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Isolation and Characterization of *Escherichia coli* in Street Fast Foods in Rural Areas

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Abstract: *Isolation and characterization of E. coli in fast foods concerns due to their presence indicates fecal contamination of the food. To identify, characterize and RFLP pattern analysis of E. coli isolated from vended fast foods in rural areas of Delhi-NCR. Genomic DNA was used to perform RFLP pattern analysis. Eighteen out of 24 (75%) analyzed samples of fast foods had E. coli contamination. The highest number of E. coli was isolated from chicken biryani and golgappa (83.33 %) and burger and vegetables ready to eat fast food (66.66 %) samples were also significantly E. coli positive. RFLP profiling of E. coli isolates was observed.*

Keywords*: Vended fast foods, Escherichia coli, Genomic DNA, RFLP profiling*

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I. Introduction

Food safety is an essential component of public health, linking health to different food production and agriculture sectors[1]. Most common bacterial pathogen is Escherichia coli which is capable of causing intestinal disease. Some *E. coli* are nontoxic and are found naturally in the intestine of humans[2]. *Escherichia coli* strain O157 is a part of enterohemorrhagic *Escherichia coli* and has been identified as the cause of numerous outbreaks by causing, hemorrhagic colitis, hemolytic uremic syndrome, diarrhea[3]. In developing countries street food and junk food plays an important role[4]. Food sector has experienced important growth during the earlier period because of social and economic changes in developing countries. They give food to large number of people daily with an extensive range of ready-to-eat foods and sometimes prepared these foods in the public places or streets, reasonably low-priced and easily offered[5]. Safe water for food and human consumption is important, but there is a limited supply. Developing countries has water shortages during summer season in many colonies and depends on other sources of water including boreholes and water tankers. Microbiological assessment of drinking water and food is important to reduce exposure to cause intestinal disease[6].

A food borne pathogen *E. coli* O157:H7 was first recognized in America[7] after an occurrence of hemorrhagic colitis following the ingestion of undercooked hamburgers at a fast-food restaurant chain[8]. The present research work in rural areas represents the data of *E. coli* in fast food products. Mostly microorganism present in different types of food items is non-toxic[9]. So it is very important to identify the toxic pathogen in order to build up an appropriate test to detect pathogen. Culturing technique is a conventional method to identify food born pathogen by plating to isolate pure culture. Genomic DNA isolation technique can arrange within pool culture of bacteria. Food borne pathogen along with dissimilar bacterial species can detect by RFLP technique. The main objective of this work was to find out the presence of *E. coli* in many street fast food or ready to eat food samples in rural areas in Delhi-NCR.

II. Materials and Methods

Sample collection: Approximately 100 g of each 24 samples of street fast food of 4 categories (6 samples of chicken biryani, 6 samples of burger, 6 samples golgappa and 6 samples of vegetables) were collected between July 2018 and August 2018 from different places in rural areas of Delhi-NCR. All samples were tested within 24 h of collection. Ten grams of each food sample was mixed with one eighth strength Ringers Solution. The sample was homogenized with an electric hand blender at 5000 rpm for 10-12 minutes (10^{-1} dilution), followed by serial dilutions up to 10^{-6} dilution.

Isolation and test of E. coli: The most probable number (MPN) method was used for determining E. coli counts [10,11]. Fermentation tubes (10 ml) of lauryl tryptose broth medium were poured with different concentration. The tubes were inoculated with 5 ml, 1 ml with 5 ml, and 1 ml and 0.1 ml amount of sample and incubated at 37°C for 24 h. All tubes under experiment producing gas after 24 h of incubation, was further tested for conformation. In this analysis, dilutions of the each sample were made using peptone water. 1ml of each sample was pipetted into one sterile test-tube containing 9 ml of peptone water, making 1:10 dilution, second

test-tube making 1:100 dilution and third test-tube making 1:1000 dilution respectively. From all the three dilutions, 1ml was transferred into already prepared Mac Conkey Broth containing each 9 ml (triplicate) with inverted positioned Durham's tubes. The tubes were covered with cotton wool and incubated at 37°C for 24 hours.

A very minute quantity from each culture showing production of acid and gas was transferred to Brilliant Green Bile Broth (Oxoid) and incubated for 48 h at 37°C and 44.5°C. Streaking on the eosine methylene blue (EMB) agar plate was done for further confirmation that was performed[10]. One or more plates containing EMB agar medium were streaked from presumptive positive test tubes in such a way that discrete colonies may appear. The plates were incubated at 37°C for 24 h. Typical nucleated colonies with or without metallic sheen indicates positive results. Few suspected E. coli colonies from each sample were selected. Further biochemical tests were done for the identification of Escherichia coli according to [12]. IMViC test was performed to distinguish between E. coli and Enterobacter aerogenes [10]. Two isolates identified as E. coli were further characterized based on DNA polymorphism by RFLP.

Genomic DNA extraction: Standard and improved phenol chloroform method was used to extract genomic DNA[13] with a few changes. Different samples were pre-treated properly for the collection of organism cell. In regard to pure bacteria culture, 1.4 ml bacteria suspension was collected by centrifugation at 380 rpm for 10 min at 4°C; precipitation was mixed well with 0.4 ml TE (10 mmol-l Tris-HCl, 1 mmol-l EDTA, and pH 8.0). After adding 50 µl of 10% SDS and 50µl proteinase K, pellete was incubated at 42°C for 20 min in water bath. All nucleic acid came out of solution soon after adding 0.15 ml of 5M NaCl and 0.25 ml of ice cold isopropanol. The supernatant was transferred to a new tube and 0.7 vol of phenol/chloroform (1: 1) was added. After gentle mixing, the mixture was centrifuged at 16.1 rcf for 10 min at 4°C. The upper phase was transferred to a new tube containing 2/3 vol of isopropanol. The mixture was cooled at -20°C for 30 min and then centrifuged at 16.1 rcf for 10 min at 4°C. The resulting pellet was dissolved with 100 µl TE buffer after washing twice with 70% ice cold ethanol. The quantity and quality of the purified DNA were determined by measuring at A260 and by calculating the ratio of A260/A280, respectively by spectrophotometer. Finally, agarose gel electrophoresis of extracted DNA was performed using 0.8% UltraPureTM agarose (Invitrogen).

Restriction analysis: To get total 20 µl of volume, 1 µl of genomic DNA was mixed with 15 µl of distilled water and 2 µl of enzyme-assay buffer and finally with 2 µl of restriction enzyme (GeNei TM) i.e. BamHI (10 U-µl), EcoRI (20 U-µl), EcoRV (10 U-µl) and HindIII (20 Uv) added in different tubes. After incubation for 1 h at 37°C, complete digestion was checked on 3% agarose gel with using 100 bp-1 kb reference ladders.

III. Results

100 g of each 24 samples of street fast food of 4 categories (6 samples of chicken biryani, 6 samples of burger,6 samples golgappa and 6 samples of vegetables) was given in table no 1.

Table no 1. E. coli +ve and -ve samples of different fast food

Samples	Total Samples	E. coli +ve	E. coli -ve
Chicken biryani	6	5	1
Burger	6	4	2
Golgappa	6	5	1
Vegetables	6	4	2

E. coli was isolated from 18 out of 24 (75%) food samples analyzed in this study; no *E. coli* isolates were obtained from the remaining 6 samples. *E. coli* was detected in 5 out of 6 (83.33%) chicken biryani and golgappa samples and in 4 out of 6 (66.66 %) samples of burger and vegetables ready to eat foods. Genomic DNA extracted from E. coli samples collected in this research work. The A260 value of two DNA sample was 1.8296 and 1.7887. The A260/A280 ratio was 1.954 and 1.846 respectively. Some alterations in the phenol/chloroform method were done in this work. These were the addition of brine solution and ice cold isopropanol before phenol: chloroform extraction of samples. Above given two steps enhanced the yield of high-quantity and high-quality genomic DNA and was agreeable to further molecular characterization with RFLP. The reliability of the extracted DNA and restriction fragment pattern was analyzed by agarose gel electrophoresis (Figs. 1). Most digested DNA fragments size was in the range from 300 to 3000 bp (Fig. 2). A powerful marker for molecular finding of pathogenic organism is RFLP digestion pattern. PCR amplification of the gene with the suitable primer would be specific for particular organism. In future, the specific gene of E. coli may be amplified and sequencing of the gene could expose the improved consideration of pathogenicity.

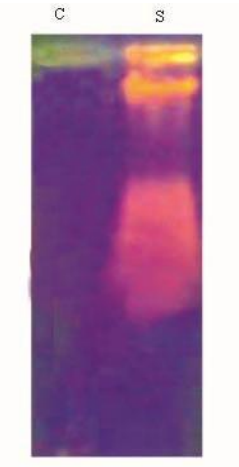

Fig. 1. Agarose gel electrophoresis pattern of the extracted DNA

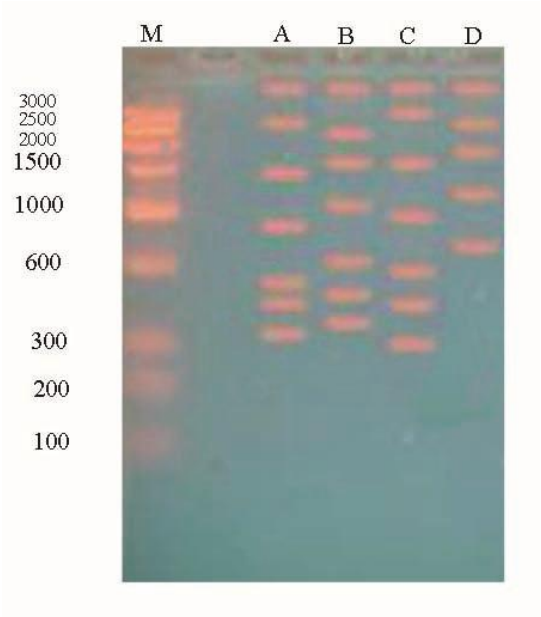

Fig. 2. RFLP profiling of sample after electrophoresis. LaneA:BamHI, laneB:EcoRI, laneC: EcoRV, laneD:HINDIII,

IV. Discussion

In this research work 75% food sample found as *E. coli* positive. The frequency of *E. coli* was found to be highest in chicken biryani and golgappa. *E. coli* was most frequently detected in convenient foods and 50% samples were found to be *E. coli* contaminated[14]. Presence of *E. coli* in food may indicate fecal contamination which might be due to insufficient cooking, use of raw vegetables, cross contamination between raw and cooked food and contaminated ingredients[15]. So, presence of *E. coli* in 75% ready to eat food samples in the present study might be representing fecal contamination. People who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene. Pathogenic bacteria including *S. aureus, E. coli* and *Salmonella* in restaurants would transfer to the cooked foods by contaminated staffs' hands or dishes[16]. Total *E.coli* was found to be present in all samples, indicating an alarming situation of health hazard. Many rural areas are endemic zone for diarrhoeal diseases every year, more than 3 % of death of children below 6 years of age is attributed to diarrhea.

V. Conclusion

Ready to eat foods vended in street fast foods in rural areas in Delhi NCR had unacceptable levels of contamination with *E. coli*. Unhygienic practice may represent the risk factors connected with contamination of packed food. Food-borne disease is an urgent public health problem and needs search association. RFLP pattern

analysis might be useful for molecular recognition of pathogenic organism among different species if coupled with PCR.

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