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Isolation of *Escherichia Coli* from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam

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Abstract: Hundred raw milk and sixty milk product samples namely Gulabjamun, Mawa and Dahi were randomly collected from different localities/sources of Tandojam for the isolation of *E.coli*, a notorious contaminant. All the samples were inoculated on different bacteriological media and a number of biochemical tests were performed for the confirmation of the isolate. The results revealed that out of 100 milk samples 57% showed growth of *E. coli*. The highest number of milk samples contaminated with *E. coli* were recorded in milk samples obtained from milk vending shops and houses. Among the 60 milk product samples 31(51.66%) showed growth of *E.coli*, the highest rate of contamination was found in Mawa/Khoa samples.

Key words: Raw milk, milk product, *E.coli*, public health

Introduction

The quality of milk is determined by aspects of composition and hygiene. Due to its complex biochemical composition and high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Therefore in the processing of milk, some of them may produce undesirable effects and some micro-organisms produce food infections which can either carry the pathogens that will increase the likelihood of infection of the consumer`s food. The contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually milk is contaminated with different kinds of microorganisms at milk collecting places. Milk is a major part of human food and plays a prominent role in the Pakistani diet. Approximately 50 percent of the milk produced, is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as Ice cream, Butter, Khoa, Paneer, Rabri, Kheer, Burfi and Gulabjaman (Anjum *et al.*,1989). 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. Under such conditions many microorganisms can find access to the milk products. Among all micro-organisms *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). Martin *et al.*, (1986) reported two cases of hemolytic uraemic syndrome which provide evidence that raw milk may be a vehicle of transmission of *E.coli* O157: H7, both affected person consumed raw milk. 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. coli* (EEC) can cause severe diarrhoea and vomiting in infants and young children (Anon, 1975). In 1971 USA faced out break of food poisoning in which 387 persons were suffered with Enteropathogenic *E.coli* due to the consumption of imported French cheese (Marrier, 1973).

E.coli was isolated from milk products like Mawa/Khoa, Cream, Dahi, Cheese, Butter and Gulabjaman (Bhat *et al.*, 1948; Kumar and Sinha, 1989; Kulshrestha, 1990).

Considering the above facts the present study was designed to isolate the *E. coli* from milk and milk products sold under market conditions at Tandojam.

Materials and Methods

All the samples were collected in sterilized container at random from different localities of Tandojam town, and were brought to Dairy Technology Laboratory, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, for the isolation of *E. coli*. All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and bio-chemical examinations. The samples were inoculated on MacConkey Agar (Difco laboratories, USA) and incubated aerobically at 37 °C for 24 hours. The plates were observed for the growth of *E. coli*. A single, isolated colony was picked and sub-cultured again on MacConkey agar for purification of the isolate. Simultaneously another single colony with similar characters was picked for the preparation of smear and stained with Gram's stain for the examination of staining and morphological characters of the isolate using bright field microscope. The cultural characteristics of the isolates were confirmed by inoculating the pure colonies on Blood Agar, Nutrient Agar, Nutrient Broth and Violet Red Bile Agar (Table 1). Biochemical tests were performed to confirm the *E. coli* using catalase test, Simmon's Citrate Agar, sugar fermentation on Triple Sugar Iron Agar, Gelatin liquefaction, Indole Production, Nitrate reduction, Urease production, Voges proskaur, Methyl red and Presumptive test (Table 2).

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

Media used	Culture character
Mac Conkey Agar	Smooth, circular pink colonies with spreading growth.
Blood Agar	Non hemolytic, grey white moist, glistening, opaque, circular, convex colonies with entire edge.
Nutrient Agar	Colorless and yellowish white, circular, smooth colonies with entire edge.
Nutrient Broth	Organism showed uniform turbidity.
Violet Red Bile Agar	Small, circular pink colonies.

Table 2: Biochemical reactions of *E.coli*

No. of Isolates	Biochemical Test	Reaction
88	Catalase	+ve
	Simmon's citrate	-ve
	TSI	A/A+gas
	Gelatin liquefaction	-ve
	Indole Production	+ve
	Nitrate Reduction	+ve
	Urease	-ve
	Voges Proskaur	-ve
	Methyl Red	+ve
	Presumptive test	+ve

Results

The results of the present study are summarized in the Table 3. According to these results the highest *E. coli* contamination was recorded from the samples of raw milk obtained from milk vending shops and houses in 13 out of 20 samples each (65%), followed by raw milk samples obtained from milk vendors on donkey that

Table 3: Summarizes the over all percentages of *E. coli* contamination in milk samples

Source/locality	No. of samples collected	No. of samples found positive	(%)
Dairy farm	20	9	15
Milk vendors on cycle	20	10	50
Milk vendors on Donkey	20	12	60
Milk vending shops	20	13	65
Houses	20	13	65
Dahi	20	11	55
Gulabjamun	20	8	40
Mawa	20	12	60
Total	160	88	

is 12 out of 20 (60%), raw milk obtained from milk vendors on cycle showed 10 out of 20 samples (50%) and raw milk collected from dairy farms showed 9 out of 20 samples (45%) contaminated with *E.coli*. Where as in case of 60 milk product samples 51.66% were found positive for *E. coli* contamination. However the highest number 12 (60%) of Mawa/Khoa samples were contaminated with *E.coli* in contrast to dahi 11(55%)followed by Gulabjaman samples 8 (40%).

Discussion

The literature reviewed in the present study provided evidence that *Escherichia coli* is frequently occurring organism in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic. The milk sold in raw form poses a great hazard to public health without adopting hygienic measures because of possibilities of contamination with *E.coli*. The raw milk falls on an easy prey to bacterial contamination because of high ambient summer temperatures of Pakistan. The results of milk samples showed that 57 out of 100 samples were contaminated with *E.coli* are in agreement with the results of Naqvi, 1972; Martin *et al.*, 1986; Hanjra *et al.*, 1989; Ahmed and Sallam, 1991; Sharma and Joshi, 1992; Adesiyun, 1994.

The results of the milk product samples revealed that highest percentage of Mawa/Khoa samples were contaminated with *E. coli* as compared to Gulabjaman samples. However, both milk products (i.e Mawa/Khoa and Gulabjaman) available at Tandojam market were highly contaminated with *E. coli*. Indeed, indigenous sweet products are commonly manufactured and consumed in Pakistan; the method of production, handling, transportation and marketing of these products are entirely depend upon traditional system. Such system could pose favourable environment for bacterial contamination. The unclean hands of worker, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination of milk products and the post manufacturing contamination.(Bhat *et al.*,1948; Marrier, 1973; Tariq Masud *et al.*, 1988; Kumar and Sinha, 1989; Grewal and Tiwari, 1990; Kulshrestha, 1990). Where as the traditionally made dahi available in the market of Tandojam was also found contaminated with *E.coli*. The results of the study are supported by Tariq Masud *et al.* (1988). Although *E.coli* is frequently occurring organisms in milk and its products, the incidence of the species of *E. coli* itself in milk and milk products as a possible cause of food borne disease is insignificant because *E. coli* normally is a ubiquitous organisms (Hahn, 1996). Important, however, is the occurrence of pathogenic strains of *E. coli* in milk products which could be hazardous for consumers.

Conclusion: The results obtained in this study concluded that milk and milk products available to the consumer have a high *E.coli* contamination. Thus, the results of the present study warn the need for more strict preventive measures. For this, regular sterilization of dairy equipment, washing of utensils, milker's hands, udders, eradication of diseased animals, pasteurization/boiling of milk is required before collection and distribution for consumption and product making. In this respect immediate cooling to 5 °C and/or pasteurization of milk could be more effective. The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels.

Thus present study suggest to isolate and characterize the different strains of *E.coli* which may cause the pathogenicity in milk products and also to investigate the HACCP of such strains in milk products.

References

Adesiyun, A.A., 1994. Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad. Intr. J. Food Microbiol., 21: 253-261.

Ahmed, A.M. and S.S. Sallam, 1991. Prevalence of *E. coli* serotypes in raw milk and some dairy products. Assiut. Vet. Med. J., 25:93-97.

Anjum, M.S., K. Lodhi and A.A. Raza, 1989. Pakistan's Dairy Industry: Issues and Policy Alternatives. Special Report Series No. 14. Pak Econom. Analysis Network Project Islamabad

Anonymous, 1975. *E. coli* Enteritis. Lancet, 1131-2

Bhat, J.V, K. Sethna and F. Fernandes, 1948. Ind. J. Dairy Sci., 49.

Difco, 1969. Difco Manual, Difco Laboratories Incorporated Detroit Michigan, 48201, U.S.A.

Diliello, L.R.,1982. Methods in Food and Dairy Microbiology. AVI Publishing Co. Inc. Westport Connt. USA:39

Grewal, J.S. and R.P. Tiwari, 1990 Microbiological quality of rasmalai. J. Food Sci. and Tech., Mysore, 27: 178-179.

Hahn, G., 1996.Pathogenic bacteria in raw milk- situation and significance. In: Bacteriological quality of raw milk. Int. Dairy Federation, Brussels (Belgium), pp: 67-83.

Hanjra, S.H., M.A. and B.B. Khan, 1989. Market quality of milk in Pakistan, Dairy Technology in Pakistan. PARC, Islamabad, 58.

Kulshrestha, S.B., 1990. Prevalence of enteropathogenic sero groups of *E. coli* in milk products samples from Bareilly and their multiple drug resistance. Ind. J. Dairy Sci., 43: 337-378.

Kumar, V. and R.N. Sinha, 1989. Incidence of coliforms in indigenous milk products. Ind. J. Dairy Sci., 42: 579-580.

Marrier, R., 1973. An outbreak of enteropathogenic *E. coli* food borne disease traced to imported French cheese Lancet, 1376-8.

Martin, M.L., L.D. Shipman, M.E. Potter, L.K. Wachsmuth, J.G.Wells, K. Hedberg, R.V. Tauxe, J.P. Davis, J. Arnoldi and J. Tilleli, 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of hemolytic uraemic syndrome. Lancet., 8514, 1043.

Naqvi, Z.H., 1972. Studies on the microflora composite samples of milk routinely supplied to market with particular emphasis on their identification and source of origin. M.Sc. Thesis W.P.A.U., Lyallpur.

Sharma, D.K. and D.V. Joshi, 1992. Bacteriological quality of milk and milk products with special reference to Salmonella and its public health significance. J. Food Sci. and Tech. Mysore, 29: 105-107.

Tariq Masud, I.H. Athar, M. Azar and M. Amin Shah, 1988. Microbiological studies on indigenous dahi with special reference to public health. J.A.H.P. Vol., 8-13