

Bacteriological analysis of street-vended fruit juices available in Rishikesh, Uttarakhand

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ABSTRACT

Background: A descriptive study was carried out due to absence of knowledge of relation between hygiene status of vendor and extent of contamination while simultaneously adding to the pool of similar studies, which would in turn help government formulate an appropriate policy. **Methodology:** A sample size of 80 fruit juices were collected from 55 vendors and cultured using CLED agar; relevant biochemical tests were run and isolates confirmed using Chromogenic Agar CPS3Id. The CFU of isolates obtained from one vendor were averaged to obtain average CFU for the vendor. The vendors were interviewed and investigator's observations were also recorded. Both were used to formulate an ingenious hygiene score scale, using which vendor hygiene score (VHS) was calculated. VHS and average CFU for the vendor were statistically analyzed and Spearman's rho was calculated along with the scatter plot. **Results:** *Klebsiella* sp. (59.05%) and non-*Candida albicans* (14.28%) are the major bacterial and fungal contaminant respectively. The median VHS is -9, which is unsuitable for consumption according to HSS. Spearman's rho was -0.736, suggestive of a statistically significant negative nonlinear correlation between VHS and extent of microbial contamination in street-vended fruit juices. **Conclusion:** The plethora of microbial contamination of street-vended fruit juices is a lucrative dimension for research in fruit juice microbiology to improve general public health, reduce disease mortality and morbidity, reduce economic losses, and to protect the general public from the risk of bioterrorism by stringent surveillance in background of limited employment opportunities and constrained livelihoods of the vendors.

Keywords: Fruit juices, hygiene, public health

Introduction

India produces about 9 million tons of fruits every year. The total market potential for fruit juices includes both packed and freshly made fruit juices.^[1] Fruit juice is considered the most popular nonalcoholic beverage among all age groups due to its fresh flavor and nutritive value.^[2]

These juices provide a lot of growth factors—vitamins and minerals to the body, which has increased their consumption in the recent times.^[3] Juices are fat-free and contain naturally occurring phytonutrients contributing to better health, e. g.,

vitamin C in orange juice acts as an antioxidant photochemical and improves the blood lipid profiles in hypercholesteromic patients and play important role in detoxification.^[1,4,5] The major ingredients of juice are water, sugar, and fruit pulp.^[2] They are prepared by extracting the liquid pulp of mature fruits and finally mixing it with sugar, water, and ice. The final product is unfermented and unpasteurized for consumption.^[3]

Juice can be contaminated at any step of preparation. There are several factors that act as source of contamination like use of unhygienic water, flies, airborne dust, contaminated raw materials and equipments, improper handling, and unhygienic working environment.^[3,6-10] Such juices are the potential sources of microbes like *Escherichia coli* O157:H7, *Salmonella* species, *Shigella* sp, *Staphylococcus aureus*, yeast.^[6] and their to survive in acidified vegetable products and fruits is of concern because of previously documented outbreaks associated with fruit juices^[7,8]

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In pregnant females, the heavily infected fetus may lead to spontaneous abortion, still births, or sepsis in infants.^[2]

Numerous outbreaks of foodborne illness are reported associated with consumption of fruit juices in India and other places. Most fruits contain bacterial counts upto 1.0×10^5 CFU/cm² on their surface.^[9] *E. coli* O157:H7 outbreak was documented in Washington, DC in 1996 after drinking unpasteurized apple juice. *Listeria monocytogenes* is also identified as one of important pathogens of these foodborne illness. An outbreak associated with drinking unpasteurized orange juice in Florida, USA in 1995 and in Australia in 1999. A cholera epidemic occurred in Pune, India, related to street-vended sugarcane juice contaminated with *Vibrio cholera*.^[3] The present study aimed to establish the hygienic status of street-vended juices and their impact on street foods contamination with a view to assess their safety for human consumption and as possible sources of bacterial and fungal pathogens. Presence of pathogenic microorganisms in fruit juices is likely to be associated as a potential source of foodborne illness and may be outbreak.

In our setup we are less aware of food safety and hygiene. Fruit juices are taken or counted upon as a healthy drink but which becomes reverse with such hidden microorganism.

A precise and well-defined monitoring and surveillance system needs to be implemented on urgent grounds to address food safety.

Study could potentially contribute toward formulating safety guidelines that should be practiced by vendors and consumers to reduce burden of foodborne/gastrointestinal disorders. Hospitals may also have an idea of common pathogenic bacteria in street-vended fruit juices in this region.

The study could potentially also contribute toward data gathered along with similar studies carried out in various cities of India and help the Government to frame requisite policies regarding food hygiene for such commonly consumed food item.

Material and Methods

A descriptive study spanning two months (May-June 2017) was carried out in Rishikesh with sample size of 80 fruit juices; collected from vendors who gave consent and gave freshly prepared fruit juice. Vendors who gave previously prepared fruit juice were not included in the study. Approval from Ethics committee was obtained Date of Approval from Ethics Committee - 29 April 2017.

Collection of samples

During the study, 55 locations in the Rishikesh catering to different age groups and communities were chosen for collection of samples and 80 samples of fresh fruit juices were collected during May-June 2017. Five varieties of fruit juices namely: pomegranate, sugarcane, mix fruit, orange, bel were chosen based upon consumer demand.

All samples were collected in sterile containers and transported to laboratory for analysis within 1 hour of procurement.

Sample analysis and processing

For analysis, 1 ml of sample was diluted as a 1:10 dilution with 9 ml of buffered peptone water. From this diluted sample 0.5 ml was inoculated in Macconkey broth and incubated for 4-5 hours. The microbial growth observed as turbidity was subcultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37 degree Celsius for 24 hours. Enrichment for *Salmonella* and *Shigella* was done as per the standard protocol.

Identification was done by findings of gram-stained smears, culture characteristics on CLED, and battery of biochemical tests and rapid tests. Further identification was made by culture characteristics on CLED agar. Various biochemical tests were carried out for confirmation of bacterial pathogens as per the standard protocol. The isolates were then confirmed by culture on chromogenic CPS3 Id plate (Biomereux, France) and incubated at 37 degree Celsius for 24 hours.

The tentative isolates of *Salmonella* and *Shigella* were inoculated on *Salmonella* and *Shigella* agar (SSA) and confirmed. Species identification of *Candida* isolates was confirmed by Germ tube test as *Candida albicans* and non-candida albicans.^[10,11]

Along with fruit juice sample, information or data on place, hygienic status of vendor and their servants, their hygiene practices and hygienic condition of vending site were collected. All data were analyzed with the Statistical Package for Social Sciences 22(SPSS version 22) for Windows (SPSS Inc.; Chicago, IL, USA) software and MS Excel (Microsoft).

Using SPSS Version 22 a correlational analysis of the average CFU of the vendor and VHS was carried out. Since an assumption of a linear correlation between the variables under question was considered, Pearson's correlational coefficient and Spearman's rho was calculated for above two variables along with graphical analysis using a scatterplot.

Results

A total of 80 samples were analyzed; 71 samples were found contaminated and 105 microbial pathogens were isolated [Figure 1]. Among isolated organisms, *Klebsiella spp* (59.05%) was the most dominant followed by *E. coli* (15.23%), *Non-candida albicans* (14.28%), *Citrobacter spp* (4.76%), *P. aeruginosa* (1.9%). On the other hand of bacterial spectrum, *Staphylococcus aureus*, *Streptococcus spp*, and *Enterococcus spp* constituted the outliers of bacterial genera unimodal distribution with respective frequency of 0.95%. Two of the isolates (1.9%) gave microbial characteristics that cannot be identified as routinely reported bacteria from laboratory at the place of study. No *Salmonella* and *Shigella* isolates were observed.

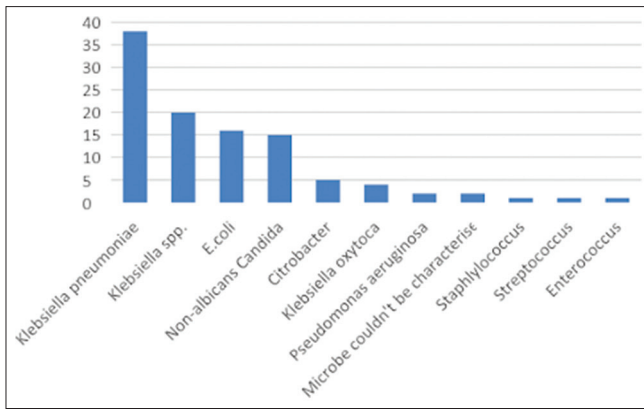


Figure 1: Graphical representation of number of microorganisms isolated

Hygiene analysis

There are no clear guidelines for unpasteurized fruit juices by Food Safety and Standard authorization of India (FSSAI) so there is need for ingeniously conceptualized Hygiene Score scale (HSS). This scale evaluated the hygiene status of vendors based on 20 parameters and allotted a score to every vendor, allowing for a quantitative analysis of vendor's hygiene score.

The three measures of central tendency for Vendor Hygiene Score (VHS) record -5.7 , -7 and -9 as mean, mode, and median respectively. They are interpreted as falling under categories of unsuitable for consumption and may be suitable for consumption respectively using HSS.

Correlational analysis

The Spearman's rho used as coefficient of correlation was calculated to be -0.736 . The magnitude of 0.736 corresponds to a strong correlation between these variables.

Scatter plot+

There is no perfect inverse relationship between the microbial contamination of street-vended fruit juices to the hygiene status of the vendor, cart/stall/shop and surroundings, technique of juice extraction as evaluated by the HSS [Figure 2].

Discussion

Fruit juices are very popular among the people of all ages around the world due to their high nutritive value and fresh flavor. Fruit juices have both positive and negative effects. They contain various organisms including many harmless yeasts and saprophytic bacteria. By detecting this bacterial load in the juice, it apparently gives an idea about the quality of the sample.^[12] The concerns over their safety and quality of fruit juices have been raised in spite of their potential benefits. Freshly squeezed fruits have little or no process steps that reduce pathogen levels, if contaminated.^[13]

In addition, lack of appreciation of basic safety issues by vendors augments the microbial loads. These include use of crude

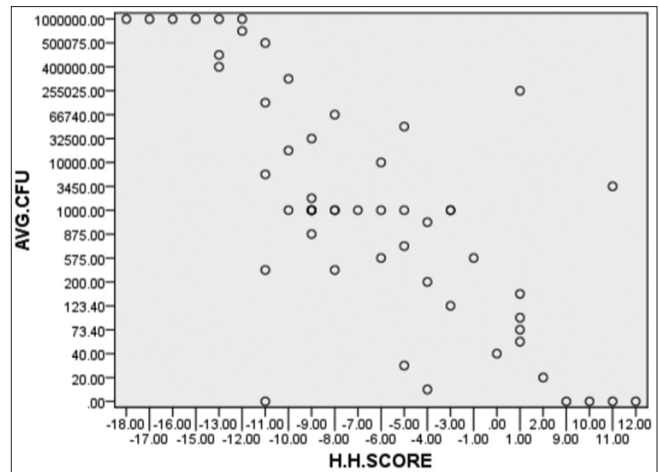


Figure 2: Scatter plot showing relationship between CFU and VHS

stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies, and airborne dust.^[14]

A number of studies from different countries have shown presence of *E. coli*, coliforms, and a variety of microorganisms like *S. pyogenes*, *Streptococcus equi*, *P. aeruginosa*, *Staphylococcus* spp, *Micrococcus* spp.^[3] This is an indication of unsanitary conditions, unhygienic practices during or after production, and poor quality of source of water used. The presence of yeasts is due to its preference for sugar and low pH, which help in its survival.^[6] The presence of *S. aureus*, the entry of the latter in juices, may be attributed to contact with the outer surface of fruits during juicings, survival, and growth of foodborne pathogens on surfaces of fruits and vegetables (on account of favorable pH) have been demonstrated and it indicate severe contamination through handling as reported by Sandeep et al.^[15]

In a study done in Netherlands, unpasteurized fruit juices were introduced as a risk factor for intestinal diseases.^[16] Another study about fruit juices microbial quality in street vendors of India showed juices contain high microbial load of coliforms, fecal coliforms, *S. aureus*, and *Vibrio*.^[3]

A study in North west of Iran showed that fruit juices were contaminated with various microorganisms.^[17]

All fruit juice samples were devoid of *Salmonella* and *Shigella*. Presence of *Klebsiella* spp in the juice is a potential source pool of *Klebsiella* infections, the gravity of the problem being compounded by the huge antibiotic resistance in it. Since pathogenic *E. coli* has been associated with outbreaks of gastroenteritis and diarrhea, the population of Rishikesh seems to be protected by the same unless exaggerated by an extrinsic factor.

To our knowledge, there was no specification set for the permissible level of microbes in fruit juices being served in India. However, the recommended specifications for fruit juices served in the Gulf region suggest that the maximum count

permitted for total colony count of coliforms, yeast, and molds are 1×10^4 , 100, and 1.0×10^3 CFU/ml, respectively (Gulf Standards, 2002).^[18] Presence of *S. aureus*, an enterotoxin producer, can cause serious health problems. *Pseudomonas aeruginosa*, another bacterium detected during the present study, is an important opportunistic pathogen and can cause food spoilage.^[19]

The HSS highlights the fact that overall hygiene maintained by the vendors provide a fertile platform for microbial entry into fruit juice and hence extent of contamination. There are many other factors contributing to the extent of fruit juice contamination.

The broad picture surfacing up is that street-vended fruit juices are significant and potential sources of various microorganisms well in or above their infective doses which may result in outbreaks, severe clinical infections in immunocompromised and immunosuppressed patients. *E. coli* O157:H7, *Salmonella*, and *Cryptosporidium* infections associated in recent years with the consumption of unpasteurized apple and orange juices have increased awareness of the presence of pathogens associated with fruits previously considered too acidic to serve as vehicles for pathogenic bacteria.^[20] So there is an urgent need for all the stakeholders to take preventive interventions at the earliest and formulate a dedicated policy to improve the microbiological safety profile of the street-vended fruit juices.

Conclusion

The fruit juices investigated in this study had higher microbial load than the specifications set for fruit juices in some parts of the world. It is clear that the colony counts of the microbial groups in our fruit juices exceeded the standard by considerable margin. These high counts, however, may pose hazard to the health of consumers especially if pathogenic species are present in the fruit juices to be consumed. Street vendors were mostly uninformed of good hygienic practices (GHP) and causes of diarrhea diseases which could increase the risk of street food contamination. They were also unaware of food regulations as well as lacking supportive services such as water supply of good and adequate quality, waste disposal systems which enhance their ability to provide safe food.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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