

Original Research Article

Bacteriological analysis of street vended fruit juices available in Vidarbha

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A B S T R A C T

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Traditionally, fruit products have been regarded as microbiologically safer than other unprocessed foods. However, many outbreaks of human infections have been associated with the consumption of contaminated fruit juices. The objective of this study was to evaluate the microbiological safety and quality of fruit juices being served in various cities of Vidarbha. A study aimed at examining the quality and safety of freshly squeezed fruit juices, in various cities of Vidarbha, based on standard techniques (e.g. culturing on selective media), showed that in most localities the street vended fruit juices remained hygienically poor since bacterial loads (Total viable counts and Total coliforms) on the whole are abnormally high. The samples were collected from various places and processed with in an hour in laboratory. The bacterial isolates were identified on the basis of their cultural, morphological & biochemical reactions. Total 115 samples of fruit juices sold by local vendors were analyzed and from which 98 organisms were isolated & identified on the basis of morphological, cultural & biochemical characteristics. In different fruit juice samples sold by local vendors four types of pathogenic bacteria were found. Based on the presence of Microorganism, it is concluded that fruit juices in certain areas inside the various city (e.g. Bus Stand, Railway Station, Vegetable market) are highly impacted and unfit for human consumption. The occurrence of pathogenic *E. coli*, *S.aureus*, *Salmonella* and *Shigella* is alarming enough for an immediate action by the suitable agency. It is suggested that regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

Introduction

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In many tropical countries they are common man's beverages and are sold at all public places and roadside shops. However in view of their ready consumption, quick methods of cleaning,

handling and extraction they could often prove to be a public health threat.

There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere (Health Canada, 2000; Parish,

1997; Sandeep *et al.*, 2001). Sources of contamination however vary. Most fruits contain bacterial counts up to 1.0×10^5 CFU/cm² on their surface (Splittstosser, 1979; Harrigan 1998). Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notably *E.coli* O157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* (Buchmann *et al.*, 1999; Ryu *et al.*, 1998; Uljas *et al.*, 1998; Sandeep *et al.*, 2001).

Although the infectious dose for these contaminating bacteria in fruit juices is not yet well established, based on the standards provided for drinking water (ICMR, 1975; ISI standards, 1973; WHO, 1984; Gray, 1994; USA, EPA, 1999), the numbers required to cause illness could be low particularly with reference to faecal coliforms and streptococci. In various cities of vidarbha such as Wardha, yavatmal, Amravati, Bhandara, Akola, Chandrapur and Gondia sample was collected. There is always a great demand for fresh fruit juices. Being tropical in location hot weather continues for a greater part of the year (February-July) increasing the need for these commodities. The consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices processed under hygienic condition could play important role in enhancing consumer's health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (2, 3). In absence of good manufacturing practice, however, the

nutritional richness of fruit juices makes the product good medium for microbial growth, vehicle of food borne pathogens and associated complications (1). Fruit juices contaminated at any point of processing could be the source of infectious pathogens. Study conducted on the microbiological safety of some fruit juices showed *Salmonella* in apple and orange juices. The prominent pathogens involved in un-pasteurized juice outbreaks have been identified as *E. coli*, *S. aureus*, *Shigella* and *Samonella* spp. The objective of this study was, therefore, to evaluate the physico-chemical quality and microbiological safety of some fruit juices being served in various cities of Vidarbha.

Materials and Methods

Sample Collection

Total 115 (105 locally made and 10 branded) fruit juice samples were collected from various places of Wardha, Yavatmal, Amravati, Bhandara, Akola, Chandrapur & Gondia. During the study, 5 locations in the city catering to different age groups and communities were chosen for collection of samples. These are: Area 1, Bus stand, 2, Railway station, 3, Vegetable Market, 4, Main Market and 5, Civil Hospital region. Samples of fresh fruit juices were picked up from at least 3 street fruit juice vendors in each zone where the sale was 50-100 each/day. 5 varieties of fruit juices namely orange, apple, mango, pineapple and lime were chosen based on the consumer demand. All samples were collected in sterile containers kept at 4⁰C and analyzed within an hour after procurement.

Sample analysis

For analysis 25 ml of the sample was

diluted as 1:10 with 250 ml of buffered peptone water and was filtered through sterile Whatmann No. 1 filter paper to remove the solid particles if any. 100 µl of filtrate was used for inoculation.

Enrichment for *Salmonella*

In case of *Salmonella*, fruit juice sample was enriched in the ratio of 1:10 in selective Tetrathionate broth by incubating at 37°C for 24 hrs (Ven kessel *et al.*, 2004).

Bacterial isolation and antimicrobial susceptibility testing

Fruit juice samples received at the microbiology Laboratory were plated on Mac-Conkey agar and incubated at 37°C for 24 hours. A significant bacterial count was taken as count equal to or in excess of 10⁵ per milliliter. Identification of pure isolates was done by observing morphological, cultural and biochemical characters according to Cheesbrough (2002,2004). The isolates were identified by Bergey's Manual for Determinative Bacteriology (Bunchanan and Gibbons, 1974).

Antibiotic sensitivity testing was performed using the Kirby–Bauer disc diffusion method. Determining sensitive and resistant bacteria to antibiotics by measuring the diameter of inhibition zone by mm and then compared with the standard diameters that installed in the standard scales.

The resistant pattern of each strain to the commonly used antibiotics was determined and the concentrations used to detect antibiotic sensitivity of the bacterial isolates include: Gentamicin (CN) (10µg), Amoxicillin (AMX) (30µg), Streptomycin (S) (30µg), Norfloxacin (NB) (30µg),

Chloramphenicol (CH) (20µg), Ciprofloxacin (CPX) (10µg), Amoxicillin/clavulanate (25µg), Pefloxacin (PEF) (10µg) and Tarivid (OFX) (30µg) using disc diffusion method according to (NCCLS, 2001).

Results and Discussion

Preliminary phyto chemical screening

Out of 115 samples, 105 locally made fruit juice samples showed the presence of pathogenic bacteria and the 10 fruit juice samples of reputed company were free from pathogenic organisms (Table1). The fruit juices were normally supplied at moderate temperature 10-15°C, pH varying between 3.8 and 7.6 depending on the type of the fruit. For consumption raw juices are normally diluted at 1: 0.1-1.0 ratio with water. For example orange and lime has 1:1 water, pineapple and apple 1: 0.5 & 0.3 and Mango 1:0.1. Consistency of the juices varied from totally pulpy nature (Mango) to watery condition (orange). A total of 115 (105 locally made and 10 branded) samples were examined. A summarized account of the results obtained for the microbiological analysis of locally made juices is given in Tables 2; Figure.1 Over all 93.31% (98/105) of the samples (locally made) examined showed pathogen contamination. Pathogenic *E.coli* was seen in 33.33%, *Shigella* in 17.14%, *S. aureus* in 23.80%, *Salmonella* in 19.04% of the samples. Observations also showed that pathogenic bacterial counts were significantly high in pineapple, orange, lime followed by mango.

Perhaps attributable to the quantity of water used for dilution. High pH, high ambient temperatures (> 28°C) appeared to favour the bacterial growth and reduce the shelf life of the juice.

Table.1 Organisms Isolated from Fruit Juice Sample available in Vidharba

Total Fruit Juice Sample	Contaminated		Non Contaminated	
	Local Brand	Company Brand	Local Brand	Company Brand
115	105	10	00	10

Table.2 Average Percentage of Microorganisms in Fruit Juices sold by Local Vendors

Isolated food poisoning Bacteria	No. of Isolates	Percentage
<i>E.coli</i>	35	33.33 %
<i>Shigella</i>	18	17.14 %
<i>S.aureus</i>	25	23.80%
<i>Salmonella</i>	20	19.04 %
Total	98	93.31 %

Figure.1 Average Percentage of Microorganisms in Fruit Juices sold by Local Vendors

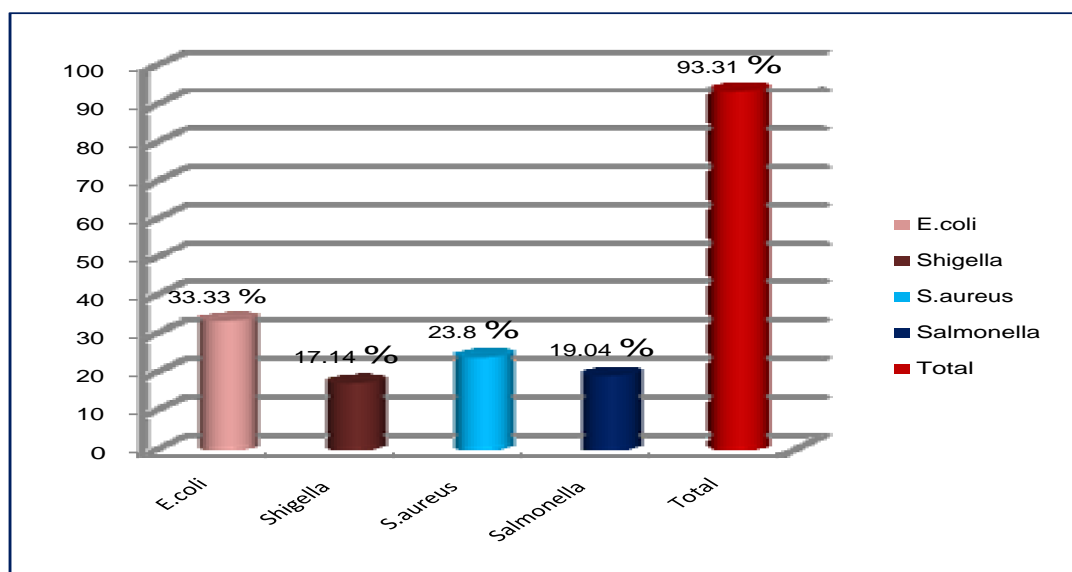


Table.3 Antibacterial Sensitivity pattern of Pathogenic Bacteria

No.(%) of Resistant of Strains				
Antimicrobial Agent	<i>E.coli</i>	<i>Shigella</i>	<i>S.aureus</i>	<i>Salmonella</i>
Septtrin	70	90	71.8	69.2
Chloramphenicol	30	00	28.2	92.3
Norfloxacin	10	00	25.6	30.8
Ciprofloxacin	00	00	30.8	00
Amoxicillin	70	70	55.1	69.2
Amoxicillin/Clavulanate	70	30	7.7	77
Gentamicin	60	00	11.5	84.6
Pefloxacin	70	20	00	7.7
Ofloxacin	20	20	16.7	23.1
Streptomycin	80	10	10.3	77

Area wise, juices obtained from localities 1,2 and 3 (Bus stand, Railway Station and vegetable market) showed high contamination. For comparison and as controls a parallel study was undertaken on canned and preserved fruit juices sold in the market. The results showed that there is no bacterial growth and the juices appeared clean and safe for human consumption. Over all the results of the study indicate that all street vended fresh fruit juices in many parts of the city showed contamination.

It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises. The location by the side of a busy road with heavy vehicular traffic (airborne particles) and over crowding seem to add to the contamination. Such locations should be avoided for establishing a street vended juice shop.

Lack of sanitary conditions in street vended juice shops and the occurrence of pathogenic *E. coli*, *Shigella*, *S. aureus* and *Salmonella* is alarming enough for an immediate action by the suitable agency. These pathogenic organisms have also shown to be highly resistant to antibiotics tested, indicating a possible cause of public health hazards (Table 3). Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

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