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COLIFORM ORGANISMS AND ELECTROPHORETIC PATTERN OF ENTEROPATHOGENIC ESCHERICHIA COLI STRAINS RECOVERED FROM KAREISH CHEESE

(With 3 Tables and 1 Figure)

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الميكروبات القولونية والهجرة الكهربية لعترات الايشيرشياكولاي الممرضة فى الجبن القريش

أحلام اللبودى

تم جمع عدد خمسين عينة من الجبن القريش من الأسواق المختلفة فى مدينة الاسكندرية وذلك لعد وعزل وتصنيف الميكروبات القولونية التى اتضح وجودها بنسبة ٩٢٪ فى العينات المختبرة ، بمتوسط قدره ٦,٩ × ١٠^٧ لكل ١٠٠ جم وقد أمكن عزل عترات الايشيرشياكولاى بنسبة ٣٤٪ وكذلك تم عزل مجموعة ميكروبات الانتيروباكتر والستروباكتر وكذلك الكلبسيلا بنسب مختلفة . وقد تبين من نتائج التصنيف السيرولوجى لعترات الايشيريشياكولاى التى عزلت من عينات الجبن القريش أنها تنتمى الى ثمانية أنواع سيرولوجية ممرضة : $O_{124}:K_{72}:B_{17}$; $O_{26}:K_{60}:B_6$; $O_{55}:K_{59}:B_5$; $O_{119}:K_{64}:B_{14}$; $O_{86}:K_{61}:B_7$; $O_{126}:K_{71}:B_{16}$ and $O_{125}:K_{70}:B_{15}$ ولقد أوضح نموذج الفصل الكهربى للبروتينات بواسطة الهجرة الكهربية من جدار خلايا العترات المختلفة لميكروب الايشيريشياكولاى الممرض ان اجزاء عديدة من هذه البروتينات اختلفت فى الأوزان الجزيئيه ومكان الهجره للعترات المختلفه مما جعل كل جزء من هذه البروتينات له تركيز خاص وصفة مميزة ويمكن أتخاذ ذلك كطريقة لتصنيف عترات الايشيريشياكولاى الممرضة. هذا وقد نوقشت الأهمية الصحية والاقتصادية للميكروبات المعزولة وكذلك الاجراءات التى يمكن اتخاذها لتحسين حالة المنتج.

SUMMARY

Fifty random samples of kareish cheese were collected from different markets in Alex. City, were examined for incidence and enumeration of

coliform organisms. Ninty-two percent. of the examined kareish cheese samples proved to be contaminated with coliform organisms with a mean value (MPN / 100g) of $6.9X10^7$. Escherichia coli could be isolated from 34.0 % of examined samples, while Enterobacter Spp; Citrobacter Spp. and Klebsiella Spp. could be also isolated at varying percentages. The serological typing of isolated *E. Coli* strains were: O_{124}: K_{72} : B_{17} ; O_{26}: K_{60}: B_6 ; O_{55}: K_{59}: B_5 ; O_{119}: K_{64}:B_{14} ; O_{86}: K_{61}: B_7 ; O_{126}: K_{71}: B_{16} and O_{125}: K_{70}: B_{15}. The electrophoretic pattern of the proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) prepared from the cell wall of the different isolated strains of *E. Coli* revealed that several bands differ in migration position and molecular weights which differ among the different strains. Each one of them has a specific characteristic feature in the protein bands and concentration.

Key words: *Kareish cheese - E.coli - Electrophoresis*

INTRODUCTION

The presence of coliform bacteria in dairy products has been reported earlier by many workers (Singh and Ranganathan 1977, 1978 and Pandey and Mandal, 1980).

Coliform bacteria rank the first among food contaminats as they are widely spread in nature. It has been considered as an indicator organisms for pinpointing the unhygienic conditions during production and processing. In the recent years much attention has been paid towards Enteropathogenic Escherichia Coli because of its importance as an organism of true faecal origin with the possible presence of associated enteric organisms such as Salmonella and Shigella (Taylor et al., 1982); Moreover members of such strains have been implicated in several diseases among consumers (Chhabra et al., 1982 and Joshi and Kahlan, 1984).

Kareish cheese is considered as one of the most popular local type of soft cheese in Egypt. Several outbreaks of acute food poisoning resulted from the consumption of different types of soft cheese contaminated with Enteropathogenic *E. Coli* were recorded by Mariene et al. (1992). Consequently contamination of dairy products with such microorganisms from a variety of sources during production and handling constitutes a public health hazard. Therefore, this investigation was conducted to

throwlight on the incidence of Coliforms especially enteropathogenic strains of E. Coli in Kareish cheese in Alexandria markets, and to evaluate the electrophoretic patterns of the protein prepared from the cell wall of the different serotypes of enteropathogenic Escherichia coli.

MATERIAL and METHODS

Collection and Preparation of the samples:

Fifty random samples of kareish cheese were collected from different dairy markets in Alexandria City Egypt. Each sample was handled and prepared for examination according to the Standard Methods for the Examination of Dairy Products (A.P.H.A., 1985).

Examination for determination of coliforms:

Prepared samples were bacteriologically examined for enumeration of coliforms (MPN /100 gm) according to the technique recommended by A.P.H.A (1985).

The isolated and purified colonies were identified morphologically and biochemically according to Krieg and Holt (1984).

Serological identification of E. Coli isolates:

The slide agglutination technique was adopted for serotyping of E. Coli strains, using available Coli antisera of Boehringwerk AG., Marburg Germany.

Electrophoretic pattern of isolated serotypes of E. Coli:

This work was done in the Dept. of Dairy Science, Faculty of Agriculture, Alexandria University.

Preparation of samples for gel electrophoresis:

Enteropathogenic *E. Coli* serotypes (O_{111} K_{58} ; O_{124} k_{72} ; O_{26} K_{60}; O_{55} K_{59} ; O_{119} K_{64} ; O_{86} K_{61} ; O_{126} K_{71} ; O_{125} K_{70}) isolated from the examined

kareish cheese samples, were cultured in Brain heart infusion (BHI) broth and incubated at 37°C for 24 h., then centrifuged at 5000 xg for 20 min. at 4°C. The obtained pellets were resuspended in 0.5 molar tris HCl (pH = 6.8). Equal volume from each culture was mixed with buffer (2% SDS, 10% glycerol, 0.002% bromophenol blue, 1% mercaptoethanol). Each mixture was boiled at 100°C for 5 minutes, then cooled and centrifuged at 10.000 r.p.m/5 minutes.

Gel electrophoresis:

Analytical slab gel electrophoresis of the different strains of E. Coli was conducted in polyacrylamide gel containing 0.1% sodium dodecyl sulphate (SDS – PAGE) according to the conventional method which involved denaturation of protein by heating for 5 min. in 1% SDS in boiling water bath prior to applying them to the gel. After electrophoresis, protein were localized in gel using coomassie blue R-250, 0.1% as described by Laemmli (1970).

Protein molecular weight determination:

Cell wall proteins of different E. Coli strains were subjected to SDS–PAGE technique to determine their relative molecular weights as described by Yashida (1988). After electrophoresis, both length of the slab gel and the distance moved by the dye (bromophenol) were measured, also after staining and destaining. The length of the gel and the positions of the protein zones were recorded. Mobility of the protein fraction was determined using the following equation:

$$\text{Mobility} = \frac{\text{Distance of Protein migration}}{\text{Length after destaining}} \times \frac{\text{Length before staining}}{\text{Distance of dye migration}}$$

Mobility was platted against the known molecular weights expressed on a semi–logarithmic scale standard low molecular weight protein were purchased from Bio–Rad, CA, USA: Phosphorylase b: 97 KDa; bovine serum albumin: 67 KDa, OV albumin: 43 KDa; Carbonic anhydrase 31 KDa; Trypsin inhibitor 21 KDa and lysozyme 14.4 KDa.

RESULTS

The obtained results were recorded in Tables 1,2 and 3 and in Fig. 1

Table 1: Statistical analytical results of Coliforms Count (MPN/100 gm) in the examined kareish cheese samples:

Total No of Sample	Positive Samples N0	%	Min.	Max.	Average
50	46	92	86	$1.13X10^9$	$6.9X10^7$

Table (2): Incidence of Isolated coliform organisms recovered from the examined kareish cheese samples.

Isolates	No. of samples	%
Escherichia coli	17	34.0
Enterobacter cloacae	23	46.0
Ent. Aerogenes	9	18.0
Ent. Agglomerans	7	14.0
Citrobacter diversus	3	6.0
Cit. Amalanoticus	2	4.0
Cit. freundii	16	32.0
Klebsiella oxytoca	8	16.0
Kl. Ozoenae	6	12.0
Kl. Pneumonae	5	10.0

Table 3: Escherichia Coli Serotypes recovered from the examined samples of Kareish cheese:

Serotype	No	%
O_{111}: K_{58}: B_4	3	17.65
O_{124}: K_{72}: B_{17}	3	17.65
O_{26}: K_{60}: B_6	2	11.76
O_{55}: K_{59}: B_5	2	11.76
O_{119}: K_{64}: B_{14}	1	5.88
O_{86}: K_{61}: B_7	1	5.88
O_{126}: K_{71}: B_{16}	1	5.88
O_{125}: K_{70}: B_{15}	1	5.88
Untypable	3	17.65

DISCUSSION

Coliform organisms may exist in cheese in large numbers. They have been used as indicator of unsanitary manufacturing practice. It was only regarded as a general inhabitant of human intestinal tract. Nowadays it constitutes a great public health significance (Vijay and Sinha, 1989).

Results in Table, 1 shows that 92% of kareish cheese samples proved to be contaminated with coliform organisms with a mean value of $6.9x10^7$/ 100 g.

E. Coli represented in 34.0% from the examined Kareish cheese samples (Table, 2).

Frequency distribution of isolated coliform organisms revealed that Enterobacter Spp., Citrobacter Spp., and Klebsiella Spp., represented in varying percentages (Table, 2).

Lower incidence of Coliforms and *E. Coli* were recorded by EL–Bassiony (1975) and Collins - Thompson et al., (1977). Nearly similar findings were reported by Al-Ashmawy et al. (1977); Shelieh et .al (1987); Ahmed et al. (1988) and Moussa, et al. (1989).

The relatively high coliform counts obtained in this work shows to what extent the kareish cheese was exposed to contamination during various stages of production and handling. This may be also due to the fact that kareish cheese is usually sold fresh to consumers, besides to the organisms can grow readily in the product.

Serological typing of isolated *E. Coli* strains recovered from the examined kareish cheese samples belonged to 8 different serotypes O_{111}:K_{58}:B_4 (3 strains); O_{124}:K_{72}:B_{17} (3 strains); O_{26}:K_{60}:B_6 (2 strains); O_{119}:K_{64}:B_{14}; O_{86}:K_{61}:B_7; O_{126}:K_{71}:B_{16} and O_{125}:K_{70}:B_{15} (One strain each), (Table, 3).

These findings substantiate what have been found by Fantasia et al. (1975); Frank and Marth (1978); Kornacki and Marth (1982); Ahmed et al. (1988) and Massa et al. (1997).

The public health importance of E. Coli has been reported by many authers as enteropathogenic serotypes have been incriminated in cases of acute gastroenteritis in infants. The responsible serotypes for such syndrome are groups O_{86}, O_{119}, O_{126}, O_{55}, O_{111}, O_{125} and O_{86}. Other

serotype of *E. Coli* O_{124} was the causative agents of dysentry like syndrom or cholera like syndrome (Kornacki and Marth, 1982).

Food-borne outbreaks could also be due to Klebsiella organism (Frazier, 1967). While Citrobacter and Enterobacter species have been associated with cases of enteritis and urinary tract infection.

Fig. 1, shows the electrophoretic pattern of the proteins prepared from the cell wall of 8 isolates of *E. Coli*. Several bands differ in migration positions and molecular weights were obtained in each pattern. These bands considerably differ among the different strains. For example in electrophoretic pattern of isolate O_{55}., two protein bands of molecular weight 115 nad 148 KDa were absent comparing to the other strains.

Also, the protein bands of 40 and 34 KDa were more concentrated in O_{111}; O_{119}; O_{86} and O_{125} strains. Moreover, there was a protein band of 20 KDa wich was absent from the electrophoretic pattern of O_{111}; O_{114} and O_{55} compared with the other strains.

In conclusion, the results obtained indicates that kareish cheese samples were seriously contaminated by coliform organisms and *E. Coli* which reflex the unsatisfactory measures of such product. It could be concluded that the electrophoretic pattern method may be used as a successful technique for differentiation of enteropathogenic serotypes of *E.Coli* strains. The strict hygienic measure must be applied to avoid public health hazard.

From the results obtained from the electropheretic patterns of cell wall proteins of *E.Coli* strains, it could be concluded that this method may be used as a successful technique for the differentiation among them.

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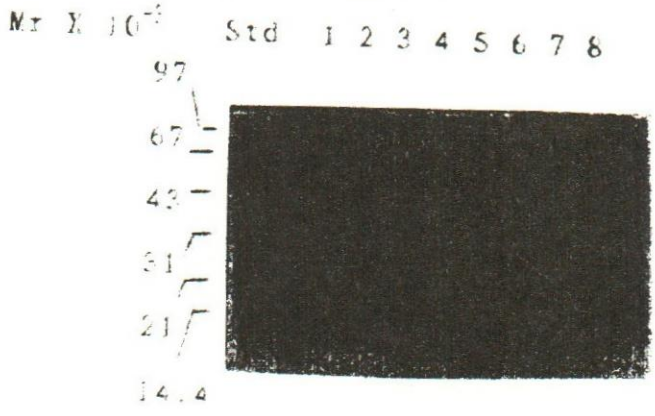

Fig. (1). SDS–PAGE (10% T) of cell wall proteins prepared from different strains of E. Coli.

Std.: standard low molecular weight proteins.

Lane 1: E Coli serotype O_{1}:K_{85}:B_{4}. Lane 2: E.Coli serotype O_{124}:K_{72}:B_{12}
Lane 3: E Coli serotype O_{26}:K_{60}:B_{6}. Lane 4: E Coli serotype O_{55}:K_{59}:B_{5}
Lane 5: E Coli serotype O_{119}:K_{69}:B_{14}. Lane 6: E.Coli serotype O_{86}:K_{61}:B_{7}
Lane 7: E Coli serotype O_{126}:K_{71}:B_{16} Lane 8: E.Coli serotype O_{125}:K_{70}:B_{15}