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ISOLATION OF ENTEROPATHOGENIC AND ENTEROTOXIGENIC ESCHERICHIA COLI FROM MEAT AND CHEESE

By

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INTRODUCTION

Enteropathogenic Escherichia coli (EPEC) of certain serovars are well recognized pathogens as causes of infantile diarrhoea and/or gastrointestinal illness in adult humans (Bray, 1945; DuPont et al., 1971; Edelman and Levine, (1983). Recently, enterotoxigenic E.coli (ETEC) strains are now considered as a common causes of traveller's diarrhoea and/or endemic infantile illness and gastrointestinal illness in human in many countries following consumption of incriminated foods and water (Sack, 1975; Gorbach et al., 1975; Lee and Kean, 1978; Kristine et al., 1985). Such strains are known to produce a heat-labile enterotoxins (LT) that is antigenic and similar to cholera enterotoxin and/or a heat-stable enterotoxin (ST) that is of small molecular weight and non-antigenic (Sack, 1975; Frank et al., 1977). The abilily of enterotoxin production by E.coli is usually plasmid mediated, and this property may be easily lost in some strains, especially on subcultivation. propably due to loss of these plasmids (Evans et al., 1977).

ETEC strains have been shown to possess 0 antigens of various serovars; not all of which are included among the so-called classical enteropathogenic serovars of E. coli (Ørskov et al., 1976; Merson et al. 1979).

Moreover, ETEC are found to adhere to the intestinal epithelium of small bowel as they posses pilus-like adhesions factors (Colonization factor antigens) (Evans et al., 1978; Smyth et al., 1979; Ørskov and Ørskov, 1977).

Since the routine examination of E. coli contamination in food and water, and E.coli enteritis in many developing countries depends largely upon the use of serotyping, the present study was intiated to study the ability of the isolated serovars to produce enterotoxins and to agglutinate erthrocytes as indicative of colonization factors.

MATERIAL AND METHODS

Collection of samples:

A total of two hundred random samples of dairy products (Processed cheese and soft white cheese, 50 samples each) and meat products (raw minced meat and fresh sausage 50 samples each) were collected from different markets and shops in Giza and Cairo cities, and transferred directly in sterile plastic pags to Bacterial Toxins Unit, Animal Health Research Institute, , Dokki, Cairo, with minimum delay, where they were examined bacteriologically.

Isolation of Typical E.coli:

1 ml of the homogenised samples was used to inoculate E.coli broth (Difco) tubes, each with an inverted gas tube. After incubation at 44.5°C for 24-48 hours, tubes showing gas production were recorded as positive. A loopful from positive tubes were streaked directly onto the surface of levine's eosin-methylene blue agar (Difco) and Endo's agar plates and incubated at 37°C for 24-48 hours. Colonies resembling E.coli were selected and maintained on veal infusion agar slants for further identification.

Biochemical Identifications:

The isolated strains were confirmed by biochemical tests as outlined by standard techniques of Edwards and Ewing (1972) and biotyped according to Stiles and La-iking (1981) and Mehlman and Romero (1982).

Sero-typing (O:K-Serovars):

All the isolates were subjected to serological typification by the slide agglutination test using standard E.coli polyvalent and monovalent (Ok-agglutinating sera) (Wellcome Laboratories) for typing the enteropathogenic strains (Cruickshank et al., 1975).

Detection of pathogenic potenial (Virulence factors) of isolated straims:

1- Detection of fimbrial Haemagglutinins in vitro:

As indicative of colonization factor antigens or adhesive properties. The test was carried out using mannose resistant haemagglutinis (MRHA) of human (H) and bovine (B) erythrocytes according to the method of Smyth et al., 1979).

2- Growth of isolated E.coli strains for production of heat labile (LT) and heat stable (ST) enterotoxins and their detection:

Ten ml of Casamino acids-yeast extract medium (Evans, et al 1973; Alderete and Robertson, 1977) in a 250 ml Erlenmeyer flask were inoculated with 2 ml from the tested bacterial strain grown over night in nutrient broth. The flasks were then shaken (200 rpm) at 37°C for 24 hours on a rotary shaker. The culture was centrifuged (250 xg, 30 min, 4°C) and the supernatant was divided into 2 portions. The first portion for LT test was stored frozen at-70°C and tested within 1 week of preparation by serological procedure using Oxoid VET-RPLA, a Kit for the detection of E.coli heat labile enterotoxin by reversed passive latex agglutination. The second portion was heat treated (80°C, 30 min) before storing at-70°C for detection ST enterotoxin which were usually performed within

1 week of preparation using suckling mouse test. According to the method of Giannella (1976), three 2-to 3-days old mice were injected through the body wall directly into the milk filled stomach with 0.1 ml each of heat treated culture supernatant fluid containing one drop of 0.2 % (W/V) Evans blue per 1 ml of test material added as a marker just before testing. After 4 hs. the mice were killed, and the pooled intestinal weight-to-remaning-body-weight ratio of the three mice was determined. Samples yielding ratios ≥ 0.090 were considered as positive results.

RESULTS

1-Isolation and biotyping:

Out of 200 samples of meat and milk products analysed, E.coli isoltes were detected in 96 (48.0 %) samples (32 raw minced meat, 24 raw sausage, 15 processed cheese and 25 soft white cheese). A total of 93 of E.coli isolates (46.5 %) recovered from raw minced meat (32 isolates), raw sausage (22), processed cheese (14) and soft white cheese (25)were identified as E.coli biovar I.Of the 93 strains identified as biovar I only 89 isolates produced gas at 45.5°C in EC broth medium. Two strains (8.3%) of E.coli isolated from raw sausage and one strain (6.7 %) from processed cheese were identified as biovar II.

2- Serotyping of isolated E.coli strains:

Out of the 96 isolates of E.coli,only 35 isolates could be typed serologically **(Table 1)**. These strains revealed ten different serovars namely; 0119:K69 (9 strains), 026: K60 (6 strains), 0124: K72 (4 strains), 0128: K67 (4 strains), 0111: K58 (3 strains), 055: K59 (3 strains), 086: K 61 and 0114 K90 (2 strains each) and one strain from each 0125: K70 and 0127 K63. The serovar 0119: K69 was recovered from white cheese (5 strains),processed cheese(3 strains) and raw minced meat (one strain). The serovars 026: K60 and 055: K59 were isolated only from meat products only, mean while the serovars 0111: K58 and 0128:K67 were found only in miced meat. Whereas, the serovars

Table 1 : Enteropathogenic strains of Escherichia coli (EPEC) isolated from meat and cheeses.

Source	No. of examined samples	Positive *E. coli* isolates No.	%	Enteropathogenic serovars O:K (B) serovar	No.	%
Raw minced meat	50	32	64	26 : 60 (6)	3	9.38
				55 : 59 (5)	1	3.12
				111 : 58 (4)	3	9.38
				119 : 69 (14)	1	3.12
				128 : 67 (12)	4	12.50
				UN	20	62.50
Raw sausage	50	24	48	26 : 60 (6)	3	12.50
				55 : 59 (5)	2	8.33
				ON	19	79.17
Processed cheese	50	15	30	119 : 69 (14)	3	20.00
				125 : 70 (15)	1	6.67
				127 : 63 (8)	1	6.67
				UN	10	66.66
White cheese	50	25	50	86 : 61 (7)	2	8.00
				114 : 90 (1)	2	8.00
				119 : 65 (14)	5	20.00
				124 : 72 (17)	4	16.00
				UN	12	48.00
Total	200	96	48		35+	36.46 +
					61-	63.54 -

UN : untypable

Table 2 : Results of testing of E.coli strains isolated from meat and cheese for enterotoxin production (LT, ST or both) and mannose-resistant haemagglutination (MRHA) of human and bovine erythrocytes to detect the colonization (adhesive) properties.

O-Serovar of E.coli	No. of strains	Enterotoxin production			MRHA				Source
		LT	ST	LT+ST	H+B+	H+B-	H-B+	H-B-	
0 26	6	0	0	0	0	0	0	0	M , S
0 55	3	0	0	0	0	3	0	0	M , S
0 86	2	1	0	1	0	0	0	0	W
0 111	3	3	0	0	2	1	0	0	M
0 114	2	0	0	2	0	0	0	0	M
0 119	9	0	0	0	3	6	0	0	M , C
0 124	4	0	0	0	0	0	0	0	M
0 125	1	1	0	0	0	0	0	0	C
0 127	1	0	0	0	0	0	0	0	C
0 128	4	2	0	2	0	0	0	0	M
Untyped	61	6	2	1	0	0	0	0	M, S, C, W

LT = Heat-labile-enterotoxin
ST = Heat-stable "

MRHA = Mannose resistant haemagglutination test
H = Human erythrocytes
B = bovine erythrocytes.

M = Minced meat
S = Sausage
C = Processed cheese
W = White cheese

0125: K70, 0127: K63 were recovered from processed cheese and the serovars 0114: K90, 0124: K72 were obtained only from the soft white cheese.

3-Enterotoxin production:

Out of the 96 E.coli strains obtained from meat and milk products samples, 21 strains were found to be enterotoxigenic E.coli (ETEC), 13 (13.54 %) cultures were LT producers, 2 strains produced ST only and 6 strains were LT and ST producers (Table 2). Only 12 E.coli strains that belonged to the classic EPEC serovars 0/86: K61,0111:K68, 0114: K 90, 0125: K70 and 0128: K67 were found to be enterotoxigenic (ETEC). E.coli 0111: K58 and 0125:K70 were found to produce LT only. Strains of 086: K61 (2 strains) and 0128: K67 (4 strains) produced LT and LT & ST, while the 2 strains of 0114: K90 produced LT+ST. None of the later 0:K classic EPEC serovars produced ST only. On the other hand, of 61 unty pable E.coli strains, only 9 (14.75 %) were found to be enterotoxigenic, 6 strains produced LT only, 2 strains produced ST only and one strain produced LT & ST **(Table 2).**

4- Colonization (adhesive) properties:

The results of Mannose-resistant haemagglutinins (MRHA) testing are demonstrated in (Table 2). Among the examined E.coli strains, only 3 distinct serovars, namely,055:K59, 0111:K58 and 0119:K69 were MRHA positive. MRHA-positive using human and bovine erythrocytes were shown by strains of the serovars 0111: K58 and 0119: K69, while serovar 055: K59 showed MRHA-Positive with human erythrocytes only. On the other hand, E.coli strains of 0111: K58 that exhibited adhesive properties, were the only ones among the MRHA positive strains that produced LT enterotoxin only.

DISCUSSION

Since the public health authorties began to re-evaluate the role of E.coli in food and water illness(Marier et al. 1973), the routine laboratory screening for E.coli in food is not restricted for the isolation, biochemical and

serological identifications of enteropathogenic E.coli (EPEC) incriminated in food outbreaks, but it is extended to detect enterotoxigenic E.coli (ETEC), enteroinvasine (EIEC) and particular attention was directed to the adhesive properties of such strains that cause gastrointestinal illness in infants and adult human(Rowe et al., 1977; Danielsson, 1979; Gross 1984).

The incidence of E.coli isolates was particularly high in raw minced meat and soft white cheese. High exsistance of E.coli in fresh meat products and cheese has been previously reported by some investigators (Fantasia et al., 1975; Frank et al., 1977; Newton et al., 1977; Stiles and La-iking 1981). E.coli was found to dominate in raw meat handling areas of packing plants subsequent to surface contamination but not the cocked meat,whereas the number of E.coli is dramatically reduced by heat. In addition Fantasia et al. (1975) and Frank et al (1977) emphasized that E.coli is capable of growing in soft ripened cheese when low level are present intially even under refrigeration.

The present study has demonstrated that of 96 E.coli strains recovered from meat and milk products, 35 (36.5 %) strains belonged to 10 of the classic EPEC serovars, most of which were previously reported to be incrinated in different E.coli diarrhoeal outbreaks subsequent to food or water contamination (Fantasia et al., 1975; Dean et al. 1972; Marier et al., 1973; Rowe et al., 1977; Back et al., 1980).

In our study, 21 strains of E.coli isolates were found to be enterotoxigenic (ETEC) of which 12 strains belonged to 5 of the classic EPEC serovars and 9 strains were untypable.

These findings are consistent with that reported by Bäck et al. (1980) who recorded that it was possible that some of outbreaks of diarrhoea in which certain E.coli strains belonging to the EPCE group have been incriminated,

due to LT-and/or ST-producing strains, while others have been due to EPEC strains with other virulence factors. There is some evidence that enteropathogenicity of at least some EPEC strains, which do not produce LT or ST, is due to another enterotoxin (S), which is not detected by standard tests for ST and LT (Gross, 1984) e.g. cytotoxin, verotoxin or VT which differ from LT and ST (Levine et al. 1978; Scotland et al. 1980). On the other hand, Ørskov et al. (1976) and Merson et al. (1979) pointed out that the enterotoxigenic strains (ETEC) generally belonged to serovars different from those enteropathogenic strains.

Some studies have demonstrated that the adherence to the intestinal mucosa is an important virulence factor for EPEC strains (Clausen and Christie 1982, Mathewson et al. 1985). Our study has demonstrated that 3 distict EPEC serovars namely 055: K59, 0111: K58 and 0119: K69 were found to carry the adhesive properties manifested by MRHA test. It has been reported that E.coli 0119 is one of entero-adherent E.coli (EAEC) which was isolated from infants with sever diarrhoea and failed to produce LT, ST or even VT (Rothbaum et al., 1982). These findings support our findings in which E.coli strains of 0119: K 69 recovered from meat and milk products were MRHA positive and did not produce either LT or ST. The enteroadhesive property of 0111 commonly isolated from infants with chronic diarrhoea was previously studied by Clausen and Christie (1982). The authors found that this strain was non-enterotoxigenic and non-invasive. However, in the present study E.coli strains 0111: K58 that exhibited adhesive properties, was the only one among the MRHA positive strains that LT producers.

SUMMARY

Out of 200 samples of meat and cheese, E.coli isolates were detected in 96 (48 %) samples. The isolates were subjected for biochemical and serological identifications, ability for enterotoxin production and properties of mannose resistant haemagglutination (MHRA) (indicative of

adhesive properties). 93 of E.coli isolates were biochemically identifed as biovar I and 3 strains of biovar II. Of E.coli isolates, 35 (36.5 %) possessed the classic enteropathogenic E.coli (EPEC) serovars: 0119: K69 (9 strains), 026: K60 (6), 0124: K 72 (4), 0128: K67 (4) 0111: K58 (3) 055: K59 (3), 086: K61 (2), 0114: K90 (2) and 0125: K70; 0127: K63 (1 each). 21 (21.87%). of E.coli isolates were found to be entero toxigenic (ETEC). Only 12 (12.5 %) of ETEC cultures belonged to the classic EPEC serovars 0111: K58, 0125: K67 (produced LT only), 086: K61, 0128: K67 (produced LT and LT & ST) and 0114 : K 90 (produced LT + ST), and 9 stra ns (9.37 %) of untypable cultures produced LT only (6 strains), ST (2) and LT + ST (1). Only 3 distinct E.coli serovars 055: K59, 0111: K58 and 0119: K69 were MRHA positive and the serovar 0111: K59 was the only one among MRHA positve strains that produced heat-labile enterotoxin (LT).

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