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Research Article

Bacteriological Assessment for Iced Sugarcane Juice in Korba, Chhattisgarh, India

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Abstract

Sugarcane juice is a common man's refreshing beverage that was extensively consumed during hot summers, among the all population group and has been great concern of hygienic measure as it was poorly implemented by street vendors and hawker. The present study aims to evaluate the quality of ice used in the sugarcane juice in different place of Korba, C.G. Ice samples collected were found to be contaminated by many pathogenic and non-pathogenic bacteria with very high bacterial load ($5x10^5$-$6x10^7$ CFU/ml) indicating a serious health concern for the people consuming it, in our study we have identified *Leclercia adecarboxylata, Citrobacter freundii* and *Escherichia hermannii*, which represents source of microbial contaminant from where the ice were bought are either from hospital morgue or from cold storage where meat and fishes were stored.

Keywords: Sugarcane Juice; Bacterial load; Ice; Coliform

Introduction

Sugarcane juice is a common man's refreshing beverage extensively consumed during hot summers that not only quenches the trust but also invigorates as well. Its unique properties to giving tolerance against the heat, availability at very cheaper cost and ubiquitous distribution makes it very popular drink among all class of people in every part of India. Sugarcane juice was extracted by crushing the sugarcane between the roller drums and served with or without ice, and it majorly composed of approximately 80% water and 20% total dissolved solids, 17% saccharose, 0.4% glucose and 0.2% fructose, traces of nitrogenous substances such as organic acids, vitamins A, B-complex, and C and also minerals such as iron, calcium, potassium, sodium and magnesium [1,2].

Sugarcane juice was majorly contaminated by spoilage microorganisms which primary causes chemical, physical and sensory deterioration of the drink. Significant variation in pH of the juice leads to accelerated microbial spoilage [3], the source of microbial contamination was supposed to be the raw material used such as unhygienic storage and extraction places, improper handling of sugarcane, knives, press, clothes, contact surface, ice, vendor's hands and air born contamination, causing various infectious diseases such as vomiting, nausea, abdominal cramps, typhoid, diarrhea etc [3,4], there had been good number of cases reported for occurrence of food borne disease such as *Vibrio* cholerae was considered to be organisms responsible for causing serious epidemic in Pune in 1991 due to contaminated sugarcane juice (Kusumaningrum, 2004). In-order to ensure microbiologically safe juice there must be strong and regular monitoring of processing of juice as poor hygienic conditions were predominant in the majority of places where sugarcane juice was sold [5]. It was observed that in room temperature sugarcane juice contains mainly mesophilic bacteria, whereas in refrigerated condition the growth is possible mainly due to psychrophilic bacteria. Most predominant species that expressed in juice and mixed juice were *Streptococcus* sp. Occasionally, *lactobacillus fermentum, Leuconostoc mesenteroides* and *Erwinia herbicola* were also predominant. Studies also reveal the presence of *Bacillus sps. Streptococcus sps*., and *lactobacillus cellubiosus* these are detrimental to sucrose present in the sugar cane juice. In our studies presence of *Leclercia adecarboxylata, Citrobacter freundii* and *Escherichia hermannii* was of great concern due to their source of origin indicates that the Ice used in sugarcane juice were bought from Morgue or from cold storage where meats were preserved.

Materials and Methods

Description of sampling sites

Samples of analysis i.e ICE which were used in sugarcane juice collected from 6 different locations in Korba among them 5 were from street sugarcane juice wander from transport nagar, and one sample from street sugarcane juice wander from Darri Road, Korba. Samples were collected in four replicates aseptically in sterile glass bottles, labeled properly and transported to the laboratory for analysis. Aliquots of the samples were used for selective isolation of different bacterial species based on standard microbiological procedures.

Microbiological analysis of Ice

Total plate count (TPC)

The samples were opened in a laminar airflow chamber under all aseptic measures. Total Viable Count was determined by pour plate method. Serial dilutions (10:1, 10:2, 10:3 and 10:4) were made from the ice samples and aliquots of 1ml were added to each duplicate Petri dish. Plate count agar (PCA) was added to each Petri dish and incubated at 35°C for 48 hours ±2, after incubation colonies were counted by colony counter and result was expressed as CFU/ml [6,7].

Total coliform bacteria/Fecal coliform bacteria

Most Probable Number (MPN) test was performed to assess the total Coliforms count pollution level in the selected area for total Coliform count and determined by multiple tube fermentation technique [6,7]. The technique involves three successive steps namely, presumptive test, confirmatory test and completed test. Total coliform and fecal coliform were calculated from MPN index table from a serial dilution of soil suspensions. The microbial colonies were counted in the three replica plates and the average values were calculated. The populations of microorganisms were considered from the number of microbes multiplied by the dilution factor for each sample.

Escherichia coli (E. coli)

EMB Agar was used for the enumeration of E. coli. All the tubes of E.C. broth showing gas were subculture by streaking on EMB agar plates and incubated at 35°C for 18-24 hrs. Positive plates contained typical colonies with green metallic sheen were inoculated on PCA slants (plate count agar) and incubated at 35 °C for 18-24 hrs and identified biochemically [6,7].

Pure culture of bacteria species

Pure culture of bacterial species from water samples were obtained by serial dilution methods recommended by Harley Prescott, 2002. The isolated bacterial strains were cultured in nutrient agar medium (Hi Media Laboratory, India). Serial dilution of the samples was made using sterile distilled water. 0.1 ml of this dilution was inoculated on nutrient agar and incubated at 37°C for 24 hrs. Pure cultures were isolated and sub-cultured twice in the same medium at 37°C. Pure cultures were subjected to biochemical identification tests its identification and characterization following the methods described in Bergey's Manual of Systematic Bacteriology. The bacteria that were picked to create anti bio-grams were streaked on to nutrient agar slants to make sample cultures and for PCR purpose.

Biochemical characterization of the isolates

The species were characterized by biochemical tests performed as per standard Microbiological methods [8], including Gelatin hydrolysis test, Starch hydrolysis test, Casein hydrolysis test, Catalase activity test, Glucose Fermentation test, Citrate utilization test, Nitrate reduction test, Oxidase Test, VP -Voges-Proskauer Test ("Vi"), Urease Test. Species identification was also confirmed by VITEK-2C (Biomerieux, France).

Antibiotic sensitivity

Antibiotic sensitivity of isolated microorganisms towards Ticarcillin. Piperacillin, Clavulanic acid, Ceftazidine, Cefoperazone/sulbactam, cefepime, aztreonam, doripenem, imipenam, meropenam, amikacin, gentamicin, ciprofloxacin, levofloxacin, minocyclin, tigercyclin, colistin, trimethoprim/sulpamethoxazole, were testing in VITEK-2C (Biomerieux, France) as per manufacturers instruction.

Results

25 samples used on sugarcane juice vender were collected from 5 different places in Korba, among them 4 were from transport nagar, and one sample was from Darri Road, samples were analyzed for total plate count, total coliform count and no of *E. coli* present. For the safety of public health, it was crucial to monitor the measure the complete microbial evaluation for the safe drinking purposes [9], our results showed that in all the localities the sugar cane juices remained hygienically poor based on high bacterial load (total plate count, total coliform count, total fecal coliform count, E. Coli count). It was observed that overall count were quite high and almost same everywhere (Table 1) represents that samples were equally conta-

minated and cannot be used for safe drinking purposes. The presence of coliform bacteria may also be due to the contaminated water used in ice. It was observed that significant bacterial species were present in the ice sample those are capable enough to cause severe outbreak and their origin was animal and mostly animal specifically from human diseases.

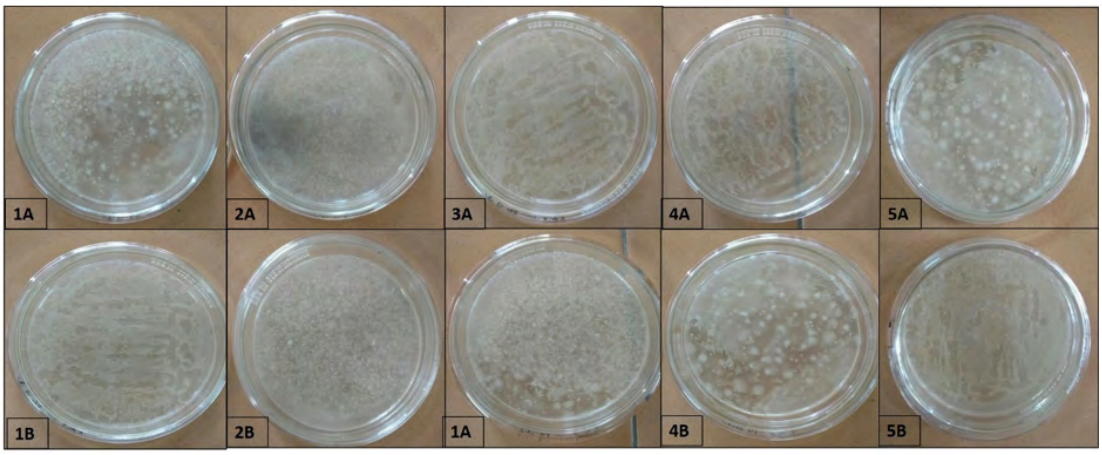

Figure 1: Bacterial colony isolated from ice samples collected from different locations of Korba.

Site of collection	Total plate count (cfu/ml)	Total coliform count (MPN/ml)	Total fecal coliform count (MPN/ml)	*E. coli*
Darri Nala	$5X10^5$	110	85	+ve
Transport Nagar A	$6X10^7$	155	100	+ve
Transport Nagar B	$4X10^7$	150	95	+ve
Transport Nagar C	$5X10^7$	149	93	+ve
Transport Nagar D	$3X10^6$	140	90	+ve

Table 1: Table representing bacterial load, total plate and coliform count, fecal count, and presence of *E. coli.*

In our study, we have isolated *Escherichia hermannii, Leclercia adecarboxylata*, and *Citrobacter freundii*. *Leclercia adecarboxylata*, also identified as *Escherichia adecarboxylata* (motile gram negative rod shaped bacteria) found to be most profoundly distributed in nature and had been isolated from food, water, and other environmental sources as well as from various clinical specimens, including blood, faces, sputum, urine, and wound pus [10]. It was also reported in many cases such as immuno compromised individuals with infections of a polymicrobial nature [11], trauma patient as rare pathogen in endocarditis [12], catheter-related bacteremia [13], bacteremia and cellulitis in children suffering from leukemia [14-16].

Citrobacter freundii, (facultative anaerobic gram-negative rod shaped bacteria of *Enterobacteriaceae* family which was ubiquitous in distribution and commonly found in soil, water, sewage, food and represents approximately 29% of all opportunistic infections in the intestinal tracts of animals and humans [17], It is known to cause variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients [17], it was also found to associated with neonatal meningitis and brain abscess [18] with very high morbidity.

Escherichia hermannii (Gram-negative, rod-shaped bacterium), was reported as the sole pathogen in a catheter-related bloodstream infection, it was also has been isolated from human

wounds [19], eye infections, blood [20-22], it was also found that *E. hermannii* is inherently resistant to penicillin, ampicillin, and carbenicillin but sensitive to other β-lactam antibiotics (cephalosporins, carbapenems, and monobactam) [23-25].

Conclusions

In the Present study presence of heavy bacterial contaminant in ice used in preparation of sugarcane juice, presence of organisms such as *Escherichia hermannii, Leclercia adecarboxylata*, and *Citrobacter freundii* represents the Ice used were bought from the places such as morgue, meat house or meat cold storage, which were responsible to cause critical health problem.

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