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Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh

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Abstract

Present investigation attempted to resolve the microbiological attributes of the fruit juices collected from different areas around Dhaka city. Twenty six vendor fruit juices and 15 packed juices were examined for the presence of total bacterial load, coliforms and staphylococci. Samples were found to harbor viable bacteria within the range between 10^2 -10^7 cfu/ml. Thirty samples exhibited the presence of staphylococci. Total coliforms were detected in 31 samples within the range of 10^2 -10^6 cfu/ml which were further detected as *Escherichia coli* and *Klebsiella* spp. Fecal coliforms were found in 4 vendor fruit juice samples (10^2 cfu/ml), while in the industrially packed samples, they were completely absent. Drug resistance among the isolates was found against ampicillin, ciprofloxacin, amoxicillin, erythromycin, chloramphenicol, ceftriaxone, piperaciline, trimethoprime-sulfomethoxazole, nalidixic acid and vancomycin. Overall, the study demonstrates that the quality of the both packed and fresh juices was unsatisfactory and hence the products need to be microbiologically controlled in order to ensure the overall health safety.

Introduction

Fruit juices are nutritious drinks with great taste and health benefits (Suaad and Eman, 2008). There are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe (Mosupye and Holy, 2000; Muinde and Kuria, 2005; Chumber *et al*., 2007). Several factors 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 airborne dust. Such juices have been shown to harbor bacterial pathogens notably *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* (Buchmann *et al*., 1999; Sandeep *et al*., 2004; Barro *et al*., 2006).

Water used for juice preparation can be a major source of microbial contaminants including coliforms, faecal coliforms, faecal streptococci, etc (Tasnim *et al*., 2010). Changes in pH may also promote the growth of pathogens (FDA, 2001). While the quality of fruit juices is strictly being maintained in the developed countries under several laws and regulations, unfortunately, in many developing countries including Bangladesh, the manufacturers are not much concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks becomes a serious problem (Tasnim *et al*., 2010).

In Dhaka city, there is a high demand for both packed and fresh fruit juices especially during summer. While most restaurants and café serve juices in apparently hygienic conditions, unfortunately in the roadside shops, recreational areas (parks), and in the busy market places, the microbiological quality of the supplied juices remains questionable. Along these lines of evidences, a prompt assessment of juices was undertaken in this study to assess their microbiological safety for the sake of the better management of public health.

Materials and Methods

Collection of samples

A total of 41 samples of fruit juices collected from

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10 locations around Dhaka City. Ten varieties of fruit juices (papaya, orange, grape, apple, sugarcane, wood apple, pineapple, lemon, mango and strawberry) were chosen based on the consumer demand. Samples were tested within an hour after procurement.

Isolation and estimation of microorganisms from juice samples

Serial dilutions of samples were made up to 10^{-7} with sterile normal saline. 0.1ml of each dilution was evenly spread on the nutrient agar medium and incubated at 37°C for 24 hours. Plates were screened for the presence of discrete colonies after incubation period and the actual numbers of bacteria were estimated as colony forming unit per ml (cfu/ml). Quantitative analysis for the presence or absence of specific microorganisms was done by plating on selective media. Total coliform count (TCC), fecal coliform count (FCC) and total staphylococcal count (TSC) were performed in similar manner as described above using MacConkey agar, membrane fecal coliform (mFC) agar and Mannitol Salt Agar (MSA) medium, consecutively. Isolates were then identified according to the Bergey's manual of determinative bacteriology (Buchanan and Gibbon, 1984) and manual for the identification of medical bacteria (Cowan, 1975). Estimation of bacterial load was performed by standard method (ICMSF, 1998). The microbiological condition of safety and hygiene were then assayed by comparing the obtained results with the limit of Gulf standard (Table 1), known as the recommended microbiological standard for fruit juices in the Gulf region (Basar and Rahman, 2007; Tasnim *et al*., 2010; Rahman *et al*., 2011).

Determination of antimicrobial susceptibility

Isolates were tested against 11 common antibacterial drugs by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark) as described previously (Bauer *et al*.1966; Acharjee *et al*., 2012). Briefly, a single colony of each isolate was introduced into 2 ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface. Antibiotics impregnated discs (ampicillin 10 µg, amoxicillin 10µg, ciprofoxacin 5 µg, ceftriaxone 30µg, nalidixic acid 30 µg, imipenem 10 µg, erythromycin 15 µg, chloramphenicol 30 µg, trimethoprime-sulfomethoxazole 25µg, gentamycin 10µg, vancomycin 30µg and piperaciline 10 µg) were then placed onto the surface of the inoculated plates. After incubation, diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant.

Results and Discussion

Colony morphology, phenotypic and biochemical traits of the isolates

Following incubation for 24 hours, typical pink, circular, convex colonies on MacConkey Agar, blue colonies on mFC agar and yellow colonies on Mannitol Salt Agar were initially considered as coliforms, fecal coliform and *Staphylococcus* spp, consequtively. Isolates from MacConkey- and mFC agar media were observed as Gram negative, single, short rods, i.e., characteristic of coliforms whereas isolates from MSA were Gram positive in a cluster arrangement which were typical for *Staphylococcus* spp. Based on the biochemical characteristics, isolates were confirmed as *E. coli*, *Klebsiella* and *Staphylococcus* spp.

Total viable count (TVC)

Most of the fruit juice samples showed equal or much higher viable bacterial count than the permitted count (Table 1 and Table 2). The highest bacterial load ($2.8x10^7$ cfu/ml) for vendor fruit juice sample was found in a sugarcane juice (sample V-21), collected from Rampura, and the lowest load was $1.9x10^3$ cfu/ml found in an apple juice (sample V-17) collected from Baily road (Table 2). On the other hand, The highest total bacterial load ($2.66x10^6$ cfu/ml) for packed fruit juice sample was found in an orange juice (sample P-12), collected from Farmgate, and the lowest load was observed to be $1.59\ x10^2$ cfu/ml in a mango juice (sample P-15) collected from Rampura (Table 3).

Variations in TVC of the both types of fruit juices may be due to the unhygienic maintenance during preparing the juice. Rahman *et al*. (2011) reported that total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice, as the highest count was found as $2.4x10^4$ cfu/ml and $3.2x10^3$ cfu/ml in fresh and packed juice, respectively which was found to be lower than our study. Tasnim *et al*. (2010) also found the load of viable bacteria in processed juice samples within the standard limit in the average of 10^3 cfu/ml. Bagde and Tumane (2011) found that total bacterial counts in juice samples ranged between $2.0\ x10^6$ to $1.0\ x10^5$ cfu/ml in Nagpur, India.

Prevalence of coliforms

Most of the fruit juices in our study were found to be unfavorable for consumption because many of them showed the presence of coliforms (*E. coli* and *Klebsiella* spp.). The presence of coliform in fruit juice

Table 1. The recommended microbiological standards for any fruit juice; all numbers are as per ml of juice consumed (Gulf Standards, 2000)

Parameter	Total viable count	Coliform	Fecal coliform	Staphylococci
Maximum bacterial load anticipated	5.0×10^3	10	0	100
Maximum bacterial load permitted	1.0×10^4	100	0	1.0×10^3

Table 2. Bacterial load in vendor fruit juice samples (n=26)

Sample No.	Type of Juice	Sampling area	Total Viable Count (TVC) cfu/ml	Total Coliform Count (TCC) cfu/ml	Fecal Coliform Count (FCC) cfu/ml	Total Staphylococcal Count (TSC) cfu/ml
V-1	Papaya	Mouchak	$1.4x10^5$ (0.0735)	$1.76x10^3$ (0.0322)	0 (0)	$1.95x10^4$ (0.0202)
V-2	Papaya	Mouchak	$1.98x10^6$ (0.0202)	$1.59x10^4$ (0.0495)	$1.95x10^2$ (0.0202)	$2.4x10^4$ (0.0071)
V-3	Papaya	Mouchak	$3.1x10^7$ (0.001)	$3.14x10^3$ (0.001)	0 (0)	$1.54x10^3$ (0.0606)
V-4	Papaya	Siddeswari	$3.1x10^7$ (0.001)	$6.95x10^3$ (0.001)	0 (0)	$8.95x10^3$ (0.001)
V-5	Papaya	Siddeswari	$3.9x10^6$ (0.001)	$1.42x10^4$ (0.0735)	0 (0)	$5.6x10^3$ (0.001)
V-6	Papaya	Siddeswari	$4.86x10^6$ (0.001)	$2.99x10^3$ (0.001)	0 (0)	$1.83x10^3$ (0.0322)
V-7	Papaya	Motijheel	$9.3x10^6$ (0.001)	$2.66x10^5$ (0.003)	0 (0)	$6.36x10^4$ (0.001)
V-8	Orange	Moghbazar	$2.2x10^7$ (.0202)	$2.3x10^4$ (0.0202)	0 (0)	$1.5x10^3$ (0.0606)
V-9	Orange	Moghbazar	$1.52x10^7$ (0.0606)	$1.47x10^4$ (0.0606)	0 (0)	0 (0)
V-10	Orange	Moghbazar	$3.9x10^5$ (0.001)	$1.5x10^3$ (0.0606)	0 (0)	$1.7x10^2$ (0.0401)
V-11	Orange	Malibagh	$5.8x10^5$ (0.001)	$6.95x10^4$ (0.001)	0 (0)	0 (0)
V-12	Orange	Malibagh	$1.58x10^6$ (.0495)	$6.5x10^3$ (0.001)	0 (0)	$1.6x10^5$ (0.0495)
V-13	Orange	Moghbazar	$8.9x10^5$ (0.001)	$1.5x10^3$ (0.1469)	0 (0)	$6.9x10^3$ (0.001)
V-14	Grape	Baily Road	$4.99x10^4$ (0.001)	$1.5x10^3$ (0.1469)	0 (0)	$1.8x10^2$ (0.0202)
V-15	Grape	Baily Road	$4.99x10^4$ (0.001)	$3.1x10^3$ (0.001)	0 (0)	0 (0)
V-16	Apple	Baily Road	$2.15x10^6$ (0.0158)	$1.7x10^3$ (0.0401)	0 (0)	0 (0)
V-17	Apple	Baily Road	$1.9x10^3$ (0.0202)	$1.95x10^2$ (0.0202)	0 (0)	$1.95x10^2$ (0.0202)
V-18	Sugarcane	Motijheel	$8.5x10^5$ (0.001)	$6.99x10^3$ (0.001)	0 (0)	$1.42x10^2$ (0.0735)
V-19	Sugarcane	Farmgate	$1.8x10^7$ (0.0322)	$1.58x10^6$ (0.0495)	0 (0)	$8.76x10^3$ (0.001)
V-20	Sugarcane	Banglamotor	$8.16x10^6$ (0.001)	$5.9x10^5$ (0.001)	$7.95x10^2$ (0.001)	$1.54x10^5$ (0.0606)
V-21	Sugarcane	Rampura	$2.8x10^7$ (0.0202)	$1.53x10^5$ (0.0606)	0 (0)	$6.95x10^5$ (0.001)
V-22	Sugarcane	Mirpur	$2.5x10^7$ (0.0154)	$2.54x10^5$ (0.0054)	$1.95x10^2$ (0.0202)	0 (0)
V-23	Wood apple	Farmgate	$1.1x10^6$ (0.01251)	$7.67x10^5$ (0.001)	0 (0)	$3.23x10^3$ (0.001)
V-24	Wood apple	Mirpur	$2.72x10^6$ (0.003)	$8.39x10^4$ (0.001)	0 (0)	$7.95x10^7$ (0.001)
V-25	Pineapple	Banglamotor	$8.99x10^6$ (0.001)	$7.95x10^5$ (0.001)	$1.9x10^2$ (0.0202)	$1.49x10^4$ (0.0606)
V-26	Lemon	Rampura	$3.94x10^5$ (0.001)	0 (0)	0 (0)	$4.19x10^4$ (0.001)

V= Vendor
All data were statistically analyzed and were found significant ($p<0.1$). Respective p-values have been indicated in parentheses.

Table 3. Bacterial load in packed fruit juice samples (n=15)

Sample No.	Type of Juice	Sampling area	Total Viable count (TVC) cfu/ml	Total Coliform Count (TCC) cfu/ml	Fecal Coliform Count (FCC) cfu/ml	Total Staphylococcal Count (TSC) cfu/ml
P- 1	Strawberry	Mouchak	4.5×10^5 (0.001)	1.8×10^3 (0.0322)	0 (0)	1.45×10^4 (0.0606)
P-2	Strawberry	Mouchak	2.95×10^4 (0.0011)	0 (0)	0 (0)	1.45×10^4 (0.0606)
P-3	Strawberry	Baily Road	1.95×10^4 (0.0202)	0 (0)	0 (0)	4.5×10^3 (0.001)
P-4	Apple	Baily Road	5.99×10^5 (0.001)	0 (0)	0 (0)	8.54×10^4 (0.001)
P-5	Apple	Siddeswari	4.64×10^3 (0.001)	0 (0)	0 (0)	1.99×10^3 (0.0202)
P-6	Apple	Siddeswari	1.95×10^4 (0.0202)	0 (0)	0 (0)	4.54×10^3 (0.001)
P-7	Grape	Motijheel	7.44×10^5 (0.001)	8.95×10^3 (0.001)	0 (0)	0 (0)
P-8	Grape	Motijheel	2.47×10^5 (0.0011)	8.99×10^2 (0.001)	0 (0)	0 (0)
P-14	Grape	Banglamotor	3.4×10^3 (0.001)	0 (0)	0 (0)	0 (0)
P-10	Mango	Moghbazar	2×10^3 (0.0202)	0 (0)	0 (0)	0 (0)
P-11	Mango	Farmgate	6.89×10^5 (0.001)	6.6×10^3 (0.001)	0 (0)	8.5×10^3 0.001)
P-13	Mango	Banglamotor	4.99×10^3 (0.001)	0 (0)	0 (0)	0 (0)
P-9	Mango	Moghbazar	2.45×10^4 (0.0054)	1.9×10^3 (0.0202)	0 (0)	2.2×10^2 (0.0202)
P-15	Mango	Rampura	$1.59X10^2$ (0.0495)	0 (0)	0 (0)	0 (0)
P-12	Orange	Farmgate	2.66×10^6 (0.003)	3.6×10^4 (0.001)	0 (0)	8.99×10^5 (0.001)

P= Packed
All data were statistically analyzed and were found significant ($p<0.1$). Respective p-values have been indicated in parentheses.

Table 4. Antimicrobial susceptibility pattern of different bacterial isolates in the fruit juice samples (n=41)

Antibiotics	Bacterial isolates					
	E. coli n=14		*Klebsiella* spp. n=27		*Staphylococcus* spp. n=30	
	R	S	R	S	R	S
AMP (10µg)	17%	83%	74%	26%	93%	7%
CIP (5µg)	61%	39%	86%	14%	ND	ND
PIP (10µg)	24%	76%	88%	12%	75%	25%
CEF (30µg)	57%	43%	97%	3%	ND	ND
AMO (10µg)	26%	74%	72%	28%	92%	8%
IPM (30µg)	0%	100%	0%	100%	ND	ND
CHL (10µg)	33%	67%	21%	79%	ND	ND
TMP-SUL(25µg)	16%	84%	12%	88%	19%	81%
NALI (30µg)	71%	29%	61%	39%	ND	ND
VAN(30µg)	ND	ND	ND	ND	63%	37%
ERY (15 µg)	ND	ND	ND	ND	18%	82%

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

AMP= Ampicillin, AMO= Amoxicillin, CIP= Ciprofloxacin, CEF= Ceftriaxone, NALI= Nalidixic acid, IPM= Imipenem, ERY= Erythromycin, CHL= Chloramphenicol, TMP/SUL= Trimethoprime-sulfomethoxazole, VAN= vancomycine, PIP= Piperaciline
ND= Not done, n=Number of isolates , R= Resistant, S= Sensitive

is not allowed by safe food consumption standard (Andres *et al*., 2004). The highest coliform count for vendor fruit juice and packed juice samples were 1.58 $x10^6$ cfu/ml (sample V-19, collected from Farmgate, Table 2) and $3.6x10^4$ cfu/ml (sample P-12, collected from Farmgate, Table 3), respectively. In Bangladesh, Ahmed *et al*. (2009) showed the presence of *E. coli* ranging from 43 to >2400/100 ml in different types of vended squeezed fruit juices in Dhaka city. In India, the fruit juices were heavily contaminated by *E. coli* (Bagde and Tumane, 2011). Moreover, 4 vendor fruit juice samples (v-2, v-20, v-22 and v-25) exhibited the presence of fecal coliform in the present study (Table 2).

A new aspect of our investigation comparative to the previous related ones is the study of antibiogram of the pathogenic isolates found in the local juice samples. We found the *E. coli* isolates highly resistant against ciprofloxacin (61%), nalidixic acid (71%) and ceftriaxone (57%) as found from antibiotic susceptibility test (Table 4). *Klebsiella* spp. showed higher resistance against ampicillin (74%), ciprofloxacin (86%), piperaciline (88%), amoxicillin (72%), ceftriaxone (97%) and nalidixic acid (61%). Such drug resistance properties may render these pathogens cause serious health hazards because of ineffective treatment of the sufferers by the commonly prescribed antibiotics.

Prevalence of Staphylococci

Coagulase-positive staphylococci may cause human disease through the production of toxins. Effective levels of toxin formation require a high number of microorganisms (approximately 10^5-10^6 micro-organisms per ml of food) (IDF, 1994). A few reports have shown the prevalence of staphylococci in fruit juice samples (Ahmed *et al*., 2009; Tambekar *et al*., 2009). In our study, staphylococci were found in 30 out of 41 tested samples. The highest total staphylococcal count for vendor fruit juice sample ($6.95x10^5$ cfu/ml) was found in a papaya juice (sample V-21), collected from Rampura (Table 2). On the other contrary, The highest total staphylococcal count for packed fruit juice sample ($8.99x10^5$ cfu/ml) was found in an orange juice (sample P-12), collected from Farmgate (Table 3). Putting forward to public health risk, high rates of drug resistance were observed for *Staphylococcus* spp. against ampicillin (93%), piperaciline (75%), amoxicillin (92%) and vancomycin (63%) (Table 4).

Interestingly, coliform and pathogenic staphylococci were absent in four packed juice samples (samples no- P-7, P-8, P-10, P-13, P-14 and P-15) in our study (Table 1), and hence these samples were considered to be safe. Notably, these samples were prepared under good sanitation practices and stored in appropriate storage conditions. Besides, our results (Tables 2 and 3) showed the safer consumption of commercially packed juice than the freshly packed juice marketed locally. This might be due to the usage of automated machine directing aseptic processing as well as for the application of some preservatives. But some preservatives of higher concentrations can be harmful for our health (Bashar and Sabita, 2007). Therefore, further studies on the optimization of preservative concentrations should be performed.

Conclusions

Present study exhibited the microbiological status of available local fruit juices to ensure food safety for a precise control over public health risk. Although the microbial growth was found less frequently among packed fruit juice than the vended fruit juice samples, the microbial load in most cases were still above the standard limit for consumption. Additionally, the study of antibiogram to detect the drug resistant pathogens in fruit juices added new insight to the existing knowledge which was not conducted previously in Bangladesh. From the data presented in the current study, it can be concluded that the microbiological quality of most of the vendor and packed juice samples collected from different areas of Dhaka city were not satisfactory as fecal coliform, *E. coli*, *Klebsiella* spp. and *Staphylococcus* spp. were detected from the samples. The lack of knowledge on safe fruit juice preparation as well as the contamination sources can contribute to the elevation of pathogens in prepared juices. It is therefore, essential for the people who handle and prepare juices, to be properly trained on safe fruit handling technique. Regular monitoring of the quality of fruit juices for human consumption is recommended to avoid any future bacterial pathogen outbreak.

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