MOLECULAR DETECTION AND CHARACTERIZATION OF ESCHERICHIA COLI ISOLATED FROM RAW MILK SOLD IN DIFFERENT MARKETS OF BANGLADESH

M. A. Islam, S. M. L. Kabir* and S. K. Seel

Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

The study was intended for molecular detection of E. coli isolated from raw cow’s milk. A total of 20 milk samples were collected from different upazila markets of Jamalpur, Tangail, Kishoreganj and Netrokona districts of Bangladesh. Milk samples were cultured onto various culture media for the isolation of bacteria. The isolated bacteria were identified by studying staining characteristics, cultural properties on different selective media, biochemical tests, catalase and coagulase test, and finally by PCR. Out of 20 samples, 15 (75%) milk samples were found positive for E. coli. 15 Escherichia coli isolates were amplified by 16S rRNA gene based PCR. Antimicrobial sensitivity test was carried out to ascertain the susceptibility of the organism to various antibiotics. Its results showed that the E. coli isolates were resistant to amoxycillin (86.67%) and erythromycin (73.33%) but sensitive to azithromycin (53.33%), ciprofloxacin (86.67%), gentamicin (86.67%), norfloxacin (80%) and streptomycin (66.67%).

Key words: Escherichia coli, 16s rRNA gene, raw milk

INTRODUCTION

Milk is considered as one of the high nutritional quality foods. Whole milk is the milk as it comes from the cow and contains about 3.5% milk fat. Research continues to expand the positive role of milk and milk products on individual’s health. Evidence goes well beyond bone health to include its effects on immunity, mild hypertension, reducing selected cancers (Giovannucci et al., 1998), supporting weight management strategies and increasing satiety in dieters, among other positive effects. However, it is an excellent medium for growth and transmission of different bacterial pathogens to humans (Donkor et al., 2007). Escherichia coli is a Gram-negative, rod-shaped bacterium, a member of the family Enterobacteriaceae and commonly found in the lower intestine of warm-blooded organisms (Singleton, 1999). It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk and contaminated raw vegetables and sprouts. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. EHEC produces toxins, known as vero-toxins or Shiga-like toxins because of their similarity to the toxins produced by Shigella dysenteriae. The pathogenic strains of E. coli that when ingested, causes gastrointestinal illness in healthy humans and animals. Most strains of E. coli live as commensals, many perhaps all are opportunistic pathogens of human and animals (Levine, 1984). Though it is a part of normal enteric microflora, but it is capable of producing serious diarrheal diseases, as well as other systemic diseases, especially infection of the urinary tract. E. coli and other facultative anaerobes constituted about 0.1% of gut flora (Eckburg et al., 2005) and oro-fecal transmission is the major route through which pathogenic strains of the bacterium cause disease. Over the last half-century it has become increasingly obvious that there are a number of different pathogenic groups of E. coli. Pathogenic E. coli strains are categorized into pathotypes on the basis of their virulence genes. At least six known pathotypes associated with gastrointestinal infections have been recognized, apart from those opportunistic “nonpathogenic strains” causing urinary tract infections, diarrhea, sepsis, and meningitis in humans and a number of similar diseases in animals. Although most sporadic cases and outbreaks have been reported from developed countries, human infections associated with Shiga toxigenic E. coli (STEC) strains have also been described in Latin America, India and other developing countries (Kaddu-Mulindw et al., 2001; Leelaporn et al., 2003). In Bangladesh, the predominant group of E. coli associated with childhood diarrhea is enterotoxigenic E. coli; accounting for approximately 20% of all diarrheal cases (Qadri et al., 2005).

*Corresponding e-mail address: lkabir79@yahoo.com

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Several works have been done throughout the world regarding the milk and microorganisms of contaminated milk (Hassan et al., 2014). In Bangladesh, a few works have been done on isolation and molecular characterization of E. coli from raw milk of cattle and buffalo (Alaine et al., 2006; Islam et al., 2008; Hossain et al., 2011, Hossain, 2015). Moreover very few works have been reported in Bangladesh on molecular detection of E. coli with other pathogenic organisms in cow’s raw milk in a specific time period. So, keeping the above facts in mind, the present study was designed with an objective to molecular detection and characterization of bacteria isolated from raw milk samples. We studied the antimicrobial susceptibility patterns of isolated bacteria with the detection of E. coli using 16s rRNA gene.

MATERIALS AND METHODS

Sample collection
A total of 20 cow’s raw milk samples were collected from different upazila markets of Jamalpur, Tangail, Kishoreganj and Netrokona districts of Bangladesh using falcon tube. The samples were collected from January 2015 to June 2015 and investigation was carried out following collection. The collected milk samples were immediately transported on ice to the Microbiology Laboratory at the Department of Microbiology and Hygiene of BAU and District Veterinary Hospital, Sirajgonj for bacteriological analysis.

Isolation and identification of E. coli
The collected milk samples were performed 10 fold dilution using 0.1% peptone water and diluted samples were inoculated onto MacConkey (MC) agar by pour plate method and incubation at 37°C for 24 hours. Then sub cultured onto eosin methylene blue (EMB) agar, MC agar by streaking to obtain pure culture. These isolates were preserved for further bacterial identification. The isolates were identified as E. coli on the basis of Gram staining, colony morphology on MC agar, EMB agar, biochemical characterization of the isolates (using sugar fermentation test, indole and MR-VP test), catalase and coagulase tests. Further the isolates were confirmed by amplification of E. coli specific 16S rRNA gene.

Bacterial Genomic DNA extraction:
A pure bacterial colony of E. coli was mixed with 100 μl of distilled water which were boiled for 10 minutes then immediately kept on ice for ice shock. Finally centrifugation was done at 10000 rpm for 10 minutes. The supernatant were collected and used as DNA template for PCR (Engler and Kelley, 2000).

Identification of E. coli by PCR
Escherichia genus specific PCR was performed to amplify 16S rRNA gene of E. coli. Two different primers pairs were used for this purpose, 16S rRNA gene (F 5’-GACCTCGGTTTAGTTACAGA-3’ and R 5’-CACACGCTGACGCTGACCA-3’) according to the methods described by Schippa et al. (2010). Each 20 μl reaction mixture consists of 3 μl genomic DNA, 10 μl PCR master mixtures (Promega, USA), 1 μl of each of the two primers with the final volume adjusted to 20 μl with 5μl of nuclease free water. Amplification was done by initial denaturation at 95°C for 5 minutes, followed by denaturation at 94°C for 45 sec, annealing temperature of primers was 55°C for 45 sec and extension at 72°C for 1 minutes. The final extension was conducted at 72°C for 5 minutes. The total reaction was performed at 30 cycles. The amplified PCR products were resolved by electrophoresis in 2% agarose gel at 100v for 30 minutes, stained with ethidium bromide and finally visualized under UV trans-illuminator.

Antibiotic Sensitivity Test
All isolates of E. coli were tested for antimicrobial drug susceptibility against antimicrobial agents by disc diffusion method according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007). Sensitivity pattern of the isolates were determined against ciprofloxacin, azithromycin, gentamicin, amoxycillin, streptomycin, erythromycin and norfloxacin. Antimicrobial testing results were recorded as sensitive, intermediate sensitive and resistant according to zone diameter interpretative standards provided by CLSI (2007).
RESULTS AND DISCUSSION

20 raw milk samples were analyzed in the laboratory by different cultural, biochemical, staining and molecular methods to detect *Escherichia coli* in raw milk. Out of 20 samples, 75% (n=15) were contaminated with *E. coli*. In MC agar plates, the samples showed bright pink or red colonies in MC agar were identified as *E. coli* (Figure 1). In EMB agar colonies produced by *E. coli* are smooth, circular, black color colonies with metallic sheen (Figure 2). The microscopic examination of Gram’s stained smears from MC agar and EMB showed that the isolated bacteria were Gram negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain (Figure 3). *E. coli* showed negative result to catalase and coagulase test. DNA extracted from *E. coli* isolates was used in the PCR assay. PCR primers targeting 16S rRNA gene of *E. coli* amplified 585 bp fragments of DNA confirmed the identity of *E. coli*. Result of PCR for *E. coli* is shown in Figure 4. Isolated *E. coli* were analyzed for the antibiotic susceptibility. The results of antimicrobial susceptibility test showed that 8 (53.33%) isolates were susceptible to azithromycin, 10 (66.67%) isolates were susceptible to streptomycin, 13 (86.67) isolates were susceptible to gentamicin, 12 (80%) isolates were susceptible to norfloxacin, 11 (73.33%) isolates were susceptible to tetracycline and 10 (66.67%) isolates were susceptible to streptomycin. Furthermore, 12 (86.67%) isolates were resistant to amoxycillin and 11 (73.33) isolates were resistant to erythromycin (Figure 5).
The present study was structured for identification and characterization of *Escherichia coli* from cow’s raw milk sample. Total 20 samples were collected and analyzed. Out of 20 samples, 15 samples were revealed the positive result for *E. coli*. *E. coli* was identified and confirmed by cultural examination, morphological studies, staining characters and biochemical tests and finally PCR were performed for the amplification of specific gene (16s rRNA gene) of isolated bacteria. Milk is the best media for the growth of many bacteria in which some of them are pathogenic. As we know fresh milk is enriched with pathogenic and non pathogenic bacteria which can be transmitted to human by milking and consumption of milk. *Coliform* bacteria present in the fresh raw milk might be hazardous if proper boiling of milk is not done during consumption. It causes disease if proper hygienic procedure is not maintained during milking. In most cases, milk containing *E. coli* was obtained from animals with subclinical mastitis. Zafolon *et al.* (2008) studied at Nova Odesa, São Paulo and showed that the prevalence of *E. coli* was 57.3%. The results obtained in our study are likewise higher when compared to those formerly documented (Soomro *et al.*, 2002; Kumer and Prasad, 2010; Hossain, 2015). Based on observations made throughout the collection of samples, we concluded that the improper hygiene practice and poor management before and during milking may have contributed to the contamination of milk with *E. coli*, and the communal farms are more vulnerable in this case. The incidence at a considerable high percentage indicates the alarming situation both for dairy farming and for public health. The presence of *E. coli* in the milk sample is an appealing as well as an important finding of this study. Results of antimicrobial susceptibility test showed that most of the isolates of *E. coli* were sensitive to azithromycin, streptomycin, gentamicin, norfloxacin and ciprofloxacin but resistant to amoxycillin and erythromycin, which was closely related with Memon *et al.* (2013) and Bedada *et al.* (2011).

CONCLUSION

The results of this study concluded that raw milk available for consumers have a high *E. coli* contamination. Thus the results of the present study warn the need for more precaution. The present research was undertaken with a view to isolating and characterizing *Escherichia coli* present in cow’s raw milk sample collected from different upazila markets of Bangladesh. Out of 20 samples, 15 (75%) milk samples 15 (75%) milk samples were found positive for *E. coli*. 15 *Escherichia coli* isolates were amplified by 16S rRNA gene based PCR. Resistant pattern against broad spectrum antibiotic (e.g., amoxycillin) delimitate an alarming situation which needs special consideration.

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Molecular detection and characterization of *Escherichia coli*

REFERENCES


