

Isolation of Escherichia Coli from Indigenous Sweet Milk Products In Relation to Public Health Sold at Sweet- Meat Shops of Jalandhar City. India

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Abstract

This study was designed with aim to isolate Escherichia coli from indigenous sweet milk products i.e. Rasgulla and Rasmalai sold at sweet meat shops of Jalandhar city. Total 55 samples out of which 40 samples of Rasgulla and 15 samples of Rasmalai were randomly collected from the sweet meat shops of the different localities of Jalandhar city. The results of the present investigation revealed that out 55 samples, 16 samples (29.09%) were found contaminated with Escherichia coli. The highest rate of contamination was recorded from the samples of the Rasmalai that were 8 samples (53.33%), followed by Rasgulla 8 samples (20%). This organism is significant from public health point of view as they have been associated with the onset of food poisoning in human beings. However a large volume of these products are produced in unorganized sector, unbranded, with little precautions of food safety and quality. This investigation is a factual documentation of such a finding and suggestions as to the methods needed to improve the safety and quality.

Key words: Escherichia coli, Milk products, Food Poisoning, Public Health, Jalandhar City.

Introduction

Indigenous milk products refer exclusively to dairy products of a particular region or country. In the present context, these are products of Indian origin (Kumbhar et al. 2009). The significance of sweet milk products in human nutrition is now well established as it is considered as the best, ideal and complete food for all age groups. History tells that the first ever preparation of Rasgulla was by Nabin Chandra Das in Calcutta (Presently Known as Kolkata) in 1868 (Kamat 2005). It became an instant hit, even with the British, due to its cheesy, pleasantly sweet taste and texture. These days, preparation of Rasgullas is standardized and made easier. Any condensed milk is turned into cheese (Chhana) by adding citric acid or lemon juice and then simmered into light sugar syrup. Similarly Rasmalai consists of sugary white, cream or yellow colored balls of cheese soaked in malai (Clotted cream) flavoured with cardamom. As Indian scenario, milk and milk products is rated as one of the most promising sectors which deserves appreciation in a big way. About 45% of milk production is consumed as fluid milk. About 35% is processed into butter

or ghee; about 7% is processed into Paneer (cottage cheese) and other cheeses, about 4% is converted into milk powder; and the balance is used for other products such as Dahi (yoghurt) and sweet meats.

Indigenous sweet milk products like Rasgulla and Rasmalai are highly susceptible to variety of microorganism because of high nutritive value and complex chemical composition (Kumar and Prasad 2010; Grewal and Tiwari 1990). The manufacture of these products is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products (Soomro et al. 2002). Under such conditions many microorganisms can find access to the milk products. Among all microorganisms Escherichia coli is frequently contaminating organism, and is reliable indicator of fecal pollution generally in insanitary conditions of water, food, milk and other dairy products (Diliello. 1982). Two cases of hemolytic uraemic syndrome has been reported which provide evidence that raw milk may be a vehicle of transmission of *E. coli* O157: H7, both affected person consumed raw milk (Martin et al. 1986). Recovery of *E. coli* from food is an indicative of possible presence of enteropathogenic and/or toxigenic micro-organism which could constitute a public health hazard. Enteropathogenic *E.*

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coli (EEC) can cause severe diarrhea and vomiting in infants and young children (Anonymous 1975). According to the South Carolina department of Health and Environmental Control report an estimated 73,000 cases of *E. coli* infection and 61 deaths occur in USA each year (Anonymous 2006). In Germany 30 people have recently died at the hands of *E. coli*. Contaminated sprouts are to blame for the *E. coli* according to German health official (Georgi 2011).

Food borne pathogens are virtually inescapable reaching every aspects of life (Elhaleem and Elkarim 2011). Furthermore microbial contaminants are extremely difficult to pinpoint precision of their presence and role in food system (Biswas et al. 2011). The pattern of acute poisoning may be different even within the region or a country (Akhlaghi et al. 2009). It is therefore essential to increase the number of studies carried out on the importance of food poisoning (Sahingoz and Sahin 2009). *E. coli* was isolated from milk products like Mawa/ Khoa, Cream, Dahi, Cheese, Butter and Gulabjaman (Bhatt et al. 1948; Kumar and Sinha 1989; Kulshrestha 1990). In the context, this study was carried out to isolate the *E. coli* form indigenous sweet milk products sold at sweet meat shops of Jalandhar city.

Material and Methods

Collection of Sample: A total 55 samples of indigenous sweet milk products i.e. Rasgulla and Rasmalai sold at sweet meat shops from the different localities of Jalandhar City were randomly collected in the month August 2011. While collecting the samples, the investigator also considered the serving practice of the vendors, like, use of apron, covering of hair, handling of money and usage of gloves while serving. The samples were collected in the sterile sampling bottles that were kept in a cold box and were immediately shifted to the laboratory of Microbiology, Department of Biotechnology, Lovely Professional University, India.

Isolation and Identification of the *Escherichia coli*. Each 1g sample was enriched in 9ml Fagi Broth (Titan Biotech LTD.), a selective broth for the cultivation of *E. coli* and incubated at 37° C for 24 hrs. All the samples positive for *E. coli* growth was confirmed by spreading the 100µl of the inoculum form the Fagi Broth tubes on the Eosin Methylene Blue Agar (CDH Pvt. LTD.) and incubated at 37°C for 24 hrs. The plates were observed for the growth of *E. coli*. A single Colony was picked and sub cultured on Eosin Methylene Blue Agar for the purification of the isolate. Gram's staining technique was used for the study of the morphology of the isolates. The culture characteristics were confirmed by inoculating the pure colonies on Endo DEV Agar (Titan Biotech LTD.), Nutrient Agar (CDH Pvt. LTD), and Nutrient Broth (Titan Biotech LTD.) Table1. Biochemical tests were performed to confirm the *E. coli* using Catalase test, Simmons Citrate

Agar (Titan Biotech LTD.), Starch Agar (Titan Biotech LTD.), Indole production, Voges- Proskauer test , Urease Production, Nitrate Production, Methyl red and Presumptive test (Breed et al. 1957; Cappuccino and Sherman 2002) Table 2.

Table: 1 Culture Characteristics of *E. coli* on different media

Media Used	Culture Character
Eosin Methylene Blue Agar	Blue- Black colonies with green metallic sheen growth
Endo DEV Agar	Green metallic sheen colonies with deep pink growth
Nutrient Agar	Colourless and Yellowish white, circular, smooth colonies with entire edge
Nutrient Broth	Grayish Uniform turbidity having no Pellicle

Table 2: Biochemical reactions of *E. coli*

No. of Isolates	Biochemical Test	Reaction
16	Catalase Test	+ve
	Simmons Citrate	-ve
	Indole Production	+ve
	Methyl Red	+ve
	Voges- Proskauer	-ve
	Starch Test	-ve
	Urease	-ve
	Presumptive Test	+ve
	Nitrate Reduction	+ve

Results and Discussion

The results of the present study are summarized in the Table 3. According to these results the 16 samples out of 55 showed the contamination of *E. coli* (29.09%). The highest contamination was recorded from the samples of the Rasmalai which showed 8 out of 15 samples positive (53.33%), followed by 8 out of 40 Rasgulla samples (20%). The literature reviewed in the present study provided evidence that *Escherichia coli* is frequently occurring organism in indigenous sweet milk products such as Rasgulla and Rasmalai. The method of their manufacturing, handling, sale and transportation of these products are

entirely based on the tradition. As observed during sample collection, the handling of sweets with bare hands, non-usage of aprons, absence of hair covering and handling of money during serving might also contribute to poor hygienic conditions. Such system could pose favourable environment for bacterial contamination. Poor personal hygiene, the use of unhygienic water, unhygienic surroundings act as source of contamination (Chukuezi 2010; Mensah et al. 2002). In 2011 total 142 cases of food poisoning has been reported in which 1 death case was recorded in India (Anonymous 2011). According to Microbial Food Safety Regulation of India, E. coli must be absent in 1g sample of Khoya/ Chhana/ Paneer as per the PREVENTION OF FOOD ADULTERATION RULES, 1956. So presence of the E. coli may cause food poisoning and pose a threat to public health.

The Findings of the present study revealed that the problem of E. coli contamination of indigenous sweet milk products available in the sweet- meat shops of Jalandhar City exists. Thus more hygienic preventive measures are required to reduce the bacterial contamination, so as to increase the wholesomeness of these products.

Table 3: *Escherichia coli* contamination of sweet milk products

Product/ Sample	No. of samples collected	No. of positive samples	Percentage of positive samples
Rasgulla	40	8	20
Rasmalai	15	8	53.33
Total	55	16	29.09

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