THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Microbial Contamination in Vended Street Fruit Juices in Allahabad City

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Abstract:
Fresh fruit juices are commonly consumed by people in India. Apart from their high nutritive value, if proper hygiene is not maintained they can be a major source of infection. A total of 40 samples were analyzed and dominant bacterial pathogens recorded were E.coli, Salmonella sp., L.casei, L.acidophilus. The contamination is mainly due to poor quality of water used for dilution, washing of utensils, contaminated ice, poor personal and domestic hygiene, peeling of fruits beforehand and shops in crowded places. The contamination will be more if the shop is located on a busy road with heavy traffic and dust. Food borne diseases mainly affect the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Improper washing of fruits add bacteria to extracts, leading to contamination. In addition, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings, often with swarming houseflies, fruit flies and airborne dust can act as sources of contamination. However health education of the vendors and implementation of standard hygienic practices may reduce contamination of fruit juices. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any further bacterial pathogen outbreaks. Our results demonstrate the non hygienic quality of three most popular types of street vended fruit juices and ice used for cooling of juices suggesting the urgent need for government participation in developing suitable intervention measures to improve microbial quality of juices.

Keywords: *Bacterial pathogens, gastrointestinal tract, hygienic practice*

1. 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. Although they are healthy and nutritious, the methods used for their extraction are not hygienic and the utensils are cleaned quickly and carelessly, so they pose a health risk due to contamination with various disease causing microorganisms (Lewis*et al*, 2004).

However, sources of contamination vary. One potential source of entry of microorganisms into fruits and fruit juices is by environmental exposure. Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink. Improper washing of fruits add bacteria to extracts leading to contamination (Buck*et al*, 2002). 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 act as sources of contamination.

Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent or limit the growth of microorganisms in juices. The most important are water activity (aw), low pH, hygiene practice and storage temperature and concentration of preservatives. The low pH of fruit juices greatly limits the number and types of bacteria that can survive or grow. Storage of products at refrigerated temperature or below is not always best for the maintenance of desirable quality of some fruits. Water used for juice preparation can be a major source of microbial contaminants.

In Allahabad city there is always a great demand for fresh juices as the climate remains hot and humid for most part of the year and street vendors serve a great portion of this demand, but their hygiene practices and microbial quality are questionable (Schaffner*et al*, 2005). In these shops, juices extracted by squeezing from a variety of fresh fruits namely apple, orange, pineapple, mosambi, guava etc. are served after considerable dilution with water and ice. In view of the high demand for fresh fruit juices during summer and overcrowding of street vended shops in many areas in the city a rapid review of the street vended fruit juices was undertaken during January to June 2014 with a view to assess their safety for human consumption and as possible sources of bacterial pathogens.

Despite periodic quality control checks and closure of shops, outbreaks of gastroenteritis caused by pathogenic *E.coli, Salmonella* and *Shigella*are not uncommon in these areas although a specific correlation has not been shown between outbreaks of gastroenteritis and consumption of these juices (Tambekar*et al*,2009). Pathogenic microorganisms can enter fruits through damaged surfaces that occur

during growing or harvest. But the main sources of contamination in fruit juices are untidy instruments and utensils, unhygienic water for dilution and unhygienic surroundings. There are various reports on the outbreaks of illness in humans associated with consumption of unpasteurized fresh fruit juices. 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 (Al-Jedah and Robinson, 2002).

Study conducted on the microbiological safety of some fruit juices showed *Salmonella* in apple and orange juices. The prominent pathogens involved in unpasteurized juice outbreaks have been identified as *E.coli, Salomonella, L.casei, L.acidophilus* species (Suneetha*et al*, 2011). The objective of this study was therefore, to evaluate the physio-chemical quality and microbiological safety of some fruit juices being served in various parts in Allahabad city.

2. Materials and Methods

2.1. Place of Work

The present study entitled "Microbial Contamination in Vended Street Fruit Juices in Allahabad City" was conducted in Department of Microbiology and Fermentation Technology, JSBB, SHIATS (Deemed University), Allahabad, during January to June 2014.

2.2. Study Sample

For the present study 40 samples of fruit juices (apple, pineapple, mosambi, orange and guava) were collected from different locations of Allahabad city.

2.3. Sample Collection

Samples of fruit juices were collected in sterile glass bottles from various different localities of Allahabad city. The collected samples were then taken to the laboratory for analysis. The samples were stored at 4^{o}C, if required.

2.4. Isolation of Microorganisms from Fruit Juices

For this 1 ml of fruit juice was mixed with 9 ml of Ringer's solution and serial dilution was made up to 10^{-5} dilutions. The samples were plated on Nutrient Agar using pour plate technique. The plates were incubated at 37^{o}C for 24-48 hours along with media control and were observed for colonies.

2.5. Identification

The identification of the isolates was done on the basis of cultural, morphological and biochemical tests as given in the Bergey's Manual of Systematic Bacteriology.

- Culture Examination

The colony characteristics (size, shape, colour, number of colonies) were observed from agar plates.

- Morphological Examination

The morphological identification of the isolates was done by the Gram's staining. For this a single colony was picked from plate and smear preparation was done on clear glass slide and there after Gram staining was done for morphological studies (shape, arrangement and Gram's reactions).

- Biochemical Examination

Following biochemical tests were performed for the identification of contaminants in fruit juices – Sugar fermentation, Catalase test and Citrate utilization test. Enteric pathogens were isolated on EMB agar.

3. Results and Discussion

The dominant bacteria in all the samples were *E.coli, Lactobacillus casei and L. acidophilus. Sallmonella sp.* was also present in many samples as indicated by growth on EMB agar. The results obtained for the microbiological analysis of the juices is given in Table 1 (showing taxonomical characterization of *Escherichia coli*), Table 2 (showing taxonomical characterization of *Lactobacillus casei*) and Table 3 (showing taxonomical characterization of *Lactobacillus acidophilus).*

S. No.	Characteristics	Observations
1	Cultural and Morphological Characteristics	
1.1	Colour of Colony	Yellow
1.2	Shape of Cells	Rod shaped
1.3	Gram's Reaction	Negative
1.4	Arrangement of cells	Chains
2	Biochemical Characteristics	
2.1	Glucose test	Positive $A^{+}G^{+}$
2.2	Oxidase test	Negative
2.3	Lactose test	Positive $A^{+}G^{-}$
2.4	Citrate test	Negative

*Table 1: Taxonomical Characterization ofE.coli * $A^{+}G^{+}$- Acid positive & Gas positive $A^{+}G^{-}$-Acid positive & Gas negative*

S. No.	Characteristics	Observations
1	Cultural and Morphological Characteristics	
1.1	Colour of Colony	White
1.2	Shape of Cells	Rod shaped
1.3	Gram's Reaction	Positive
1.4	Arrangement of cells	Bunches
2	Biochemical Characteristics	
2.1	Catalase test	Negative
2.2	Glucose test	$A^{+}G^{-}$
2.3	Mannitol test	Positive

Table 2: Taxonomical Characterization ofL.casei

S. No.	Characteristics	Observations
1	Cultural and Morphological Characteristics	
1.1	Colour of Colony	White
1.2	Shape of Cells	Rod shaped
1.3	Gram's Reaction	Positive
1.4	Arrangement of cells	Chains
2	Biochemical Characteristics	
2.1	Catalase test	Negative
2.2	Glucose test	$A^{+}G^{-}$
2.3	Mannitol test	Negative

Table 3: Taxonomical Characterization of L.acidophilus

Types	Location	Total Sample Collected	Sample no.	Type of Microbes Isolated
Apple	Mahewa	3	A1	*E.coli, Salmonella sp., L.casei*
			A2	*E.coli, L.casei*
			A3	*Salmonella sp., L.casei*
Apple	SHIATS canteen	1	A4	*Salmonella sp., L.casei*
Apple	Rambagh	2	A5	*E.coli, L.casei*
			A6	*L.casei*
Apple	Mutthiganj	2	A7	*L.casei*
			A8	*L.casei*
Pineapple	Mahewa	3	P1	*E.coli*
			P2	*E.coli*
			P3	*E.coli, Salmonella sp.*
Pineapple	SHIATS canteen	2	P4	*E.coli, Salmonella sp.*
			P5	*E.coli*
Pineapple	Rambagh	2	P6	*E.coli*
			P7	*E.coli, Salmonella sp.*
Pineapple	Mutthiganj	1	P8	*E.coli, Salmonella sp.*
Guava	Mahewa	2	G1	*E.coli*
			G2	*E.coli, Salmonella sp.*
Guava	SHIATS canteen	0	-	-
Guava	Rambagh	3	G3	*E.coli*
			G4	*E.coli*
			G5	*E.coli, Salmonella sp.*
Guava	Mutthiganj	3	G6	*E.coli*
			G7	*E.coli*
			G8	*E.coli, Salmonella sp.*
Orange	Mahewa	3	O1	*L.acidophilus*
			O2	*L.acidophilus, E.coli*
			O3	*L.acidophilus, E.coli*
Orange	SHIATS canteen	2	O4	*L.acidophilus, Salmonella sp., E.coli*

			O5	*L.acidophilus, E.coli*
Orange	Rambagh	2	O6	*L.acidophilus*
			O7	*L.acidophilus, E.coli*
Orange	Mutthiganj	1	O8	*L.acidophilus, E.coli*
Mosambi	Mahewa	2	M1	*E.coli, Salmonella sp.*
			M2	*E.coli*
Mosambi	SHIATS canteen	1	M3	*E.coli*
Mosambi	Rambagh	3	M4	*E.coli, Salmonella sp.*
			M5	*E.coli, Salmonella sp.*
			M6	*E.coli, Salmonella sp.*
Mosambi	Mutthiganj	2	M7	*E.coli*
			M8	*E.coli*

Table 4: Microorganisms Isolated from various Fruit Juices from different localities of Allahabad

In Table 4 it is shown that the dominant microorganisms which were isolated were found to be *Escherichia coli, Salmonella sp., Lactobacillus casei* and *Lactobacillus acidophilus*. These are the microorganisms which are present as contaminants in the vended fruit juices from various localities of Allahabad city. *E.coli* though occurs as a member of the normal intestinal flora some strains of *E.coli* and *Salmonella sp.* are pathogenic and can cause gastroenteritis showing diarrhea and dysentery like symptoms. As *E.coli* is present as the normal intestinal flora so in order to identify the causative agents of an enteric disease, the first step must be the separation of intestinal pathogen from the other gram negative bacilli which comprise the normal flora of the intestine, EMB agar medium is differential for final identification of pathogens.

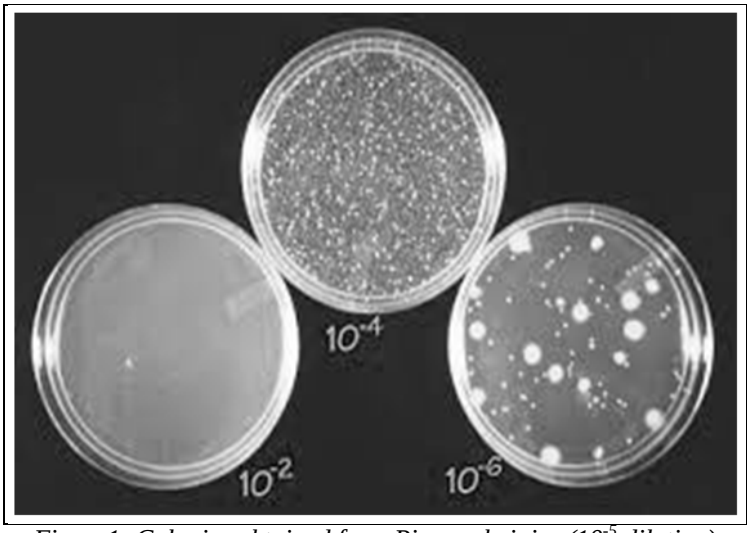

Figure1: Colonies obtained from Pineapple juice (10^{-5} dilution)

The colonies obtained in the pineapple plate at 10^{-5} dilution represents the presence of *E.coli* this shows that after fermentation the growth of *E.coli* was observed and analyzed.

Figure 2: Colonies obtained from Apple juice

Serial Dilution of apple juice was performed and colonies of *L.casei* were obtained on agar plates at 10^{-2}, 10^{-4} and 10^{-6} dilution.

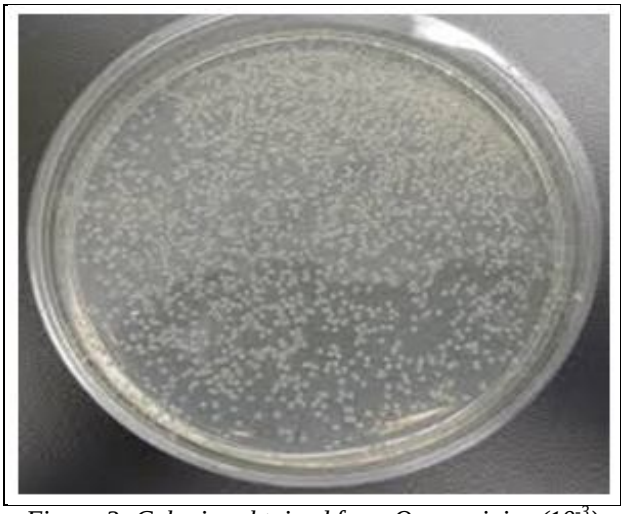

Figure 3: Colonies obtained from Orange juice (10^{-3})

In the present study serial dilution of Orange juice was performed and colonies of *L.acidophilus* were obtained at 10^{-3} dilution.

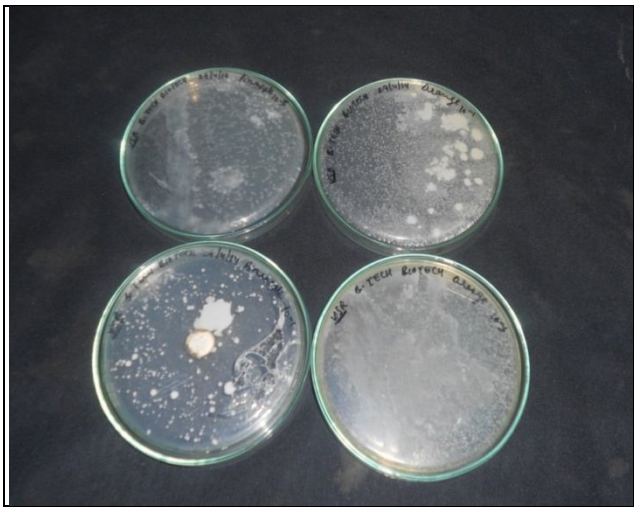

Figure 4: Colonies obtained from Orange and Pineapple Juice

In the present study different colonies were observed in pineapple and orange juices after incubation of 24 hours at 37^{o} C.

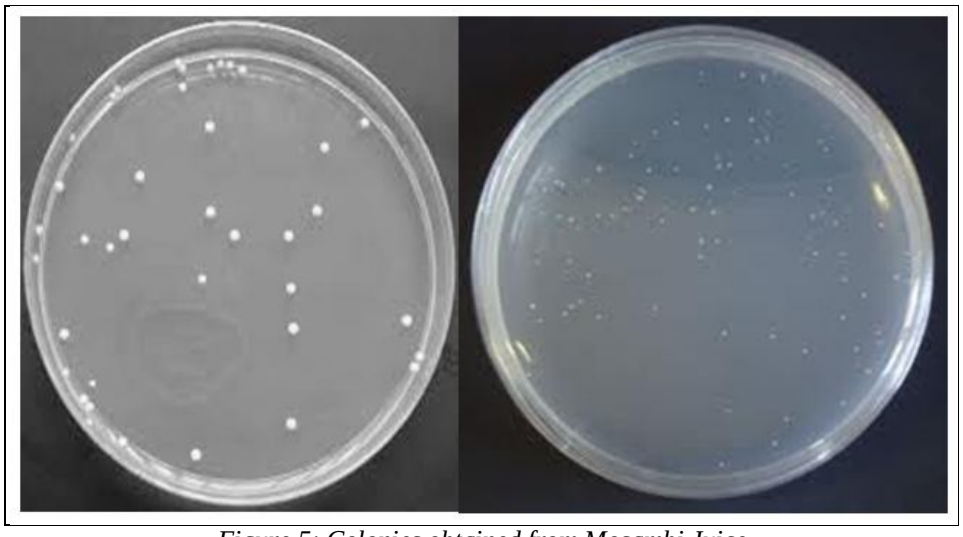

Figure 5: Colonies obtained from Mosambi Juice

Types	Sample Code	*E. coli (CFU/ml)*	*Salmonella sp. (CFU/ml)*	*L. casei (CFU/ml)*	*L. acidophilus (CFU/ml)*
Apple	A1	$25x10^5$	Present	$5.0x10^5$	-
	A2	$30\ x10^5$	-	$2.3x10^5$	-
	A3	-	Present	$3.4x10^5$	-
	A4	-	Present	$5.6x10^5$	-
	A5	$28x10^5$	-	$4.9x10^5$	-
	A6	-	-	$3.4x10^5$	-
	A7	-	-	$5.2x10^5$	-
	A8	-	-	$3.7x10^5$	-
Pineapple	P1	$33x10^5$	-	-	-
	P2	$42x10^5$	-	-	-
	P3	$29x10^5$	Present	-	-
	P4	$46x10^5$	Present	-	-
	P5	$38x10^5$	-	-	-
	P6	$44x10^5$	-	-	-
	P7	$39x10^5$	Present	-	-
	P8	$32x10^5$	Present	-	-
Guava	G1	$14x10^5$	-	-	-
	G2	$16x10^5$	Present	-	-
	G3	$17x10^5$	-	-	-
	G4	$19x10^5$	-	-	-
	G5	$14x10^5$	Present	-	-
	G6	$12x10^5$	-	-	-
	G7	$15x10^5$	-	-	-
	G8	$13x10^5$	Present	-	-
Orange	O1	-	-	-	$1.5x10^5$
	O2	$23x10^5$	-	-	$2.7x10^5$
	O3	$28x10^5$	-	-	$3.0x10^5$
	O4	$21x10^5$	Present	-	$2.8x10^5$
	O5	$27x10^5$	-	-	$7.0x10^5$
	O6	-	-	-	$4.3x10^5$
	O7	$26x10^5$	-	-	$2.5x10^5$
	O8	$23x10^5$	-	-	$3.6x10^5$
Mosambi	M1	$29x10^5$	Present	-	-
	M2	$35x10^5$	-	-	-
	M3	$39x10^5$	-	-	-
	M4	$43x10^5$	Present	-	-
	M5	$27x10^5$	Present	-	-
	M6	$51x10^5$	Present	-	-
	M7	$45x10^5$	-	-	-
	M8	$49x10^5$	-	-	-

Table 5: Total Viable Count of Microorganisms Isolated from various Fruit Juices

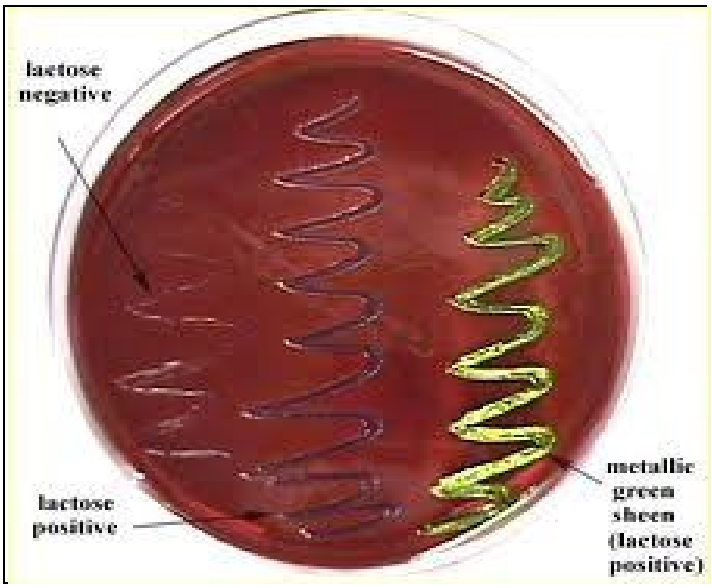

Figure 6: EMB plate-E.coli colonies with a green metallic sheen and Salmonella sp. colonies are uncoloured and transparent.

In Table 5 it is shown that the total viable count of microorganisms which were isolated from various street fruit juices ranges between $1x10^5$ CFU/ml -$50x10^5$ CFU/ml. In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised as freshly prepared juices have no process or steps to minimize the microorganisms if they are contaminated (Mahale*et al.*, 2008). Pineapple juice shows maximum *E.coli* contamination and presence of *Salmonella sp*. followed by apple and mosambijuice in which the microbial count was lesser than pineapple juice. (Al-Jedah and Robinson, 2002) had reported on a number of factors responsible for contamination of fruit juices. Prior to processing, most fruit contains bacterial counts of 1 X 10^5CFU/ml on their surface. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads (Mahale*et al.*, 2008). These include unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis *et al.*, 2006).
In Figure 6 it is shown that the *E.coli* which ferment lactose and form coloured colonies and *Salmonella* do not ferment lactose and produce colourless and transparent colonies.

4. Summary and Conclusion

A total of 40 samples were analyzed and dominant bacterial pathogens recorded were *E.coli, Salmonella sp., L.casei, L.acidophilus.* From this overall study it may be concluded that all street vended fruit juices in many parts of the city were contaminated by various types of microorganisms. Again it may be concluded that consumption of fresh fruit juices cannot be stopped on unhygienic grounds and not the street vendors prohibited from selling such items. Regular monitoring and precaution should be taken by Government agencies to maintain the quality of fruit juices for human consumption.

5. Acknowledgements

The authors are thankful to Head, Dept. of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad for providing necessary facilities to conduct this experiment.

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