El-Mossalami et al. (1989) (12%). In many countries *Salmonellae* are very important causative agents of zoonotic and foodborne disease (ICMSF, 1978). *Salmonella typhimurium* is the commonest serotype infecting man and animals (Pains and Manzini, 1966; Roberts et al., 1975; Edel et al., 1978 and Bachhil and Jaiswal, 1988). Whereas, Darwish et al. (1986) could detect *S.typhi* in beefurger samples.

Meat products may be contaminated also with *E.coli* from food handlers, utensils, air, soil and water under inadequate hygienic circumstances during manufacturing, packing and marketing of these products (Frazier and Westhoff, 1978). *E.coli* biotype I was detected at all stages of meat handling and recognized as an indicator of direct or indirect fecal contamination of meat (Stiles and Ng, 1981). Enteropahtogenic *E.coli* (EEC) can be defined as any strain of *E.coli* that has the potential to cause diarheal disease. EEC can be further subdivided into four categories including the classical enteropathogenic *E.coli* (EPEC), faculatively enteropathogenic *E.coli* (FEEC), enterotoxigenic *E.coli* (ETEC) and enteroinvasive *E.coli* (EIEC) (Kornacki and Marth, 1982). The incidence of *E.coli* isolates in beefburger was 27.72, 30, 25, 16 and 12% as reported by Duitschaever et al. (1977), Darwish et al. (1986), Tolba (1986) and Darwish et al. (1991), respectively. However, in sausage the prevalence of *E.coli* was recorded in different percentages by El-Khateib (1982) (36.11%), Gobran (1985) (50%), Tolba (1986) (52.59%), Abdel-Aziz (1987) (50%), Niazi and Refai (1988) (44%), Eman (1990) (48%) and Darwish et al. (1991) (40%). On the other hand, Roushdy (1971), Gobran (1985), Darwish et al. (1986), Niazi and Refai (1988), Eman (1990) and Darwish et al. (1991) found that the incidence of *E.coli* in the examined minced meat samples was 24, 52, 20, 64, 84, 48 and 44%, respectively. Many researchers concluded that EPEC isolated from different meat products was incriminated in different diarrhoea and gastrointestinal outbreaks in adult human (Marier et al., 1973 and Edelman and Levine, 1983).

MATERIALS and METHODS

1- Collection of samples:

A total number of 100 random samples of various types of packed meat products were collected from different shops and supermarkets at Assiut City. The types of samples were 30 from each beefburger and sausage and 40 from minced meat samples.

The samples were obtained in their intact original package and transferred as quickly as possible to the laboratory for bacteriological investigation to evaluate samples under investigation for the presence of *Salmonellae* and *E.coli*.

2- Preparation of samples:

Twenty five grams of each sample were weighed aseptically into a sterile blender jar and 225 ml of buffered peptone water were added. The samples were homogenized at low speed for 2 minutes. Subsequent 10 fold serial dilutions of the homogenate were prepared with buffered peptone water up to 10^{-6} from the original dilution (1:10) as recommended by ICMSF (1978).

3- Laboratory technique:

Detection of *Salmonella*: The method for detection of *Salmonella* recommended by ICMSF (1978) and FAO (1979) based on giving the chance for the few numbers of normal or of stressed *Salmonella* organisms to grow first in a non-selective liquid medium at 37°C as pre-enrichment, then subcultured into a liquid selective medium and incubating it at 42 to 43°C was followed. The later was inoculated into a solid selective medium and after incubation at 37°C the plates were examined for the presence of colonies. These colonies are then examined for the biochemical and serological characteristics of *Salmonella* species.

Pre-enrichment: 25 g sample were blended with 225 ml buffered peptone water and transferred aseptically to a sterile 500 ml flask then incubated at 37°C for 16-20 h.

Enrichment: 10 ml of each pre-enrichment medium were transferred to 100 ml tetrathionate broth medium and another 10 ml to 100 ml of selenit broth medium, previously warmed to 42-43°C and incubated at 42-43°C for 48 h.

Plating out on selective media: Brilliant green agar, MacConkey agar and *Salmonella – Shigella* agar media were streaked from each enrichment flask and incubated at 37°C for 24 h, then examined for typical colonies of *Salmonella*.

Confirmation: Typical or suspected colonies were selected from each selective medium and streaked on nutrient agar medium which incubated at 37°C for 24 h. Morphological, biochemical and serological confirmation was performed.

Detection of *E.coli*: The technique was carried out according to FAO (1979).

Presumptive test: One ml from the previously prepared dilutions was inoculated separately into each of three Lauryl Sulphate Tryptose Broth fermentation tubes with inverted Durham's tubes. The tubes were incubated at 35-37°C for 24 and 48 h. After 48 h, tubes showing gas production were recorded as positive.

Confirmation test: A loopful from each positive tube in the presumptive test was transferred separately into each of three E.C. Broth tubes with inverted Durham's tubes. The tubes were incubated at 45±5°C for 48 h. Tubes showing gas production were considered positive.

E.coli count (MPN/g): From each gas positive tube of E.C. Broth, a loopful was streaked on Levine's Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 35-37°C for 24±2 h. Isolated typical colonies or colonies most likely to be *E.coli* were subjected to further identification by biochemical tests including indole, methyl red, Voges Proskauer and citrate (IMViC reaction). The MPN of *E.coli* per gram of the examined sample was determined (A.O.A.C., 1980).

Identification of the *Enteropathogenic E.coli* (EPEC): Serological identification of the *Enteropathogenic E.coli* (EPEC) was carried out as recommended by Edwards and Ewing (1972) and Bailey and Scott (1978).

RESULTS

Table 1: Incidence of *Salmonella* serotypes isolated from the examined packed meat products samples.

Meat products	No. of samples examined	Positive samples	
		No.	%
Beefburger	30	5	16.67
Sausage	30	1	3.33
Minced meat	40	-	-

Table 2: *Salmonella* serotypes isolated from the examined packed meat products.

Serotypes	Antigenic Structure	Frequency of isolation			Total
		Beefburger	Sausage	Minced meat	
S.typhimuirum	1, 4(5), 12:1:1,2	2	-	-	2
S.typhi	9, 12(Vi) :d -	3	-	-	3
S.paratyphi A	1, 2, 12:a: (1,5)	-	1	-	1

Table 3: Incidence of *E.coli* (Biotype I and II) isolates recovered from the examined packed meat products.

Meat products	No. of examined samples	Positive samples		*E. coli* isolates			
		No.	%	Biotype I		Biotype II	
Beefburger	30	2	6.67	1	50.00	-	-
Sausage	30	5	16.67	2	40.00	2	40.00
Minced meat	40	7	17.50	5	71.43	2	28.57
Total	**100**	**14**	**14.00**	**8**	**57.14**	**4**	**28.57**

Table 4: Incidence of isolated Enteropathogenic *E.coli* (EPEC) from examined packed meat products.

Meat products	No. of examined sampled	*E.coli*		*Enteropathogenic E. coli*		Untypable	
		No.	%	No.	%	No.	%
Beefburger	30	2	6.67	1	50.00	1	50.00
Sausage	30	5	16.67	4	80.00	1	20.00
Minced meat	40	7	17.50	7	100.00	-	-
Total	100	14	14.00	12	85.71	2	14.29

Table 5: Enteropathogenic strains of *E.coli* (EPEC) isolated from packed meat products.

Meat product	No. of positive samples	Enteropathogenic serovars		
		O : K ((B) serovar	No	%
Beefburger	2	$O_{35} : K_{59} (B_5)$	1	50
		Untypable	1	50
Sausage	5	$O_{111} : K_{58} (B_4)$	1	20
		$O_{128} : K_{67} (B_{12})$	1	20
		$O_{26} : K_{60} (B_6)$	1	20
		$O_{126} : K_7 (B_{16})$	1	20
		Untypable	1	20
Minced meat	7	$O_{111} : K_{58} (B_4)$	2	28.57
		$O_{86} : K_{61} (B_7)$	2	28.57
		$O_{125} : K_7 (B_{16})$	2	28.57
		$O_{26} : K_{60} (B_6)$	1	14.29
Total	14	Typed	12	85.71
		Untypable	2	14.29

DISCUSSION

Meat and meat products play a role in most reported outbreaks of foodborne disease. Certain pathogenic micro-organisms in or on food of animal origin still constitute a particular hygienic risk.

Salmonellae are important pathogens in both animal and man. They are ubiquitous micro-organisms that have been found in most of the animal species in most of the geographic areas of the world. *Salmonellosis* today is one of the most important foodborne disease which caused a significant public health problem and foods of animal origin particularly meat and meat products are still the major source of human *Salmonellosis*.

It is evident from the present investigation that the incidence of *Salmonella* in examined beefburger and sausage was 5(16.6%) and 1(3.33%) respectively. At the same time, it was found that *Salmonella* could not be detected in the examined minced meat samples (Table 1). On the other hand, it is evident from Table (2) that *Salmonella* serotypes recovered and identified from beef burger samples were two strains of *S.typhimurium* and three strains of *S.typhi*, while *S.paratyphi* A strain was recovered only from the examined sausage samples. The antigenic structure of the identified *Salmonella* serotypes was reported also in Table (2).

Many investigators failed to isolate *Salmonella* from the examined hamburger like Karim (1976, Ibrahim (1981), Tolba (1986), Abdel-Aziz (1987) and Khalafalla (1988), whereas, other workers could detect the organism in beefurger samples in lower incidence than that in the obtained results (Darwish et al., 1986) (5%), Ahmed, 1988 (6%) and El-Mossalami, 1989 (6%).

Very high incidence of *Salmonella* in sausage was recorded by Weissman and Carpenta (1969) (38%), Roberts et al. (1975) (29.65%) and Abdel-Aziz (1979) (28%) which seem to be higher than the present result. *Salmonella* could be recovered also from sausage in higher percentage than the obtained results by many investigators as Sadek (1963) (4%), Pains and Manzini (1966) (10%), Kool and Bes (1973) (12%), Tolba (1986) (10%), Abdel-Aziz (1987) (10%), Ahmed (1988) (8%), Khalafalla (1988) (8%), Nabbut (1988) (11.2%) and El-Mossalami (1989) (8%).

Regarding minced meat samples, the present study failed to detect the presence of the organism in any of the examined samples, this finding was in acceptance to Roushdy (1971), El-Mossalami and

Roushdy (1973), Foster et al. (1987) and Hefnawy (1980) who failed also to isolate *Salmonella* from the examined minced meat.

In contrast, other authors could recover the organism from examined minced meat samples in variable percentages as El-Agroudi and Sadek (1963) (7.3%), Edel et al. (1978) (24.7%), Vassiliadis et al. (1978) (18.25%), Deseo and Engeli (1979) (0.5%), Darwish et al. (1986) (5%), Ahmed and Abdel Aziz (1988) and El-Mosalami (1989) (12%).

Concerning *Salmonella* serotypes, *S.typhimurium* was the commonest strain isolated from similar meat products by most investigators. Sadek (1963) succeeded to isolate *S.typhimurium* from the examined sausage, while Darwish et al. (1986), could detect S.typhi in the examined beefburger samples. *S.typhimurium* was recovered also from minced meat by El-Agroudi and Sadek (1963), Edel et al. (1978) and Bachhil and Faiswal (1988), while, *S.typhi* was detected in 2% of the examined minced meat samples (Gobran, 1985).

The concept of hazard analysis critical control point is of value in the control of *Salmonella* contamination in the food service industry. This involves using process analysis and sampling to identify sources of contamination to determine the chances of *Salmonella* organisms surviving heat and other processes, and to assess the probability of their growth at each stage of food processing. Special control measures should then be established at the critical points that have been thus identified, and microbiological or other appropriate monitoring can be concentrated at these points (WHO, 1980).

The native habitat for *E.coli* is the enteric tract of man and animals, thus its presence in foods generally indicates direct or indirect pollution of fecal origin (ICMSF, 1978).

Meat products may be contaminated with *E.coli* from food handlers, food utensils, air, soil and water under incomplete hygienic circumstances during manufacturing, packing and marketing of these products (Frazier and Westhoff, 1978).

From the results achieved in Table (3), it can be concluded that *E.coli* was isolated from 6.67, 16.67 and 17.50% of the examined beefburger, sausage and minced meat, respectively. The present study indicates also that the incidence of *E.coli* isolates was particularly low in beefburger which seem to be lower than the results reported by Duitschaever et al. (1977) (27.72%); Darwish et al. (1986) (30%); Tolba (1986) (25%); (16%) and Darwish et al. (1991) (12%). On the other hand, very high existence of *E.coli* in hamburger has previously been reported by Abdel-Aziz (11987) (70%).

It is evident also from Table (3) that the incidence of *E.coli* in the examined sausage samples was lower in comparison with the findings recorded by El-Khateib (1982) (38.11%); Gobran (1985) (50%); Tolba (1986) (52.59%); Abdel-Aziz (1987) (50%); Niazi and Refai (1988) (44%0; Iman (1989) (68%); Eman (1990) (48%) and Darwish et al. (1991) (40%).

Concerning the incidence of *E.coli* in the examined minced meat samples, many investigators reportered higher results than the obtained findings, as reported by Roushdy (1971) (24%); Gobran (1985) (52%); Darwish et al. (1986); Niazi and Refai (1988) (64%) and Darwish et al. (1991) (44%).

The presence of *E.coli* as enteropathogens in raw minced meat and fresh sausage provides an evidence of pollution of fecal or water origin and reflects the unsatisfactory hygienic condition during manufacturing and handling of these products by hman carriers (Mehlman et al., 1976 and Niazi and Refai, 1988).

It is evident from the present study that out of 14 *E.coli* isolates, 8(57.14%) were identified as typical *E.coli* (biovar I) where (1/2, 50%) was recovered from beefburger, (2/5, 40%) from sausage and (5/7, 71.43%) from the examined minced meat samples. The other *E.coli* isolates 4(28.57%) could be identified as atypical *E.coli* (biovar II) which constituted 2/5, (40%) from sausge and 2/7, (28.57%) from minced meat samples, Table (3).

From the results achieved in Tables (4 and 5), it was found that out of 14 isolates of *E.coli* recovered from different examined packed meat products samples, 12 strains (85.71%) were serologically typed as Enteropathogenic *E.coli* (EPEC). These strains revealed 6 different serovars namely, $O_{35} : K_{59} (B_5)$ (one strain), $O_{128} : K_{67} (B_{12})$ (one strain), $O_{86} : K_{61} (B_7)$ (two strains), $O_{26} : K_{60} (B_6)$ (two strains, $O_{111} : K_{58} (B_4)$ (three stains) and $O_{126} : K_7 (B_{16})$ (three strains).

These findings are nearly extent to those reported by Gobran (1985); Niazi and Refai (1988) and Darwish et al. (1991).

Many investigators concluded that EPEC isolated from different meat products was incriminated in different infantile diarrhoea and gastrointestinal outbreaks in adult human (Dupont et al., 1971; Marier et al., 1973 and Edelman and Levine, 1983).

In conclusion, considerable interest has been shown by public health officials regarding *E.coli* in food and water. The implication of *E.coli* particularly *E.coli* biotype I (biovar I) as indictor of faecal contamination (Stiles and Ng, 1981) varies with the food type and

handling that food has been received (ICMSF, 1980). Many workers, have stated that *E.coli* as one of predominant Enterobacteriaceae should be taken into account when considering the sanitary standards and hygiene of food handling particularly minced meat, sausage, beefurger and local manufactured products either frozen or fresh (Stiles and Ng, 1981; Gobran, 1985 and Niazi and Refai, 1988).

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