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**PREVALENCE AND SURVIVAL OF ENTEROPATHOGENIC  
ESCHERICHIA COLI (EEC) IN YOGHURT**  
(With 4 Tables)

By

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تواجد ومدى بقاء ميكروب الاشيرشيا كولاي الممرض ( EEC ) فى  
الزبادى

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أجريت هذه الدراسة لمعرفة مدى تلوث الزبادى المنتج محلياً فى مدينة أسيوط  
بميكروب الاشيرشياكولاي الممرض ( EEC ) . وقد تم جمع عدد 30 عينة من الزبادى  
عشوائياً من محلات البقالة والسوبر ماركت المختلفة وفحصت معملياً للتعرف على مدى وجود  
هذا الميكروب . وقد بين الفحص أن 46.6% من العينات تحتوى على ميكروب الـ E.coli  
وكان عدده يتراوح بين 10 ، 100 حتى 1000/جرام . وقد وجد أن عدد 9 عترات من  
عترات الـ E.coli المعزولة كانت من النوع الممرض والسبب لحالات التسمم  
الغذائى Enteropathogenic E.Coli وبدراسة مدى نمو وبقاء ميكروب الاشيرشيا  
كولاي الممرض ( EEC ) أثناء عملية تصنيع وتخزين الزبادى . قمناً بحقن ميكروب  
EEC ( 0/25/B15 ) فى لبن معقم تم صنع بعد ذلك إلى زبادى تم حفظه فى الثلاجة عند  
درجة 4± درجة مئوية لمدة عشرة أيام . وبقياس عدد ميكروب الاشيرشياكولاي والرقم  
الهيدروجينى ( pH ) أثناء التصنيع ودورياً أثناء التخزين تبين أن تناقص  
الميكروب المحقون مباشرة أثناء التصنيع وكذلك تدريجياً أثناء فترة التخزين فى الثلاجة  
حيث لم يمكن إكتشافه فى اليوم العاشر وكان عدده أقل من 10 خلايا / جرام . وأن تناقص  
الرقم الهيدروجينى من 6.5 إلى 4.5 بعد التصنيع ثم تناقص تدريجياً أثناء التخزين حتى  
وصل إلى أدنى حد له فى العاشر وكان 3.9 . وقد تم مناقشة خطورة هذا الميكروب على الصحة  
العامة وكذلك مايجب إتباعه عند تصنيع الزبادى للحصول على منتج خالى من ميكروب  
الاشيرشياكولاي الممرض .

**SUMMARY**

30 yoghurt samples were collected randomly from Assiut city markets, and examined for presence of enteropathogenic E.coli (EEC). E.coli could be isolated from 46.6% of the 7 examined yoghurt samples. The highest frequency distribution of E.coli (23.3%) lies within the range of 10-100/g. Nine out of 14 E.coli strains isolated from yoghurt samples (64.3%) were serotypes of EEC. They were serotyped as 026/B6, 025/K11, 044/ B74, 0111/B4, 0124/B17, 0125/B 15 and 0126/B16. Survival of EEC (Serotype 0125/B15) in yoghurt kept at 5±1°C was also studied. EEC survived until the end of the tenth day of storage at a population of less than 10 cells/g. The pH value of yoghurt decreased from 6.5 to 4.5 by the end of preparation and reached to 3.9 at the end of refrigerated storage.

## INTRODUCTION

Contamination of dairy products by *Escherichia coli* has been used as an index of unsanitary manufacturing or handling practices. Growth of *E. coli* in dairy products can cause defects in texture and flavor (YALE, 1943 and ERNSTROM, 1954). However, since the implication of certain strains of *E. coli* "designated" as enteropathogenic (EEC) in certain outbreaks of foodborne illness (COSTIN *et al.*, 1964 and MARIER *et al.*, 1973), presence of *E. coli* in dairy products has become a public health concern.

CORSLEY *et al.* (1955) stated that there is a relationship between pathogenicity and serotypes of *E. coli*. The pathogenicity of enteropathogenic strains of *E. coli* (EEC) has been well documented (COSTIN *et al.*, 1964 and OGAWA *et al.*, 1968). These strains can produce either a cholera-like (toxigenic) or a shigella-like (invasive) illness (OGAWA *et al.*, 1968 and SACK, 1975).

Several investigators have surveyed dairy products including yoghurt for presence of *E. coli* and EEC (PAPAVASILLIOU, 1957; MURRAY, 1960; JONES *et al.*, 1967; MOURSRY, 1969; SINGH and RANGANATHAN, 1974; STEFANOVIC and BACIC, 1981; GAD EL-RAB, 1983; ABBAR and MOHAMED, 1987 and AHMED *et al.*, 1988). Although there are no documented outbreaks of foodborne illness caused by EEC and traced to consumption of yoghurt, several studies have been conducted to characterize survival of EEC in fermented skim milk and yoghurt (GOEL *et al.*, 1971; FRANK and MARTH, 1977; PRA-SAD *et al.*, 1980 and MOHANAN *et al.*, 1985).

This study was performed to assess the presence of enteropathogenic *E. coli* (EEC) in yoghurt available in Assiut city markets, also to determine the safety of yoghurt prepared from milk artificially contaminated with EEC.

## MATERIALS and METHODS

### I- Occurrence of EEC in yoghurt :

#### 1- Collection and preparation of samples :

Thirty random samples of yoghurt were collected from different groceries and supermarkets in Assiut city. Each sample was handled and prepared directly for examination according to standard methods (MARTH, 1978).

#### 2- Enumeration and isolation of *E. coli* :

*E. coli* were estimated by a 3-tube Most Probable Number (MPN) technique according to A.O.A.C. (1975). Confirmatory tests were done on each isolate presumed to be *E. coli* as described by FISHBEIN *et al.* (1976).

#### 3- Analysis for enteropathogenic *E. coli* (EEC) :

Selection of *E. coli* for serological analysis was carried out according to the methods recommended by FISHBEIN *et al.* (1976). *E. coli* strains were screened serologically in the Dept. of Bacteriology, Faculty of Medicine, Assiut University.

### II- Survival of EEC in yoghurt :

#### 1- Cultures :

Enteropathogenic *E. coli* serotype O125/B15 originally isolated from yoghurt samples, was cultured in brain heart infusion broth (Oxoid) and incubated at 37°C

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for 24 hours. Starter cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) grown in sterile skim milk, were obtained from the Department of Food Science, Faculty of Agriculture, Assiut University.

## 2- Preparation and sampling of yoghurt :

Yoghurt was prepared from sterile milk. The milk was inoculated with EEC strain 0125/B15 at 45°C immediately after addition of the starter to provide  $2 \times 10^6$  cells/ml. Addition of starter cultures was done according to LAMPERT (1975). The infected yoghurt with its control were kept at 5±1°C. Samples to determine the EEC count and pH value were taken from milk after inoculation, from prepared yoghurt and daily thereafter up to 10 days. The samples were prepared for examination according to standard methods (MARTH, 1978).

## 3- Enumeration of EEC :

The method suggested by SPECK *et al.* (1975) was employed. Samples were surface plated onto Trypticase Soy Agar plates (Oxoid). The plates were held for one hour at room temperature followed by adding a layer of Violet Red Bile Agar (Oxoid), then they were incubated at 37°C for 24 hours.

## 4- pH determination :

The pH value of yoghurt was determined by using an Orion pH meter model 701, equipped with standard combination electrode.

## RESULTS

The obtained results were recorded in Tables 1, 2, 3 & 4.

**Table (1):** Counts and distribution of *E.coli* in yoghurt samples.

Counts/g	No. of the samples	%
/ 10	3	10
10-100	7	23.3
100-1000	1	3.3
1000	3	10
Total	14	46.6

**Table (2):** Occurrence of *E.coli* in yoghurt samples.

Positive samples		No. of isolated strains	Untypable <i>E.coli</i>		EEC	
No.	%		No	%	No.	%
14	46.6	14	5	35.7	9	64.3

**Table (3):** Enteropathogenic *Escherichia coli* (EEC) recovered from yoghurt samples.

No. of strains	Types of EEC
2	026/B6
2	025/K11
1	044/B74
1	0111/B4
1	0124/B17
1	0125/B15
1	0126/B16

**Table (4):** Survival of enteropathogenic *Escherichia coli* strain 0125/B15 during preparation and storage of yoghurt at  $5 \pm 1^\circ\text{C}$ .

Days of storage	Counts of EEC/g	pH value
Inoculum of milk	$2 \times 10^6$	6.5
0 time (prepared product)	$1 \times 10^6$	4.5
1	$1 \times 10^6$	4.3
2	$3 \times 10^4$	4.3
3	$2.9 \times 10^4$	4.28
4	$1 \times 10^4$	4.15
5	$1 \times 10^4$	4.14
6	$1 \times 10^4$	4.14
7	$1 \times 10^4$	4.1
8	$5 \times 10^3$	4.0
9	$1 \times 10^3$	4.0
10	$\angle * 10$	3.9

\* No colonies could be detected on the plate.

## DISCUSSION

### I- Occurrence of *E.coli* and EEC in yoghurt samples :

It is evident from Table 1, that *E.coli* could be detected in 46.6% of the examined yoghurt samples. The numbers of *E.coli* in the positive samples varied among 10, 100 and up to 1000/g. The highest frequency distribution of *E.coli* (23.3%) lies within the range of 10-100/g. Nine of 14 *E.coli* strains isolated from yoghurt samples were serotypes of EEC (Table 2). They were serotyped as 026/B6 (2 strains), 025/k11 (2 strains), 044/B47, 0111/B4, 0124/B 17, 0125/B15 and 0126/B16 (one strain each) (Table 3). Nearly a similar incidence of *E.coli* was obtained by GAD EL-RAB (1983), although he recorded higher levels of *E.coli*/g of yoghurt. A higher incidence (55%) of *E.coli* was obtained by MOURSY (1969), while a lower finding was recorded by STEFANOVIC and BACIC (1981) that *E.coli* could be detected in 10% of the examined yoghurt samples. No EEC could be detected between *E.coli* isolated from kefir and cultured creams (KHAZANOVA *et al.*, 1964); however, several investigators have surveyed milk and milk products for the presence of EEC (MURRAY, 1960; SINGH and

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RANGANATHAN, 1974; ABBAR and MOHAMED, 1987 and AHMED et al., 1988).

### II- Survival of EEC in yoghurt :

As recorded in Table 4, EEC decreased in numbers from  $2 \times 10^6$  to  $1 \times 10^6$ /g during preparation of yoghurt, and continued to lose its viability during the days of refrigerated storage ( $5 \pm 1^\circ\text{C}$ ). After 10 days of storage the viable counts of EEC became undetectable ( $< 10$  cells/g). A sharp drop in the pH value of yoghurt from 6.5 to 4.5 occurred by the end of its preparation, and a low value of 3.9 was reached by the end of the storage. It is obvious from the obtained results that the failure of EEC to grow in yoghurt and the loss in its viability may be due to the low pH value of yoghurt (4.5-3.9), and the competition of starter cultures (*L. bulgaricus* and *Strept. thermophilus*). These findings agree with the conclusion of PARK et al. (1973) that lactic acid fermentation in milk controls growth of *E. coli* mainly through lowering of pH. Furthermore, BIELECKA (1985) added that *Lactobacillus bulgaricus* had the greatest inhibitory effect on survival of EEC in yoghurt and that was also, confirmed by MOHANAN et al. (1985). However, in a previous study conducted by FRANK and MARTH (1977) EEC survived for about 17 days in refrigerated fermented skim milk. Also, it was found that *E. coli* increased in numbers from  $10^4$  to  $9.0 \times 10^5$ /g during preparation of Dahi and then decreased to  $9 \times 10^3$ /g after 10 days of storage at  $4-5^\circ\text{C}$  (PRASAD et al., 1980). The variation between these data and the obtained results can be attributed to the fact that the survival of *E. coli* in fermented dairy products is highly variable depending on starter cultures used, pH value, temperature of storage and composition of the product (GOEL et al., 1970 and PARK et al., 1973).

In conclusion, contamination of yoghurt by EEC from the view point of public health should not be ignored. Strict hygienic measures during preparation of yoghurt accompanied by rapid development of lactic acid fermentation by good fresh starter cultures and use of clean milk, are essential for making the product unfavorable for growth and survival of EEC.

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