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Human Bacteria in Street Vended Fruit Juices: A Case Study of Visakhapatnam City, India

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Contamination of ready-to-eat foods and beverages sold by street vendors and hawkers rendering them unacceptable for human consumption has become a global health problem. A study aimed at examining the quality and safety of freshly squeezed fruit juices, in a metropolitan city (Visakhapatnam) in south India, 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 (HVC 0.88-33.6$\times10^4$ CFUs/100 ml; TC 0.8-22.2$\times10^4$ CFUs/100 ml). Based on the presence of faecal coliforms (0.4-11.0 CFUs/100 ml) and faecal streptococci (0.0-6.6 CFUs/100 ml), it is concluded that fruit juices in certain areas inside the city (e.g. R.T.C. Complex, Fishermen's colony, Vegetable market) are highly impacted and unfit for human consumption. Overall, it is contended that contamination is mainly due to poor quality of water used for dilution, prevailing unhygienic conditions related to washing of utensils, maintenance of the premises, and location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and over crowding. The occurrence of pathogenic *E. coli*, *Streptococcus faecalis*, *Salmonella typhi* and *Salmonella typhimurium* 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.

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/cm^2 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 Visakhapatnam (Lat. 17°41'34"N; Long. 83°17'45") (Andhra Pradesh, India) there is always a great demand for fresh vegetable and fruit juices. Being tropical in location hot weather continues for a greater part of the year (February-September) increasing the need for these commodities. While most restaurants and cafes serve juices in apparently hygienic conditions, in the roadside shops and recreational areas (beaches, parks) and busy market places (shopping malls, bus stations etc.) their microbiological quality remains questionable. In these shops, juices extracted by squeezing from a variety of fresh fruits namely oranges, grape, pomegranate, apple, pineapple, watermelon, papaya, carrot etc. are served after considerable dilution with water and ice. Despite periodic quality control checks

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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. In view of the high demand for fresh fruit juices during summer and over crowding of street vended shops in many areas in the city a rapid review of the street vended fruit juices was undertaken during June–September 2004 with a view to assess their safety for human consumption and as possible sources of bacterial pathogens. The program was carried out by the students of the Alcorn State University as a part of the Minority International Research and Training (MIRT) sponsored by NIH, USA in summer 2004.

MERTERIALS AND METHODS

Collection of Samples. 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, R.T.C. Complex (commuter dominated commercial region), 2, M.V.P.Colony (student dominated regions), 3, R.K.Beach (recreational area), 4, Jalaripet (fishermen colony) and 5, Market region (the vegetable market place). Samples of fresh fruit juices were picked up from at least 3 shops in each zone where the sale was 50-100 each/day. 5 varieties of fruit juices namely orange, grape, mango, pineapple and pomegranate were chosen based on the consumer demand. All samples were collected in sterile containers kept at 4^{o}C 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.

Bacterial enumeration and confirmation. Isolation and enumeration of bacteria were made using the growth in selective media such as nutrient agar for Total Viable Counts (TVC), Sorbitol MaConkey agar (United States Pharmacopoeia, 1980; M298, Himedia, Mumbai) for pathogenic *E. coli 157:H7*, and P-A coliform (APHA, 1998-9222 A, B, C) for total coliforms (TC) and *E. coli* like organisms, Thiosulphate Citrate Bile Sucrose agar (TCBS agar) (Kobayashi, et al*.,* 1963; M189, Himedia, Mumbai) for total vibrios (TV), *Vibrio cholerae* like organisms (VCLO) and *Vibrio parahaemolyticus* like organisms (VPLO), M-faecal coliform agar (MFC agar) (Geldrich et al., 1974; M0311, Himedia, Mumbai) for faecal coliforms, Xylose Lysine Deoxycholate agar (XLD agar; M1108, Himedia, Mumbai), *Salmonella-Shigella*-agar (S-S agar M108, Himedia, Mumbai) for *Salmonella*, M. *Enterococcus* Agar (MEA agar) (Burkwall and Hartman, 1964; M1108, Himedia, Mumbai) for *Streptococcus faecalis*. All the selective media were obtained from Himedia Laboratories Limited, A-406 Bhavaeswar plaza; LBS Marg, Mumbai, India and protocols followed were as per the guidelines given by the National Institute of Nutrition, Hyderabad, India (personal communication). Since this is a one time study, 3-6 samples were collected from each location and surface plates were made in triplicates in appropriate selective media. Bacterial isolation was by both spread plate method and serial dilution technique. For bacterial enumeration spread plates were used to determine number of Colony Forming Units (CFU). For computation, average number per plate is divided by sample volume and is expressed as CFU/100 ml/37^{o}C-44.5^{o}C/48 hours (APHA, 1998). In all the cases, counts were made up to 96 h. Since the bacterial populations tend to be highly skewed, geometric mean (Mean), standard deviation (SD) and confidence levels (conf. levels) were calculated for all the samples using standard formulae. For confirmation of the pathogens, typical colonies were inoculated into Rapid Microbial Limit Test kits which are a combination media in liquid and solid phase in a single bottle for simultaneous enrichment, isolation and confirmation of pathogens. These MLT kits are recommended in diagnostic microbiology for accurate identification of pathogen and are supplied by Himedia Laboratories Limited, Mumbai, India. Biochemical tests were performed using conventional methods to confirm pathogen identification whenever necessary.

RESULTS

The fruit juices were normally supplied at moderate temperature 10-15^{o}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 has 1:1 water, pineapple and grape 1: 0.5 & 0.3 and Mango 1:0.1 Pomegranate is diluted with milk (1:0.5). Consistency of the juices varied from totally pulpy nature (Mango) to watery condition (orange). A total of 108 samples were examined. A summarized account of the results obtained for the microbiological analysis of the juices is given in Tables 1 and 2. Total Viable Counts (TVC) were high (0.8-33.6$\times 10^4$ CFUs/100 ml) in all the samples. Total coliform counts varied between 0.8-22.2$\times 10^4$ CFUs/100 ml, faecal coliforms between 0.4-11.0, *faecal streptococci* between 0.0-6.6 and *Vibrio* between 0.8-9.4$\times 10^4$ CFUs/100 ml. (Table1). Over all 66.6% (72/108) of the samples examined showed pathogen contamination. Pathogenic *E. coli* was seen in 27.7%, *Shigella* in 16.6%, *Salmonella* in 38.8% and *S. faecalis* in 6.2% of the samples. *S. typhi* and S. *typhimurium* were encountered in 3 samples one each of grape, mango and orange. *V. cholerae* was not encountered in any of the samples. Observations also

Table 1. Pathogenic bacteria (×10^{4}CFUs/100ml) range and mean in fresh fruit juices sold in street vended shops in Visakhapatnam city, India

Type of Juice	TVC	Total Coliforms	Pathogenic *E. coli*	*Salmonella*	*Shigella*	*Vibrio*	FS	FC	pH
Grape									
Range	11.8-	9.6-10.8	2.8-3.6	3.8-4.8	2.4-3.2	0.8-1.8	(5.8-	5.6-	4.5-
Mean	17.8	10.1	3.2	4.2	3.0	1.0	6.6)	7.2	6.8
SD	14.5	0.43	3.03	0.41	0.48	0.41	6.4	6.24	
Conf. limit	2.52	0.13	0.91	0.12	0.14	0.12	1.65	0.32	
	0.69						0.49	0.09	
Pine apple									
Range	18.8-	11.4 -22.2	8.8-10.6	6.6-8.2	1.2-2.6	1.6-9.4	5.4-	10.4-	5.2-
Mean	33.6	17.4	10.2	7.8	1.8	4.7	6.2	11.0	6.6
SD	25.4	4.12	0.77	0.81	0.71	3.15	5.6	18.7	
Conf. limit	6.29	1.24	0.23	0.24	0.21	0.95	5.06	0.32	
	2.11						1.53	0.09	
Mango									
Range	6.6-	5.6-9.8	1.2-3.2	1.4-2.2	0.4-1.2	0.8-1.4	1.0-	6.4-	4.5-
Mean	10.6	7.15	2.2	1.7	0.9	1.2	3.2	8.2	5.6
SD	8.6	1.27	0.73	0.30	0.32	0.32	2.0	7.0	
Conf. limit	1.67	0.23	0.22	0.09	0.09	0.10	0.86	0.84	
	0.49						0.26	0.25	
Orange									
Range	16.8-	8.6-10.4	2.4-4.2	6.0-7.4	2.6-3.2	1.0-2.4	1.6-	5.0-	3.8-
Mean	24.4	9.48	3.9	6.4	2.9	1.8	2.0	6.6	4.6
SD	20.9	0.58	0.84	1.52	0.22	0.54	1.8	6.0	
Conf. limit	3.1	0.11	0.25	0.46	0.07	0.16	0.67	0.22	
	1.03						0.20	0.07	
Pomegranate									
Range	0.8-	0.8-4.2	0.6-2.2	1.0-2.0	0.4-1.2	1.4-2.0	3.6-	7.2-	6.6-
Mean	4.8	2.4	1.35	1.8	0.6	1.7	6.8	8.4	7.6
SD	2.47	1.09	0.62	0.38	0.32	0.22	4.8	9.6	
Conf. limit	1.84	0.21	0.11	0.11	0.09	0.07	0.92	1.38	
	0.52						0.27	0.42	

Table 2. Faecal coliforms (FC) and faecal streptococci (FS) (×10^4 CFUs/100ml) in fresh fruit juices sold through street vended shops in different areas in Visakhapatnam

Area	Grape			Pine apple			Mango			Orange			Pomegranate		
	TVC[1]	FC[1]	FS[1]	TVC	FC	FS	TVC	FC	FS	TVC	FC	FS	TVC	FC	FS
1 (R.T.C. Complex)	12.8	5.6	5.2	33.6	8.2	5.6	9.0	5.8	1.0	18.6	5.5	1.8	3.06	1.02	0.8
2 (M.V.P. Colony)	11.8	4.28	3.08	-	-	-	6.6	4.4	0.6	22.1	6.0	1.8	1.2	0.82	0.16
3 (R.K.Beach)	13.6	3.01	2.06	18.8	6.6	3.4	8.2	6.4	0.5	16.8	5.0	1.6	0.8	0.4	0.0
4 (Jalaripet)	17.8	7.6	4.2	26.4	11.0	4.8	10.6	8.2	3.2	22.5	6.4	2.0	-	-	-
5 (Market place)	16.4	10.4	6.6	22.8	10.4	6.2	-	-	-	24.4	6.6	1.89	4.8	1.3	1.0
Mean	14.5	6.18	4.23	25.4	9.05	5.0	8.6	6.2	1.3	20.9	5.9	1.82	2.47	0.89	0.49
SD	2.52	2.91	1.77	6.29	2.03	1.2	1.67	1.6	1.3	3.1	0.7	0.15	1.84	0.38	0.48

[1]TVC: Total Viable Counts; FC: Faecal Coliforms; FS: faecal streptococci.

showed that pathogenic bacterial counts were significantly high in pineapple, orange and grape followed by mango. Perhaps attributable to the quantity of water used for dilution. Pomegranate showed least contamination. This is evidently due to the use of milk for dilution of the raw juice. High pH, high ambient temperatures (> 28^{o}C) appeared to favour the bacterial growth and reduce the shelf life of the juice. Area wise, juices obtained from localities 1, 4 and 5 (R.T.C Complex, Fishermen's colony, vegetable market) catering to the needs of fishermen, vegetable vendors and commuters from the nearby villages showed high faecal coliform and streptococcal counts indicative of faecal contamination. It is contended that the unhygienic location of the shops namely heavy vehicular traffic near bus station (Area 1), by the side of sewage collection point in Jalaripet (Area 4) and decomposing vegetable waste in the vicinity (Area 5) are responsible for the 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 with faecal coliforms and faecal streptococci. 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) (R.T.C. Complex) or by the side of the waste disposal system (Fisherman's colony) 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* O157:H7 *&*, *Shigella* and *S. typhimurium* is alarming enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

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