

Animal Health Research Institute  
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# **GROWTH AND SURVIVAL OF *ESCHERICHIA COLI* CARRYING MULTIDRUG RESISTANCE PLASMIDS IN MINCED MEAT AT DIFFERENT HOLDING TEMPERATURES AND DURING ITS THERMAL PROCESSING WITH REFERENCE TO THEIR PUBLIC HEALTH HAZARD**

(With One Table and One Figure)

BY

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نمو وبقاء ميكروب الايشيريشيا كولاي الحامل للبلازميدات الخاصة بمقاومته العديد من المضادات الحيوية أثناء حفظ اللحم المفري في درجات الحرارة المختلفة وكذلك أثناء شيه مع الإشارة إلى خطورته على الصحة العامة

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تم اختيار عترة من الميكروب القولوني الممرض وحقنها في اللحم المفري. ثم تقسيم اللحم المفري المحقون إلى مجموعتين متساويتين. المجموعة الاولى تم تقسيمها إلى ثلاثة أقسام متساوية وحفظت عند درجات الحرارة ٢٠-٢٥ م° ، ٤ م° ، ٢٠- م°. وبالكشف الدوري على اللحم المفري عند درجة ٢٠-٢٥ م° بعد : صفر ، ١٢ ، ٢٤ ، ٣٦ ، ٤٨ ساعة وكذلك المحفوظة عند درجة ٤ م° بعد : صفر ، ١ ، ٢ ، ٣ ، ٤ ، ٥ ، ٦ ، ٧ ، ١٤ يوم والمحفوظة عند درجة ٢٠- م° بعد : صفر ، ١ ، ٢ ، ٣ ، ٤ ، ٥ ، ٦ أسبوع لمعرفة عدد الميكروب القولوني الممرض تبين أن الميكروب ينمو بزيادة كبيرة في اللحم المفري المحفوظ عند درجة ٢٠-٢٥ م° بينما يتناقص العدد في اللحم المفري المحفوظ عند درجة ٢٠- م° حتى يختفي. والمجموعة الثانية من اللحم المفري الذي تم حقنه تم اختبارها بتأثير حرارة الشى على الميكروب القولوني الممرض وجد أن أقصى درجة حرارة التجارب أثناء عملية الشى تتراوح بين ٤٥-٥٠ م°. وجد أن الميكروب القولوني الممرض لا يستطيع مقاومة حرارة الشى ويختفي نهائيا عند درجة ٥٠ م°. وقد تمت مناقشة الأهمية الصحية لوجود هذا الميكروب وسمومه على الصحة العامة.

## **SUMMARY**

Minced meat was experimentally inoculated by Enteropathogenic *Escherichia coli* (O<sub>114</sub>K<sub>90</sub>) strain and then divided into two parts: the

first of which was subdivided into three equal parts and kept at different holding temperatures: 20-25°C (room temperature) for 48 hours; 4°C for 14 days and -20°C for 6 weeks respectively. An obvious increase in *E.coli* count in minced meat samples kept at 20-25°C had occurred, while a noticeable reduction in counts had occurred in samples kept at -20°C and finally not detected at the end of the experiment. The second part was performed to find out the effect of thermal processing of minced meat on *E.coli*, the maximum internal temperatures of the sample during grilling on coke fire ranged from 45°C-50°C. The strain of *E.coli* could not resist the heating process of minced meat and was completely destroyed. Public health importance of the obtained results was discussed.

**Key word:** *E. coli* carrying plasmids, minced meat.

## INTRODUCTION

*Escherichia coli* is considered as a commensal in the alimentary tracts of most domestic and wild animals as well as man. Its persistence in the environment is limited so its presence is often used as an indicator of fecal contamination of water or food (Synge, 2000). Although many strains of *E.coli* are harmless inhabitant of the gastrointestinal tract, some can cause disease.

Enteropathogenic *E.coli* (EPEC) strains causing either watery or bloody diarrhea. The former associated with attachment, physical alteration of the integrity of the intestine. EPEC are highly infectious for infants, the dose is presumably very low. EPEC enteritis now appear to be relatively of little importance is poor (Susan and Cameron, 1994 and FDA, 2000).

The upper temperature of growth of mesophilic and psychrotrophic food-borne bacteria is important, since food is often held warm at temperatures near the maximum temperature of growth of these bacteria. In addition, an elevated incubation temperature (45°C) is recommended for the enrichment of various samples for the isolation of *E.coli* and fecal coliforms (Weiss *et al.*, 1983).

Heating foods will reduce the microbial population; the degree of reduction depends on the magnitude of the heat treatment, i.e., time and temperature. The heating is completed, stringent hygienic measures must be implemented to prevent recontamination of the food with psychrotrophic spoilage or pathogenic microbes. (Marth, 1998).

Freezing has been established as an excellent method of preserving quality in foods. It preserves the taste, texture and nutritional value of foods better than any other method and as a result an extensive quantities of food are now frozen world wide (Marilyn and Yen-Con, 1997).

The importance of temperature control during meat production and storage to prevent the out growth of *E.coli* strains and indicate that proper sanitation and processing practices that prevent and reduce contamination on carcasses with *E.coli*. (Berry and Koochmaraie, 2001)

The present study was conducted to determine the role of different holding temperatures as well as the effect of thermal processing of minced meat on viability and survival of *E.coli* O<sub>114</sub> K<sub>90</sub>.

## **MATERIAL and METHODS**

### ***Escherichia coli* culture:**

Enteropathogenic *E.coli* (O<sub>114</sub>K<sub>90</sub>) strain carrying multidrug resistance plasmids isolated in a previous study done by Refaie *et al.*, (2002). Stock culture was maintained on nutrient agar slant and grown for 18-24 hours in nutrient broth (Difco).

### **Preparation of samples:**

Minced beef meat samples were prepared under sterile conditions in the laboratory. The minced meat was examined for the presence of *E.coli* according to (ICMSF, 1978).

### **Inoculation and enumeration:**

The tested strain was counted after inoculation into nutrient broth and incubation for 18-24 hours by diluting 10 folds on Eosin Methylene Blue (EMB) agar plates (Biolife, Milano-Italy).

The prepared *E.coli* strain culture was (the approximate count was 10<sup>7</sup> cells/ml) well mixed with minced meat under sterile conditions (Ware, *et al.*, 1999 and Berry and Koochmaraie, 2001) and then divided into two parts: The first part was subdivided into three equal parts and kept at different holding temperatures (20-25°C for 48hrs.; 4 °C for 14 days and -20°C for 6 weeks) representing the normal keeping and refrigeration conditions. 10 gm from each inoculated minced meat at different holding temperatures were weighed and mixed with 90 ml sterile 0.1% peptone water in a blender jar and was mixed for 2.5 minutes (2000 rpm) to provide a dilution 10<sup>-1</sup>. Serial dilutions were prepared from the original dilution then streaked on EMB agar plates and incubated at 37°C for 18-24 hours. The second part was grilled on

coke fire for 15-20 minutes, the maximum internal temperature of inoculated minced meat was recorded. The grilled minced meat samples were then inoculated into nutrient broth (Difco) for 18-24 hours and streaked on EMB agar plates to examine its viability.

*E.coli* counts were determined at time of inoculation (time 0), then after 12, 24, 36 and 48 hours for minced meat samples kept at room temperature (20-25°C), 1, 2, 3, 4, 5, 6, 7 and 14 days for those held at 4°C and at time of inoculation 1,2,3,4,5 and 6 weeks for minced meat held at -20°C.

## RESULTS

The obtained results are recorded in table 1.

## DISCUSSION

The growth and survival of the *E.coli* strain in minced meat during storage as calculated from confirmed colonies on EMB agar are presented in Table (1) and Fig.1.

The counts of *E.coli* strains after inoculation held at 20-25°C were  $12 \times 10^5$ ,  $15 \times 10^6$ ,  $14 \times 10^7$ ,  $3 \times 10^8$  and  $13 \times 10^8$ /g at time of inoculation, 12, 24, 36, and 48 hours, respectively.

The results indicated that large increase had occurred in counts which agreed with the findings recorded by (Bentley *et al.*, 1989; Palumbo, *et al.*, 1995 and Siragusa, 1998).

Palumbo *et al.*, (1995) suggested that any food containing pathogenic *E.coli*, including the hemorrhagic strains, hold within 10-49°C range could generate an increased health hazard to consumers.

The counts of *E.coli* organisms in minced meat held at 4°C were  $12 \times 10^5$ ,  $11 \times 10^5$ ,  $16 \times 10^5$ ,  $13 \times 10^5$ ,  $8 \times 10^5$ ,  $15 \times 10^4$ ,  $12 \times 10^4$ ,  $5 \times 10^3$  and  $5 \times 10^3$ /g at time of inoculation, 1, 2, 3, 4, 5, 6, 7 and 14 days respectively. The results indicated that the counts of *E.coli* during this storage increased slowly then decreased and finally remain constant in number which agreement with observations of (Palumbo, 1986; Dorsa *et al.*, 1998 and Berry and Koohmaraie, 2001).

Low-temperature holding of foods (refrigeration at 5°C) was until recently, the primary means utilized to restrict the growth of pathogens in foods. The viable count remained constant, i.e., they survived (Palumbo *et al.*, 1997).

The counts of *E.coli* strains in minced meat held at -20°C were  $12 \times 10^5$ ,  $1 \times 10^4$ ,  $8 \times 10^3$ ,  $9 \times 10^2$ ,  $3 \times 10^2$ ,  $1 \times 10^2$  and 0/g at time of

inoculation, 1, 2, 3, 4, 5 and 6 weeks, respectively. The results revealed the growth pattern of *E.coli* organisms highly affected during frozen storage. The initial population drastic decrease in cell count during the first two weeks of storage, after that the viable cell count of *E.coli* showed a gradual decrease. Finally the viable cells at the end of the experiment which lasted for 6 weeks after which the organism was failed for detection. Nearly similar findings were recorded by (Sage and Ingham, 1998 and Barkocy-Gallagher, *et al.*,2002). Sage and Ingham (1998) reported that freezing and subsequent thawing of ground beef may provide an additional margin of safety against *E.coli* infection by killing a proportion of cells that may be present.

Grilling was done on coke fire for 15-20 minutes with maximum internal temperature 45-50°C. After grilling *E.coli* organisms were completely destroyed, these results were in accordance with the results obtained from (Palumbo *et al.*,1995; Dorsa *et al.*,1997 and Marth 1998). Heat do appear to add a degree of safety to beef products when *Escherichia spp* are initially present, even at high levels (Dorsa *et al.*,1997).

In conclusion, the results presented in this study indicated that *E.coli* increased in minced meat at 20-25°C and could be regarded as a public health hazard. *E.coli* strain can grow to high and hazardous level in minced meat stored above 4°C in the first days then decreased in number in prolonged time. Finally *E.coli* organisms were highly affected during frozen storage (-20°C) which decreased in number and at the end of experiment *E.coli* organisms failed to be detected.

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Table:1 *Escherichia coli* counts of stored minced meat at different temperatures

Hours/ 20-25°C	0		12		24		36		48					
	$12 \times 10^5$		$15 \times 10^6$		$14 \times 10^7$		$3 \times 10^8$		$13 \times 10^8$					
Days/ 4°C	0	1	2	3	4	5	6	7	14					
	$12 \times 10^5$	$11 \times 10^5$	$16 \times 10^5$	$13 \times 10^5$	$8 \times 10^5$	$15 \times 10^4$	$12 \times 10^4$	$5 \times 10^3$	$5 \times 10^3$					
Weeks/ -20°C	0		1		2		3		4		5		6	
	$12 \times 10^5$		$1 \times 10^4$		$8 \times 10^3$		$9 \times 10^2$		$3 \times 10^2$		$1 \times 10^2$		0	



**Fig. 1: *Escherichia coli* counts of stored minced meat at different temperatures**

