



Microbiological Quality and Safety Assessment of Sugar Cane Juice and Ice Sold by Vendors in Faisalabad City, Pakistan

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Abstract

The risk of illness associated with untreated freshly squeezed juices sold by street vendors is increasing gradually. Street vended juices are widely consumed by millions of people in developing countries.

Epidemiological data indicated that unpasteurized sugar cane juices indisputably resulted in increased numbers of reported outbreaks. In Faisalabad city there is a constant great demand for fresh fruit juice. To determine the microbiological quality, a total of sixty five samples of sugar cane juice and thirty samples of ice sold by street vendors on four different popular roads of Faisalabad city were collected and analysed by standard methods for the detection of coliform, fecal coliform and *Escherichia coli*. All the collected samples of sugar cane juice and ice were hygienically poor as shown through high bacterial load ranging from 2×10^3 - 3×10^7 CFU/mL and 2×10^3 - 3×10^5 CFU/mL respectively. The Consequence of this study indicated the prevalence of different pathogenic microorganisms (Total coliform, fecal coliform, *Escherichia coli*) in many tested samples. Results demonstrated the low quality, safety of street vended sugar cane juices and ice added in it for cooling purpose. Appropriate actions must be taken immediately by respective authorities to improve quality and safety of juices to prevent any type of outbreak.

Keywords: Food Safety; Vendors; Pathogens; Contamination; Beverage; Hygiene; Ice

Introduction

Street vended juices have food safety issues related to them because they are usually prepared under unhygienic conditions. Microbiological hazards that can cause serious health problems are often associated with consumption of these juices [1]. In Faisalabad city there is a constant great demand for fresh fruit juice. Street vended juices are widely consumed by millions of people in developing countries. The demand for the production of safe food with good nutritional profile and extended shelf life is ever growing in market [2]. Sugar cane juice is an affordable source of refreshment and energy. It is an important tool for better health due to its nutritive profile.

Sugar cane juice has many health benefits including rising innate immunity to infections [3]. In many tropical countries, it is sold in all municipal areas, parks, bus stands and all busy market places. On vendors it is simply prepared by extracting generally by mechanical means. The final product is untreated juice ready for consumption. The cane may contain high microbial load on its

surface as it is transported un-hygienically from field to the point of extraction. Inappropriate washing of sugar cane before extraction adds bacteria into extract leading to contamination. This critical process of extraction of sugar cane juice with no hygienic measures causes the occurrence of food borne diseases.

Ice added for cooling purpose of juice is sometimes contaminated with pathogens due to the contaminated water source which is used in its manufacturing or poor hygiene in its handling and transportation. In different parts of the world, outbreaks have been reported due to the contaminated ice [4,5]. Therefore, sugar cane juice sold by vendors and ice added to it for cooling can pose health hazards. Spoilage microorganisms are the primary reason for the chemical, physical and sensory deterioration of sugar cane juice [2]. Possible sources of microbial contamination have been acknowledged as poor hygienic handling, raw material, ice, inappropriate cleaning of the sugar cane press knives, interaction surfaces, sellers hands and airborne contamination [6].

Contaminations from raw material have been identified to be a cause of infectious diseases such as typhoid, abdominal cramps, diarrhoea etc. [6,7]. These juices with no additional treatments have been a possible source of bacterial pathogens particularly *Salmonella* and *E. coli* (O157H7) [9]. In 1991 sugar cane juice contaminated with *Vibrio cholera* was linked to a cholera epidemic in Pune city India. The added ice was found contaminated with *Vibrio cholera* in this case [7-9].

In interpretation of the threat posed by bacterial pathogens in sugar cane juice and its flourishing demand, this study was conducted to assess the microbial quality and safety of street vended sugar cane juices and ice. In this paper, standard microbiological methods were used to evaluate the safety and quality of sugar cane juices and ice samples.

Materials and Methods

Collection of samples

Samples of street vended sugar cane juices and ice were collected from four selected areas in the Faisalabad city, Pakistan (Table 1). Samples were collected under aseptic conditions in sterile bottles. Collected samples were placed at 4°C and evaluated within an hour of procurement.

Area	No of samples
Jhang Road	19
Jaranwala Road	13
Sargodha Road	18
Satyana Road	15
Total	65

Table 1: Sampling plan.

Microbiological analysis of sugar cane juice

Total plate count

Total plate count (TPC) of sugar cane juice samples was determined by using pour plate method. Petri dishes, pipettes and test tubes were sterilized before use. Serial dilutions (10^{-1} - 10^{-4}) of sugarcane juices were prepared. Aliquots of 1 mL were taken from each tube by using a pipette of 1 mL and added to each sterilized petri dish.

After addition of Plate Count Agar (PCA) all petri dishes were incubated at $35^{\circ}\text{C} \pm 1$ for 48 hrs. Colony counter was used to count colonies after incubation. Result was stated as CFU/mL [10].

Total coliform and fecal coliform count

1 mL from the prepared serial dilutions was inoculated into the set of three tubes each containing 10 mL of Lactose broth with in-

verted Durham tubes. These tubes were incubated at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for period of 24 - 48 hrs. Tubes with gas production/turbidity were detected as positive and used for confirmatory test. Positive tubes were further sub-cultured into Brilliant Green Lactose bile broth (BGB) and E.C broth having 10 mL volume with Durham tubes. (BGB) tubes after incubation ($35^{\circ}\text{C} \pm 1/48\text{h}$) and E.C tubes ($44^{\circ}\text{C} \pm 1/48\text{h}$) were observed.

Tubes with gas production were identified positive for coliforms. Total Coliform and fecal coliform count was calculated by MPN tables.

E. coli detection

Eosin-methylene Blue agar (EMB) was used for the detection of *E. coli* in the samples. Positive tubes from E.C broth were streaked on EMB agar plates and incubated at $35^{\circ}\text{C} \pm 1$ for a period of 18 - 24 hrs. Plates containing green colonies with metallic sheen were considered as positive.

Yeast and mold count

Test tubes and petri plates were sterilized in laminar air flow. Potato dextrose agar was used for the enumeration of yeast and mold. Serial dilutions were prepared by 1 mL of sugar cane juice sample and 9 mL of saline water.

0.1 mL of the sample from prepared dilutions was poured in the petri plates and mixed well. Plates were incubated at $28^{\circ}\text{C} \pm 1$ for 48 hrs and after incubation yeast and mold colonies were counted with colony counter and expressed in table 2.

Microbial analysis of ice samples

Thirty samples of ice were collected aseptically from different sugar cane juice vendors located in the Faisalabad city. Collected samples were analysed after they were allowed to melt under aseptic conditions at 5°C. The ice samples were microbial examined to determine total plate count, coliform count, fecal coliform count and *E. coli* using the same methods as for sugar cane juice samples within an hour of collection.

Results and Discussion

Microbial profile of sugarcane juice sample

Sugar cane juice is a refreshing and nutritive drink. Due to its short shelf life it is mostly sold fresh. Freshly squeezed sugar cane juices have no processing step that can reduce pathogenic levels in juice. The way to overcome such hazards is that people related with food handling must be trained [11]. The purpose of this study was to analyse the microbial status of street vended sugar cane juices in different areas of Faisalabad city. Collected samples of sugar cane juices showed high bacterial load ranging from 8×10^4 - 3×10^7

CFU/mL as shown in table 2. A study conducted on the microbiological quality assessment of sugarcane juice sold in Peshawar City, Khyber Pakhtunkhwa-Pakistan showed similar results [6]. Maximum Total Plate count (TPC) was found in Sargodha road area (3×10^7) and minimum in Jaranwala road area (8×10^4) CFU/mL. Traffic load and sewage water lines associated with the cleaning of utensils were the main cause for the higher TPC of Sargodha road area. The main source for the higher levels of microbes is contaminated water used in preparation of juice [12,13]. Jaranwala road is posh area as compared to Sargodha road area of Faisalabad city because of this hygienic measures were usually maintained to some extent due to this (TPC) was competitively low in this region.

Recent studies reported that fresh fruit juices with the presence of coliform were also associated with other harmful bacteria like *Enterobacter*, *E. coli* and *Klebsiella* which are responsible for severe infections [14]. The presence of coliforms in a sample indicates the existence of harmful, disease-causing microorganisms (Pathogens). Samples of sugar cane juice analysed for total coliform bacteria showed variation between < 26 to > 90 MPN/mL (Table 2). The highest detected value for fecal coliform bacteria was 46 MPN/mL and lowest 12 MPN/mL in the area Jhang road and Jaranwala road respectively. *E. coli* is considered as a factor for the identification of poor sewerage system or when the water from sewerage got mixed with the drinking (usable water). All the samples of sugar cane juice examined to detect *E. coli* and samples collected from Jhang and Sargodha road area showed highly positive results (Table 2). Furthermore, *Salmonella* was absent in all analysed samples of sugar cane juice.

Yeast and mold may also be hazardous to human health due to their ability to produce toxic metabolites. Yeast and mold may cause allergic reactions. Some species of fungi may elicit infections. Sargodha Road showed highest count of yeast and mold as compared to other areas. As shown through different studies unhygien-

ically processing of juice may be the major cause of contamination [15,16]. Occurrence of pathogenic bacteria at high rates in juices is an alarming situation which can place the consumers at high risk of illness.

Microbial profile of ice samples

Sugar cane juice is generally served with added ice for the sake there was a need for the analysis of ice samples in this study. Studies from different countries have shown that harmful microorganisms can be present in ice and many of them can even survive in ice, although as ice melts in drinks microbes are able to survive there too [4]. In this study ice samples collected from different sugar cane juice vendors in Faisalabad city showed high bacterial load ranging from (2×10^3 - 3×10^5) CFU/mL as shown in the table 3. The maximum TPC (3×10^5) CFU/mL in the area of Sargodha road may be due to the poor hygienic practices or use of contaminated water source in manufacturing of ice. Satyana road area showed minimum TPC (2×10^3) CFU/mL.

Ice samples analysed for total coliform count showed maximum number of total coliform bacteria (110 MPN/100 mL) in Sargodha road area (Table 3). Some of the collected ice samples were confirmed with the presence of fecal coliform and also showed positive result for *E. coli* (Table 3). This indicates the unsanitary conditions, unhygienic practices during or after formation of ice and use of contaminated water source for the production of ice.

Conclusion

Result of this study reveals that unhygienic conditions and poor qualities of water are the main factors for the contamination of sugarcane juice. Food hygiene standards must be implemented in a proper way on permanent basis for street vendors. Immediate action should be taken by the respective authorities to prevent any type of outbreak. Furthermore, additional treatments must be introduced for street vendors to make sugar cane juice safer for public health.

Area	No of samples	TPC (Total Plate Count) (CFU/mL)	Total Coliforms (MPN/mL)	Fecal Coliform (MPN/mL)	<i>E. coli</i>		Yeast and mold (CFU/mL)
					+ve	%age	
Jhang Road	19	2×10^6	90	46	6	31%	4×10^6
Jaranwala Road	13	8×10^4	26	12	0	0%	5×10^3
Sargodha Road	18	3×10^7	90	21	8	44%	3×10^7
Satyana Road	15	4×10^5	60	24	4	26%	2×10^5

Table 2: Microbiological profile of sugarcane juice sold by street vendors in different areas of Faisalabad city.

*Note: CFU: Colony Forming Unit, MPN: Most Probable Number.

Area	No of Samples	Total Plate Count (CFU/mL)	Total Coliform Bacteria (MPN/100mL)	Fecal Coliform Bacteria (MPN/100mL)	E. coli	
					+ve	%age
Jhang Road	8	2×10^4	24	2.0	2	25%
Jaranwala Road	5	6×10^3	7.5	0.3	0	0
Sargodha Road	9	3×10^5	110	2.8	3	33%
Satyana Road	7	2×10^3	15	0.3	0	0

Table 3: Bacteriological profile of ice used in street vended sugarcane juice.

*Note: CFU: Colony Forming Unit; MPN: Most Probable Number.

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