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Microbial Safety of Street Vended Fruit Juices in Dhaka City of Bangladesh

Muhammad Ekhlas Uddin[1*], Tanzina Akter[2,3], Md. Anowar Khasru Parvez[2], Shamsun Nahar[2], Safirun Pervin[1], Bishajit Debnath[1] and Suvamoy Datta[1]

[1]Department of Microbiology, Primeasia University, HBR Tower, 9 Banani C/A, Dhaka-1213, Bangladesh.
[2]Department of Microbiology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[3]Environmental Biotechnology Division, National Institute of Biotechnology, Ashulia, Savar, Dhaka-1349, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MEU, TA and SD designed the study. Author BD managed the experimental process and analyses of the raw data. Authors MEU and TA wrote the protocol and the first draft of the manuscript. Authors SP, MAKP and SN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Freshly squeezed fruit juice is a nutritious drink. Its appealing look and attractive taste make it a popular drink among many different aged people. But the risk due to fresh juice consumption is very important.

Aims: The present study was undertaken to investigate the microbiological quality of freshly squeezed juices and to determine their safety for human consumption.

Place and Duration of Study: This study was conducted in Microbiology Department of Primeasia University, Banani, Dhaka-1213, Bangladesh during February, 2015 to April, 2015.

Methodology: Four types of fruit juices such as Lemon, Sugarcane, Mango, and Papaya juice were collected from Banani, Tejgaon, Mirpur, Badda and Uttara. Total viable bacteria and fungi counts were estimated by using nutrient agar (NA) and Sabouraud's dextrose agar plates respectively. Pathogenic bacteria were isolated and identified using selective media and various biochemical tests. Kirby-Bauer disc diffusion method on Mueller Hinton agar media was used for

**Corresponding author: E-mail: ekhlasmbio@gmail.com;*

the determination of sensitivity of the isolated strains to commonly prescribed antibiotics.
Results: Among all the samples, Lemon juice from Uttara, Sugarcane juice from Mirpur and Uttara and Papaya juice from Tejgaon were of poor quality due to having all five types of pathogenic proliferation of bacteria including *Escherichia coli*, *Klebsiella* spp, *Staphylococcus* spp, *Vibrio* spp, and *Salmonella* spp. Ampicillin was found to be the least effective antibiotics against the isolates whereas Nalidixic acid was highly sensitive against *klebsiella* spp.
Conclusion: The result revealed that most of the samples had high microbial load, and were not suitable for consumption.

Keywords: Fruit juice; total viable bacteria; coliform; Escherichia coli.

1. INTRODUCTION

Fruit juices are very popular drinks. They contain essential antioxidants, vitamins, minerals, and naturally occurring phytonutrients which play important role in the prevention of different types of diseases such as Alzheimer disease, cataracts, and some of the functional declines associated with aging [1]. Vitamin C which is naturally present in fruit juices helps to form collagen, cartilage, muscle, blood vessels and to absorb iron [2]. Blood lipid profiles of hyper-cholesterolemia patient can also be improved by consuming fruits juice [3]. Besides, fruits juice inhibits breast cancer, congestive heart failure (CHF), and urinary tract infection [3]. Fruit juices are commonly sold as beverages at all public places and roadsides shops in tropical countries. The local people of the tropical countries consume street vended fruit juices to quench thirst. Consumer prefer fresh-cut fruits juices rather than the processed one because they belief that the fresh fruit juice contains the original nutritional value. In addition, they are easily accessible, convenient and cheaper than the whole fruits [4].

However, in the absence of good hygienic practices, the high concentration of various vitamins, amino acids, sugars and minerals of freshly squeezed fruit juices provide suitable medium for growth and survival of bacteria and different types of parasitic and saprophytic fungi. Food borne illness associated with the consumption of fruit juices has been reported earlier [5,6,7,8]. It has also reported that a parallel correlation with consumption of fruit juice and food-borne illnesses [9,10,11].

Dhaka is a densely populated city with a temperature reaching 30-42℃ in the months of March to June. During these months of summer, people comprising a huge portion of total population (approximately 94%) of all income and age groups including tourists consume these fresh pressed and squeezed juices [12]. In order to meet the needs of busier lifestyles and to get freshness, consumption of fresh fruits is increasing continuously in Dhaka, the capital city Bangladesh. Usually acidic pH (<4.5) of fruit juices prevents microbial growth but the presence of pathogenic microorganisms such as *E. coli*, *Salmonella* spp, *Shigella* spp, *Citrobacter* spp, *Vibrio* spp, *Aeromonas* spp, *Proteus* spp, *Bacillus cereus* in street vended fruit juices collected from various parts of Dhaka city has been reported [6,9]. Presence of these pathogenic microorganisms poses a significant threat and causes economic loss to the consumer. In order to mitigate this situation, the present study was undertaken to check the microbiological quality of freshly squeezed juices and their safety for human consumption in terms of bacterial and fungal pathogens.

2. METHODS AND MATERIALS

2.1 Study Area and Sample Collection

Five locations in Dhaka City were chosen for our study namely Banani, Tejgaon, Mirpur, Badda and Uttara. Four types of fruit juices such as Lemon, Sugarcane, Mango, and Papaya juice were collected during February, 2015 to April, 2015. Four samples for each site, therefore a total of 20 samples were collected. 250 ml for each sample were collected in sterile container, kept in ice-boxes and transported immediately to the Centre for Excellence Laboratory (CEL), Microbiology Department of Primeasia University, Dhaka-1213, Bangladesh.

2.2 Estimation of Total Viable Bacteria and Fungi

For the calculation of total viable bacteria (TVB) and the total fungal count (TFC), 100 µl of each sample was taken onto fresh nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates by spread plate technique (Cappuccino and Sherman, 1996). NA Plates were incubated at 37℃ for 24 hours and SDA plate was kept at 25℃ for 48 hours.

2.3 Estimation of *E. coli, Klebsiella* spp. and *Staphylococcus* spp.

For the estimation of total coliform and fecal coliform, MacConkey agar and Eosin Methylene Blue agar plates were used. From the dilution 10^{-2} and 10^{-4}, 100 µl of each sample was plated and incubated at 37℃ for 24 hours. For total *Staphylococcus* spp. count, same procedure was applied on fresh Mannitol Salt Agar (MSA).

2.4 Isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.

Five (5) ml of sample was transferred into 45 ml of alkaline peptone water (APW) and selenite cysteine broth (SCB) for the enrichment of *Vibrio* spp. and *Salmonella spp*, respectively and incubated at 37℃ for 5 hours. Then the samples were diluted up to 10^{-5} and 100 µl of each sample from dilution 10^{-2} and 10^{-4} were plated on Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar and *Salmonella-Shigella* (SS) agar and for the isolation of *Vibrio* spp. and *Salmonella* spp., respectively. All the plates were incubated at 37℃ for 48 hours.

2.5 Biochemical Characterization

For the biochemical characterization of the isolates, different tests including Indole, Voges-Proskeur (VP), Methyl red (MR), Motility, H_2S production, Oxidase, and Citrate tests were done.

2.6 Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar against the commercially available common antibiotics following the standard protocol. Commercially available laboratory grade antibiotic discs of Sulfomethoxazole-trimethoprim (30 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Nalidixic Acid (30 µg), Gentamicin (10 µg), Ceftriazone (30 µg), Ampicillin (10 µg) and Netilmicin (30 µg).

3. RESULTS

The current study was performed to determine bacterial quality and handling practices that predispose unpasteurized fruit juices to contamination in Dhaka city. All the 20 samples of fruit juice were found to be heavily contaminated with numerous microorganisms. A battery of biochemical test were performed to identify bacteria and the results were presented in Table 1.The biochemical tests were conducted three times independently, and the results were found to be reproducible. One representative data has been shown. The TVC was in a range between 5.10×10^{6} *CFU/ml* to 9.60×10^{8} *CFU/ml* whereas TFC was between 1.28×10^{5} *CFU/ml* to 6.40×10^{5}*CFU/ml* which indicated the presence of both bacteria and fungi spp. in huge amount (Table 2). Half of the total samples harbored fecal coliform *E. coli* in a range between 1.00×10^{2} *CFU/ml* to 4.21×10^{3} *CFU/ml*. Lemon juice from Banani, Tejgaon, Mirpur, Sugarcane juice from Tejgaon and Uttara, Mango juice from Tejgaon and Badda, Papaya juice from Banani, Badda and Uttara were free from *E. coli* contamination. *Klebsiella* spp. and *Salmonella* spp. were found in majority of fruit juices up to 4.76×10^{5} *CFU/ml* and 4.50×10^{3} *CFU/ml,* respectively, excepting Lemon juice from Badda and Papaya juice from Uttara where no *Klebsiella* spp. or *Salmonella* spp. were found.

Cholera causing potential agent *Vibrio* spp. were found in almost all samples excepting Lemon juice from Banani, Tejgaon, Mirpur, Badda, Sugarcane juice from Banani and Papaya juice from Uttara. Among 14 positive cases of *Vibrio* spp. Sugarcane juice from Uttara and Papaya juice from Mirpur had highest count having 6.60×10^{4} *CFU/ml* and 6.28×10^{4} *CFU/ml,* respectively. Among all these 20 juice samples Papaya juice from Uttara was safe enough compared to others as it lacked most of the pathogenic microorganisms.

Results from antibiogram test of the isolated microorganisms were shown in Table 3. *Salmonella* spp. showed highest resistance against Nalidixic acid and Gentamicin which were 98% and 72%, respectively, followed by Ciprofloxacin (70%), Ampicillin (65%) and Ceftriaxone (35%) (Table 3). Another member *Klebsiella* spp. were found to be less sensitive against Sulfomethoxazole-trimethoprim and Ciprofloxacin having 10% and 22% sensitivity, respectively whereas highest susceptibility was found against Nalidixic acid that was 90%. Most potent fecal coliform, *E. coli* showed moderate level of sensitivity against sulfomethoxazole-trimethoprim (55%). Additionally, Ampicillin was found to be less effective against *E. coli* as it showed about 95% resistance against it. *Vibrio* spp. was 75% and 60% resistant against Tetracycline and Gentamicin, respectively. Pathogenic *Staphylococcus* spp. showed highest resistance against Netilmicin (90%) followed by Ampicillin (84%) (Table 3).

Table 1. Biochemical characteristics of isolates from street vended fruit juices

Isolated Strains	TSI			H_2S reaction	Indole test	MR test	VP test	Citrate test	Motility test	Oxidase test
	Slant	Butt	Gas							
E. coli	Y	Y	+	-	-	+	-	-	+	-
Salmonella spp.	R	Y	-	+	-	+	-	-	+	-
Klebsiella spp.	Y	Y	+	-	-	-	-	+	-	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-

Note: TSI =Triple Sugar Iron, Y=Yellow (Acid), R=Red (Alkaline), MR=Methyl red, VP=Voges-Proskauer

Table 2. Microbial analysis of different street vended fruits juices

Type of Juice	Sampling area	TVB *(CFU/ml)*	TFC *(CFU/ml)*	*E. coli (CFU/ml)*	*Klebsiella spp. (CFU/ml)*	*Staphylococcus spp. (CFU/ml)*	*Vibrio spp. (CFU/ml)*	*Salmonella spp. (CFU/ml)*
Lemon juice-1	Banani	2.84×10^{8}	3.26×10^{6}	0	4.76×10^{5}	2.52×10^{5}	0	0
Lemon juice-2	Tejgaon	4.04×10^{8}	4.30×10^{6}	0	9.00×10^{3}	3.00×10^{4}	0	1.00×10^{2}
Lemon juice-3	Mirpur	2.60×10^{8}	2.70×10^{6}	0	2.10×10^{4}	3.30×10^{5}	0	0
Lemon juice-4	Badda	9.60×10^{8}	2.80×10^{6}	1.80×10^{3}	0	2.00×10^{5}	0	0
Lemon juice-5	Uttara	1.96×10^{8}	3.30×10^{5}	4.21×10^{3}	1.40×10^{4}	6.00×10^{4}	5.00×10^{3}	3.00×10^{2}
Sugarcane juice-1	Banani	6.44×10^{8}	3.20×10^{6}	1.56×10^{2}	0	3.00×10^{4}	0	1.00×10^{3}
Sugarcane juice-2	Tejgaon	3.72×10^{8}	2.00×10^{6}	0	0	2.00×10^{4}	4.00×10^{3}	6.10×10^{2}
Sugarcane juice-3	Mirpur	1.20×10^{8}	2.88×10^{6}	3.00×10^{2}	2.40×10^{4}	1.00×10^{4}	7.60×10^{2}	2.00×10^{2}
Sugarcane juice-4	Badda	2.92×10^{8}	2.88×10^{6}	2.00×10^{2}	4.00×10^{4}	1.00×10^{5}	7.32×10^{3}	0
Sugarcane juice-5	Uttara	8.40×10^{8}	4.48×10^{6}	0	1.50×10^{4}	3.40×10^{5}	6.60×10^{4}	1.00×10^{2}
Mango juice-1	Banani	1.00×10^{8}	6.40×10^{5}	1.00×10^{2}	2.00×10^{4}	0	7.20×10^{3}	0
Mango juice-2	Tejgaon	2.00×10^{8}	5.60×10^{5}	0	3.20×10^{4}	0	8.92×10^{3}	1.10×10^{2}
Mango juice-3	Mirpur	2.00×10^{8}	2.70×10^{5}	2.00×10^{2}	1.00×10^{4}	0	9.52×10^{3}	2.00×10^{2}
Mango juice-4	Badda	1.33×10^{6}	1.28×10^{5}	0	1.10×10^{5}	0	6.00×10^{3}	4.50×10^{3}
Mango juice-5	Uttara	3.60×10^{8}	2.08×10^{6}	2.00×10^{2}	8.80×10^{4}	3.24×10^{5}	5.84×10^{3}	0
Papaya juice-1	Banani	1.02×10^{8}	2.50×10^{5}	0	2.00×10^{3}	2.36×10^{5}	9.67×10^{3}	0
Papaya juice-2	Tejgaon	5.32×10^{8}	1.96×10^{6}	3.00×10^{3}	3.20×10^{5}	4.21×10^{5}	6.20×10^{3}	1.00×10^{2}
Papaya juice-3	Mirpur	1.04×10^{8}	1.12×10^{6}	1.00×10^{2}	5.80×10^{4}	0	6.28×10^{4}	1.85×10^{3}
Papaya juice-4	Badda	6.30×10^{7}	2.90×10^{5}	0	1.30×10^{4}	0	7.20×10^{2}	9.00×10^{2}
Papaya juice-5	Uttara	5.10×10^{6}	0	0	0	1.00×10^{4}	0	0

Table 3. Antibiotic susceptibility patterns of isolate bacteria from street vended juice in the Dhaka city

Isolated organisms	Antibiotics Sensitivity Pattern															
	SXT		CIP		TET		NA		GEN		CEF		AMP		NET	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Salmonella spp.	ND		70%	15%	ND		98%	2%	72%	28%	30%	60%	65%	25%	ND	
Vibrio spp.	ND		ND		75%	15%	ND		60%	30%	ND		ND		ND	
Klebsiella spp.	90%	10%	78%	22%	ND		10%	90%	30%	70%	ND		70%	30%	ND	
Staphylococcus spp.	ND		30%	70%	ND		ND		25%	75%	ND		84%	16%	90%	9%
E. coli	45%	55%	ND		ND		ND		20%	80%	ND		95%	5%	ND	

Note: SXT =Sulfomethoxazole- trimethoprim, CIP =Ciprofloxacin, TET =Tetracycline, NA =Nalidixic acid, GEN=Gentamicin, CEF = Ceftriazone, AMP= Ampicillin, NET =Netilmicin; R= Resistant, S= sensitive and ND= not done

4. DISCUSSION

The presence of *Klebsiella* spp. in fruit juice is alarming and agreed with the previous result of Haryani et al. [13] where he found *Klebsiella* spp. in the street food in Malaysia. Fuentes et al. [14] and Ghenghesh et al. [15] also showed the presence of *Klebsiella* spp. in fruit juice along with other pathogenic proliferation. The presence of *Klebsiella* spp. can lead to cross contamination with other street food as well as might pose serious health-concerns. The occurrence of high microbial load in the street vended foods might be due to improper washing of fruits, preservation without adequate refrigeration, use of crude stands and carts, and to the unhygienic surrounding environment as described by Lewis et al. in 2006 [16].

The high microbial load of *E. coli* observed in this study is quite similar with the study of Subbannayya et al. [17] for street vended juices in India. The main source of *E. coli* contamination might be through contaminated water used to wash dishes or to dilute juices. According to Tambekar et al. [18] the presence of *E. coli* and other coliform bacteria could be because of inadequate hand washing by food workers and unhygienic processing practices. Furthermore, the absence of *E. coli* and *Salmonella* spp. in other juice samples might be due to the quality of potable water used for preparing the juice and the minimal use of contaminated animal manure during fruit growth stages.

In the current study, almost all juice samples from all sites were found to be contaminated with *Staphylococcus* spp. This was similar with a previous work done in India by Bagde NI and Tumane PM [19]. Presence of *Staphylococcus* spp. in fruit juices might be due to contaminated hands of food handlers and dirty clothing, which ultimately indicates improper hygienic practices while food processing [20,21].

The current study was a preliminary investigation, where only a number of pathogenic microorganisms were screened. However, while collecting juice samples, it was found that cutting board, knives, spoons, glass and jugs were not frequently washed and a chance of cross contamination was possible. Cross contamination can be avoided if utensils or equipments used in juice processing are washed properly with detergents and water frequently [22]. It can be said that the results obtained in this study might be useful for monitoring the microbial quality of different fruit juices to avoid any future food-borne disease outbreaks as well as be useful in implementing a proper HACCP approach with good GMP practices.

5. CONCLUSION

The general findings showed that, the juices were prepared and served in unhygienic environments. This was supported by the laboratory results which showed significantly high total viable counts (TVC) as well as other pathogenic bacterial count including coliform, fecal coliform, *Vibrio* spp. etc. in ready to drink juices. With such kind of findings, it suggests that, low and middle income people who are the main customers of these locally vended juices are at high risk of acquiring food-borne diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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