Bacteriological Study on Coliform Organisms from Ethiopian Traditional Cheese West Showa Zone, Ethiopia

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Abstract: The study was conducted between September 2008 and April 2009 based on the objective of isolation and identification of *Enterobacteriaceae* and determining the prevalence of coliforms including *E. coli*O_{157} H_7 in Ethiopian cottage cheese (Ayib) samples collected aseptically from purposively selected two local open markets in Holeta and Ada Berga districts. 225 cottage cheese samples were collected from one local open markets in each district and an overall coliforms prevalence of 27.8% (95%CI=23.7-31.9) was identified. The prevalence of coliforms in Ada Berga and Holeta open markets accounted for 28.9% (95%CI=26.76-31.09) and 26.7% (95%CI=22.61-30.79) respectively. The 225 samples of cottage cheese collected from Holeta local open market showed a prevalence 10.7 (24/225), 8.0 (18/225), 5.3 (12/225), 2.7 (6/225) and 2.2 (5/225) for *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Enterobacter cloacae* and *E. coli*O_{157} H_7 respectively . Similar samples size of cottage cheese collected from Ada Berga local open market showed occurrence of *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Enterobacter cloacae and E. coli*O_{157} H_7 at 12 (27/225), 8.9 (20/225), 3.6 (8/225), 4.4 (10/225) and 3.6% (8/225) respectively. There was no statistical significance (χ^2=0.227, dF =1, p-value= 0.559) in the prevalence of coliforms between the selected study areas. It can be included that there is a need for the regulatory authorities and producers to ensure the proper handling of the byproduct to prevent the public from tremendous health problems.

Key words: *Enterobacteriaceae* · Holeta · Ada Berga · Prevalence · Ayib

INTRODUCTION

Indigenous foods are produced in homes, villages and small cottage industries at lower prices within the means of a majority of consumers in the world. An examination of these foods will provide clues how production and preservation can be expanded and thereby contributing to improved nutrition and reduction of poverty in African countries. Man has long preserved dairy products during high milk production period through curdling, cheese making, butter making and create the potential for trade [1].

Cheese is an excellent source of protein, fat and minerals such as calcium, iron, phosphorus, vitamins and essential amino acids and therefore it is an important food in the diet of youngsters and elderly. Cheese produced is generally consumed very soon after manufacture, primarily because of the poor shelf life under ambient conditions [2]. Traditional cheese is mainly produced from raw non-pasteurized milk. In general, raw milk contains 30% of undesirable organisms in total bacterial count hence it requires great attention during production of dairy products [3]. A number of standard tests are employed to determine the quality of dairy products. Among them enumeration of coliforms is an important test to evaluate the sanitation of foods.

Coliforms refer to those members of the Enterobacteriaceae that ferment lactose such as *Escherichia, Klebsiella* and *Enterobacter* species [4]. Coliforms are normal flora of the gastrointestinal tract which invade sterile parts of the body after consumption of defective foods and cause serious infections which are said to be "opportunistic pathogens" [5]. *Escherichia coli* are of interest among coliforms when present it indicates recent faecal contamination with the possibility of accompanying enteric pathogens of which *E. coli*

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O_{157}:H_7 is one of the most serious of known coliform food borne pathogens [6, 7]. Considering the above facts, the study was conducted to isolate, identify and to determine the prevalence of coliforms and possibly *E. coli* O_{157}: H_7 in cottage cheese.

MATERIALS AND METHODS

Sample Collection: A total of 450 cottage cheese samples each weighing 100gm was collected aseptically in a sterile universal bottle from 2 local open markets in Holeta and Adeaberg districts of West Showa zone, Ethiopia during the period (September 2008-April 2009). All samples were transported in icebox to the Microbiological Laboratory of Faculty of Veterinary Medicine, Addis Ababa University for microbiological analysis.

Isolation and Identification of Coliforms: General guidelines of detection, isolation and identification of Enterobacteriaceae from dairy products were applied in this study [8]. Different culture media such as MacConkey agar (DIFCO, USA) and Eosin methylene blue agar (DIFCO, USA) were used to isolate coliforms and *E. coli*. Similarly, Sorbitol MacConkey agar (SMAC, Oxoid CM0813, England) was used to isolate O_{157}: H_7.

Enrichment Technique: Twenty five grams (25gm) of each labelled cheese sample was diluted in 225 ml lactose broth and vortexed using a stomacher 400 (Seward Medical, England) at high speed for two minutes and incubated for 18 – 24 hrs at 37°C.

Plating: A loopful of inoculums' from the enriched broth was streaked on MacConkey agar (DIFCO, USA) for primary screening of coliforms based on colony

morphology after 24hrs of incubation at 37°C. Eosin methylene blue (EMB) agar (DIFCO, USA) and Sorbitol MacConkey agar were also used to characterize *E. coli* and *E. coli* O157:H7 respectively.

Data Management and Analysis: The data were entered and analyzed in MS Excel and SPSS version 15.0, 2006 software. Difference among and between proportions of the groups was analysed using Chi-square ($\chi2$) test and confidence interval for prevalence. Descriptive statistics such as percentage and frequency distribution were employed to describe or present the nature and characteristics of the data.

RESULTS

A prevalence of coliforms of 26.7% (60/225) was detected in the samples collected from Holeta local open market. Similarly, 28.9% (65/225) coliform prevalence was found in samples obtained from Ada Berga local open market (Table 1). Among the 60 positive samples of coliforms at Holeta region; *E. coli*, *K. pneumoniae, E. aerogenes and E. cloacae* were detected at 24/225, 18/225, 12/225 and 6/225 respectively. The prevalence of *E. coli O157:H7* was 2.2% (5/225) and 3.6% (8/225) in Holeta and Ada Berga local open markets, respectively (Table 2).

Out of 65 positive samples for coliforms at Ada Berga district; *E. coli, K. pneumoniae, E. aerogenes and E. cloacae* were isolated at 27/225, 20/225, 8/225 and 10/225 respectively (Table 2). The results showed higher prevalence of coliforms at AdaBerga than Holeta region. As well as the study revealed that there was no statistical significant difference ($\chi^2 = 0.277$, df= 1, P value = 0.599) in

Table 1: Prevalence of coliforms in the examined samples

Study area	Nº of samples (n)	Nº of positive	Prevalence of coliforms (%)	χ^2-value	P-value	95% Confidence interval
Holeta	225	60	26.7	0.277	P=0.599	22.6-30.8
Ada Berag	225	65	28.9			26.8-31.0
Total	450	125	27.8			23.7-31.9

Table 2: Proportion of coliform organisms in the study samples

Isolates	Nº of isolates (%)		Overall prevalence (%)
	Holeta	Ada Berga	
E. coli	10.7	12	22.7
K. pneumoniae	8.0	8.9	16.9
E. aerogenes	5.3	3.6	8.9
E. cloacae	2.7	4.4	7.1
E. coli $O_{157:}$ H_7	2.2	3.6	5.8

Table 3: Prevalence of different coliform organisms in the study areas

Study area	Isolates	N° positive	Prevalence	χ^2-value	GhP- value
Holeta	*E. coli*	24	10.7%	0.199	P=0.656
Ada Berga		27	12.0%		
Holeta	*K. pneumoniae*	18	8.0%	0.115	P = 0.735
Ada Berga		20	8.9%		
Holeta	*E.aerogens*	12	5.3%	0.837	P = 0.360
Ada Berga		8	3.6%		
Holeta	*E.cloacae*	6	2.7%	1.037	P = 0.309
Ada Berga		10	4.4%		
Holeta	*E. coli* $O_{157:}$ H_7	5	2.2%	0.173	P = 0.398
Adaberg		8	3.6%		

the prevalence of coliforms between the two districts (Table 1). Table 3 displays the prevalence of *E. coli, K. pneumoniae, E. aerogenes, E. cloacae* and *E. coli O157:H7* in the collected samples from Holeta and Ada Berga regions.

DISCUSSION

Although coliforms are frequently occurring organisms in milk and milk products, the incidence of this organisms itself as possible cause of food borne disease is insignificant, because these organisms are normally ubiquitous organisms so that they are an indicator of faecal contamination [9]. The importance, however, is the occurrence of pathogenic strains of *E. coli* in milk and milk products which could be hazardous for the consumer. In Ethiopia Yilma *et al*. [10] isolated Citrobacter, Enterobacter, Klebsiella, Providencia, Salmonella, Serratia, Hafnia, Klyuvera and Erwinia from traditional dairy products such as cheese. In addition important human pathogenic species such as *Enterobacter sakazakii* and *Klebsiella pneumoniae* were also identified in the same product.

The study revealed overall prevalence 27.8% (125/450) for coliforms which is a little higher than findings of Padilla and Fernandes [11] (24%). However, the current finding is lower than the results of Adenike *et al*. [12] who indicated 35% prevalence rate coliforms of which 23% was *E. coli* isolates.

A study done by Adenike *et al*. [12] in Ethiopia showed the isolation rate of *E. coli* (24.3%) and *K. pneumoniae* (18.8%) from fermented dairy products is higher as compared to the current findings. This variation in the results of coliform prevalence status could be due to the difference in cheese manufacturing, location, hygienic handling while milk collection, packaging, preservation and transportation. According to El Safey and Abdul-Raouf [13] 26% incidence of *E. coli* from soft

domietta cheese, soft karish cheese and ras cheese, which is nearly in accordance with the overall prevalence of *E. coli* isolates (22.7%) found in present study. Moreover, a study done in Egypt, on 60 karish cheese samples demonstrated that *E. coli* was isolated from 75% of the samples [14].

In one study a prevalence of 9.7% of *K. pneumonia* in milk, ice-cream, cheese and jammed samples was reported [14]. However, current prevalence of *K. pneumonia* (16.9%) is higher as compared to this finding. The present study also recorded an overall prevalence of *E. coli* 22.7%. The study is not in accordance with the study conducted by Jacques *et al*. [16] who reported the prevalence of *E. coli* as 34% in semi soft cheese.

Ladan and Reza [17] isolated *E. coli* from 98% of soft-cheese samples and 19.48% of isolates belonged to EPEC serogroups. Araujo *et al*. [18] detected high prevalence of faecal contamination in 95.5% of cheese samples in Brazil and EPEC was isolated from 21.1% of the samples. Similarly, Abbar and Kaddar [19] reported that 40.5% of cheese samples in Iraq were contaminated with EPEC strains.

The study revealed an overall prevalence of *E. coli O157 :H7* (5.8%) and several coliform species such as *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Enterobacter cloacae* at overall prevalence of 28.7% are found to be occurred in processed samples. This indicated that there is a great risk of contamination of cheese with these organisms. Therefore, reducing contamination of milk from different sources should be used to produce wholesome cheese.

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