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How Far These Road Side Vended Fresh Fruit Juices are Safe for Consumption ?

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ABSTRACT

Background: Fruits or the extracted fresh juices are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits. Until recently, roadside vended unpasteurized fruit juices have been considered non-hazardous with respect to microbiological pathogens due to their acidic nature. However, in light of the many Indian and global outbreaks of food borne associated illnesses with these products, it is apparent that certain bacterial, viral and parasitic pathogens can survive these acidic conditions and remain infectious. The most likely mechanisms by which fresh fruit juice, and the fruit it is processed from becomes contaminated with pathogenic microorganisms are through direct contact with animal or human faeces, or indirect contact with contaminated water, ice, soil, processing equipment, infected food handlers or through the containers they serve.

Objective: The present idea of this study is to assess the microbiological quality of the road side vended unpasteurized fresh fruit juices and estimate their safety for consumption.

Method: A cross-sectional study was conducted to evaluate the microbiological quality of locally prepared fresh fruit juices by choosing 12 crowded fresh fruit juice shops on the road side in the city for collection of samples. Ten varieties of fruit juices were included namely orange, grape, mango, pineapple, pomegranate, watermelon, musk melon, sapota, banana and papaya. These samples were collected aseptically and processed for possible contamination by bacteria or fungi.

Results: A total of 120 locally prepared fresh fruit juice samples were analysed, from which 162(78.02%) bacterial strains and 43(20.98%) fungal strains were isolated. All most all the samples were contaminated by one or more than one microorganism. Among the bacterial isolates, Esch. coli was the major isolate with 25.37% and among fungal, Aspergillus Spp. (6.83%). Papaya and sapota were highly contaminated while grape and promegranate were the least contaminated. Various parameters were observed at the juice centres for possible contamination of these fresh extracted fruit juices.

Conclusion: It is an alarming situation and local health authorities should take some concrete action, make guidelines to prevent potential food poisoning from juices that contain pathogenic bacteria. This is high time that government should frame a licensing system under the supervision of local health authorities to check the possible contamination of these fresh fruit juices vended by road side/street vendors.

KEYWORDS : : Bacterial, Fresh fruit juices, Fungal, Roadside vended, Unpasteurised .

INTRODUCTION

In majority of tropical countries fresh fruit juices are common man's beverages and well recognized for their flavour, taste, refreshing feeling, urgent thirst relief, nutritive value, mineral and vitamin content. Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits or their juices.[1] These beverages are available in any corner of all public places and roadside shops as there is no need of any licence from the health authorities to run these shops. However in view of their quick methods of extraction , preparation , handling and ready consumption, they could often prove to be a public health threat as the knowledge of the safety of drinking these juices is very little for the public. There are reports of food borne illness associated with the consumption of fruit juices at several places in India and other parts of the world.[2,3,4,5,6,7] Sources of contamination however vary. Most fruits contain bacterial counts up to 1.0×10^5 CFU/cm^2 on their surface.[8,9] Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with contaminated ice , prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens with a Total Viable Counts (TVC) of (0.8-33.6×10^4 CFUs/100 ml) in all the samples. Total coliform counts varied between 0.8-22.2×10^4 CFUs/100 ml, faecal coliforms between 0.4-11.0x10^4, faecal *Streptococci* between 0.0-6.6x10^4 and *Vibrio* between 0.8- 9.4×10^4 CFUs/100 ml. Over all 66.6% (72/108) of the samples examined were contaminated[6] where as Lakshmi Reddi et al[7] showed the contamination 96.6% (145/150 samples). Hence, outbreaks of food borne gastroenteritis is common in these areas by consuming these fruit juices contaminated with these pathogenic bacteria. Frequent consumption of fruits and fruit juice causes dental decay, and may be a more significant factor in the development of dental caries (cavities) than eating candy. Fruit juice causes dental decay because it naturally contains acids, which chemically dissolve the enamel off the surface of the tooth, and sugars that the bacteria

in the mouth ferment to create even more tooth-destroying acids.[10] Among yeasts Pichia, Candida, Saccharomyces, and Rhodotorula are commonly encountered genera responsible for spoilage of juice.[11] Certain common moulds such as Penicillium Spp., Aspergillus Spp., Eurotium, Alternaria, Cladosporium, Paecilomyces,and Botrytis have also been reported in spoilage of fruit juices.[11,12,13] Fruit juices have pH in the acidic range (<4.5) serving as important barrier for microbial growth. However, food borne pathogens such as Esch. coli and Salmonella survive in acidic environment of fruit juices due to acid stress response. A peculiar and rare transmission of Chaga's disease (American trypanosomiasis by *Trypanosoma cruzi)* was reported by Nobrega AA et al[14] by consumption of of Acaí Palm Fruit beverage in Brazil. Pereira KS[15] et al also reported similar findings in Caracas, Venezuela. Indeed, fruit juice intake has been consistently associated with reduced risk of many cancer types[16,17,18,19] might be protective against stroke[20] and delay the onset of Alzheimer's disease.[21] Oxidative stress is relieved by fresh fruit juices, there by helps against neurodegenerative diseases including Lou Gehrig's disease (aka MND or ALS), Parkinson's disease, Alzheimer's disease,Huntington's disease, Depression and Multiple sclerosis.[22] In view of the high demand for fresh fruit juices during summer (February- July) and over crowding of road side vended shops in many areas in the city and to identify the microbiological quality of street vended fruit juices , this present study was undertaken during April and May 2015 with a view to assess their possible sources of bacterial and fungal contamination and the safety for human consumption.

MERTERIALS AND METHODS

A cross-sectional study was conducted to evaluate the microbiological quality of locally prepared fresh fruit juices by choosing 12 crowded fresh fruit juice shops on the road side in the city for collection of samples. Samples of 100 ml fresh fruit juices were picked up from each shop where the sale was 100-150 glasses per day. Ten varieties of fruit juices were included namely orange, grape, mango, pineapple, pomegranate, watermelon, musk melon, sapota, banana and papaya. The pH of all samples were measured using digital pH meter (Global Instruments, Mumbai, India.) All samples were collected in sterile containers kept in refrigerator at 4°C and analyzed within an hour after procurement. The samples were analysed in the department of Microbiology by taking 25 ml of each sample and diluted as 1:10 with 250 ml of buffered peptone water and was filtered through sterile Whatmann No. 1 filter paper to remove the pulp. An amount of 100 µl of filtrate was used for inoculation and isolation of bacteria were made using the growth in regular/selective media such as MacConkey agar , EMB agar, TCBS agar, SS agar, Mannitol Salt Agar and an enriched media like Blood agar. Sabourauds dextrose agar (SDA) was also inoculated for possible fungal isolates. All the media were obtained from Himedia Laboratories, Mumbai, India and protocols followed were as per the guidelines given by the National Institute of Nutrition, Hyderabad, India (personal communication). Gram staining, motility and certain biochemical tests were performed using conventional methods to confirm the bacterial identification whenever necessary using standard laboratory techniques.[6,23] Fungal identification was done using colonial morphology on SDA and wet mount examination with lactophenol cotton blue stain.[24] Further, the antibiotic susceptibility pattern of all isolated bacterial strains was determined by Kirby-Bauer disc diffusion method on Muller-Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials.[25,26] The panel of antibiotics used were amikacin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, lomefloxacin, penicillin and sparfloxacin obtained from Himedia, Mumbai, India. Methicillin resistance was tested for all S.aureus isolates using cefoxitin (30 µg) disc.[27] Different parameters as mentioned in table- 1 were observed at every vending shop during the preparation of the juices as a customer for the possible way of contamination of microbial agents and analysed.

RESULTS:

The fresh fruit juices were normally supplied at moderate.temperature 10-15°C, pH varying between 3.8 and 7.4 diluted with water and ice depending on the type of the fruit. For consumption, raw juices are normally diluted at 1: 0.1-1.0 ratio with water. Consistency of the juices varied from totally pulpy nature (Mango, Sapota and Banana) to watery condition (Orange, Grape and Water melon). In the present study, a total of 120 samples obtained from 12 road side vended shops were examined for possible contamination by various microbial agents. The pH of the fresh fruit juices were estimated by using a digital pH meter (Globe Instruments, Mumbai, India) immediately after inoculation of the sample. A summarized account of various parameters were shown in Table-1 for a possible way of contamination of these juices. The percentage of bacterial and fungal contamination of fresh fruit juices was shown in Table -2. A total of 205 microbial agents isolated from 10 types of fruit juices from 12 vendors. Hence 205 microbes isolated from 120 samples giving an isolation rate of 58.54%. Among them, 162(79.02) were bacterial strains and 43(20.98) were fungal. *Esch. coli* formed the major isolate with 25.37% followed by *Staphylococcus aureus* (11.71%). *Aspergillus Spp.* (6.83%) formed the major isolate followed by *Penicillium Spp*. (05.85%) among fungal isolates. Papaya, sapota contaminated with more number of microbes and grape, pomegranate with least number. (Table-3). Antimicrobial sensitivity pattern of bacterial isolates was shown in Table-4. All isolates were showing multi drug resistance becoming resistant to more than 6 antimicrobials. In the present study, among 24 strains of *Staphylococcus aureus,* 13(54.17%) strains were Methicillin Resistant *Staphylococcus aureus* (MRSA)

Table-1: Parameters observed at the site during preparation of fresh fruit juices by Roadside Vendors

S.No.	Parameter	Number	Percent
1	Hand Washing before preparing juice	8	66.67
2	Washing of utensils	12	100.00
3	Type of glasses used (Disposable)	8	66.67
4	Wiping utensils after washing	12	100.00
5	Washing of fruits	6	50.00
6	Storage of cut fruits: (Closed Vessel)	2	16.67
7	Kind of water used for dilution of fruit juices (Mineral Water)	2	16.67
8	Usage of Ice	12	100.00
9	Methods of Breaking of Ice using Rubber tube.	10	100.00
10	Vessels to be closed with lids	5	41.67
11	Covered waste bin at vending unit	0	0.00
12	Vendor's clothes (Dirty)	7	58,33
13	Long finger nails	8	66.67
14	Handles juice with bare hands (Without disposable gloves)	10	83.33
15	Handles money while serving	12	100.00
16	Smoking:	2	16.67
17	Pan/Gutka/Nut powder chewing	3	25.00
18	Scalp scratching	7	58.33
19	Wiping hands with his dress/Towel frequently	8	66.67
20	Any anti-fly measures	0	0.00

Table-2: Bacteria/Fungi isolated from different fresh fruit juices

S.No.	Bacterium/Fungus Isolated	Number Isolated	Percent
GRAM POSITIVE			
1.	Staphylococcus aureus	24	11.71
2.	Coagulase Negative Staphylococci (CoNS)	13	06.34
4.	Aerobic Spore Bearers (ASB)	13	06.34
3.	Enterococcus faecalis	07	03.41
5.	Micrococci	10	04.88
GRAM NEGATIVE			
1	Esch.coli	52	25.37
2	Klebsiella spp.	23	11.22
3.	Pseudomonas aeruginosa	09	04.39
4.	Proteus Spp.	11	05.37
	FUNGI		
1.	Yeasts/Candida Spp.	6	02.93
2.	Aspergillus Spp.	14	06.83
3.	Penicillium Spp.	12	05.85
4.	Mucor Spp.	07	03.41
5.	Rhizopus Spp.	04	01.95

Table 3: Bacterial/Fungal isolates from different fresh fruit juices

Fruit Juice	pH	GRAM POSITIVE (n=66) (40.74%)					GRAM NEGATIVE (n=96)) (59.26%)				FUNGI (n=43)				
		S.aureus	CoNS	ASB	E.faecalis	Micrococci	Esch.coli	Kleb. Spp.	P.aeruginosa	Proteus Spp.	Yeast / Candida Spp.	Aspergillus Spp.	Penicillium Spp.	Mucor Spp.	Rhizopus Spp.
Banana (*Musa acuminate)*	4.6-5.4	4(1.95)	1(0.49)	2(0.98)	2(0.98)	3(1.46)	6(2.93)	2(0.98)	2(0.98)	3(1.46)	-	2(0.98)	-	2(0.98)	-
Grape (*Vitis vinifera)*	4.6-6.9	-	2(0.98)	-	-	2(0.98)	3(1.46)	4(1.95)	-	2(0.98)	-	2(0.98)	-	-	-
Mango (*Mangifera indica)*	4.6-5.8	6(2.93)	3(1.46)	2(0.98)	1(0.49)	1(0.49)	6(2.93)	3(1.46)	1(0.49)	1(0.49)	2(0.98)	2(0.98)	1(0.49)	-	-
Muskmelon (*Cucumis melo)*	5.8-6.4	-	1(0.49)	1(0.49)	-	2(0.98)	3(1.46)	5(2.44)	-	1(0.49)	1(0.49)	1(0.49)	1(0.49)	-	1(0.49)
Orange (*Citrus sinensis)*	3.6-4.4	-	-	4(1.95)	-	-	4(1.95)	1(0.49)	-	-	1(0.49)	1(0.49)	1(0.49)	2(0.98)	-
Papaya(*Carica papaya)*	5.3-6.2	4(1.95)	1(0.49)	2(0.98)	3(1.46)	1(0.49)	8(3.90)	6(2.93)	3(1.46)	2(0.98)	-	4(1.95)	6(2.93)	1(0.49)	1(0.49)
Pineapple (*Ananas comosus)*	5.1-6.8	-	1(0.49)	1(0.49)	-	-	6(2.93)	-	-	-	1(0.49)	-	1(0.49)	1(0.49)	1(0.49)
Pomegranate (*Punica granatum)*	6.6-7.4	4(1.95)	1(0.49)	-	-	-	7(3.41)	-	1(0.49)	-	-	-	1(0.49)	1(0.49)	-
Sapota(*Manilkara zapota)*	4.8-6.8	6(2.93)	3(1.46)	1(0.49)	1(0.49)	1(0.49)	9(4.93)	2(0.98)	2(0.98)	2(0.98)	1(0.49)	2(0.98)	1(0.49)	-	1(0.49)
TOTAL		24 (11.71)	13 (6.34)	13 (6.34)	07 (3.41)	10 (4.88)	52 (25.37)	23(11.22)	9(4.93)	11(5.37)	6(2.93)	14(6.82)	12(5.85)	7(3.41)	4(1.95)

Table-4: Antimicrobial sensitivity pattern of bacterial isolates from different fresh fruit juices

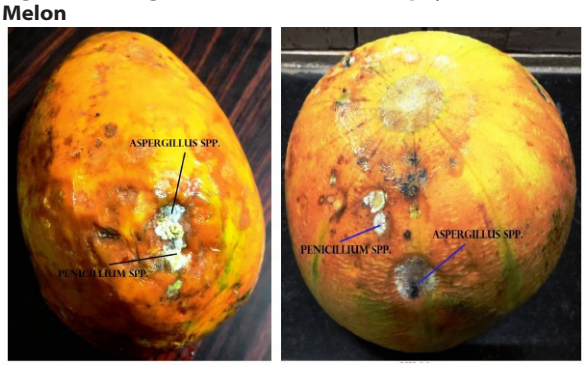
S.No.	Antimicrobial	Disc Content	GRAM POSITIVE (n=66)					GRAM NEGATIVE (n=96)			
			S.aureus (n=24)	CoNS (n=13)	ASB (n=13)	E.faecalis (n=07)	Micrococci (n=10)	Esch.coli (n=52)	Kleb. Spp. (n=23)	P.aeruginosa (n=09)	Proteus Spp. (n=11)
1	Amikacin	30 µg	16(66.67)	10(76.92)	10(76.92)	03(42.86)	07(70.00)	36(69.23)	16(6957)	04(44.44)	06(54.55)
2	Ampicillin	10 µg	2(08.33)	04(30.77)	04(30.78)	0(00.00)	0(00.00)	06(11.54)	06(26.09)	0(00.00)	0(00.00)
3	Cefotaxime	30 µg	10(41.67)	07(53.34)	06(46.15)	02(28.57)	03(30.00)	32(61.54)	08(34.78)	02(22.22)	02(18.18)
4	Ceftazidime	30 µg	12(50.00)	06(46.15)	05(38.46)	01(14.24)	02(20.00)	26(50.00)	12(52.17)	03(33.33)	03(27.27)
5	Ceftriaxone	30 µg	14(58.33)	08(61.53)	07(53.84)	02(28.57)	04(40.00)	33(63.46)	14(66.87)	04(44.44)	04(36.36)
6	Ciprofloxacin	5 µg	10(41.67)	03(23.08)	02(15.38)	01(14.24)	02(20.00)	12(23.08)	06(26.09)	01(11.11)	02(18.18)
7	Gentamicin	10 µg	17(70.83)	10(84.62)	07(53.84)	04(57.14)	06(60.00)	38(73.08)	17(73.91)	05(55.55)	07(63.64)
8	Lomefloxacin	10 µg	12(50.00)	06(46.15)	08(61.54)	02(28.57)	03(30.00)	20(38.46)	10(43.49)	02(22.22)	03(27.27)
9	Penicillin	10U	0 (00.00)	0(00.00)	04(30.77)	0(00.00)	0(00.00)	0(00.00)	0(00.00)	0(00.00)	0(00.00)
10	Sparfloxacin	5 µg	13(54.17)	03(23.08)	06(46.15)	03(42.86)	04(40.00)	21(40.38)	12(52.17)	02(22.22)	04(36.36)

DISCUSSION

In spite of the potential benefits offered by fresh fruit juices like their flavour, taste, refreshing feeling, urgent thirst relief, nutritive value, mineral and vitamin content fruit juices, concerns over their safety and quality have been raised. Freshly squeezed fruit juices have little or no process steps that reduce pathogen levels if contaminated. In the present study, various parameters responsible for contamination of fresh fruit juices of various fruits were shown in Table-1. These fresh fruit juices which are on high demand, vendors tend to peel and keep them in an uncovered container much before time of consumption and such fruit juices generally attract house flies because of their sweet odour. So, these swarming house flies can act as source of contamination.[5,7,28] Improper washing of fruits adds these bacteria to juices leading to contamination. In addition lack of appreciation of basic safety issues and ignorant of good hygienic practices by road side vendors contribute to these microbial loads. These include mainly unavailability of running water or mineral water for dilution of crude fresh fruit juices and washing of hands , utensils are usually done in one or two containers and sometimes without soap. Adding to this, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust also contributes to the microbial load.[7,29] Similar findings were observed in our present study (Table-1). A number of studies from India and abroad have shown that microbial quality of ice added to fresh fruit juices for its taste, refereshness, urgent thirst relief could be a cause of concern. The microbial safety of commercial ice used in drinks was evaluated by Agbaje Lateef *et al* in Nigeria[29] and found that microbial loads of these ice samples ranged from 1.88-3.20 X 10^4 cfu/ml which was largely above the recommended loads of more than 500 and 1000 cfu/ml for ice obtained from manufacturing plants and retail outlets respectively. In majority of coastal areas where fish were exported in huge amounts use ice. The unused remaining ice from these areas will be sold to the vendors for a little money. Hence these highly contaminated ice blocks may be a source of microbial contamination.[29,30] Added to this, the big ice blocks will be made into small pieces using a big rubber tube by tying both ends and beaten by a wooden stick by keeping over the dust on road side and poured into in the thermocol box by opening one end of the tube directly where the dust on the outer walls of the tube also falls. All most all samples were contaminated by one or more microbial agent in our present study. Papaya, sapota were contaminated with more number of microbes (13 out of 14 microbes 92.86%) and grape and pomegranate with least number (6 out of 14 microbes 42.86%). Papaya and sapota juices were highly contaminated because it is maximally consumed and already peeled out quite before the juice preparation inviting contamination from so many sources as notified in Table-1. In all the juices *Esch.coli* (25.37%) and *S.aureus*(11.71%) were the predominant organisms followed by *Klebsiella spp.(* 11.22%), *Aspergillus Spp.* (6.83%), Aerobic Spore Bearers (ASB) and Penicillium Spp.(5.85%). (Table 2). Least contamination in grape and pomegranate juices was recorded as these fruits were peeled on time of juice preparation. Similar findings were observed by other workers regarding the predominance of *Esch.coli* followed by *S.aureus*.[6,7,30,31] *Salmonella Spp.,Shigella Spp.* and *Vibrio cholerae* were not isolated in the present study where as Joy E Lewis et al [6] isolated all the three organisms. Tambekar et al.,[31,] Ankur Titarmare et al.,[32] and Bello Olorunjuwon et al[33] isolated only *Salmonella Spp.,* where as Lakshmi Reddi et al[7] isolated only *Shigella Spp. Vibrio cholera* was iso-

lated by Durgesh et al[29] and Mosupye et al.[34] A number of studies from different countries including India have shown the presence of *ESch.coli* coliforms and a variety of microorganisms like *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, *Micrococcus spp. Streptococcus faecalis, Proteus Spp.* along with *Vibrio cholera, Salmonalla Spp.* and *Shigella Spp.* indicating unsanitary conditions, unhygienic practices during or after production and poor quality of source of water used. If the source water is of poor quality used for preparation of ice, harmful microorganisms may persist in ice since the process of freezing cannot destroy them.[30,35,36,37] When ice is thawed the surviving microorganisms though may be injured, tend to recover their viability so that when the ice melts into the juices, they may be able to survive these too (Food and Environmental Hygiene Department (FEHD)2005).[38]. A total of 43 fungal strains yielding yeasts/Candida Spp. and 4 Spp. of moulds in the present study (Fig.1)

Figure 1: Fungal contamination of Papaya and Musk Melon



PAPAYA MUSK MELON

Similar findings were observed by Bello et al., [33] and Kamal Rai et al.[39] Spoilage by moulds in fruit juices is characterized by loss of juice cloud.[40] Among these, some moulds produce mycotoxins which are of great threat to human health. Major mycotoxins associated with fruit juices are byssochlamic acid , patulin , ochratoxin, and citrinin. [41,42] Antimicrobial sensitivity of all bacteriological isolates was done (Table-4) and it was evident that all isolates were multidrug resistant showing resistance to more than 6 drugs. Similar findings were observed by many workers[43,44,45,46,47] *Staphylococcus aureus* is an entero toxin producer can cause food poisoning .The presence of *S. aureus* may be explained by the fact that human beings that are processors or vendors carry this organism on/in several parts of their bodies as normal flora and responsible for contamination.[43] Among 24 strains of *Staphylococcus aureus* 13(54.17%) strains were Methicillin Resistant *Staphylococcus aureus* (MRSA) in our study. Many of these MRSA isolates are becoming multidrug resistant and superbugs and are susceptible only to glycopeptide antibiotics such as vancomycin.[48]

CONCLUSION

Fresh fruit juices contain microorganisms which are potentially hazardous to public health. The presence of Esch. coli, Coliforms and *Staphylococcus aureus like* pathogenic microorganisms in juices is a clear indication leading to food borne diseases. Juices were spoiled with high level of moulds and yeasts which is attributable to low pH of juices may be responsible for health hazards as certain mycotoxins are produced by certain moulds. The selling and consumption of fresh fruit juices cannot be stopped on unhygienic grounds, and road side vendors cannot also be prohibited from selling these items, since it is a source of their livelihood and a source of health and nutrition for the consumers. It is an alarming situation and local health authorities should take some concrete action by making guidelines to prevent potential food poisoning from juices that contain pathogenic bacteria. This is high time that government should frame a licensing system under the supervision of local health authorities and create awareness to check the possible contamination of these fresh fruit juices vended by road side/street vendors.

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