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ASSESSMENT OF MICROBIOLOGICAL QUALITY OF DIFFERENT TYPES OF FRUIT & SOFT DRINKS SAMPLES OF DHAKA CITY

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Fruit juice is widely consumed everywhere in the world because of its authentic taste and is popular among both adults and children. Several studies have been conducted on common juice available in Dhaka to detect the level of microbial contamination. Consequently, the current study was done to evaluate the microbiology of a total of 25 samples and compare drinks from three different categories (street vending, packaged drinks, and soft drinks) that were collected from various locations throughout the city of Dhaka. Among all the samples fresh juice samples were found to be highly contaminated with different microbes where the total viable bacterial count was within a range of 10^3 to 10^6 CFU/ml. However, other pathogenic microbes like *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp., and *Salmonella* spp. also present in fresh juice but were totally absent in other samples. The result indicates that street juice samples may be prepared under unhygienic environments and which may serve as a reservoir of various pathogenic bacteria. The study reveals that government should take necessary action to ensure public health safety.

Keywords: Food safety, Fruits juice, Food contamination, Consumer risk, Microbiological quality

INTRODUCTION

Fruit juices are very popular among people of all ages throughout the year, especially during the hot seasons. Because fresh flavor and nutritional benefits make it the most popular non-alcoholic drink across all age groups (1). The body receives a substantial amount of growth substances from these juices, including vitamins and minerals, which has led to an increase in their consumption in past few years (2). Juices are fat-free and contain naturally occurring phytonutrients that promote better health. For example, orange juice's vitamin C functions as an antioxidant photochemical and improves the blood lipid profiles in hypercholesterolemic patient's also crucial pieces in detoxification (3, 4, 5). Juice's main components are fruit pulp, sugar, and water. Any stage of juice preparation can result in contamination. Use of poor sanitation, insects, air pollutants, and poisoned raw meat, poor handling, unsanitary materials, and equipment are just a few of the things that might cause contamination (2, 7-11). Environmental sources of contaminating organisms of juices are carefully considered as these microbes invade the drink preparation during processing, packaging, and handling (Rahman et al., 2011). These juices may contain bacteria such as *Escherichia coli* O157:H7, yeast, *Shigella* sp., *Salmonella* species and *Staphylococcus aureus* (7). Microbial deterioration is extremely typical in fruit juices (20). *Escherichia coli*, *Micrococcus* spp., *Staphylococcus aureus*, and other bacteria were recovered from fruit juice samples. *Pseudomonas* spp., *Shigella* spp., *Salmonella* spp., *Bacillus* spp., *Streptococcus* spp., *Enterobacter aerogenes*, *Aspergillus*, *Rhizopus*, *Saccharomyces*, *Penicillium*, and *Rhizopus* spp. were among the fungi that were isolated. Parasites were identified in *Fusarium* spp. and *Neurospora* spp. were hookworms, *Trichuris trichiura*, *Ascaris lumbricoides*, *Entamoeba hartmani*, *Giardia lamblia*, and several *Schistosoma* species (21). The severely infected fetus in pregnant women may result in spontaneous abortion, stillbirths, or neonatal sepsis (6). Yeasts that cause spoilage, including *Saccharomyces cerevisiae*, *Candida lipolytica*, and *Zygosaccharomyces* spp., are able to survive under acidic condition. Fruit juices have a bacterial load before pasteurization that is comparable to the organisms associated mainly with fruits at harvest plus contamination that were introduced post-harvest. Pasteurization removes infections and other heat-sensitive bacteria; as a result, it will significantly lower the microbial burden and prolong the product's expiry date. In the literature, there are numerous accounts of bacterial development in fruit juices, however the majority of those concerns with unpasteurized or contaminated juices causing human sickness (12, 13).

There are also some studies available on the subject of pasteurized fruit juices being contaminated with fungi (14). In sound apples, yeast populations might range from 1000 CFU/g to 10 rotten ones have million CFU/g (15). Several factors, including pH, temperature, and preservative content, may be crucial in stopping microbial development (16-18). To increase the quality of fresh fruit juices and prevent contamination,

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microbiological quality tests and preventative strategies are essential. Street vendors ought to receive training based on the required standard (Bangladesh Standards and Testing Institution) BSTI or (Food Safety and Standards Authority of India) FSSAI operating procedures to prevent microbiological food poisoning and contamination can be minimized to a large extent. In tropical nations, fruit juices are frequently offered as beverages at all public locations including roadside stands. People living there in tropical nations use fruit drinks sold on the street to quench their thirst. Customers choose freshly cut fruit juices as opposed to processed ones because of a belief that the juice of fresh fruit has original nutrient content. Furthermore, they are inexpensively, conveniently, and readily available than whole fruits (22). In the months of March through June, the temperature in the crowded city of Dhaka can rise to 30 to 42 degrees Celsius. In these months of summer, the majority of the population including tourists of all ages consume these pressed and squeezed fresh juices (23). Moreover, packaged juice and other soft drinks are always available in different markets which also have high demand throughout the year.

Therefore, the current investigation was conducted to evaluate the microbiological quality of different fresh and package juices and to evaluate the practice, attitude, and knowledge amid stands selling fresh fruit juices by the side of the road (19).

MATERIALS AND METHODS

Samples and Sampling Sites. Total of 25 samples of three categories (Fresh juice, n=15; package fruit drinks, n=05 and soft drinks, n=05) were collected from the local market (Siddeswari, Mailbag, Shantinagar) and super shops (Near Bailey Road) early in the morning and transported to the laboratory as soon as possible following standard methods as suggested by American Public Health Association (24).

Microbiological analysis of each sample. 10 ml of each of the samples was homogenized in 90 ml saline and diluted to 10^{-5} following the standard methods then the volume of 0.1 ml from each sample suspension was spread onto nutrient agar (NA) and incubated at 37℃ for 24 hours for enumerating total viable bacteria (TVB). Sabouraud dextrose agar (SDA) (Oxoid Ltd., Basingstoke, Hampshire, England) was inoculated followed by incubation at 25℃ for 48 hours for the isolation of fungi. On the other hand, for the isolation of *Escherichia coli*, *Klebsiella* spp., 0.1 ml of each sample suspension was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar and incubated at 37℃ for 24 hours (24, 25, 26). For enumerating total fecal coliform (FC), 0.1 ml of each sample suspension was spread onto membrane fecal coliform (mFC) (Oxoid Ltd., Basingstoke, Hampshire, England) agar and incubated at 45℃ for 24 hours. 0.1 ml of each sample suspension was spread on mannitol salt agar (MSA) (Oxoid Ltd., Basingstoke, Hampshire, England) for the estimation of *Staphylococcus aureus*, and the plates were incubated at 37℃ for 24 hours. (24-26). For the enumeration of *Pseudomonas* spp., 0.1 ml of each sample suspension was spread onto *Pseudomonas* agar (Oxoid Ltd., Basingstoke, Hampshire, England) and plates were incubated at 37℃ for 24 hours. For the estimation of *Listeria* spp., 0.1 ml of each sample suspension was spread onto *Listeria* Identification agar (LIA) agar base (Oxoid Ltd., Basingstoke, Hampshire, England) containing *Listeria* Supplements and the plates were incubated at 37℃ for 24 hours (24-26).

Enrichment of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. The *in vitro* cultivation of the species of *Salmonella*, *Shigella*, and *Vibrio* often appears difficult or with faulty results (false-negative) due to their viable but non-culturable (VBNC) attributes (25-26). Therefore, samples were enriched prior to isolating these bacteria (7, 24). Enrichment was performed for *Salmonella* spp. and *Shigella* spp., in the selenite Selenite Cystine Broth (SCB). 1 ml of homogenized sample suspension was transferred to SCB followed by incubation at 37℃ for 4 hours and serial dilutions were made up to 10^{-5}, and from 10^{-3} dilution 0.1 ml was spread onto Salmonella Shigella (SS) agar (Hi media, India) followed by the incubation at 37℃ for 24 hours. For the enrichment of *Vibrio spp.*, 0.1 ml of the homogenized sample suspension was transferred to alkaline peptone water (APW) and incubated at 37℃ for 4 hours and serial dilutions were made up to 10^{-5} and from 10^{-3} dilution 0.1 ml was spread onto TCBS (Oxoid Ltd., Basingstoke, Hampshire, England) agar followed by the incubation at 37℃ for 24 hours (25-26). Finally, all isolates were confirmed by a number of biochemical tests, like the triple sugar iron (TSI) test, motility indole urease (MIU) test, methyl-red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, catalase test, and oxidase tests (24-26).

RESULTS

Prevalence of bacteria and fungi. The current study revealed that most of the fresh juice samples were found to be contaminated with a higher microbial count, on the other hand, packaged juice and soft drinks demonstrated lower microbial count. The total viable bacteria count (TVBC) was observed within a range of 10^3 to 10^6 CFU/ml in all juice samples collected from the local market. The Highest TVBC is observed in Papaya (3.2×10^7 CFU/ml) and Sugar cane (2.6×10^6 CFU/ml) showed the maximum total fungal count both samples are collected from a local shop (Table 1). In the case of package juice and soft drinks samples, most of them were found to be contaminated under 10^4 CFU/ml. Where *E. coli*, *Staphylococcus* spp., *Vibrio* spp., and *Salmonella* spp. were predominantly found in most of the fresh juice samples within a range of 10^1 to 10^4 CFU/ml. *Salmonella* spp. were present in only two samples and *Vibrio* spp. were present in three samples. However, Fecal coliform and *Shigella* spp. could not be detected in fresh juice samples. The highest *E. coli* (1.5×10^3 CFU/ml) were found in freshly made sugarcane juice of local. Similarly, other bacterial count such as *Staphylococcus* spp., *Vibrio* spp., *Pseudomonas* spp. and *Salmonella* spp. were comparatively higher in sugarcane juice samples of the local market. Whereas packaged fruit drinks and soft drinks samples were free from pathogenic bacterial except two of the pack juice contained *Staphylococcus* spp. (Table 1, 2, 3 and 4).

Antibiogram results of isolates. Results from the antibiogram of the isolated microorganisms are shown in Table 5. *E. coli* collected from juice samples show moderate sensitivity against used antibiotics, Ampicillin 25 µg (90%) and Gentamycin 10 µg (80%) were sensitive against *E. coli*. In case of *Pseudomonas* spp. Gentamycin 10 µg (66.6%) was most sensitive. However, *Staphylococcus* spp. and *Salmonella* spp. were found to most resistant bacteria among all, they show almost 50% to 80% resistances against most of antibiotics. Another isolate *Vibrio* spp. was found to be less resistant against most of the antibiotics having 33.3% to 66.6%.

DISCUSSION

The most contaminated juice samples were street-vended juices compared to packaged or soft drinks. According to the Gulf Standards (2000), the permitted total viable microbial limit for all non-alcoholic beverages (fruit drinks & soft drinks) should be less than 10^4 CFU/ml (31). But here the TVBC counts of most of the fresh juice samples exceed the range which could be due to mishandling and mistreatment during the preparation and storage of these items which are often linked to causes outbreaks of microbial infections and

diseases. Interestingly, freshly prepared lemon juice and soft drinks have low microbial counts which may be due to the low pH (around 2.5 to 3.5 pH) of the juice items (32). The disease agents spread by juice drink not only harm large groups of people but also sometimes results in serious disability and death.

Table 1: Microbial Load in different fresh fruit juice samples (CFU/ml).

Sample	**TVBC**	**TF**	***E. coli***	***Staphylococcus* spp.**	***Vibrio* spp.**	***Pseudomonas* spp.**	***Salmonella* spp.**
Papaya (n=3)	5.6×10^6	3.2×10^3	3.5×10^2	3.5×10^3	3.2×10^2	2.0×10^2	1.2×10^1
Watermelon (n=3)	1.0×10^5	7.5×10^3	1.8×10^1	6.0×10^2	5.0×10^2	1.5×10^3	0
Lemon (n=3)	5.0×10^3	4.5×10^2	3.0×10^1	0	0	6.0×10^1	0
Orange (n=3)	6.0×10^3	8.0×10^2	0	9.0×10^2	0	6.0×10^1	0
Sugar cane (n=3)	4.6×10^6	4.2×10^4	1.5×10^3	3.5×10^3	3.2×10^2	3.0×10^2	3.2×10^2

Note: TVBC: Total Viable Bacterial Count, TF: Total Fungi, *Shigella* spp. and Fecal Coliform totally absent in all the samples.

Table 2: Microbial Load in different package fruit drinks samples (CFU/ml).

Sample	**TBVC**	**TF**	***E. coli***	***Pseudomonas* spp.**	***Staphylococcus* spp.**	***Samonella* spp.**	***Vibrio* spp.**
Sample 1	1.2×10^4	1.1×10^2	0	0	1.3×10^2	0	0
Sample 2	1.4×10^2	0	0	0	0	0	0
Sample 3	1.8×10^3	1.2×10^2	0	0	0	0	0
Sample 4	1.2×10^2	1.1×10^2	0	0	1.0×10^1	0	0
Sample 5	1.0×10^4	0	0	0	0	0	0

Note: TVBC: Total Viable Bacterial Count, TF: Total Fungi, *Shigella* spp. and Fecal Coliform totally absent in all the samples.

Table 3: Microbial Load in different soft drinks samples (CFU/ml).

Sample	**TBVC**	**TF**	***E. coli***	***Pseudomonas* spp.**	***Staphylococcus* spp.**	***Salmonella* spp.**	***Vibrio* spp.**
Sample 1	2.0×10^2	0	0	0	0	0	0
Sample 2	1.6×10^3	1.1×10^1	0	0	0	0	0
Sample 3	2.3×10^3	0	0	0	0	0	0
Sample 4	1.2×10^3	0	0	0	0	0	0
Sample 5	1.5×10^3	0	0	0	0	0	0

Note: TVBC: Total Viable Bacterial Count, TF: Total Fungi, *Shigella* spp. and Fecal Coliform totally absent in all the samples.

Tables 4: Confirmative biochemical tests for the isolates.

Assumed Organism	**TSI**			**H_2S reaction**	**Indole test**	**MR test**	**VP test**	**Citrate test**	**Catalase**	**Oxidase test**
	Slant	**Butt**	**Gas**							
Escherichia coli	Y	Y	+	-	-	-	-	+	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+
Salmonella spp.	R	Y	-	+	-	+	-	-	+	-

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

Table 5: Antibiotic susceptibility patterns of isolated bacteria from juice samples.

Antibiotics name	***E. coli (n-4)***		***Pseudomonas* spp. *(n-5)***		***Staphylococcus* spp. *(n-6)***		***Salmonella* spp. *(n-2)***		***Vibrio* spp. *(n-3)***	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Penicillin (100µg)	ND	ND	ND	ND	100%	0%	80%	20%	66.6%	33.3%
Ampicillin (25µg)	90%	10%	100%	0%	80%	20%	60%	40%	ND	ND
Amoxycillin (30µg)	80%	20%	66.6%	33.3%	100%	0%	ND	ND	ND	ND
Ciprofloxacin (5µg)	80%	20%	66.6%	33.3%	80%	20%	50%	50%	ND	ND
Erythromycin (15µg)	ND	ND	ND	ND	50%	50%	ND	ND	ND	ND
Sulfomethoxazole-trimethoprim (25µg)	80%	20%	ND	ND	ND	ND	50%	50%	ND	ND
Imipenem (10µg)	80%	20%	ND	ND	ND	ND	50%	50%	33.3%	66.6%
Nalidixic acid (30µg)	ND	ND	ND	ND	ND	ND	80%	20%	ND	ND
Tetracycline (10µg)	ND	ND	ND	ND	ND	ND	ND	ND	33.3%	66.6%
Netilmicin (30µg)	ND	ND	66.6%	33.3%	80%	20%	ND	ND	ND	ND
Gentamycin (10µg)	20%	80%	33.3%	66.6%	20%	80%	00%	100%	33.3%	66.6%

Note: S: Sensitive, R: Resistance, ND: Not Done.

The presence of *E. coli* and another enteropathogenic microorganisms in fresh juice samples might be of inadequate hand washing practices by food workers during preparation and repetition of unhygienic processing practices (27, 28, 30, 36). *Staphylococcus* spp. and *Pseudomonas* spp. can contaminate most of the fresh juice by reason of the lack of knowledge in cleaning and safe fruit juice preparation which can be avoided by properly training the food handlers on safe fruit handling techniques (28, 29, 36). A different study on street food in Dhaka city also found similar results (30, 36). The microbiological quality of packed fruit juices indicates the good practice during production and is properly maintained by the manufacturers. Moreover, in most of the street vended juice samples were found to be contaminated with many pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus, Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *and Vibrio* spp. These microorganisms can cause various food-borne diseases such as food poisoning, diarrhea, dysentery, cholera, typhoid and pneumonia (35, 36). Such results may be due to unwanted unhygienic conditions and lack of appropriate knowledge; therefore, it can be prevented by proper training and monitoring. Different studies on food samples revealed that drug resistance microorganisms in food samples are increasing constantly which is a great threat for the near future (33-35). Similarly, pathogenic *Staphylococcus* spp. and *Salmonella* spp. from our study showed the highest resistance against most of the common antibiosis used.

CONCLUSIONS

The present study revealed the microbiological status of packed fruit juice products. Overall, the microbiological status of fresh street vended juice products were not so good. Because most of them exceed the microbial limit, indicated some unhygienic handling which is alarming for consumers. To reduce microbial contamination besides improving the quality of drinks; GHP, GAP, and HACCP need should be followed to avoid the contamination. However, It is necessary to continuously monitor by the government-authorized institutes (like BSTI) to control the microbial and chemical quality of the juices, as well as public awareness about by the appropriate authority of the adulteration fruit juices, state. The public awareness should also be created and their consequences on human health.

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