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Microbiological quality assessment for drug resistant pathogenic microorganisms from the fresh vended fruit juices

Nurunnahar Akter Lucky, Ifra Tun Nur and Tasnia Ahmed*

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

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Fruit juices are very popular due to their freshness and taste. These juices contain all the nutritive values those were present in whole fruits. Fresh juices are healthful drink for people of all ages. But the condition is quite opposite if they become contaminated with pathogenic bacteria during the juice processing. Because of the nutritive properties juice can give enough opportunities to the microorganisms to survive and multiply. In our current study we included eight types of fresh juices to study the microbial load. All the samples were heavily contaminated. Total bacterial and fungal count was found to be up to 3.0×10^7 cfu/ml and 2.6×10^6 cfu/ml, respectively. *Salmonella* spp. and *Shigella* spp. was present only in one sample. *Staphylococcus aureus* and *Pseudomonas* spp. were the most prominent in all of the eight samples. *Vibrio* spp. was found in six samples ranging from 2.0×10^2 cfu/ml to 2.9×10^4 cfu/ml. All the pathogenic isolates showed drug resistance towards the most commonly prescribed antibiotics indicating the risk of difficulty in eradicating diseases.

Key words: Fruit juices; Contamination; Drug resistance

Fruit juices are very popular among the people of all ages throughout the year, especially during the hot seasons. They are prepared by squeezing the fruit pulp and mixing it with significant amount of water, ice, syrup and other ingredients depending on the recipe (1). Fresh fruit juices are the non-alcoholic beverage consumed globally. For enjoying the freshness of the fruit, unpasteurized fresh made juices are most popular among the people. As they contain lots of vitamins, minerals (calcium, phosphorous, sodium), several bioactive compounds (flavanone glycosides, hydroxycinnamic acid, antioxidants) which help to improve human health status, maintain blood lipid profile in hypercholesterolemia patients, inhibition of breast cancer, urinary tract infections, congestive heart failure, (2-9). These juices are generally sold in busy areas like shopping malls, schools, universities, hospitals, offices and so on (10-14). If the fruits used in preparing juice have damages skin surface, they may become contaminated with environmental microflora among which pathogenic microbes can be present. Moreover, ingredients used in preparing juice, ice, water, equipments used during the preparation, hygienic condition of the juice maker, surface where the fruits are processed etc can affect the quality of juice and if not properly maintained, can transfer pathogenic microorganisms to the final juice. If this happens, the consumers will suffer from food borne gastrointestinal disorders and even death sometimes (9, 15-23). Some pathogenic microbes found in fruit juices include *Escherichi coli* 0157:H7, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp., *Streptococcus* spp. etc. (21-23). The current study was designed to determine the presence of pathogenic bacteria found in fresh vended fruit juices and their drug resistance traits sold in different areas of Dhaka city to identify the risk of public health.

*Corresponding Author: Mailing address**.** Tasnia Ahmed, Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh, Bangladesh; E-mail: tasnia2009@yahoo.com.

MATERIALS AND METHODS

Study area and sample processing. Fruit juices sold in different areas were subjected to microbiological analysis. The study included eight different juices such as sour mango, apple, wood apple, papaya, mixed fruit, banana-milk, sugarcane and orange juice. Fresh fruit juices from vendors were collected during the time span of February 2015 to April 2015. Samples were collected aseptically and transferred to the microbiological laboratory for microbial assay. The juices were homogenized in normal saline and serially diluted up to 10^{-5} (24).

Microbiological assay. 0.1 ml of homogenized fruit juices were inoculated onto nutrient agar (NA), Sabouraud dextrose agar (SDA), Pseudomonas agar, MacConkey agar, mannitol salt agar to detect total viable bacterial count, total fungal count, *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus* respectively. For detection of VBNC (viable but non culturable) bacteria, 1ml of fruit juice was introduced into 9ml of alkaline peptone water (APW) and selenite cysteine broth (SCB) for enrichment and after that inoculated onto Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar and *Salmonella-Shigella* (SS) agar for the detection of *Vibrio* spp., *Salmonella* spp., and *Shigella* spp. (25, 26). SDA plates were incubated at 25 ^oC for 48 hours and all the other plates were incubated at 37 ^oC for 24 hours.

Study for antibiotic susceptibility. To demonstrate the drug resistance pattern of the pathogenic isolates found in the fresh fruit juices were subjected to observe their drug resistance traits against most commonly used antibiotics. Suspensions were prepared using the isolates and after getting the desired turbidity inoculated onto Muller-Hinton agar (MHA) plates using a sterile cotton swab. After that some pre-selected antibiotic discs were placed over the media and incubated at 37 ^oC for 24 hours. After the incubation period plates were observed to determine the zone of inhibition indicating the susceptibility against the antibiotic. Antibiotics which were used for this segment of our study include Streptomycin (10 µg),

Vancomycin (30µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Gentamicin (10µg), Kanamycin (30µg), Amoxicillin (10µg), Nalidixic acid (30µg), Ampicillin (10µg), Erythromycin (15µg) and Chloramphenicol (10µg). Normal saline was used as negative control.

RESULTS AND DISCUSSIONS

In Bangladesh, the outbreak of food associated diseases is a common scenario due to lack of proper knowledge, adequate consciousness and lack of law enforcement. Food items especially those available from the vendors have been already showed that they often contain a good account of pathogenic microbes which can cause public health problems (25, 27- 33.). Fruit juices available in the streets are one of the most popular drinks during the hot seasons. People choose to drink the juices because of their freshness and the food values (34). But the final product which they drink are often contaminated with various pathogens. The vendors don't have the proper knowledge about the transmission of microorganisms from their simple actions (23-37). For example, the hygienic condition of the vendor himself is one critical factor. Then the quality of the fruits used for preparing juice determines the final quality of juice. The lower the quality of both outside and inside of a fruit is, the greater the chance of low quality juice. Vendors use low cost ice slab which they process to use with juice. Huge amount of such ice and water is used in juice making which bring a huge load of microorganisms. Additionally the equipments used during juice preparation is also important factor. Contamination can occur during any stages of these procedures (38, 39). Our ongoing study headed to determine the pathogenic load of microorganisms with their drug resistance traits.

In current study we have identified a number of pathogenic isolates from all of the eight fruit juices. Total viable bacteria was found to be present between 2.1×10^6 cfu/ml to 3.0×10^7 cfu/ml. The total fungal count was between the range of 1.5×10^5 cfu/ml to 2.6×10^6 cfu/ml. *Escherichia coli* was present in four samples out of eight indicating the fecal contamination due to unhygienic preparations and contaminated water source (40, 41). The range of *E. coli* was within 8.0×10^3 cfu/ml (orange juice) to 1.8×10^5 cfu/ml (papaya juice). *Salmonella* spp. (3×103 cfu/ml in papaya) and *Shigella* spp. (2.8×10^2 cfu/ml in mixed fruit juice) was found to be present only in one sample. Unfortunately *Vibrio* spp. was present in six samples out of eight indicating the risk caused by VBNC bacteria. *Staphylococcus aureus* (from 6.0×10^3 cfu/ml to 2.5×10^5 cfu/ml) and *Pseudomonas* spp. (from 2.0×10^3 cfu/ml to 2.8×10^5 cfu/ml) was present in all the samples. As fruit juices are very common, adequate knowledge should be provided to the common people raising the awareness about the health hazards and the food laws

TABLE 1. Microbiological analysis of fresh juice samples

	Juice Sample	Microbial load (cfu/ml)								
		TVBC	**Total Fungi**	***E. coli***	***Klebsiella* spp.**	***Pseudomonas* spp.**	***Staphylococcus* spp.**	***Vibrio* spp.**	***Salmonella* spp.**	***Shigella* spp.**
S1	Sour mango	2.3×10^6	1.5×10^5	0	0	1.8×10^4	6.0×10^3	2.0×10^2	0	0
S2	Banana milk	2.9×10^7	1.3×10^6	7.0×10^4	5.1×10^4	2.2×10^4	2.0×10^4	2.9×10^3	0	0
S3	Papaya	2.1×10^6	1.3×10^6	1.8×10^5	4×10^4	2.6×10^4	1.2×10^4	5.7×10^3	3×10^3	0
S4	Apple	2.1×10^6	2.1×10^6	0	1.2×10^4	2.1×10^5	8.8×10^4	2.9×10^4	0	0
S5	Sugercane	3.2×10^6	2.6×10^6	0	2.0×10^3	2.3×10^5	2.5×10^5	0	0	0
S6	Wood apple	3.0×10^7	2.8×10^5	8.4×10^3	7.0×10^3	2.0×10^3	8×10^3	1.6×10^4	0	0
S7	Orange	2.3×10^6	6.3×10^5	8.0×10^3	1.2×10^4	2.8×10^5	1.1×10^5	6×10^2	0	0
S8	Mixed fruit	2.2×10^6	8.0×10^5	0	1.5×10^4	2.0×10^4	8.0×10^3	0	0	2.8×10^2

TVBC = Total viable bacterial count

TABLE 2. Antibiotic susceptibility of the isolates

Isolates Antibiotics	*Klebsiella* spp N=8		*Pesudomonas* spp. N=9		*Staphylococcus* spp. N=7		*E. coli* N=3		*Salmonella* spp. N=3		*Shigella* spp. N=2		*Vibrio* spp. N=8	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
AMP (10µg)	10%	90%	90%	10%	100%	0%	55%	45%	50%	50%	30%	70%	40%	60%
CIP (5µg)	20%	80%	80%	20%	25%	75%	50%	50%	45%	55%	35%	65%	75%	25%
KAN (30µg)	30%	70%	10%	90%	100%	0%	25%	75%	30%	70%	10%	90%	30%	70%
CEF (30µg)	40%	60%	35%	65%	75%	25%	ND	ND	35%	65%	40%	60%	30%	70%
AMO (10µg)	30%	70%	85%	15%	75%	25%	50%	50%	85%	15%	50%	50%	35%	65%
IPM (30µg)	25%	75%	40%	60%	25%	75%	45%	55%	20%	80%	30%	70%	10%	90%
STR (10µg)	25%	75%	20%	80%	0%	100%	30%	70%	40%	60%	30%	70%	45%	55%
VAN (30µg)	90%	10%	70%	30%	100%	0%	20%	80%	50%	50%	75%	25%	20%	80%
GEN (10µg)	10%	90%	20%	80%	0%	100%	45%	55%	30%	70%	45%	55%	40%	60%
NALI (30µg)	30%	70%	15%	85%	75%	25%	85%	15%	25%	75%	40%	60%	10%	90%
ERY (15 µg)	40%	60%	35%	65%	75%	25%	60%	40%	ND	ND	20%	80%	50%	50%

AMP= Ampicillin, AMO= Amoxicillin, CIP= Ciprofloxacin, CEF= Ceftriaxone, NALI= Nalidixic acid, IPM= Imipenem, ERY= Erythromycin, VAN= Vancomycin, GEN= Gentamicin, STR= Streptomycin
Sensitive- S; Registrant- R

should be enforced strictly on the preparation procedures. This must be helpful in decreasing food borne illness among the general people.

Due to the increase in drug abuse, pathogenic isolates are becoming familiar towards the most common and available antibiotics (42-45) which can be observed by their resistant patterns during the antibiotic susceptibility tests. Not only this, the situation is becoming worse due to the transfer of the resistant genes to the susceptible isolates rendering them resistant also. *Klebsiella* spp. is almost resistant to Vancomycin and showed susceptibility toward the other used antibiotics (Table 2). *Pseudomonas* spp. are resistant against Ampicillin, Ciprofloxacin, Vancomycin, Amoxycillin. *Staphylococcus aureus* is totally resistant against Ampicillin and Vancomycin where as susceptible to Streptomycin and Gentamycin. *E. coli* is very much sensitive to Streptomycin, Vancomycin; *Salmonella* spp. to Imipenem, Kanamycin; *Shigella* spp. to Kanamycin, Erythromycin and *Vibrio* spp. to Nalidixic acid and Imipenem. So it is very important to use drugs appropriately prescribed by the registered physicians to avoid the chance of resistance due to drug abuse.

CONCLUSION

Fruit juices are very popular drink with adequate amount of vitamins and minerals which is very important for our health. But this nutritious substance can also be harmful for the health if harbors adequate amount of pathogenic bacteria. Pathogenic bacteria can be introduces from the raw fruits, ingredients, equipments and multiply rapidly using the nutrition of the fruit juice. Food law enforcement should be strict enough to control the condition specially in case of street vending.

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