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Microbiological Analysis of Street Vended Sugarcane Juice from Bhilai City, India

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Due to reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere, a study was undertaken to assess the safety of street vended fruit juices particularly sugar cane juice as possible sources of bacterial pathogens. Bacteriological examination of sugar cane juice was done by multiple-tube fermentation test. In the present investigation freshly squeezed juices of sugarcane, showed occurrence of high microbial loads consisting of number of pathogens like Coliforms, Fecal coliforms, *E.coli*, *S.aureus*. The presence of *E.coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many other types of disease-causing organisms. This can be prevented by checking the water source of the vendors.

Key words: *E. coli*, Contamination, Sugarcane Juice, Bhilai.

Due to reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere, a study was undertaken to assess the safety of street vended fruit juices particularly as possible sources of bacterial pathogens.

Fresh squeezed juice of sugarcane juice by street venders in Bhilai city were analyzed for their microbial contents during the months of April 2010 to August 2010. The viable counts of all 5 samples were approximately 100 ml with significant load of coliforms, fecal coliforms and staphylococcal counts.

In developing countries fruits and vegetables juices sold by street venders are widely consumed by millions of people. These juices provide a source of readily available affordable source of nutrients to many sectors of the population[1]. Un-pasteurized juices are preferred by the consumers because of the fresh flavour attributes and hence, in recent times, their demand has increased. They are simply prepared by extracting the juices[2, 3, 9].

Fruit juices are well recognized for their mineral and vitamin content and high nutritive value, in many tropical countries juices are the common man's beverages and are sold mostly in public places and roadside shops. There have been reports of food borne illness associated with the consumption of fruit juices in India and elsewhere[4,5]. Food borne diseases mainly affect the gastrointestinal tract and are transmitted through consumption of contaminated food or drinks. Street vended fruit juices could prove to be a public health threat[6-9].

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In Bhilai city (C.G., India) there is constant great demand for fresh fruit juice , while most restaurants and cafe serve juice in apparently hygienic conditions, juices sold at the roadside shops and recreational areas, parks and busy market places (shopping malls, bus station etc.) are of questionable microbial quality. In these shops, juices are extracted by squeezing from a variety of fresh fruits e.g. tamarind water, sugarcane juice, drinking water, jaljeera, orange, apple, grape, pineapple etc. are served after dilution with water and ice. Despite periodic quality control checks of fruit juices by Food and Drug Department, outbreak of gastroenteritis caused by pathogenic *E. coli*, Salmonella and Shigella are common, though a specific correlation has not been shown between infection and juice consumption[9,10] .Gastroenteritis has been linked to a range of micro -organisms including bacteria (such as Camphylobacter, Salmonella and Shigella species) and protozoa (Giardia & Entamoeba), (Phillip, 1991) whilst most cases of gastroenteritis caused by such pathogens are linked to the contamination of food or water supplies, in recent years infection through recreational contact with sewage contaminated water has been recognized as a serious problem[10,11].

In view of the high demand for fresh fruit juices during summer in Bhilai city, a study of the street vended juices was undertaken during April to August 2010 with a view to assess safety for human consumption and as possible sources of bacterial pathogens.

E. coli is a type of coliform bacteria commonly found in the intestines of humans and warm-blooded animals. Most strains of *E. coli* do not cause illness in healthy humans and are actually beneficial to the synthesis of vitamins.

Some strains, however, cause cramps and diarrhea in humans. One particular strain named O157:H7 produces a powerful toxin that can cause severe illness. Health organizations across the world have selected *E. coli* as the most reliable indicator for the bacteriological quality of drinking water [12, 13, 14]. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many other types of disease-causing organisms.

Presence of *E. coli* bacteria does not necessarily mean that your drinking water contains the strain of *E. coli* O157:H7. Heath organizations do not believe it necessary for a private well owner to test specifically for *E. coli* O157:H7 under normal circumstances. If *E. coli* O157:H7 is present in your well, it is highly likely that other strains of *E. coli* are also present. If a well has *E. coli* present, regardless of strain, you should not drink the water unless it is effectively disinfected. Max. acceptable concentration for *E. coli* for drinking water has been set at no organisms detectable/100 mL.

Sugarcane contains 18-20% solids and the juice obtained from it is a popular beverage. This natural product is reported to impart health benefits to the consumers. However, the major problem encountered is the lack of hygiene during crushing operation resulting in the contamination of the juice with a heavy load of micro-organisms which arises due to improper cleaning of the sugarcanes and handling of the finished product[9]. Raw sugarcane juice is a carbohydrate-rich, low acid food and is therefore susceptible to the growth of moulds and yeasts, of spoilage bacteria and also of pathogenic bacteria. Therefore, such freshly crushed juice cannot be preserved even for a few hours as it is known to ferment very quickly[10]. There is also no published information/patent available on processing of sugarcane juice using microfiltration employing membranes[11, 14, 15] .

Bacterial contamination cannot be detected by sight, smell or taste. The only way to know if a water supply contains bacteria is to have it tested. The Environmental Protection Agency (EPA) requires that all public water suppliers regularly test for coliform bacteria and deliver water that meets the EPA standards[15].

MATERIALS AND METHODS

The present investigation deals with the detection of coliforms to determine the purity of Sugarcane juice using multiple tube fermentation tests.

Sources of sample

The sources of sample to be tested were collected from 10 different hawkers of the Bhilai city of Chhattisgarh state. The samples were tested in RCDSR Microbiology, Biochemistry lab. If the sample were positive, they were further put to confirmatory test, if negative then discarded.

The samples were used for coli forms Bacteriological Index.

Collection of sample

All the samples were collected from 05 different hawkers of Bhilai city and were subjected for coli form test. The samples were collected in evening hours when the hawkers had just started his sale. The samples were collected in the sterilized vials.

Sanitary analysis of water

Multiple – Tube Fermentation Test or Most Probable Number (MPN) - This test is the most oftenly used technique for the sanitary analysis of water. The test is used to detect coli forms (Coliforms are defined as facultative anaerobic, gram negative, non – sporing, rod shaped bacteria that ferment lactose with the production of acid and gas within 24 hrs of incubation at 35°c) that make up approximately 10% of the intestinal microorganisms of humans and others animals and have found widespread use as indicator organisms of fecal contamination[15-20].

The test is performed sequentially in three stages: Presumptive, Confirmed and Completed test. Lactose broth tubes are inoculated with different water volume in the presumptive test. A pH indicator such as bromocresol purple is also added to lactose broth for the detection of acid. Tubes that are positive for gas production are inoculated into brilliant green lactose bile broth in the confirmed test and positive tubes are used to calculate the most probable number (MPN) of coliform in the water sample following the statistical table. The completed test, involving the inoculation of EMB agar plate, nutrient agar slant and brilliant green lactose bile broth and preparation of a gram stain slide from NA slant, is used to establish that coliform bacteria are present in the sample. The complete process, including the confirmed and completed tests requires at least 4 days of incubation and transfers [15-21, 26-31].

Presumptive coliforms test

The presumptive coliform test is used to detect coliforms in a water sample. In this test lactose fermentation tubes are inoculated with different water volumes and production of acid and gas from the fermentation of lactose in any of the tubes is a presumptive evidence of coliforms in the water sample. The lactose broth used in this test is selective for the isolation of coliforms because of the addition of bile and lauryl sulphate or brilliant green. A pH indicator such as bromocresol purple is also added to lactose broth for the detection of acid. The colour of the indicator changes to yellow with the production of acid from lactose[15-20, 26-31.]

Confirmed coliforms test

This test is used to confirm the presence of coliforms and to determine the MPN value in water samples showing positive or doubtful presumptive test. In the confirmed test, water samples from all the positive presumptive lactose broth tubes are inoculated into tubes of brilliant green lactose bile broth and incubated at 35°c for 48 hrs. Positive confirmed tubes are used to determine MPN. A statistical method is used to estimate the population of coliforms, which means that the result obtained is expressed as the most probable number (MPN) of coliforms. A count of number of lactose fermentation tubes/brilliant green lactose bile broth showing production of gas following the incubation period is taken and MPN is found by matching the results with those provided in the statistical table [15-21, 26-31].

Completed coliforms test

Completed test is used to establish the presence of coliform bacteria and as a confirmatory test for the presence of *E. coli* in a water sample. In the completed test, the samples from the positive brilliant green lactose bile broth from the confirmed test are streaked onto a selective differential medium for coliforms and inoculated into lactose broth tube as well as streaked on a nutrient agar plate to perform gram-staining. The medium commonly used is Eosin Methylene Blue (EMB) that is selective in nature because of the dye methylene blue which inhibits the growth of gram-positive bacteria, allowing the growth of gram-negative bacteria. EMB is differential in nature; the lactose fermenting bacteria gives coloured colonies (a positive confirmed test) due to the formation of a complex in EMB that precipitates out onto the coliform colonies. Non-lactose fermenters produce colourless colonies on EMB agar. If there is production of acid and gas in the inoculated lactose broth and there are rod shaped bacteria showing Gram-negative reaction, these confirm the presence of *E. coli* in the sample and are considered a positive completed test [15-21, 26-31].

Gram-stain technique

Gram-positive organisms are blue; gram-negative organisms are red. Results are acceptable only when controls have given proper reactions[22,23].

Various modifications of the Gram stain technique exist. Use of the modification by Hucker for staining smears of pure culture; include a gram-positive and gram-negative culture as controls, was followed.

IMViC Test [24-26]

The IMViC tests consist of four different tests:

1 Indole production;
2 Methyl – red;
3 Voges – Proskauer;
4 Citrate utilization.

Observation

1. All the lactose fermentation tubes were found positive with the production of acid (yellow colour) and gas after 24 - 48 hours of incubation.
2. All the Brilliant Green lactose fermentation tubes were found positive with the production of acid (yellow colour) and gas after 24 - 48 hours of incubation.
3. EMB agar plates were found positive with bluish mucoid colonies. Gram stain was done on the organisms found on the slant and the slide was found positive.
4. After performing Gram Stain the samples were found negative with rod shaped reddish to pink colored bacilli.
   Gram Reaction – Negative
   Morphology – Rod shaped
   Arrangement – Single

5.a) The sample tubes developed cherry red colour in the reagent "layer", showing presence of *E. coli* in Indole test.

b) The sample tubes developed red colour that shows a positive MR test but no colour change in VP test, showing presence of *Escherichia coli*

c) In Simmon's Citrate slant there is no growth and there is no change in the colour of the medium (i.e. green) i.e. the samples are citrate negative hence confirms the presence of *E. coli.*

RESULTS AND DISCUSSION

MPN result of the present study shows that water and juices collected from Supela were most contaminated and that of Risali was found to be least contaminated.

The MPN count of coliform bacteria in Sugarcane juice was found in the range of 110 to 160 so as per the described limit of WHO Sugarcane juices are under the category of polluted. The presence of coliforms shows the danger of fecal pollution and consequent hazard of contracting disease through pathogenic organisms. On the EMB plate metallic sheen colored colonies, in gram staining, presence of gram negative bacteria and the biochemical test shows the presence of *E. coli* bacteria in all the samples[15-21, 24-26].Study Concluded that typical enteropathogenic *E. coli* strain is a leading cause of infantile diarrhea in developing countries, whereas they are rare in industrialized countries. Moreover, *E. coli* is also responsible for causing a number of other health disorders: urinary tract infections, pulmonary infections, abscesses, skin-wound infection, etc. In the present study we can conclude that Sugarcane juice formed the

Table 1. MPN count of different samples of Sugarcane juice

Sample	MPN value /100 ml
Sample no. – 1	13
Sample no. – 2	14
Sample no. – 3	13
Sample no. – 4	11
Sample no. – 5	09

Table 2. The pollution status of drinking water on the basis of *E. coli* contents (WHO 1984)

E. coli in per liter	Water pollution status
10,000	Heavily polluted
1000	Polluted
100	Slightly polluted
10	Satisfactory
3 or less	Potable

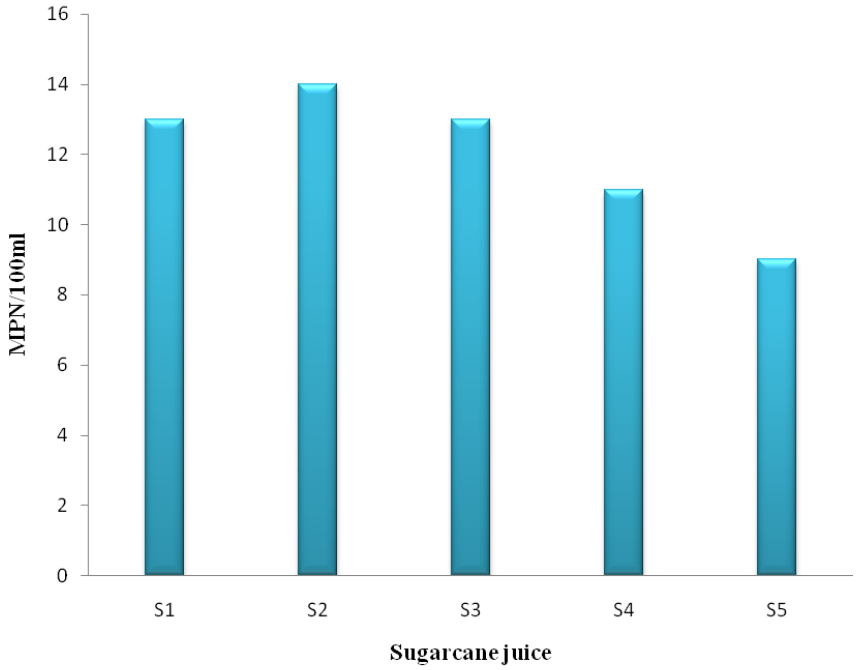

Fig. 1. Graph shows MPN count of different Sugarcane juice sample

favorable substrate for bacterial growth, this is due to presence of water in the sugarcane juice that provides favourable media for growth of *E. coli* but at the same time high sugar content formed the basis of low concentration of bacteria.

The results of the present findings clearly demonstrated that, the road side ready fresh juices did not meet public health standards and many kinds of enteropathogenic bacteria were found like, *Escherichia coli.* Such foods lead to hazardous effects to the consumers. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for juice preparations, especially street vended fruit juices.

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