Prevalence of Enteropathogenic *Escherichia coli* Isolated from *Chhana* Based Indian Sweets in Relation to Public Health

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Abstract *Chhana* based milk products viz. *rossogolla*, *kanchagolla*, *narampak sandesh* and *karapak sandesh* are very popular in eastern part of India and gaining popularity in other parts of the country. A wide variation in manufacture method, microbial quality and shelf-life of these traditional milk products were observed by previous research. The aim of the present study was to determine the prevalence of contamination of *chhana* based milk products available in Kolkata city with Enteropathogenic *Escherichia coli* (EPEC) serogroups. Random samples of different *chhana* based milk products were collected from different parts of Kolkata city in aseptic condition, cultured in selective media and examined for biochemical tests. Among 240 samples, *E. coli* was isolated from 67 (27.91%) of them. Potential EPEC was present in 52 samples (21.66%) and 55 of the isolates were EPEC. Eleven serogroups were identified viz. O26, O55, O111, O119, O114, O125, O142, O86, O126, O127, O128. Among all these serogroups, O55 (23.66%) was the most prevalent. Though recent studies on virulence factors indicate that not all strains serologically classified as EPEC are able to attaching/effacing lesion, it is believed that the isolation of EPEC serogroups from *chhana* based milk products represent a potential risk for public health particularly children, as well as an indicative of the presence of other enteropathogens. Considering the public health importance of sweetmeat consumers, the product should be prepared hygienically reducing the microbial load present in it. The

result indicates that strict preventive measures should be adopted to ensure contamination free sweetmeats for the safety of the consumers.

Keywords *Escherichia coli* · Enteropathogenic · *Chhana* · Diarrhoea · Prevalence

Introduction

Enteropathogenic *Escherichia coli* (EPEC) strains have been implicated in food-borne human illnesses, especially as an important agent of infantile diarrhoea in developing countries [1]. Enteropathogenic *E. coli* strains are genetically mutative and cause different types of diarrhoea in patients. A combination of these strains with enterohemorrhagic *E. coli* increases their ability to create systematic infections in humans [2]. The pathogenicity of EPEC strains is not completely known but most of them produce vero toxins which are different from the enterotoxigenic *E. coli* (ETEC) toxins [3, 4]. Some EPEC strains are attached to intestine epithelium [4]. Different strains of EPEC cause a different level of infection, and it has been shown that these strains are continuously changing and their genome becomes similar to hemorrhagic strains [4]. Enteropathogenic *E. coli* strains traditionally have been defined as members of specific *E. coli* serotypes that have been epidemiologically incriminated as causes of infantile diarrhea and include the somatic (O) serogroups: O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 [5]. Disease due to EPEC can be severe, refractory to oral rehydration, protracted, and lethal [6–10].

Coliforms could be found in milk and milk products and are used as a hygienic indicator for such products [11]. Sweetmeat prepared from milk is an integral part of the

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culinary habits of people all over India. But these products are extremely vulnerable to contamination with spoilage and pathogenic organisms as well as toxic metabolites of microbial origin if subjected to advertent and inadvertent abuse during their production and processing. *Chhana*, a product of acid coagulation of hot milk and draining out of whey is used in preparing different kinds of sweets such as *rossogolla*, *Kanchagolla*, *Narampak Sandesh* and *Karapak Sandesh*. To improve the microbiological quality of these sweetmeats precaution against contamination must be taken at different Critical Control Points [12]. Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. *Escherichia coli* frequently contaminates food and it is a good indicator of fecal pollution [13–15]. The presence of coliforms in cheese and their relation to EPEC in soft cheeses has received considerable attention [16, 17]. Studies showed that 1–5% of food-infections were related to the consumption of milk and dairy products, that 53% of cases of food-borne infections caused by EPEC [16]. The microbiological safety and quality of foods are directly related to identify the number of microorganism present in the products. Coliforms have probably got more attention than most other groups of bacteria on account of their importance as indicator organisms for predicting unhygienic conditions during production and processing. *Escherichia coli* has attracted much attention recently as a potential pathogen since several strains of EPEC have been isolated from raw and pasteurized milk and milk products suspected to be associated with outbreak of gastroenteritis and food poisoning in human being [18].

Coliforms could be found in different *chhana* based Indian products and are used as hygienic indicator for such products [19]. Considering the above facts the present study was designed to record the serotypes and prevalence of contamination of *chhana* based Indian products viz. *rossogolla*, *kanchagolla*, *narampak sandesh* and *karapak sandesh* with EPEC strains, sold in different parts of Kolkata city.

Materials and Methods

Standard strains Standard strains of *E. coli* (MTCC-443) were procured from MTCC Chandigarh, India. All the isolates were confirmed through biochemical tests by comparing with the results of standard strains.

Collection of Samples

Two hundred and forty samples of *chhana* based milk products viz. *rossogolla*, *kanchagolla*, *narampak sandesh* and *karapak sandesh* (60 samples of each) were collected

randomly in aseptic condition from Kolkata and its suburban areas, transferred to sterile plastic container and were immediately transported to the laboratory of the Department of Dairy Microbiology. They were stored at 4°C and analyzed within 24 h. Sampling method and bacteriological examinations were carried out according to dairy products standard methods [20].

Microbiological Analysis

A Portion (10 g) from the centre of each sample was extracted aseptically and homogenized with 90 ml sterile enrichment broth (lactose broth) and incubated at 37°C for 24 h, for further biochemical analysis [21]. Coliform count was carried out by using five different dilutions, each with three replicates, as per MPN method (ISO 9308). Then the positive samples were examined for fecal coliform contamination [22]. For isolation and identification of *E. coli*, the enriched sample was cultured on selective medium eosin methylene blue (EMB) Agar and incubated at 37°C for 24 h. Morphologically typical colonies (at least four/plate) producing metallic sheen were taken into nutrient broth for further identification.

Physiological and Biochemical Analysis

Four to five suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests (Table 1). The culture characteristics of the isolates were confirmed by inoculating the pure colonies on Blood Agar, Nutrient Agar and Violet Red Bile Agar. Biochemical tests were performed to confirm the *E. coli* using catalase test, Simon's citrate agar, sugar fermentation on Triple sugar Iron Agar, Gelatin liquefaction, Indole production, Nitrate reduction, Urease production, Voges proskauer, Methyl red and presumptive test. The *E. coli* isolates thus obtained were stored at –20°C in tryptic soy broth containing 15% of glycerol. The isolates were propagated in sheep blood agar for serological tests.

Serological Analysis

For EPEC detection the isolates were screened by slide agglutination with three polyvalent O antisera (poly A: O26, O55, O111, O119; poly B: O114, O125, O142, O158; poly C: O86, O126, O127, O128). Isolates that were positive in the screening test were then agglutinated with monovalent O antisera. The strains belonging to the same serogroups and isolated from the same samples were reported only once. Positive control strains were included in each experimental run. A strain giving clump-ing with 4% saline was defined as rough.

Table 1 Culture characteristics on different media and biochemical reaction of *E. coli*

Culture characteristics		Biochemical reaction	
Media used	Observation of positive test	Tests performed	Observation for positive test
MacConkey's agar	Smooth, circular pink colonies with spreading growth	Catalase Simon's citrate	+ve –ve
Blood agar	Non hemolytic, grey white moist, glistening, opaque, circular, convex colonies with entire edge	TSI Gelatin	A/A + gas –ve
Nutrient agar	Colourless and yellowish white, circular smooth colonies with entire edge	Liquefaction Nitrate reduction	+ve
Violet red bile agar	Small, circular, pink, colonies	Urease	–ve
		Indol production	+ve
		Methyl red	+ve
		Voges proskaur	–ve
		Citrate	–ve
		Motility	+ve
		Presumptive test	+ve

Statistical Analysis

Categorical variables were compared by using Fisher's exact test. Continuous variables were compared by using analysis of variance or Student's *t* test. Correlations were evaluated by linear regression. A *P* value (two tailed) of less than 0.05 was considered significant.

Results

Results for the culture characteristics and biochemical reaction of *E. coli* colonies isolated from different *chh-ana* based milk products are depicted in Table 1. Fecal coliform was detected in all sweetmeat samples, indicating the occurrence of fecal pollution. Faecal and non faecal coliforms are confirmed from the colony characteristics on Eosine Methylene Blue Agar, MacConkey's Agar, Blood Agar, Nutrient Agar, Violet Red Bile Agar, Gram's staining and biochemical reactions (Table 1). Significant variations of total coliform count were observed among the type of sweetmeat samples ($P < 0.05$). Table 2 revealed that 62.08% of the total samples were positive for total coliform whereas 27.91% of samples were of faecal type. Total coliform count was highest in *kanchagolla* (90%), followed by *rossogolla* (78.33%) and *narampak sandesh* (53.33%). *Karapak sandesh* exhibited the lowest one. Similar trend was also registered in four types of sample for faecal coliform. Their distributions are 40, 35, 28.33, 8.33% for *kanchagolla*, *rossogolla*, *narampak sandesh* and *karapak sandesh* respectively. A total of 268 colonies were isolated from 240 samples and examined by biochemical tests, 186 colonies were detected as *E. coli* of which 55 strains were serologically identified as EPEC (Table 2). Eleven EPEC serogroups were isolated from sweet meat samples except *karapak sandesh*. Highest EPEC contamination was investigated in *kanchagolla* (47.27%) followed by *narampak sandesh* (25.45%) and *Rossogolla* (27.27%). *Karapak sandesh* samples did not exhibit any EPEC serogroup. Enteropathogenic *E. coli* serogroups included O26, O55, O111, O119, O114, O125, O142, O86, O126, O127, O128. The most common EPEC strain among the samples was O55 serogroup. Distribution of EPEC serogroups in four types of sweetmeat product are depicted in Table 3.

Table 2 Frequency of total and faecal coliforms in sweetmeat samples

Sweetmeats brands	No of samples surveyed	No. of positive samples for total coliform (%) ^a	No. of positive samples for <i>E. coli</i> (%) ^a	No. of positive samples for EPEC (%) ^a	No. of isolates taken (faecal & nonfaecal)	No. of positive isolates (faecal type)	No. of EPEC serogroup (%) ^b
<i>Kanchagolla</i>	60	54 (90)	24 (40)	22 (36.66)	96	68	26 (38.23)
<i>Rossogolla</i>	60	47 (78.33)	17 (28.33)	11 (18.33)	79	54	15 (27.77)
<i>Narampak sandesh</i>	60	32 (53.33)	21 (35)	19 (31.66)	62	45	14 (31.11)
<i>Karapak sandesh</i>	60	16 (26.66)	5 (8.33)	Nil (00)	31	19	Nil
Total	240	149 (62.08)	67 (27.91)	52 (21.66)	268	186	55 (29.56)

^a Figures in the parenthesis indicate percentage based on sample surveyed

^b Figures in the parenthesis indicate percentage based on positive faecal isolate

Table 3 Frequency distribution of Enteropathogenic *E. coli* serogroups isolated from *chhana* based milk Products

Serogroups → Samples ↓	O26	O55	O111	O119	O114	O125	O142	O86	O126	O127	O128	Total
<i>Kanchagolla</i>	2 (3.64)	7 (12.74)	2 (3.64)	3 (5.46)	2 (3.64)	1 (1.82)	1 (1.82)	2 (3.64)	1 (1.82)	3 (5.46)	2 (3.64)	26 (47.30)
<i>Rossogolla</i>	1 (1.82)	3 (5.46)	1 (1.82)	1 (1.82)	2 (3.64)	2 (3.64)	2 (3.64)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	15 (27.30)
<i>Narampak</i>	1 (1.82)	3 (5.46)	2 (3.64)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	1 (1.82)	14 (25.40)
<i>Sandesh</i>												
<i>Karapak</i>	–	–	–	–	–	–	–	–	–	–	–	–
<i>Sandesh</i>												
Total	4 (7.27)	13 (23.64)	5 (9.09)	5 (9.09)	5 (9.09)	4 (7.27)	3 (5.45)	4 (7.27)	3 (5.45)	5 (9.09)	4 (7.27)	55 (100)

Figures in the parenthesis indicate percentage based total serogroup

Discussion

The results in Table 2 indicate that 27.91% of samples were positive for *E. coli* among total samples, whereas Singh and Ranganathan [18] analyzed 100 various sweetmeat samples in which 47% were positive for *E. coli*. This variation of present study may be due to difference in storage, handling, transportation, packaging and product variability. Our results indicate that sample of hard grade variety sweetmeat viz. *karapak sandesh* has the lowest number for positive *E. coli* (8.33%) as compared to soft grade varieties viz. *kanchagolla* (40%), *rossogolla* (28.33%), *narampak sandesh* (35%). Similar findings were reported by Sen and Rajorhia [19] who observed that the microbial load in hard grade sweetmeat was minimum. Low microbial count in hard grade sandesh is due to low moisture and high sugar content as compared to soft grade variety [19]. Higher coliforms in soft grade sandesh may be due to high proportion of moisture and low amount of sugar that tremendously accelerate the growth of microbes in soft grade. Several other factors like unsanitary method of production, handling, storage and transportation are also responsible for such high microbial load in soft grade varieties of sweetmeat.

In our study *E. coli* was isolated from 27.91% of samples, and 29.56% of isolates belonged to the EPEC serogroup. Recent studies in other countries indicate similar results in other dairy products. In Brazil high levels of faecal contamination (95.5%) was detected in cheese samples, and EPEC was isolated from 21.1% of the samples [20]. Similar reports were found in Iraq where 40.5% of cheese samples were contaminated with EPEC strains [23]. In the present study we detected 11 different EPEC serogroups in four varieties of *chhana* based sweets and that O55 was the most prevalent serogroup. Recent studies in other parts of world indicate different prevalent EPEC serogroups in soft cheeses [20]. Tibana investigated 25.83% of positive EPEC serogroups among 78 of *E. coli* strains isolated from milk samples in Rio de Janeiro [24].

However, in the city of Ouro Preto, 605 strains of *E. coli* were isolated from one type of soft cheese and 9.8% were positive for EPEC serogroups [25]. Frank and Marth, in the United States, did not detect any EPEC serogroups in soft and semi-soft cheese samples [26]. In Harare, Zimbabwe 126 strains of *E. coli* were isolated from several foods and drinks consumed in a rural community, and 7.5% of them were EPEC serogroups [27]. Most prevalent serogroups found in milk and milk products in Egypt are O111, O126, O128, O26, O25 and O125 [20, 28]. In Iraq, four EPEC serogroups, including O111, O86, O125 and O119, are commonly isolated from cheese samples [29]. In the present investigation all EPEC serogroups were found in the soft variety sweetmeat samples tested and O55 was the most frequent one (23.66%). Contamination of dairy products by EPEC strains has been investigated and O126, O128, O25 and O125 were isolated [20, 29]. It was observed that O55 EPEC serogroup is one of most prevalent in children diarrhea in Brazil [30].

In conclusion, isolation of EPEC serogroups from *chhana* based sweets represents a potential, as well as indication of the presence of enteropathogens. However, more research should be carried out for genotypic characterization of the EPEC viz. typical and atypical serogroups. For typical EPEC, the only reservoir is humans; for atypical EPEC, both animals and humans can be reservoirs. Typical and atypical EPEC also differ in genetic characteristics, serotypes, and virulence properties. Atypical EPEC is more closely related to Shiga toxin-producing *E. coli* (STEC), and like STEC these strains appear to be emerging pathogens. Although recent studies on virulence factors indicate that not all EPEC strains are able to attaching/effacing lesion, it is however believed that high prevalence of contamination with EPEC strains increases the risk of infection for children due to the consumption of milk sweets. It seems that further epidemiological investigation is needed to reveal the importance of contamination in *Chhana* based milk sweets. The occurrence of a high proportion of *E. coli* in milk sweets samples may be due to the

lack of proper sanitation and improper method of processing during manufacturing of these products. It is worth to emphasize the importance of surveillance in manufacturing the Chhana based Indian milk sweets, since the presence of EPEC possibly indicates enteropathogens contamination. Therefore, stringent hygienic measures must be followed to prevent contamination of these sweets with coliforms.

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