ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(5): 1099-1105
www.allresearchjournal.com
Received: 22-03-2016
Accepted: 23-04-2016

Christo J Padamadan
M.Sc Student, Department of Microbiology, Hindusthan College of Arts and Sciences, Coimbatore, Tamil Nadu, India.

Stefi Raju V
M.Sc Student, Department of Microbiology, Hindusthan College of Arts and Sciences, Coimbatore, Tamilnadu, India.

N Hema Shenpagam
Associate Professor, Department of Microbiology, Hindusthan College of Arts and Sciences, Coimbatore, Tamil Nadu, India.

Correspondence
Christo J Padamadan
M.Sc Student, Department of Microbiology,
Hindusthan College of Arts and Sciences, Coimbatore, Tamil Nadu, India.

Isolation and identification of microorganism from street vented fruit juices and packed juice and its antibiogram

Christo J Padamadan, Stefi Raju V and N Hema Shenpagam

Abstract
The Present study was to isolate the coliforms from street vented and packed fruit juices from different area of Thrissur city. Four mentor fruit juices and four packed fruit juices were examined for the presence of coliforms. Coliforms were detected in two samples Apple juice and pineapple juice with MPN index of 140+and 140+ which were further detected as *E coli, Klebsiella* spp, *Enterobacter* spp, *Enterococci* spp. The highest pH was recorded in apple juice (street vented: 4.86, packed fruit juice: 3.10) and the lowest pH in grape juice (street vented: 2.55, packed fruit juice: 1.45). The highest titratable acidity was recorded in grape juice (street vented: 13.5%, packed fruit juice: 16.5%) and the lowest pH in apple juice (street vented: 4.02%, packed fruit juice: 10.85%). Drug resistance among the isolated was found against Amikacin, Ampicillin, Cefixime, and Cefotaxime. Overall study demonstrates that the quality of fresh juices was unsatisfactory compared to packed juices. Hence the products need to be microbiologically controlled in order to ensure the overall health safety.

Keywords: Fruit juices, health risk, coliform, antibiotics

Introduction
Fruit juices are well recognized for their nutritive value, mineral, and vitamin contents. In many tropical countries, they are common man's beverages and are sold at all public places and roadside shops (Joy Lewis *et al*., 2006) [7]. Juices have shown to be potential sources of bacterial pathogens notably E.coli 0157:H7, species of Salmonella, Shigella and Staphylococcus aureus (Sandeep *et al*., 2001).

There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere (Sandeep *et al*., 2001). Several factor can act as source of contamination such as use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and air borne dust. Also pathogenic organisms can enter fruits and vegetables through damaged surfaces such as punctures, wounds, cuts and splits that occur during growing or harvesting. Not only fresh fruit juices, packed fruit juices also contain pathogenic organisms from report (Chen *et al*., 2010) [4]. Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated food or drink.

Presence of Coliforms on the surface of fruits is indicative of faecal contamination (Reddy *et al*., 2000) [11]. Improperly prepared fresh fruit and vegetable juices are recognized as an emerging cause of food borne illness (Sandeep *et al*., 2004) [13]. The normal habitat of faecal coliforms is the intestinal tracts of man and animals and they are not known to be found in nature in the absence of faecal contamination from the sources. Some often are pathogenic and cause diseases like typhoid, dysentery and enteric fever etc. Thus the presence of organism in water and fruit juices is dangerous for human consumption (Salle, 2000) [12]. Water used for juices preparation can be a major source of microbial contaminants including Coliforms, faecal coliforms, faecal Streptococci, etc. (Tasnim *et al*., 2010) [14]. Changes in pH may also promotes the growth of pathogens (FDA, 2001) [6].

The Present study was conducted to compare the presence of coliform in packed fruit juices and street vented fruit juices. The antibiotic sensitivity patterns of the isolated coliforms to any common antibiotics were also performed.

Materials and methods

Sample Collection

The fresh squeezed juices as well as water sample (used in preparation of juices) were collected aseptically from four different public areas of Thrissur district, placed them in sterile containers, stored at 4^0C then transferred to the laboratory for sample analysis. Packed fruit juices were collected from different retail outlets.

Method

1. Determination of pH (Erkmen *et al*., 2004) [5]

pH was measured using digital pH meter after homogenizing 10ml fruit juices in 90ml distilled water.

2. Determination of Titratable Acidity

3 Drops of phenolphthalein was added to the to the juice/water solution in each beaker. 0.1M solution of NaOH was poured into the burette using a funnel until it reaches the zero mark. Slowly titrated the Na OH into the juice/water solution. The point of neutrality was reached when the indicator changes from colour less to pink.

Calculation of acid in the given fruit sample

Factor for: Citric acid : 0.0064 (Citrus fruit)
Malic acid : 0.0067 (Apples)
Tartaric acid: 0.0075 (Grapes)

Result expressed as percentage acid:

$$\text{Percentage acid} = \frac{\text{Titre} \times \text{acid factor} \times 100}{10 \text{ (ml juice)}}$$

3. MPN of Food and water (Aneja, 2002) [1].

The MPN test is a combination of presumptive, confirmed and complete tests. It is used to detect and estimate coliform population in a food /or juice sample. This method employs the use of Lactose fermenting broth for presumptive test. Eosin Methylene Blue [EMB] agar plate for confirmed and complete tests.

0.1 ml of (10-3, 10-4, and 10-5) each dilution of each samples was inoculated into three test tubes of Lactose fermentation broth with Durham's tube. After incubation period of 24 hours the number of tubes in each set of three that show positive for acid and gas production was counted. A count of number of tubes showing positive results for complete test were noted, this count gives the MPN ratio which when checked against standard probability table gives us the MPN for the sample. Calculation of MPN was done from the complete test results table ratio of the number of tubes positive in each of the three dilutions gives the MPN ratio. The same MPN ratio was checked against standardized MPN probability tables to obtain the Most Probable Number of coliforms for the sample.

A modified procedure was used to calculate the MPN of water. In this test, we used double strength (ds) and single strength (ss) Lactose fermenting broth for the presumptive and EMB agar for the confirmed and complete tests. Serial dilution of the water sample was not made. Instead, we directly used 10 ml, 1 ml and 0.1 ml to the media tubes (Aneja, 2002) [1].

4. Isolation and identification (Aneja, 2002) [1].

All the positive tubes (obtained in MPN food and water tests) were sub-cultured on to Eosin Methylene Blue [EMB] agar, MacConkey agar, Nutrient agar and incubated at 37ºC for 24 hours. Isolated colonies were identified by Grams staining, motility, cultural and biochemical tests according to Bergey's manual.

Antibiogram test

The isolated bacteria were checked for the antibiotic sensitivity to standard antibiotic by the Kirby-Bauer disc diffusion method. The diameter of zone of inhibition were measured and tabulated.

Table 1: Standard Chart of Antibiotics

Antibiotic	Zone Of Diameter		
	Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Amikacin	17	15-16	14
Ampicillin	17	14-16	13
Cefixime	19	16-18	15
Cefotaxime	23	15-22	14

Result and Discussion

Fruit juices are widely consumed by millions of people. Street vented fruit juices are well appreciated by consumers, because of their taste, low price, and availability at right time (Ohiokpehai, 2003) [10]. However, street foods are frequently associated with diarrhoeal diseases due to their improper handling and serving practices (Bello *et al*., 2013) [3]

The present study was carried to isolate and identify the coliforms in street vended fruit juices, packed fruit juices and water samples used in preparing fruit juice. A total of four street vended fruit juice, water samples and four packed fruit juice samples were analyzed. Street vented fruit juice and water sample were found contaminated. In street vented apple juice coliforms such as E coli, Klebsiella spp, Enterococci spp was isolated, from pineapple juice E coli, Klebsiella spp and Enterobacter spp was isolated. From the water sample E coli and Klebsiella spp was isolated. Whereas in packed fruit juices and street vented grape juice and orange juice none of the organisms were isolated (Table: 1). Improper washing of fruits adds these bacteria to juices leading to contamination. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and air borne dust (Lewis *et al*., 2006) [9]

Table 1: Organisms isolated from fruit juices and water samples

Fruit Juices	Street vented fruit juices	Packed fruit juices
Apple juice	*E coil, Klebsiella* spp, *Enterococci* spp	None
Grape juice	None	None
Orange juice	None	None
Pineapple juice	*E coli, Klebsiella* spp, *Enterobacter* spp	None
Water sample	*E coli, Klebsiella* spp	-

Determination of PH

The PH of the packed and street vented fruit juices were carried out using PH meter. From the PH of fruit juices investigated highest acidity was shown in grape juice followed by pineapple juice, orange juice and apple juice. (Table: 3, Fig: 1) Grape juice had lowest microbial load and

maximum load was observed in Apple juice in this study. This could be attributed to the very low PH observed in grape juice and highest PH in Apple juice. Where as in packed fruit juices the pH was low which resulted in low microbial load. The PH of fruit juices is usually too low with good potentials of inhibiting the growth of pathogenic bacteria (Uzeh *et al*., 2007).

Table 2: PH of different street vented and packed fruit juices

Fruit juices	P^H of street vended juice	P^H of packed fruit juice
Apple juice	4.86	3.10
Grape juice	2. 55	1.45
Orange juice	4.65	2.32
Pineapple juice	3.75	2.00

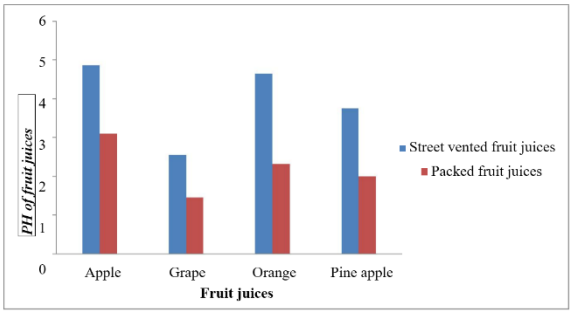

Fig 1: PH of Street vented and packed fruit juices

Determination of Titratable Acidity (Ta)

The total acidity of fruit juices is due to the mixture of organic acids, whose composition varies depending on fruit nature and maturity. The main acids encountered in fruits are tartaric, malic, citric, succinic, lactic and acetic acids. Total titrable acidity varied significantly in different types of fruit juices. Maximum content of total titrable acidity was recorded in Grape juice followed by pineapple juice and orange juice while it was minimum in apple juice (Table: 4, Fig:2)

Table 3: Titrable acidity recorded in street vented and packed fruit juice

Juices	TA of street vended fruit juices (%)	TA of packed fruit juices (%)
Apple juice	4.02	10.85
Grape juice	13.5	16.5
Orange juice	6.4	11.52
Pineapple juice	11.39	13.52

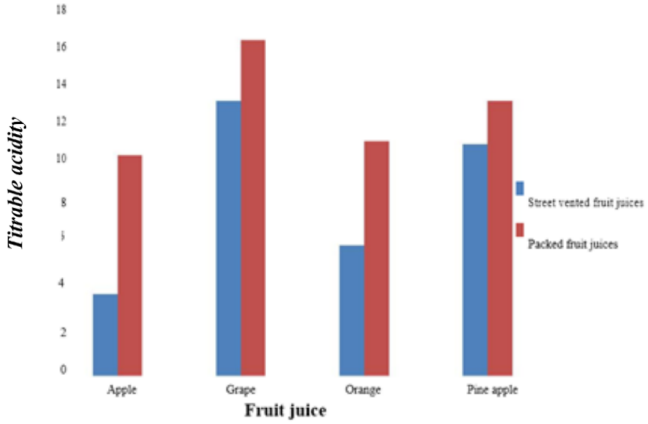

Fig 2: Titrable acidity of Street vented and packed fruit juices

MPN of Street Vended Fruit Juices

The presumptive test result of juice sample -1 showed that, all the tubes were positive for all the three dilutions (10-3, 10-4 and 10-5). This indicates that sample-1 was highly contaminated with a count of 140+ coliforms per 100 ml. In the case of sample -2, the presumptive test showed three tubes were positive in 10-3, 10-4 and 10-5 dilutions only two tubes were positive. This indicates that sample 2 is also contaminated with a count of 140+. In the case of sample -3 and sample -4 presumptive tests showed negative result in all the three dilutions (10-3, 10-4 and 10-5). This indicates that sample-3 and sample-4 was free from coliform contamination (Table 5).

Table 4: MPN presumptive test result of different street vented fruit juice samples

Samples	Incubation period (hrs.)	10^{-3}			10^{-4}			10^{-5}			MPN index
		1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	
Sample No-1 Pineapple juice	24	+	+	+	+	+	+	+	+	+	140+
Sample No-2 Apple juice	24	+	+	+	+	+	+	+	+	+	140+
Sample No-3 Orange juice	24	-	-	-	-	-	-	-	-	-	0
Sample No-4 Grape juice	24	-	-	-	-	-	-	-	-	-	0

MPN of Packed Fruit Juices

The presumptive test result of packed fruit juice sample-1, sample-2, sample-3 and sample-4 showed that, all the tubes were negative for all the three dilutions (10-3, 10-4 and 10-5). This indicates that sample-1, sample-2, sample-3 and sample-4 were free from coliform contamination (Table 6). Packed fruit juices are usually pasteurized to remove incidence of pathogenic microbes. Pasteurization of fruit juice, involve brief exposure to high temperature for example 2 to 5 minutes at 80ºC, which will destroy all vegetative cells leaving the much more heat resistant spores unaffected. Pasteurization is used to reduce bacterial count and therefore to prolong the life of the drink (Kisko and Roller, 2005) [8].

Table 5: MPN presumptive test of different packed fruit juice samples

Samples	Incubation period (hrs.)	10-3			10-4			10-5			MPN index
		1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	
Sample No-1 Pineapple juice	24	-	-	-	-	-	-	-	-	-	0
Sample No-2 Apple juice	24	-	-	-	-	-	-	-	-	-	0
Sample No-3 Orange juice	24	-	-	-	-	-	-	-	-	-	0
Sample No-4 Grape juice	24	-	-	-	-	-	-	-	-	-	0

MPN Water

The results obtained from presumptive test reports showed that all tubes of water sample were positive for all the three concentrations (ie, 10ml, 1ml and 0.1 ml). This indicates sample was contaminated with coliform with a coliform count of 38+ (Table 7).

Table 6: MPN presumptive test of water sample

Samples	Incubation period (hrs.)	Double strength medium with 10ml sample			Single strength medium with 1ml sample			Single strength medium with 0.1ml sample			MPN index
		1	2	3	1	2	3	1	2	3	
Sample No-1 water	24	-	+	+	-	+	+	-	+	+	38+

Most Probable Number [MPN] Of Water Samples

Plate 1: MPN of water sample

Isolation

Out of 8 fruit juices and water sample analyzed, 2 street vented fruit juice samples (Apple juice, pineapple juice) and water sample showed the presence of coliforms. Hence they were found to be unfavorable for consumption The presence of coliforms in fruit juice sample may be due to poor personal and domestic hygiene indicating lack of knowledge of hygienic practices and safety of food products (Bello *et al*., 2013) [3]. The presence of coliforms in fruit juice is not allowed by safe food consumption standard (Andres *et al*., 2004) [2]. The morphological and cultural characteristic of the isolated coliforms are shown in Table: 8 (Plate no: 2 -21)

Table 7: Morphological and cultural characteristics of Isolated Coliforms

Organism	Cultural Characteristics		Morphology	
	On MacConkey agar	On Nutrient agar	Gram Staining	Motility
E.coli	Lactose fermenting pink coloured colonies.	Round, creamy white, moist, smooth, opaque colonies.	Gram Negative	Motile
Klebsiella spp.	Large mucoid, lactose fermenting pink coloured colonies.	Large, opaque, moisturized, mucoid colonies.	Gram Negative	Non motile
Enterobacter spp	Lactose fermenting pink coloured colonies.	Small, opaque round colony,	Gram Negative	Motile
Enterococci spp	Lactose fermenting pink coloured colonies	Small, pinpointed, opaque colonies	Gram Positive	Non motile

The Bacterial Isolates from Street Vented Fruit Juices

Plate 2: *E. coli* on Nutrient agar MacConkey

Plate 3: *E. coli* on agar

Plate 4: *E. coli* on EMB plate

Plate 5: *Klebsiella* spp on mac Conkey agar

Plate 6: *Klebsiella* spp on Nutrient agar

Plate 7: *Enterococci* spp on nutrient agar

Plate 8: *Enterococci* spp on Macconkey agar

Plate 9: *Enterococci* spp on blood agar

The Bacterial Isolates from Water Samples

Plate 10: *E. coli* on Nutrient agar from water

Plate 11: *E. coli on* Macconkey Agar

Plate 12: *Klebsiella* spp on Nutrient agar from water

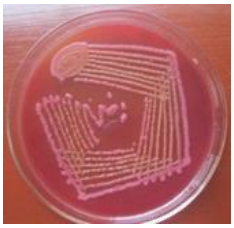

Plate 13: *Klebsiella* spp on MacConkey agar from water

Microscopic Examination Gram 'S Staining

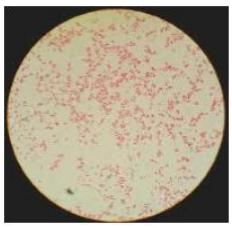

Plate 14: Microscopic examination of *E. coli*

Plate 15: *Microscopic examination of Klebsiella* spp

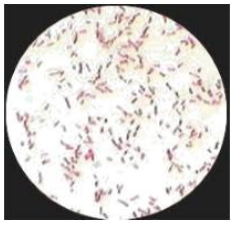

Plate 16: Microscopic examination of *Enterobacter* spp

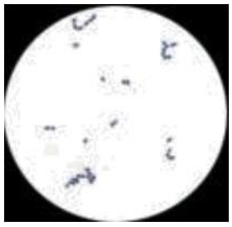

Plate 17: Microscopic examination of *Enterococci* spp

Table 8: Biochemical test of the isolated organisms.

Test	*E. coli*	*Klebsiella* spp	*Enterobacter* spp	*Enterococci* spp
Catalase	Positive	Positive	Positive	Negative
Oxidase	Negative	Negative	Negative	Negative
Indole	Positive	Negative	Negative	Negative
MR	Positive	Negative	Positive	Negative
VP	Negative	Positive	Positive	Negative
Citrate	Negative	Positive	Positive	Positive
Urease	Negative	Positive	Negative	Positive
Nitrate	Positive	Positive	Positive	Positive
Mannitol Fermentation	Positive	Positive	Positive	Negative
TSI	A/A	A/A	A/A	K/A
Glucose Fermentation	A/G	A/G	A/G	Positive
Lactose Fermentation	A/G	A/G	A/G	Negative
Sucrose Fermentation	A/G	A/G	A/G	Positive
Maltose Fermentation	A/G	A/G	A/G	Positive

Antibiogram of Isolated Coliforms

From the antibiogram study of isolated coliforms it was found that E. coli was resistant to Amikacin, Ampicillin and Cefixime and Klebsiella spp showed resistance to Ampicillin Cefixime and Cefotaxime, Such drug resistance properties may render these pathogens cause serious health hazards because of ineffective treatment of the sufferers by the commonly prescribed antibiotics.

Table 9: Antibiotic sensitivity of the isolated bacteria to common antibiotics.

Organism	Antibiotic	Zone of inhibition (mm)	Interpretation
Escherichia coli	Amikacin	12	Resistant
	Ampicillin	No zone	Resistant
	Cefixime	10	Resistant
	Cefotaxime	20	Intermediate
Klebsiella spp.	Amikacin	16	Intermediate
	Ampicillin	No zone	Resistant
	Cefixime	No zone	Resistant
	Cefotaxime	No zone	Resistant
Enterobacter spp.	Amikacin	19	Sensitive
	Ampicillin	No zone	Resistant
	Cefixime	10	Resistant
	Cefotaxime	20	Intermediate
Enterococci spp.	Amikacin	16	Intermediate
	Ampicillin	No zone	Resistant
	Cefixime	7	Resistant
	Cefotaxime	9	Resistant

Antibiotic Sensitivity of Isolated Coliform

Plate 18: Antibiotic sensitivity of *E. coli*

Plate 19: Antibiotic sensitivity of *Klebsiella* spp

Plate 20: Antibiotic sensitivity of *Enterobacter* spp

Plate 21: Antibiotic sensitivity of *Enterococci* spp

Discussion

In the current study microbial growth was found less frequently among packed fruit juice than the vented fruit juice samples. Consumption of commercially packed juice was safe than fresh juice: it is because of addition of preservatives and increase in acidity. This might be due to usage of automated machine directing aspects processing as well as application of some pusenele. The use of preservatives should not be substituted for proper hygienic and standard method of production of fruit drink because higher concentration can be harmful to our health.

Contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils, contaminated water, poor personal and domestic hygiene, peeling of fruits beforehand, shop in crowded place, dust particles in the evening and maintains of premises. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreak. Further studies on the optimization of preservative concentration should be performed.

Reference

1. Aneja KR. Experiments in Microbiology. New Age International Publications. New Delhi, India, 2002.
2. Andres SC, Giannuzzi L, Zaritzky NE. The effect of temperature on microbial growth in apple cubes packed in slim and preserved bye use of orange juice. International journal of food science and technology. 2004; 39(9):927-933.
3. Bello OO, Bello TK, Bankole SA. Occurrence of antibiotic resistant Staphylococcus aureusin some street vented foods in Ogun state Nigeria. Journal ofadvances in Biology. 2013; 1(1):21-28.
4. Chen SJ, Zhang M, Wang SJ. Physiological and quality responses of Chinese 'Suli' pear (pyrus bretschneideri Rehd) to 1-MCP vaccum infiltration treatment. Journal of Science and Food Agriculture. 2010; 90:1317-1322.
5. Erkmen O, Bozkurt H. Quality charachteristics of retailed sucuk (Turkish dry-fermented sausage).Food technology. 2004; 42:996-1006.
6. FDA. Food and Drug Administration: Internalization of microorganisms into fruits and vegetables, 2001. (Internet, www). http;//www.cfsan.fda.gov.html.
7. Joy E Lewis, Patrina Thompson, Rao BVVBN, Kalavati C, Rajanna B. Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. International Journal of Food Safety. 2006; 8:35-38.
8. Kisko G, Roller S. Carvacrol and p-cymene inactivate Escherichia coli O157: H7 in apple juice. Bmc Microbiology, 2005, 5.
9. Lewis JE, Thompson P, Rao BVVBN, Kalavati C, Rjanna B. Human Bacteria in street vended Fruit Juices:

A case study of Visakhapatnam City, India. Internet Journal of Food Safety. 2006; 8:35-38.
10. Ohiokpehai O. Nutritional aspects of street foods in Botswana. Pakistan Journal of Nutition. 2003; 2(2):76-81.
11. Reddy SM, Ram Reddy S. Microbiology A Laboratory Manual. Revised edition BSC. Publishers and Distributors, Hyderabad, 2000.
12. Salle AJ. Fundamental principles of Bacteriology. TMH edition, Tata McGraw Hill Publishing Co Ltd., New Delh, 2000, 691-699.
13. Sandeep M, Diwakar A, Abhijit G. Microbiological Analysis of Street Vended Fresh squeezed Carrot and Kinnow - Manderian Juices in Patiala City, India. International Journal of Food safety. 2004; 3:1-3.
14. Tasnim F, Hossain MA, Nusrath S, Hossain MK, Lopa D, Haque KMF. Quality assessment of industrially processed fruit juices available in Dhaka city, Bangladesh. Malaysian Journal of Nutrition. 2010; 16(3):431-438.
15. Uzeh RE, Alade FA, Bankole M. The microbial quality of pre-packed mixed vegetables salad in some retail outlets in Lagos, Nigeria. African Journal of Food Science. 2009; 3(9):270-272.