

Research article

SCREENING OF PATHOGENIC BACTERIA *E. COLI* FROM FRUIT JUICES AND MILK SHAKES COLLECTED FROM DIFFERENT LOCALITIES OF LAHORE BY MICROBIAL AND IMMUNOLOGICAL TECHNIQUES

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Few serotypes of *Escherichia coli* (*E. coli*) are most dangerous food-borne and water-borne pathogens that can also be causative agents of intestinal and extra-intestinal infections. *E. coli* is a known food-borne pathogenic bacterium that causes serious illnesses in animals as well as to human beings; such as abdominal pain, diarrhoea which usually leads to hemorrhagic diarrhoea and kidney failure etc. *E. coli* is a type of bacteria commonly found in the human intestinal tract and in human feces. This *E. coli* is only one of several mutant varieties of bacteria that are both pathogenic and antibiotic-resistant. *E. coli* is known to be present on fruits, vegetables, beef etc. There are 70 strains of *E. coli* that are associated for causing different illnesses from minor to major destruction. The present study was concerned with the screening of pathogenic bacteria with the help of microbial and immunological techniques. The pathogenic *E. coli* was isolated from fruit juices and milk shakes. Samples were cultured on three different media namely Tryptic Soy Broth (TSB) media, TSB agar media and EC media modified with novobiocin (selective media) for screening of food-borne pathogenic bacteria. The antibody was produced in rabbit and used for Dot blot and Enzyme Linked Immunosorbent Assays (ELISA) analysis of pathogenic *E. coli* 0157:H7, immunoassays results by using this polyclonal antibody were repeated by using monoclonal (commercial) anti-*E. coli* 0157:H7 to confirm specificity and reproducibility of immunoassays used in this study. It was found through the study that immuno-dot blot assay is a simple and rapid detection by which one can simply visualize reaction of the specific antibody to its antigen. ELISA are based on the measurement of an enzymatic reaction associated with immune complexes were also performed. Results were obtained from both microbial and immunological tests that confirmed the contamination of pathogenic *E. coli* in fruit juices and milk shakes. Extractions of DNA, RNA and proteins were also done which was an initial step towards future study of pathogenic bacteria at molecular level.

Key words: *Escherichia Coli*, Pathogenic, Immunological, Bacteria

Introduction

The present study was concerned with the screening of pathogenic bacteria with the help of antigen-antibody interaction. The pathogenic bacteria were isolated from fresh food samples (fruit juices and milk shake cultures

by growth in media). Then sub-cultured on nutrient agar media, MacConkey Agar (Mac Agar) and selective media for screening of food-borne pathogenic bacteria. The antibody was produced in rabbit to use it for Enzyme Linked Immunosorbent Assay (ELISA) and Dot-blot analysis. Unpasteurized milk, juice, and meat are potential sources of *E. coli*. Commercial juice should be always pasteurized, and juice concentrates are also heated sufficiently to kill pathogens. Fruits and

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vegetables should be washed thoroughly, especially those that will not be cooked. To access the food contamination in food samples, ELISA and Dot-blot immunoassay techniques were used. Molecular analysis of food borne pathogenic bacteria such as *E.coli* O157:H7 was also done which include DNA, RNA and protein isolation and analysed by agarose and SDS-Polyacrylamide gel electrophoresis.

Escherichia coli O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* that is the major cause of food borne illness (Karch et al., 2005). Infection often leads to hemorrhagic diarrhea, and occasionally to kidney failure, especially in young children and elder people. *E. coli* O157:H7 was first recognized and reported as a pathogen as a result of an outbreak of unusual gastrointestinal illness in 1982. The purpose of an ELISA is to determine if a particular protein is present in a sample and if it is present, how much (Leng et al., 2008). SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (Shapiro et al., 1967). Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein molecules using an electric field applied to a gel matrix (Berg et al., 2002). Proteins are usually analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Pollution and unhygienic conditions are the main reasons for food borne diseases. Country like Pakistan is also threatened by pathogenic bacteria that cause serious illnesses. Concerning the contamination of various foods with dangerous bacteria, it is important for us to understand some basic facts surrounding this problem. Recent outbreaks of food borne illness associated with the consumption of unpasteurized fruit juices have led to the number of disease causing bacteria that may occur in such products. The objectives of the study were microbial screening of pathogenic bacteria *E.coli* O157:H7 in samples (juices and milk shakes), antibody (anti-*E.coli*) production in rabbit to use for immunological tests, molecular analysis of food borne pathogenic bacteria. The overall goal of the project is to advance our knowledge on pathogenesis; and assessment of risk for food borne microbial infections and contamination caused by *E.coli* O157:H7.

Material and Method

Material and Chemicals used:

Yeast, TSB media, TSB agar, Sorbitol- MacConkey Agar Medium (SMAC), Crystal Violet Gram Stain, Iodine Solution, Acetone-alcohol (Decolorizer), Safranin, Sodium hydroxide NAOH, Distilled water, Agar, Glycerin, EC media modified with novobiocin, Pure cultures of pathogenic *E.coli*, Pure cultures of heat killed pathogenic *E.coli* (for antibody production), Freund's Complete Adjuvant (cat# F5881), Freund's Incomplete Adjuvant (cat# F5506), Primary antibody (anti-*E.coli* Polyclonal and Monoclonal), Anti-Rabbit Ig G (Secondary antibody), Anti-Mouse (Secondary antibody), Chloroform, Nitrocellulose membrane (Hybond-C) Milipore company, 5% skimmed milk, 1XPBS, 3,3-Diamino benzidine Tablet (DAB) (cat# D5905) Sigma company, Pure gene DNA extraction purification system (DNA extraction kit) (cat# d-5000) Genra Systems, USA., Pure script RNA purification system (RNA extraction kit) (Genra Systems, USA), Cell lyses solution [(EDTA) Ethylene diamine tetracetic acid and (SDS) sodium dodecyle sulphate], RNase, Protein precipitation solution (ammonium acetate), 70% ethanol, 100% ethanol, Protein DNA precipitation solution (citric acid, sodium chloride), RNase free water, Proteinase solution, Loading dye, Coomassie blue dye, Agarose, 0.5X TBE, Ethidium bromide (Et.br), Tris chloride buffer pH 8.8, 30% polyacrylamide/bisacrylamide, 10% SDS, 10% Aps, TEMED, Sample buffer, Running buffer, Resolving gel buffer, Stacking gel, Resolving gel, Coomassie Brilliant Blue, An ultraviolet-fluorescent dye, Nitrile rubber gloves, Bromophenol blue(As color marker dye), Rabbits (albino), Microtitter plates, Eppendorfs, Test tubes Flasks, Petri dishes, A gel rack, A comb, Power Supply, UV lamp or UV lightbox or other method to visualize DNA in the gel and ELISA reader (Bio Rad).

For the screening of pathogenic bacteria 300 samples of fresh juices, canned juices and milk shakes were collected from different juice corners of different localities of Lahore. These samples were cultured in three media namely Tryptic Soy Broth (TSB), Sorbitol Mac.Conkey TSB Agar media and EC media modified with novobiocin.

Results

Tryptic soy broth (TSB) is commonly used as enriched media. Sorbitol Mac.Conkey agar was also used to facilitate growth of target organism. Selective media are formulated with ingredients like Novobiocin which is an antibiotic that inhibit the growth of some bacteria, but

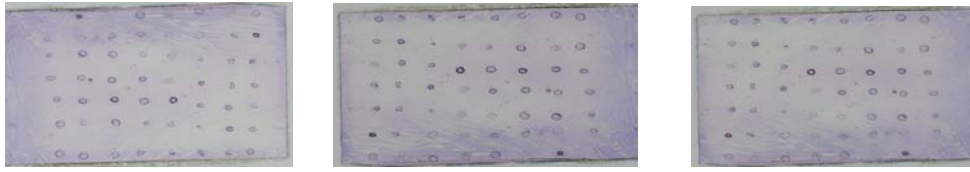


Fig 1. Three Immuno Dot-blot of samples from 1-189



Fig 2. ELISA results of fruit juices and milk shake samples (1-300)

enhance growth of the target organism. Gram staining shows rod shape, pink *E.coli*.

Titer results of produced Primary antibody

Produced polyclonal antibody authentication and titer determination was carried out by performing Dot-blot protocol.

Immuno-Dot blot

Immuno-Dot blot was done and found as simple and quick method for the screening of food borne pathogenic bacteria with antigen-antibody interaction (Fig 1).

ELISA Results

ELISA was also done which is more accurate and appropriate immunological method to check food contamination by calculating optical densities of microbially grown pure cultures of *E.coli* with the antibody against that bacteria (Fig 2).

DNA and RNA Results

Agarose gel electrophoresis was done for visualizing DNA and RNA.

Total Protein Extraction Results

SDS gel preparation was done for visualizing total protein extracted from bacterial cells grown on selective media.

Results of microbial screening and immunoassays

300 samples of fruit juices and milk shakes shows the results of microbial growth of pathogenic *E.coli* in different media, their gram staining, immuno Dot blot assay shows the reactivity of pathogenic *E.coli* with antigen-antibody that how much it is reactive to the specific antibody. ELISA gives the cut off values and the ODs of samples at 480nm that confirms the presence or absence of pathogenic *E.coli*. Sixty four different canned juices were also tested and it was found that

most of them appeared negative against microbial and immunological tests.

Discussion

The study was done for the screening of pathogenic bacteria *E. coli* from fruit juices and milk shakes collected from different localities of Lahore by microbial and immunological techniques. It was found through literature review that no study has done for screening of pathogenic bacteria from fruit juices and milk shakes in the past. Samples of fruit juices and milk shakes were cultured in different media, growth appeared that confirmed the presence of pathogenic bacteria in samples tested. The contamination is mainly due to unhygienic conditions during the preparation and handling of food items. Due to the consumption of uncooked vegetables, beef, unpasteurised milk, juices and drinking water, or handling of food by affected person are the main causes. *E. coli*-mediated illnesses are normally food- and waterborne.

Fresh juices and milk shakes are contaminated due to unhygienic conditions during their preparation as they are not pasteurized. And people like to utilize them as raw because they cannot be cooked if so they will lost their important vitamins and not effective for health as used raw. Study found that canned juices has less bacterial contamination as they are properly pasteurized and packed according to hygienic conditions. 64 different canned juices were also tested and it was found that most of them appeared negative against microbial and immunological tests. Study recommended that people should utilize canned juices.

Primary antibody (anti-*E. coli*) was produced by grown culture of heat killed pure pathogenic *E. coli*. Titer detection of that antibody (anti-*E. coli*) was also checked and found good quality that gave positive results. ELISA was also done which is more accurate and appropriate immunological method to check food contamination by calculating optical densities of microbially grown pure cultures of *E. coli* with the antibody against that bacteria. Extraction of DNA, RNA and proteins was done for molecular analysis which was an initial step towards future study of pathogenic bacteria at molecular level.

Conclusion

The study was done for the screening of pathogenic bacteria *E. coli* from fruit juices and milk shakes collected from different localities of Lahore by microbial and immunological techniques. From the study it can be concluded that few serotypes of *Escherichia coli* (*E. coli*) are one of the most dangerous food-borne and water-borne pathogens that can also be causative agents of intestinal and extra-intestinal infections. *E. coli* O157:H7 is a known food-borne pathogenic bacteria that causes serious illnesses in animals as well as to human beings; such as abdominal pain, diarrhoea which usually leads to hemorrhagic diarrhoea and kidney failure etc. The contamination is mainly due to unhygienic conditions during the preparation and handling of food items especially unpasteurised milk, juices and drinking water, or handling of food items by affected person are the main causes.

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