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Original Article

Morphological, Biochemical, Antibiogram Study and Molecular Characterization of *Escherichia coli (E. coli)* Isolates from Street Foods in and Around Dhaka City

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The potential contamination of street foods with pathogenic microorganisms has been documented and several outbreak of diseases have been traced out in relation to consumption of contaminated street foods in Bangladesh. The sanitation and hygienic quality of the food was assessed by the analysis of an indicator bacteria rather than of pathogens are used. The *E. coli* and fecal coliform are generally used as an indicator bacteria of sanitation and hygienic quality of the food. A total of 9 isolates of *E. coli* were detected from 30 samples of street foods during the month of February to May 2009. The samples were collected from Gabtali, Dhaka University Campus, Mohakhali, Sadarghat, Paltan area and other important places of Dhaka City. On the basis of the hygienic standard above results, 46.66% sample(n=30) was found within the acceptable range. Six biochemical tests (EIMViC) were performed for representative 9 isolates from 9 samples. All the isolates showed positive reaction to Catalase, Oxidase, Indole and Methyl red test and negative reaction to Voges- proskauer and Citrate test further confirming the isolates were *E. coli*. The antibiotic susceptibility pattern of the isolates was determined against six most commonly used antibiotics (Amphicillin, Gentamicin, Sulfamethoxazole, Nalidixic Acid, Kanamycin and Tetracycline). Out of 9 isolates, three isolates were found resistant against Amphicillin and others were found sensitive. All the isolates of *E. coli* were found sensitive to Gentamicin (CN). The DNA and plasmid DNA were extracted following standard procedure and showed the same banding pattern in agarose gel electrophoresis along with a 1 Kb DNA ladder and reference culture proving that the isolates were *E. coli* and formed plasmid DNA under adverse environmental condition.

Keywords: *Escherichia coli*, Antibiotics, Plasmid DNA, Street food, Reference culture

Introduction

Fecal indicators are the normal inhabitant in the large intestine of men and worm blooded vertebrate animals. Their presence in foods may indicate that fecal contamination may have occurred and even strongly correlated with the risk of pathogenic organisms being present. *Escherichia coli (E. coli)* is a Gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals[1]. Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans[1]. The fecal coliforms *(E. coli)* are presently used as an indicator of the sanitary and hygienic condition of food and related products and its presence may indicate the potential occurrence of pathogenic organisms to the men, domestic and wild animals. The species includes the intestinal diarrhoeagenic *E. coli* and extra intestinal pathogens[2-4]. Several *E. coli* pathotypes have been implicated with the diarrhoeal illness, a major public health problem worldwide, with over two million deaths occurring in each year[5]. The street food industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries. The industry feeds millions of people daily with a wide variety of food stuffs that are relatively cheaper and easily accessible. However, food borne illnesses of microbial origin are a major health problem associated with street foods[5]. Potential health risks are associated with contamination of foods by *E. coli*, *Salmonella typhi*, *Pseudomonas* sp., *Staphylococcus aureus* or *Proteus* sp. during preparation, post cooking and other handling stages[6-7]. The EHEC strains produce shiga like toxin 1 (stx1), shiga like toxin 2 (stx2) and variants thereof [8]. They are involved in the episodes of diarrhoea with various complications. Food-borne illness is a major public health problem in both developed and developing countries of the world. Considering these facts as mentioned above the present investigation was undertaken with an aim to assess the microbiological quality of street foods in and around Dhaka city. In this study, we investigated the total viable count of bacteria in food available in the streets of Dhaka city and Total Coliform (TC), Fecal Coliform (FC)

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and *E. coli* in street foods. We also investigated morphological, biochemical, antibiogram and molecular characterization of the isolates of *E. coli* from food items.

Materials and Methods

The total experiments were divided into three parts such as isolation, characterization and screening of the *E. coli* isolates along with total bacterial count. Subsequently, screening of the antibiotic sensitivity of the isolates and their molecular characterization were done. A total of 30 samples were collected from five different places of Dhaka city (Gabtali, Dhaka University Campus, Mohakhali, Sadarghat and Paltan). Lauryl Tryptose Broth (LTB), Brillant Green Bile Broth (BGBB), Eosine Methylene Blue Agar (EMB) and Muller-Hilton agar were generally used for growth and characterization of coliform bacteria. For the isolation of *E. coli* 20 gm samples were added aseptically into 180 ml Ringer solution following standard procedure of MPN method and then ten fold serial dilution was made and 1 ml of inoculum from each dilution was plated onto EMB agar plate and incubated for 24 hours or more and all the plates containing characteristic colonies were counted using a colony counter. One loop-full of bacterial suspension from EMB plate was transferred to the BGBB in the fermentation tubes and was then incubated for 48±3 hours at 35±0.5°C. A pure colony of each isolates from EMB media was picked and Gram staining was performed. Six biochemical tests were also done for the isoates giving typical colonies. Susceptibility of *E. coli* that isolated from street foods to different antibiotics was determined by standardized agar disc-diffusion method[9]. The zone diameters for individual antimicrobial agents were translated into sensitive, intermediate and resistant categories by referring to an interpreting table[10]. Plasmid DNA isolation of the samples and reference were done with or without antibiotic. Extracted DNA were analysed by agarose gel electrophoresis on 0.8% gel along with a 1 Kb DNA ladder and reference culture. After electrophoresis, the gel was stained with ethidium bromide for at least 20 minutes and the bands were visualized under UV light using Gel Doc system.

Results

Total bacterial/Viable count helps to determine the concentration of the aerobic or heterotrophic microorganisms present in the samples. The highest bacteria count (19.85×10^7 cfu/g) was in fuchka taken from Dhaka University Campus. The lowest (100 cfu/g) was in puri taken from Gabtali (Table 1). The highest total coliform count was recorded 28 MPN/g on average, which was found in 15 samples collected from different areas and lowest count was recorded Nil MPN/g in 6 samples. The highest fecal coliform count (28 MPN/g) was found in 3 samples collected from Dhaka University Campus and Gabtali and the lowest count was recorded (Nil MPN/g) in 14 samples (Table 1).

The isolates were found to be short Gram-negative rods and non-spore formers that is characteristics of *E. coli*. Extensive biochemical tests were performed in order to measure the variability of biochemical behaviour among different strains of *E. coli*. Detailed biochemical study revealed that all the strains had the biochemical characteristics typical of *E. coli* bacteria. All nine isolates produced positive reaction to catalase, oxidase, indole and methyl red tests. Negative reactions were recorded for all 9 isolates in Voges-Proskauer and citrate tests. All the strains were tested for their antibiotic sensitivity against the six commonly used antibiotics. All the isolates showed susceptibility to gentamicin (CN). The isolates showed variation in sensitivity pattern against the other antibiotics used in this study.

Table 1. *Total viable count of bacteria*

Area	Name of the sample collected	Total viable count, cfu/gm	Total coliform count. MPN/gm	Total fecal coliform count. MPN/gm
Dhaka	Velpuri	604×10^8	20	3.6
University	Singara	110×10^5	28	14
Campus	Jhalmuri	910×10^5	28	28
	Chotpoti	1985×10^4	17	9.3
	Fuchka	870×10^7	20	6.1
	Chola vorta	300	2	Nil
	Samocha	7450	4	4
	Vegetable roll	1250	2	Nil
Gabtali	Dalpuri	100	Nil	Nil
	Singara	13350	Nil	Nil
	Chola vorta	1295×10^4	28	28
	Jhalmuri	466×10^3	28	28
Paltan	Chotpoti	2395×10^4	28	Nil
	Fuchka	346×10^5	28	1.8
	Chola vorta	111×10^5	28	1.8
	Murir moa	28000	28	Nil
	Velpuri	265×10^5	28	24
	Chop	17900	Nil	Nil
	Singara	24900	Nil	Nil
Mohakhali	Singara	9650	2	Nil
	Jhalmuri	126×10^5	28	17
	Chira moa	58000	2	Nil
	Chola vorta	267×10^5	28	12
	Gilapi	3250	2	Nil
	Dalpuri	600	Nil	Nil
Sadarghat	Gilapi	0	Nil	Nil
	Velpuri	394×10^5	28	24
	Jhalmuri	289×10^5	28	6
	Chola vorta	269×10^5	28	12
	Samocha	785×10^3	28	Nil

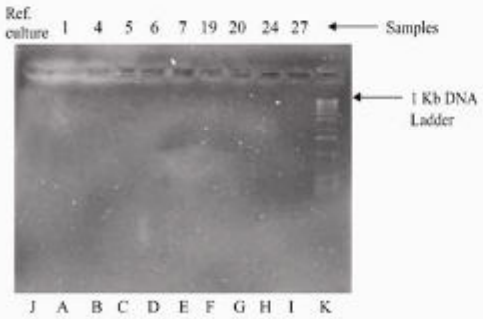
E. coli isolates displayed differential reactions to antibiotic test. All the isolates were sensitive to gentamicin. 66.7% isolates showed sensitivity to amphicillin, 99.0% to both saiphomethoxazole and kanamycin, while 77.8% isolates were sensitive to tetracycline and 99.0% of the isolates were sensitive to nalidixic acid. 11.1-33.3% of the isolates showed resistance to different antibiotics (Table 2).

Table 2. *Frequency of antibiotic resistance/ susceptibility of E. coli isolates*

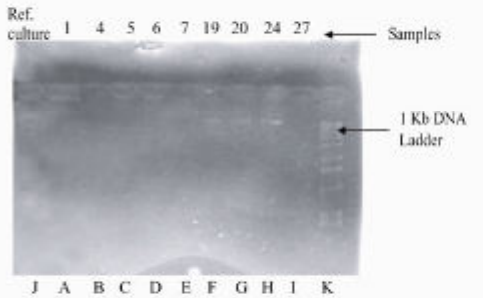
Antibiotic used	Sensitive (%)	Resistant (%)
Amphicillin (AMP)	66.67	33.33
Gentamicin (CN)	100	0
Sulphomthoxazole (SXT)	89.99	11.11
Kanamycin (K)	89.99	11.11
Tetracycline (TE)	77.78	22.22
Nalidixic acid (NA)	98.99	11.11

The plasmid DNA extracted from samples and a reference *E. coli* strains that were grown without antibiotic in LB media showed no bands of plasmid when visualized under UV light

using Gel Doc system (Photograph 1). Whereas when samples and reference strains were grown with antibiotic, all three isolates and the reference strains showed bands. This indicates that the three isolates which acquired plasmids in stressful condition and are resistant reaction to Amphicillin (Photograph 2).



Photograph 1. *Agarose gel electrophoresis of the samples and with reference culture growing without antibiotic*



Photograph 2. *Agarose gel electrophoresis of the samples with reference culture growing without antibiotic*

Discussion

The present study was targeted for the microbiological quality analysis of street foods in terms of fecal contamination and characterization of the *E. coli* found in the street food samples. In this study, a total of 30 samples were collected from the 5 different places of Dhaka city and microbiological quality analysis of street foods in terms of fecal contamination and characterization of the *E. coli* found in the street foods samples were done. In present investigation, the standard plate count of 16 (53.3%) street foods samples (Table 1) were found within the acceptable limit(less than 10^6 cfu/g), while the count was within the unacceptable limit (more than 10^6 cfu/g), for the rest 14 (45.5%) samples according to ICMSF standard. When considering the presence of coliform, 12 (40%) samples (Table 1) and was within the acceptable range (less than 10 MPN/g) and 18 (60.0%) samples were found contaminated with coliform which was above acceptable range. In all street foods samples, the presence of fecal coliform in 14 (46.66) samples were found in acceptable range (nil) while the other 16 (53.34%) samples (Table 1) were not in acceptable range. The presence of coliform and fecal coliform bacteria within the acceptable range is considered to be safe for consumption. Presence of *E. coli* in street foods samples indicates the possibility of fecal contamination from the materials and water required for preparation or it may be due to cross contamination by food handlers[11]. A total of 9 isolates in 9 samples out of 30 samples were considered in this study based on their primary identification by production of typical green metallic sheen on EMB agar. Then the 9 isolates were subjected to perform 6 biochemical tests. From the experimental findings, it was seen that all the isolates resulted typical biochemical test as *E. coli*. All 9 isolates were tested for their antimicrobial sensitivity /resistance pattern using commercial antibiotic. Antimicrobial resistance in human pathogens has become a major public health issue. In this study, the isolates were subjected to six (6) most commonly used antibiotic such Amphicillin, Gentamicin, Sulphamethoxazole, Nalidixic acid, Kanamycin, and Tetracycline. All the test strains are sensitive to Gentamicin. 4 of the isolates (44.4%) were sensitive to all the antibiotics. The other 5 isolates (55.6%) showed variation in their susceptibility pattern to the antibiotics used except Gentamicin. A total of 3 isolates (33.3%) were resistant to Amphicilin, 1 isolates (11.1%) were resistant to Sulphamethoxazole, 1 isolates (11.1%) each were resistant to Nalidixic acid, Kanamycin and tetracycline. A total of 2 isolates (22.2%) were resistant to more than one antibiotics i.e. showed multi-drug resistance. Previous studies revealed that in most developing countries especially in Bangladesh, food-borne diseases are treated by an inadequate quantity of antimicrobials, without identifying a pathogen. This is probably one of the important factors for observed antimicrobial resistances in *E. coli* isolates in this study. The molecular confirmation of the isolates as *E. coli* was detected by gel electrophoresis of the samples and a reference (*E. coli*) that were grown without antibiotic in LB media and subsequent staining no banding pattern were visualized under UV light (Photograph 1). All the 9 isolates and a reference culture (*E. coli* 0157:H7) were grown with antibiotic (Amphicillin) and among them three isolates (S-19, S-20, S-24) and a reference culture (*E. coli* 0157:H7) that were grown in LB media with antibiotic (Amphicillin) shown the same banding pattern (Photograph 2) in agarose gel electrophoresis proving that the isolates was *E. coli* and formed plasmid DNA under adverse environment whereas all the isolates and the reference culture shown no banding pattern in agarose gel electrophoresis when grown without antibiotic because no plasmid DNA formed in normal condition. Out of 30 samples, 46.7% were in acceptable range for consumption in terms of fecal contamination.

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