

Occurrence of multidrug resistance shiga-toxin producing *Escherichia coli* from milk and milk products

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Abstract

Aim: The aim of this study was to assess the hygienic quality of milk and milk products in respect of shiga toxin producing *Escherichia coli* (STEC).

Materials and Methods: 205 samples of raw milk and milk products were processed for isolation of *E. coli*. The isolates were screened by mPCR for detection of virulence gene. 52 *E. coli* isolates were tested against 15 commonly used antibiotics in the field.

Results: Of the 205 samples of milk and milk products 52 (25.36%) were positive for *E. coli*. Out of which 4% samples were positive for STEC from raw milk and 3.64% from milk products. Also, 25 of 52 (48%) of *E. coli* isolates were multidrug resistance whereas 62.5% of STEC from milk and milk products were multidrug resistance.

Conclusions: The milk and milk products production is of poor hygienic quality in and around Jammu region which needs improvement.

Key words: milk, mPCR, multidrug resistance, STEC

Introduction

Shiga toxin producing *Escherichia coli* (STEC) are widely distributed in the intestinal flora of humans and warm blooded animals. Cattle, sheep and poultry birds are thought to be the reservoir for STEC [1], although other animals including goat, pigs, fowls, cats and dogs do harbour these bacteria. It has been reported that the transmission can occur through the contaminated milk and milk products [2]. The pathogenicity of STEC is associated with a number of virulence factors, including shiga toxins (encoded by the *stx*₁ and *stx*₂ genes), intimin (encoded by the *eaeA* gene), and the haemolysin (encoded by the *hlyA* gene) [3]. During the past decade STEC have emerged as a major food borne pathogen of public health concern responsible for mild diarrhea to Haemorrhagic colitis, Haemorrhagic uraemic syndrome (HUS) [4] and Thrombocytopenic purpura (TPP) [5] in humans. STEC is highly infectious organism and the infective dose may range between 10 to 50 organisms [6]. This was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhoea traced to food restaurants [7].

However, still the significance of milk and milk products as the source of STEC needs to be elucidated, particularly in Jammu region of Jammu and Kashmir state. Considering the impact of the STEC in veterinary and human medicine as an emerging food borne

pathogen, present study was carried out with the objectives to investigate occurrence of *E. coli*, virulence gene profile of STEC and antimicrobial susceptibility of *E. coli* isolates from milk and milk products.

Materials and Methods

The samples from foods of animal origin comprising 150 of raw milk (10 ml each), 30 of cheese (10 gm each) and 25 of *kalaari* (10 gm each) were collected twice a week in and around Jammu. The samples were collected in sterile containers and transported to laboratory over ice for further processing of *E. coli*. The isolation of *E. coli* was achieved by enrichment in MacConkey broth, followed by plating on Eosin Methylene Blue (EMB) agar (Hi Media, Mumbai, India). Suspected colonies were confirmed by standard microbiological techniques [8, 9].

The mPCR for detection of *stx*₁, *stx*₂, *eaeA* and *hlyA* virulence genes of the *E. coli* isolates was done as per the method of Paton and Paton [10]. The primers used are listed in Table-1. DNA amplification was performed in a thermal cycler (Master Cycler Gradient, Eppendorf, Germany). PCR products were analyzed by agarose gel electrophoresis and the specific DNA bands were visualized using ethidium bromide staining under UV illumination.

The *E. coli* isolates obtained from the samples were examined for their antimicrobial drug susceptibility/resistance pattern by disc diffusion technique of Bauer, et al. [11]. The antibiotic discs used were obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai.

Table- 1. List of primers (5'-3') used in the mPCR reaction

Primer	Sequence (5'-3')	Amplicon size	Reference
<i>stx</i> ₁ -F	ATAAATCGCCATTCGTTGACTAC	180bp	(10)
<i>stx</i> ₁ -R	AGAACGCCCACTGAGATCATC		
<i>stx</i> ₂ -F	GGCACGTCTGAAACTGCTCC	255 bp	
<i>stx</i> ₂ -R	TCGCCAGTTATCTGACATTCTG		
<i>eaeA</i> -F	GACCCGGCACAAGCATAAGC	384bp	
<i>eaeA</i> -R	CCACCTGCAGCAACAAGAGG		
<i>hlyA</i> -F	GCATCATCAAGCGTACGTTCC	534bp	
<i>hlyA</i> -R	AATGAGCCAAGCTGGTTAAGCT		

Table- 2. Occurrence of different virulence genes among the *E. coli* isolates from milk and milk products

Types of samples analysed	No. of isolates screened	No. of different types of genes					
		<i>stx</i> ₁	<i>stx</i> ₂	<i>stx</i> ₁ & <i>stx</i> ₂	Total STEC	<i>eaeA</i>	<i>hlyA</i>
Milk	46	1 (2.17)	2 (4.35)	3 (6.52)	6 (13.04)	4 (8.69)	6 (13.04)
Milk products	6	0 (0)	2 (33.33)	0 (0)	2 (33.33)	2 (33.33)	1 (16.67)
Total	52	1 (1.92)	4 (7.69)	3 (5.77)	8 (15.38)	6 (11.54)	7 (13.46)

Numbers in parenthesis indicate percentages

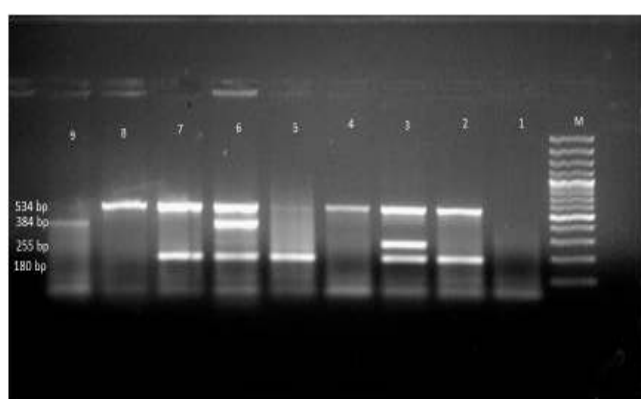


Figure-1. Agarose gel showing mPCR amplification products of *stx*₁, *stx*₂, *eaeA* and *hlyA* genes in *E. coli* isolate.

Lane M: 100 bp Molecular weight marker,
Lane 1: negative Control,
Lane 2, 7: Amplified products of *stx*₁ and *hlyA* genes,
Lane 3: Amplified products of *stx*₁, *stx*₂ and *hlyA* genes,
Lane 4, 8: Amplified product of *hlyA* gene,
Lane 5: Amplified product of *stx*₁ gene,
Lane 6: Amplified products of *stx*₁, *eaeA* and *hlyA* genes,
Lane 9: Amplified products of *eaeA* genes.

The isolates were tested against 15 commonly used antibiotics viz., Amikacin 30µg, Ampicillin 10µg, Amoxicillin 10µg, Cefotaxime 30µg, Cefixime 5µg, Chloramphenicol 30µg, Cefuroxime 30µg, Ciprofloxacin 5µg, Co-trimoxazole 25µg, Gentamicin 10 µg, Nalidixic acid 30µg, Norfloxacin 10µg, Tetracycline 30µg, Kanamycin 10µg, and Streptomycin 10 µg.

Results and Discussion

Due to ability of STEC to cause outbreaks of fatal illness, many studies have been conducted in different parts of the globe. In the present study, 46 of 150 (30.66%) milk and 6 of 55 (10.90%) milk product samples were found contaminated with *E. coli*. Similar results were obtained by Daood, [12] who reported the prevalence of *E. coli* in milk (35.45 %) and in sweet cheese (7.14 %). Also similar results were obtained by Rashid, et al. [13], who reported 33.96% in milk and 14.89% in milk product samples. However, our findings are in contrast with those of Soomro, et al. [14] whose results revealed prevalence of *E. coli* in milk and milk products to be 51.66 and 57% respectively. Similarly, Ali and Abdelgadir [15] screened 100 raw milk samples from different localities/sources of Khartoum State. Their results revealed 63 percent of the samples to be positive for *E. coli*. High prevalence of *E. coli* was reported in different types of milk by many researchers [16,17].

The presence of different types of genes among *E.*

coli isolate are shown in Table-2. Eight of 52 (15.38%) isolates from milk and milk products were possessing *stx*₁ and or *stx*₂ genes (Fig.1) as detected by PCR and designated as STEC. The results of the present study are in agreement with the findings of Rashid, et al. [13] where 16.98% of STEC were found in milk and 8.51% in milk products.

Varying prevalence of STEC in milk and milk products has also been reported by other workers. These include: 0.87% in raw milk samples of cattle [18]; 33.5% in raw milk from Malaysia [19]; 3.3% in milk samples from Brazil [20] and 0.75-16.2% in various dairy products [21]. This might be due to varying hygienic milking practices being followed. However, a total of 52 *E. coli* isolates comprising of 46 isolates from milk and 6 from milk products were tested for their antibiotic sensitivity/resistance pattern to various antibiotics. Twenty five of 52 (48%) isolates from milk and milk products showed multidrug resistance to three or more antibiotics. Chloramphenicol was the most effective with 83% sensitivity followed by Norfloxacin with 80% sensitivity observed among the isolates. Further, 74 % Ciprofloxacin and Co-trimoxazole, 60% to Gentamicin, 54% Nalidixic acid was sensitive. Whereas 68% of isolates were resistant to Amikacin, 67% to Amoxicillin, 60% to Ampicillin, Cefixime and Kanamycin.

The results of our study show varying antibiotic sensitivity pattern of *E. coli* in milk and milk products as compared to reports of other researchers. Daood [12]

studied the antibiotic resistance pattern of *E. coli* in milk and milk products. The resistance was observed against Ciprofloxacin (0%), Gentamicin and Chloramphenicol (3.28%), Tetracycline (16.39%), Amoxicillin/Clavulanic acid (92.26%), Ampicillin (95.08%), Cefoxitin (96.76%), Cephazoline (97.54%), Cephalothin (98.36%) and Sulfamethoxazole/Trimethoprim (100%). The highest resistance was reported against Tetracycline (92.2%), followed by that to Streptomycin (90.4%) and Nalidixic acid (88.3%); lower resistance to Amoxicillin/Clavulanic acid and Ceftriaxone were detected in 14.2% and in 17.7% of the isolates, respectively by Rangel and Marin [22].

Resistance to Ampicillin and Gentamicin was the most common finding (44.4%), followed by resistance to Erythromycin (33.3%), Amoxicillin (11.1%), Tetracycline (11.1%) and Nalidixic acid (11.1%) observed by Rahimi, et al. [23]. The highest resistance observed to Tetracycline, Penicillin, and Enrofloxacin with the frequency rate of 58.82, 46.07 and 45.09% respectively, as well as the lowest resistance to Nitrofurantoin and Ciprofloxacin with the frequency rate of 3.92 and 7.84% was observed by Momtaz, et al. [24]. So findings of our study differed from the findings of other workers. It could be assumed that the resistance pattern of *E. coli* had changed with respect to antimicrobial drugs being used in the area both in animals and human beings. Further the drug resistance attribute is mediated through plasmids, however, needs further confirmation.

Conclusions

On the basis of present results, it could be concluded that the microbial quality of milk and milk products sold in retail shops/local markets of Jammu area is not satisfactory. It is suggested that the raw milk and milk products should be handled under strict hygienic condition with constant microbial monitoring and stored in cool places to avoid recontamination and to safeguard the health of consumers. With a large population rearing cattle in this area, public health awareness including safe and hygienic practices while handling cattle or their products would be paramount importance in reducing the STEC infections in humans. Moreover, to prevent the reduced risk, the incorporation and consistent application of good manufacturing practices and hazard analysis of critical control points at every stages of food chain, from farm to retailer by those involved with handling and processing raw milk and milk products in both manufacturing and home environment should be followed.

Authors' contributions

JA has carried out the research work, MR guided the student to carry out the work and prepared the draft of the research work, MUR helped in collection of samples. MAB finalized the research plan and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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