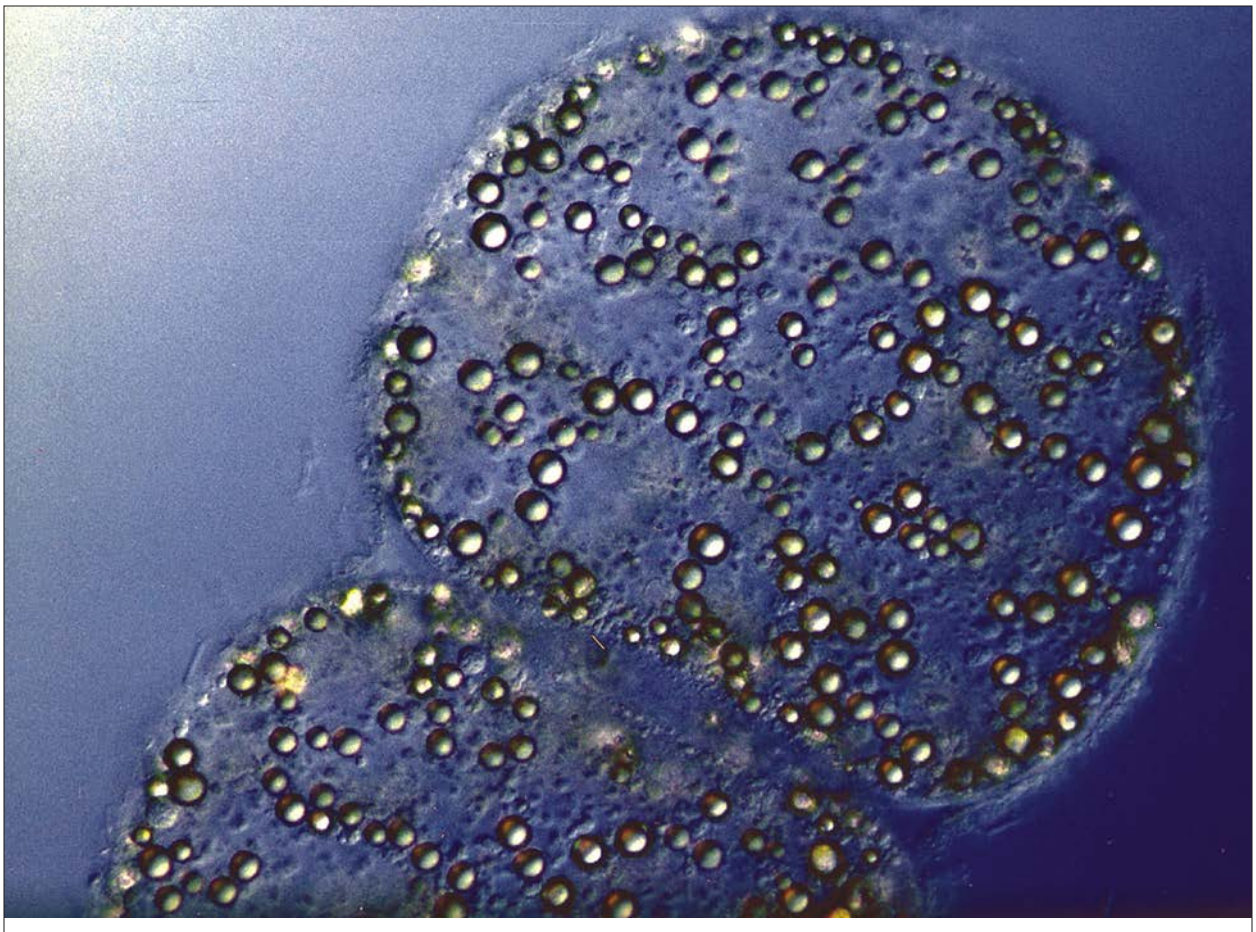

Microbiology

Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 4 (2): 27-40, 2014
ISSN 2153-0696 / DOI: 10.3923/mj.2014.27.40

Rapid Identification of Eosine Methylene Blue Positive *Escherichia coli* by Specific PCR from Frozen Chicken Rinse in Southern Chittagong City of Bangladesh: Prevalence and Antibiotic Susceptibility

[1]Nazneen Naher Islam, [1]Suza Mohammad Nur, [1]Zinat Farzana, [2]Inkeyas Uddin, [2]K.M. Kamaruddin and [2,3]A.M.A.M. Zonaed Siddiki

[1]Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, 4331, Bangladesh

[2]Poultry Research and Training Centre (PRTC), CVASU, Chittagong, 4202, Bangladesh

[3]Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, 4202, Bangladesh

Corresponding Author: Nazneen Naher Islam, Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong, 4331, Bangladesh Tel: +88(031)726311-4

ABSTRACT

E. coli is the major bacterial pathogen which can cause outbreaks of food borne illness and it is also an indicator of different fecal contamination. The present study was aimed to isolation and identification of the *E. coli* in the samples of refrigerated chicken rinse obtained from different super stores under Chittagong City Corporation, Bangladesh. Packages of chicken meat were opened aseptically strictly and total 1000 mL chicken rinse were transferred to 100 mL of PBS and incubated at 37°C for 24 h. Aliquots were transferred to Nutrient Broth medium and incubated at 37°C for overnight. After incubation the aliquots were streaked in MacConkey Agar media and incubated at 37°C for overnight. Typical *E. coli* colonies were picked from selective agar media and restreaked on EMB (Eosine Methylene Blue) medium. Several microbiological and biochemical tests were performed to confirm the suspected *E. coli*. Moreover, a PCR was developed to detect the microorganism in the chicken rinse by molecular method. The total DNA obtained by thermal extraction of microorganism was amplified using two set of primers designed for specific region of *E. coli* 23S rRNA gene and presence of amplicon 662 bp was found correlated to the result obtained from the standard culture method. All the samples were resistant to two or more than two antibiotics. The sample showed 100% resistant to Doxycycline and Oxytetracycline. But they were uniformly sensitive to Ampicilin. 100% susceptibility was found for Gentamycin group. The second susceptibility result was found in case of Chloramphenicol group. The prevalence of *E. coli* infection in frozen chicken rinse was found 77.51 and 100% by bacteriological and molecular techniques respectively in this study.

Key words: *E. coli*, PCR, chicken rinse, prevalence, antibiotic susceptibility, Bangladesh

INTRODUCTION

The consumption of meat has grown significantly during the last few years with one estimate of the global average in 2000 to be 38 kg capita^{-1} (Smil, 2002). The risk of disease from

ingesting pathogens found in raw meat is significantly higher than cooked meat, although both can be contaminated. Meat can be incorrectly or insufficiently cooked, allowing disease-carrying pathogens to be ingested. Also, meat can be contaminated during the production process at any time, from the slicing of prepared meats to cross-contamination of food in a refrigerator. All of these situations lead to a greater risk of disease however, the main source of disease caused by microbial pathogens is usually raw meat (Sammarco *et al*., 1997).

Dressed chicken is easy and convenient to the consumers, relief bothering of processing and save time. Therefore, demand for dressed chicken is increasing all over the world with the change of life style, food habit and lack of availability of manpower. In the developed country, chickens are slaughtered, processed and package at processing plant only. The consumer purchased frozen packed chicken either whole chicken or cut up parts. Though demand of dressed chicken is increasing but quality control is not at all developed in Bangladesh. Some research work noted that prechilled carcass with skin may be stored for 18-20 days at -2+0.5°C as frozen temperature (Bulgakova, 1975; Uddin, 2001), 6 months at -18°C and 1 year at -30°C in the liquid N-frozen without affecting on flavours, aroma, tenderness, juiciness, pH, moisture bindings capacity and consistency (Guslyannikov and Koreshkov, 1976). But in Bangladesh sometime the above parameters of preservation is not followed strictly.

Foodborne pathogens are a major threat to food safety, especially in developing countries where hygienist and sanitation facilities are often poor. *Salmonella enterica, Escherichia coli* O157:H7 and *Shigella* spp. are among the major causes of outbreaks of foodborne diseases. This large-scale study investigated the prevalence of these foodborne pathogens in meat (beef and chicken) and dairy products collected from street vendors butchers, retail markets and slaughter houses in Egypt (Ahmed and Shimamoto, 2014). The National Food Surveillance System in Japan was formed in 1998 to monitor the contamination of retail foods with bacterial pathogens and their result show the prevalence of the various bacterial pathogens in the retail food supplied in Japan (Hara-Kudo *et al*., 2013). Extraintestinal pathogenic *Escherichia coli* bacteria (ExPEC) exist as commensals in the human intestines and can infect extraintestinal sites and cause septicemia (Lyhs *et al*., 2012). Studies indicated that retail meat products are frequently contaminated with E. coli and that poultry and pork meat may be a potential source of ExPEC (Johnson *et al*., 2009; Manges *et al*., 2007).

Centers for Disease Control and Prevention (CDC, 2013) reported on *E. coli* infections by Farm Rich Brand Frozen Food product. Among them 82% of ill persons were 21 years of age or younger, 31% of ill persons were hospitalized. Two ill persons developed Hemolytic Uremic Syndrome (HUS), a type of kidney failure. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia (Todar, 2006). Being bound with respective receptor virulent strains of *E. coli* also can modify host's cell immune system to cause diarrhoea and many others diseases (Croxen and Finlay, 2010).

Numerous reports have been found describing the use of PCR in identification and characterization of *E. coli* isolates (Ahmed and Shimamoto, 2014; Bej *et al*., 1990; Min and Baeumner, 2002). In most laboratories of Bangladesh this is done by identification of traits of cultured bacteria which sometimes provide obscure result. For the control of disease and for

economically healthy management of meat the need for reliable and rapid methods for identification of *E. coli* is crucial. Molecular methods provide accurate confirmation of the identity of the microorganism isolated from any sample.

Recently, antimicrobial agents used in therapy and as feed supplements to promote growth in food animals may increase the spread of drug resistant bacteria from animals to humans (Chaslus-Dancla *et al*., 2000; Salauze *et al*., 1990). In Bangladesh, the management condition of poultry farm's chicken therapy is not so standard quality and there is no consistent policy of antibiotics usage; no record was kept of which drugs were used and these change from time to time depending on availability of drugs.

In this study, we reported the use of modern molecular tools like PCR as a confirmatory test for diagnosis of *E. coli* and estimate the prevalence of the organism in frozen chicken rinse from different superstores of Chittagong Metropolitan area. The traditional bacteriological and biochemical tests were also performed to identify the organism prior to PCR. Moreover, Culture Sensitivity test (CS test) was performed to reveal antibiotic sensitivity of *E. coli* isolates against 8 commercially available antibiotic discs.

MATERIALS AND METHODS

Collection of frozen chicken meat samples: A total of 37 frozen chicken carcasses were obtained randomly from 5 selected super markets in chittagong metropolitan area. All the samples were transported by ice bag and process for microbiological tests using aseptic technique within 30 min after purchase. The samples were placed in a sterile stomacher bag with 250 mL phosphate buffer saline. The contents of stomacher bag were then homogenized. In the laboratory the chicken rinse were filtrated by cheese cloth. The filtrated rinses were centrifuged at 4800x g for 25 min at 4°C. The supernatant was discarded and the pellet was collected. 0.1 mL pellet was suspended in 5 mL of nutrient broth. After inoculation of the pellet the samples were incubated at 37°C for 24 h.

Bacteriological approaches *E. coli* detection: Nutrient broth was prepared and distributed into test tubes (10 mL in each test tube). Then the test tubes with their contents were sterilized by autoclaving. After sterilization test tubes were coded according to the sample number. One mililitter of each sample was inoculated into 9 mL of corresponding number coded nutrient broth. A control was also prepared. After inoculation the test tubes were incubated at 37°C for 24 h in the incubator. MacConkey agar media was prepared into a conical flask and sterilized by boiling. After boiling the media was allowed to cool. Then the prepared media were poured into sterile petriplates and allowed to solidify. After solidification of the MacConkey plate, the plate was streaked using one loopful of test selective pre-enrichment culture from each sample. Then the plates were incubated at 37°C for 24 h in the incubator. In MacConkey plate the typical *E. coli* colony usually characterized by dry, red/pink colony. EMB (Eosine methylene blue) agar media was prepared into a conical flask and sterilized by autoclaving. After autoclaving the media was allowed to cool. Then EMB media was poured on sterile petriplates coded according to sample number and allowed to solidify. After solidifications, EMB plates were streaked by one loopful of culture from MacConkey agar of each sample then the plates were incubated at 37°C for 24 h. All *E. coli* identified were subjected to gram staining, indole test, methyl red test and several other *E. coli* specific biochemical tests.

Molecular approaches of *E. coli* detection

DNA extraction: DNA was extracted from all the *E. coli* isolated from meat samples according to the following method (Salehi *et al*., 2005). Briefly, pure bacterial culture from nutrient agar slant was subcultured in nutrient broth medium. Each mililitre broth culture was taken in separate eppendorf tube and centrifuged at 10000 rpm for 5 min. The supernatant was discarded and any remaining liquid was removed by soaking (with wipes). The pellet was collected and replenished with 200 μL autoclaved de-ionized water and the finger shaking was performed to dissolve the pellet. The cap of the eppendorf tube was pierced by sterile needle before placing it in a water bath at 100°C for 10 min. Immediately after boiling, the eppendorf tube was kept in ice for 10 min followed by centrifugation at 10,000 rpm for 10 min. Finally, around 100-150 μL supernatant containing bacterial chromosomal DNA was collected and stored at -20°C.

Polymerase Chain Reaction (PCR): Following DNA extraction, PCR analysis was done based on 23S rRNA. PCR was performed to identify *E. coli* using the following primer- (Bentley and Leigh, 1995): Forward Primer: 5'-GCT TGA CAC TGA ACA TTG AG-3' Reverse Primer: 5'-GCA CTT ATC TCT TCC GCA TT -3'. All the PCR reaction materials (Master Mix [2X], Forward and Reverse primers, template and nuclease free water) were mixed in a PCR tube and the run condition in pre-PCR step at 94°C for 2 min was applied. A total of 35 PCR cycles were run under the following conditions: Denaturation at 94°C for 45 sec, annealing at the temperature 57°C for 1 min and extension at 72°C for 2 min. After the final cycle, the preparation was kept at 72°C for 10 min to complete the Reaction. Then the PCR products were stored in the thermocycler at 4°C until they were collected. Along with each set of PCR reaction, a positive control with known *E. coli* DNA template and a negative control (water instead of extracted DNA) were used as known standards.

PCR products were electrophoresed in a 1% agarose gel containing 500 μg mL^{-1} of ethidium bromide and the gel was visualized by UV transilluminator (Biometra GmBH, Germany).

Antibiotic susceptibility tests: The isolated bacterial isolates were subjected to Culture Sensitivity (CS) test. Each of the isolates was first subcultured in 5 mL of nutrient broth in a test tube separately. The test tubes were then incubated at 37°C overnight. On the following day with the help of a autoclaved cotton bud, each of the samples was spread on Mueller-Hinton agar plate separately. Commercially available antibiotic discs were placed on the edge of the plate. Then the plate was incubated at 37°C for 24 h. After incubation, diameters of zones of inhibition were measured with respect to the data available from National Committee for Clinical Laboratory Standards (NCCLS, USA). Then the test organism was reported as 'susceptible', 'intermediate' or 'resistant'. The antibiotic discs used were Ampicilin, Amoxycilin, Cefalexin, Erythromycin, Chloramphenicol, Gentamycin, Doxycycline and Oxytetracycline.

RESULTS

Bacteriological analysis: From the collected 37 samples a total of 29 chicken rinse samples were confirmed as *E. coli* infected using the EMB (Ethyne Methylene Blue) culture. 4 samples (66.66%) of super market-MB, 10 samples (90.90%) of super market-KM, 7 samples (70%) of super market-A, 4 samples (80%) of super market-S and 4 samples (80%) of super market-WM were found as EMB positive. The level of prevalence was found in a range of 66.66-90.90%. The average prevalence of all super shop was found 77.51%. All of these data has been presented in Table 1 and Fig. 1.

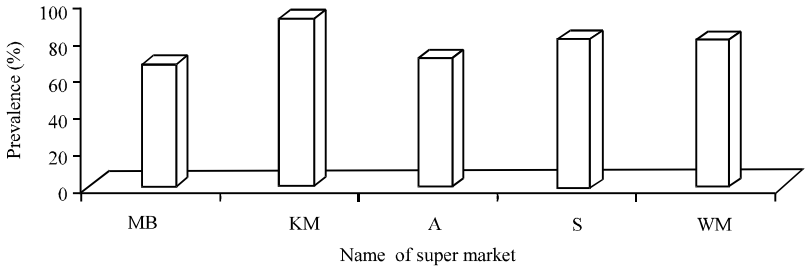

Fig. 1: Prevalence (%) of *E. coli* infection determined by Eosine Methylene Blue (EMB) test

Table 1: Prevalence (%) of *E coli* infection determined by Eosine Methylene Blue (EMB) test

Name of the super market	No. of selected frozen chicken	No. of positive EMB culture	Level prevalence (%) in EMB culture	Average (%)
MB	6	4	66.66	77.51
KM	11	10	90.90	
A	10	7	70.00	
S	5	4	80.00	
WM	5	4	80.00	
Total	37	29	387.56	

The EMB positive colonies were further characterized according to the several conventional biochemical tests. All of *E. coli* samples were motile and found positive to lactose fermentation, formation of indole and MR test but negative to VP, citrate test, urease test, oxidase test and H_2S production. All of this data has been tabulated in Table 2.

Molecular detection: Through PCR analyses, 100% (29 out of 29) of EMB positive samples were confirmed as *E. coli*. The *E. coli* strain was identified on the basis of 662 bp PCR product corresponding to the 23S rRNA gene on 1% agarose gel (Ghorbanpoor *et al.*, 2007). The results of PCR identification of eighteen *E. coli* samples have been presented in Fig. 2a-b. Therefore, more precisely it can be stated that all of the biochemically confirmed isolates were *E. coli*. The comparison study of bacteriological and PCR assay have been presented in Table 3 and Fig. 3.

Antibiotic susceptibility: A total of identified 29 *E. coli* isolates were subjected to antibiotic susceptibility test against 8 antibiotics with standard concentration i.e., Erythromycine (E^{15}), Ampicillin (AMP^{25}), Amoxycillin (AMX^{30}), Oxytetracycline (O^{30}), Chloramphenicol (CL^{30}), Cefalexin (CN^{30}), Gentamycin (GEN^{10}) and Doxycycline (DO^{25}). Table 4 and Fig. 4 recorded the results of the culture sensitivity test for detection of antibiotic sensitivity. The isolated *E. coli* samples were uniformly sensitive to Ampicillin. 13.51% were intermediately susceptible and 48.64% was found succeptible to Ampicillin. Beside this 37.83% was found resistant to Ampicilin. 29.72% resistant, 21.62% intermediately succeptible and 48.64% susceptible result obtained in case of Amoxicillin. For the case of Cefalexin, 70.27% resistant, 5.4% intermediately susceptible and 24.32% succeptible were detected. Moreover, 91.89 and 8.1% were found resistant and susceptible, respectively to Erythromycin. 100% susceptibility result was found for Gentamycin group. The second

Table 2: Identification of *E. coli* by different bacteriological and biochemical reactions

	Biochemical reactions											
Isolates	MacConkey agar plate (colony color)	EMB agar plate	Methyl-red test (MR)	Indole production	Motility	Lactose fermentation test	H_2S production	Oxidase test	Simmons citrate test	Urea hydrolysis	Voges-Proskauer test	Comments
MB1	Pink/red	Green Metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
MB2	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
MB4	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
MB5	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM1	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM2	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM3	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM5	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM6	Pink/red	Green metallic sheen-	+	+	+	+	-	-	-	-	-	*E. coli*
KM7	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*

Table 2: Countinue

	Biochemical reactions											
Isolates	MacConkey agar plate (colony color)	EMB agar plate	Methyl-red test (MR)	Indole production	Motility	Lactose fermentation test	H_2S production	Oxidase test	Simmons citrate test	Urea hydrolysis	Voges-Proskauer test	Comments
KM8	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM9	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM10	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
KM11	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A1	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A3	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A4	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A5	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A6	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
A7	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*

Table 2: Countinue

	Biochemical reactions											
Isolates	MacConkey agar plate (colony color)	EMB agar plate	Methyl-red test (MR)	Indole production	Motility	Lactose fermentation test	H_2S production	Oxidase test	Simmons citrate test	Urea hydrolysis	Voges-Proskauer test	Comments
A9	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
S2	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
S3	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
S4	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
S5	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
WM1	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
WM2	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
WM3	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*
WM4	Pink/red	Green metallic sheen	+	+	+	+	-	-	-	-	-	*E. coli*

Here, +: Positive, -: Negative result, respectively

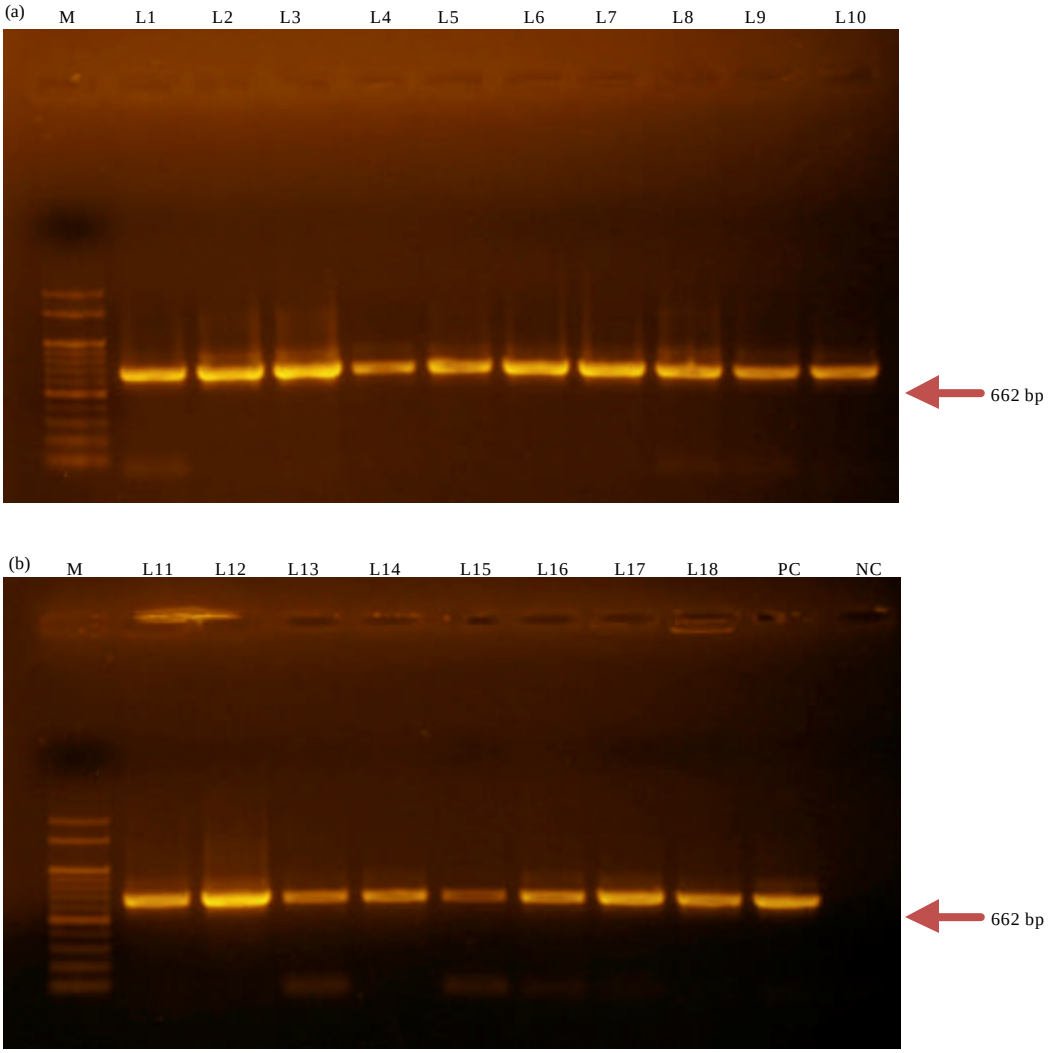

Fig. 2(a-b): Electrophoretic separation (on 1% Agarose) of 23S rRNA gene fragment, (a) 10 isolates of *E. coli* that has amplified 662 bp gene fragments. Lane M-100 bp sharp Marker DNA, Lane L1: MB 1, Lane L2: MB 2, Lane L3: MB 4, Lane L4: KM 6, Lane L5: KM 7, Lane L6: KM 8, Lane L7: KM 9, Lane L8: A1, Lane L9: A 3, Lane L10: A4 and (b) 8 *E. coli* isolates that has amplified 662 bp gene. Lane M: 100 bp sharp Marker DNA, Lane L11: A5, Lane L12: S2, Lane L13: S3, Lane L14: S4, Lane L15: S5, Lane L16: WM1, Lane L17: WM2, Lane L18: WM3, Lane PC: Positive control and Lane NC: Negative control

Table 3: Comparison of bacteriological and *E. coli* specific PCR assay for the detection of *E. coli* in chicken rinse

Catagories of *E. coli* sample	Bacterial culture	Bacterial culture positive (%)	Catagories of *E. coli* sp-PCR sample	No.of Sp-PCR samples	Sp-PCR positive (%)
Positive	29		Positive	29	
Negative	8	77	Negative	0	100
Total	37		Total	29	

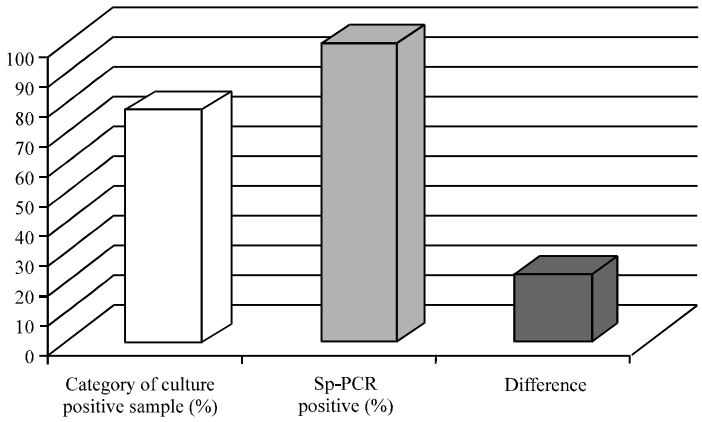

Fig. 3: Comparison of bacteriological analysis and *E. coli* specific PCR assay and their differences for detection of *E. coli* from chicken rinse sample

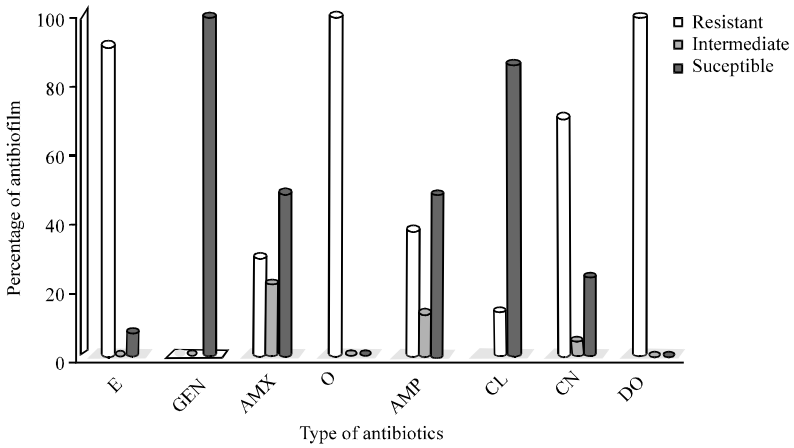

Fig. 4: Comparative analysis of antibiotic susceptibility pattern (%) of isolated and detected *E. coli* after culture sensitivity test. Here, E: Erythromycine, GEN: Gentamycin, AMP: Ampicillin, AMX: Amoxycillin, O: Oxytetracycline, CL: Chloramphenicol, CN: Cefalexin and DO: Doxycycline, respectively

Table 4: Results of Culture Sensitivity (CS) test of isolated *E. coli*

		Antibiotics							
Pathogen	Effective pattern (No.)	E	GEN	AMX	O	AMP	CL	CN	DO
E. coli	Resistant	27	0	9	29	11	0	20	29
	Intermediate	0	0	6	0	4	4	2	0
	Susceptible	2	29	14	0	14	25	7	0

E: Erythromycine, GEN: Gentamycin, AMP: Ampicillin, AMX: Amoxycillin, O: Oxytetracycline, CL: Chloramphenicol, CN: Cefalexin and DO: Doxycycline, respectively

susceptibility result was found in case of Chloramphenicol group. On the other hand both Doxycycline and Oxytetracycline were showed 100% resistant to bacterial growth (Table 4 and Fig. 4).

DISCUSSION

We collected 37 samples from different super stores in strictly maintained aseptical condition. All the samples were homogenized in 100 mL Phosphate Buffer Saline (0.085%) for per sample. After filtration and centrifugation pellet was collected and inoculate in Nutrient Broth. Upon incubation bacteriological test was performed. In our bacteriological study we have found 66.66, 90.90, 70, 80 and 80% *E. coli* prevalence from each individual super market named, MB, KM, A, S and WM, respectively. The overall prevalence was found 77.51% in bacteriological tests where as a previous study was found 0.9-12% of prevalence (Lyhs *et al*., 2012).

We performed several biochemical tests for the identification of our isolates. We found positive result such as for Indole test a red or pink colored ring at the top after adding Indole reagent. A turbidity in citrate test, gas production with color change of medium etc. were obtained through biochemical test. We also performed gram staining for *E. coli* and got rod shape of bacteria.

In this study, we aimed to evaluate the species specific PCR for detecting the *E. coli* responsible in superstores chicken samples. Therefore, a total of 29 EMB positive samples subjected to extraction of DNA by boiling method and all DNA were used as template in PCR techniques to amplify 23S rRNA gene of *E. coli* where present. The primers for *E. coli* were designed based on a DNA sequence coding for 23S rRNA gene and we found 100% positive result corresponding with 662 bp DNA on 1% agarose gel. Specific primers were proven to be specific, since on agarose gel only one band was observed for each set of the primers and no signal was detected with negative controls. All the signals were very obvious. The difference of signal cannot be explained by the amount of template DNA used for PCR because the same amount (3 μL) was used for all sample. Therefore, it is possible that the difference in the copy numbers of the coding regions for these particular probes could explain the phenomenon (Bentley and Leigh, 1995). Results of direct detection of *E. coli* by organism specific PCR (Eco 2083 and Eco 2745 used as primers) assay reveals that PCR based incidence of *E. coli* in the studied samples was 100%. This is the first report on the molecular detection of *E. coli* in chicken rinse in southern part of Bangladesh. A result of 309 bp band was obtained in a previous study conducted by Asensi *et al*. (2011) but the percentages of prevalence found by PCR was not reported. Our overall bacteriological and molecular result indicate that the level of prevalence was upper high and enough to cause infection because poultry meat has been frequently associated with food borne illness in which initial contamination is traceable to food handler (Halpin-Dohnalek and Marth, 1989).

The long time and abundant use of antimicrobial agents in therapy and as feed supplements to promote the growth in food animals are spreading the drug resistant bacteria from animals to humans (Chaslus-Dancla *et al*., 2000; Salauze *et al*., 1990). In Poultry farms of Bangladesh, there was no consistent policy of antibiotics usage; no record was kept of which drugs were used. Table 4 represents that, the sample showed 100% resistant to Doxycycline and Oxytetracycline. These have probably been caused by the severe use of Oxytetracycline and Doxycycline as a routine in poultry firm. The isolated *E. coli* samples were uniformly sensitive to Ampicilin. 48.64% of microorganisms were found susceptible and 13.51% were found intermediately susceptible to Ampicilin. 37.83% of microorganisms were found resistant to Ampicilin too. 29.72% were resistant, 21.62% intermediately susceptible and 48.64% susceptible result obtained in case of Amoxicillin whereas 70.27% resistant, 5.4% intermediately susceptible and 24.32% susceptible were found to Cephalexin. 91.89% resistant, 8.1% susceptible to Erythromycin. The 100% susceptibility was found for Gentamycin group. The second susceptibility result was found in case of Chloramphenicol group. Cefalexin has also evidently been widely used at some time. A previous study of *E. coli*

resistant result was found as-Tetracycline (96.6%), Erythromycin (66.6%), Gentamycin (50%) by Islam (2003). Comparing with these results with our ones, it is indicating that these three antibiotics have become more resistant in Bangladesh.

E. coli is the most common gram-negative pathogen in patients of all ages and the most common cause of Urinary Tract Infections (UTIs). Additionally Enterohemorrhagic *E. coli* (EHEC) is a subset of pathogenic *E. coli* that can cause diarrhoea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to Hemolytic Uremic Syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults (Todar, 2006). As passing time *E. coli* mediated infection is increasing over the world which should be taken under serious concern as a public health issue (Todar, 2006). The food poisoning from retail supermarket is raising the early prevention of it could beneficial for both customers and seller. Prevention and control can be assured by taking fruitful measures by seller. In case of customers the frozen chicken meat should be processed with special hygienic measures. Because, even at high temperature *E. coli* can produce heat labile Enterotoxin. It has been also reported that *E. coli* can survived at high temperature and pressure (Usajewicz and Nalepa, 2006).

CONCLUSION

From the above study it can be concluded that, PCR assay can be used as a rapid diagnostic method with high sensitivity for diagnosis of *E. coli* in condition of Bangladesh because high prevalence of infection by the organism was detected in chicken samples of various superstores. The high percentage of infection in both bacteriological and PCR base detection result revealed the risk of consuming frozen chicken in the study area. Uprising resistant of *E. coli* against several antibiotic drugs were found at a very high rate. However, most of the isolated *E. coli* was susceptible to a number of antimicrobial agents. As Doxy-cycline, Oxytetracycline and Erythromycin had shown good resistant to growth of *E. coli* so the abundant uses of these three antibiotics should be limited. The antibiotic resistance profile of *E. coli* isolated from livestock carcasses in this report would provide important public health information and because of its epidemiological importance, measures must be taken in this field to minimize the zoonotic disease transmissible from poultry to humans.

ACKNOWLEDGMENT

We are grateful to the respective super markets authorities of Chittagong metropolyton area for supplying us frozen meat samples.

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