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Research article

Association of food safety knowledge and practice with microbiological contamination in street vended fruit juice: A cross-sectional study in Chattogram metropolitan city

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A B S T R A C T

In Bangladesh, the cost, taste, and warm, humid climate all encourage street juice consumption. However, practices for making street juices safely have come under threat, which has led to microbial growth in the juices. The purpose of this study is to look into the practices and cleanliness of the street sellers as well as the microbiological load. A pretested questionnaire was used to evaluate 71 roadside vendors' awareness of food safety practices. For the purpose of microbiological quantification, representative samples of 10 lemon juice and 10 sugarcane juice were obtained from 20 merchants out of the 71 respondents. The range of Total Plate Count (TPC) and Total Coliform Count (TCC) for lemon juice was 8.58×10^{2}-4.38×10^{4} cfu/ml and 6-34 cfu/ml respectively. For sugarcane juice the counts were 1.24×10^{4}-8.34×10^{4}cfu/ml and 17-54 cfu/ml respectively. Some food safety and hygiene practices and knowledge showed significant associations with the microbial load in the fruit juices including lack of safety knowledge on food poisoning (70%), presence of microorganism on dirty hands (70%), elevation of contamination on food by insanitary surroundings (70%) reluctance to wearing gloves (75%), poor waste management facilities (73%). Therefore, the study recommends focused food safety training to minimize microbial contamination.

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1. INTRODUCTION

Incommensurate practice of sanitation, personal hygiene, repugnant use of raw materials, improper processing, transportation and storage of food can facilitate infiltration of pathogenic organisms into food and cause food poisoning and/or food infection. Bacteriological contamination in food can cause a variety of health issues, ranging from mild abdominal

discomfort and flatulence to extreme food poisoning and dehydration. Consumption of contaminated foods leads to significant episodes of food-borne disease (Sivapalasingam et al., 2004).

Fruit juice is a common and popular non-fermented beverage worldwide, made from extraction or pressing of the natural fruits and often blended with water, ice, sugar, and other ingredients, or in some cases, consumed raw to quench thirst. Phytonutrients, antioxidants, vitamins and minerals are vital elements of fruits and have significant association in minimizing the risk of cardiovascular disease, diabetes, cataracts and some of the functional impairments associated with age (Liu, 2003).

Chattogram is the trading hub of Bangladesh, with a population of 2.5 million. The average daily temperature of Chattogram was recorded 26 ºC over the time period 1958-2012 and the mean annual humidity was 73.7% (Shahid et al., 2016; Biswas et al., 2019). Excessive demand of fruit juices from street vending sites and restaurants appears to be primarily attributed to lifestyle changes and concomitant urbanization and to remain cool during warm conditions (Dhanesh and Indira, 2018).

However, multiple observations have verified the presence of dangerous organisms such *E. coli*, *Salmonella enterica*, *Shigella* spp, *Citrobacter* spp, and *Bacillus cereus* in the roadside fruit juices. Typically, fruit juices with lower pH (4.5) inhibit microbial proliferation (Uddin et al., 2017). Unhygienic food handling practices or a lack of awareness regarding food hygiene and contamination may be the cause. There is a mismatch between street food vendors' handling practices and understanding of food safety, according to a number of studies on the subject that have been published in different nations (Bas et al., 2006; Omemu and Aderoju, 2008; Chukuezi, 2010; Muyanja et al., 2011). A study on the knowledge, attitude and practice (KAP) of the street vendors in Bangladesh revealed that the food vendors possessed basic knowledge and attitude towards food safety but lacked a thorough understanding of handling food safely (Hossen et al., 2020). Another study on knowledge, attitude and practice of chicken vendors in Dhaka city disclosed significant correlation between education and experiences with food safety practices (Siddiky et al., 2022). Food safety knowledge positively influences food handler's attitudes, and attitudes in turn considerably contribute to hygienic conditions for food safety, according to empirical research. Additionally, food handlers' attitude partially mediates the effect of knowledge on food hygiene and disease control actions, in addition to the indirect influence of their attitude on food safety knowledge and personal hygiene (Kwol et al., 2020). The aim and objectives of this study despite having similar type of studies in Dhaka city were- a) to investigate whether the geographic area of Chattogram impacts the KAP of the food handlers along with microbial load in the juices, b) to understand whether there is any association between microbial load and KAP of the street juice handlers in the studied zone of Bangladesh.

2. MATERIALS AND METHODS

Study area

Chattogram metropolitan city was divided into 16 regions based on the geographical position of the police stations under CMP (Chattogram metropolitan police). 10 areas were selected randomly for completion of the study.

Questionnaire and data collection

The knowledge, application and attitude was assessed with a questionnaire including 30 questions with a combination of open and closed as well as fixed choice questions. The questions were prepared in accordance with Codex code of practice on general principle of food and hygiene and regulation (Alimentarius, 2003). The study was conducted in Chattogram city from May 2019 to June 2019.

Sample collection

20 out of the 71 respondents were chosen at random using the Rand between function in Microsoft Excel 2007 in order to understand the relationship between food safety, cleanliness, and practice standards, and microbiological content in the juice sample. Lemon juice and sugarcane cane juice were chosen as samples of two different juice varieties. For the assessment by random sampling, a total of 30 samples were collected: 10 samples of lemon juice, 10

samples of the raw water used to make lemon juice, and 10 samples of sugarcane juice. Within an hour after collection, all of the samples were transported to the lab using sample collection bags stored aseptically at 4 ℃.

Sample processing

All the samples were liquid in state and no additional processing was necessary. The initial homogenate for total plate count was prepared by adding 1 ml sample to 9 ml 0.1% sterile buffered peptone water (BPW) and then serial dilution was performed by 10 fold dilution technique up to 10^{-3} recommended by APHA (Khan et al., 2015).

Bacteriological analysis

Total Plate Count (TPC) and Total Coliform Count (TCC) were performed to determine the total viable organism and total coliform present in the samples. Pour plate technique was followed with slight modification recommended in a study conducted in Bangladesh (Khan et al., 2015). Plate Count Agar (PCA) was used as a medium for Total Plate Count according to the aforementioned study. MacConkey agar was used for total coliform count (Uddin et al., 2017). Incubation was done for all Petri dishes at 37°C for 24-48 hours.

Data analysis

Statistical analysis was performed in Stata®13 software. Descriptive analysis was done to check the percentage of each factor related to the knowledge, attitude and hygiene practice of the street vendors. Analysis of Variance (ANOVA) was performed to find the association of the knowledge, attitude and hygiene practice of the vendors with microbial loads of different types of juice.

3. RESULTS AND DISCUSSION

Demographic profile of the vendors

All the participants (N=71) in the study were observed for their demographic attributes. The respondents were male (100%), the age group of the participants was in the range of 14-65 years. 21% of the participants did not receive any academic education (15 out of 71).

Knowledge, attitude and practice (KAP) towards food safety and hygiene

The KAP result is showed in the Table 1 and 2. Regarding the hygiene practice, about 75% of the participants were observed selling the juice with no hand gloves, 85% of the vendors did not wear any hair net while preparing the juice, 73% of the sellers had no mask. Washing hands during money transaction and food handling was not a common practice and almost 78% of the respondents did not wash their hands after these actions. Almost 54% of the vendors were observed to have left the juice storage container without lid thus attracting various insects and flies elevating the chance of contamination. Further, 70% vendors did not enfold the raw ingredients with any cover. Almost 51% of the vendors were noticed to have used municipal water and 66% vendors collected ice from middlemen with no confirmation of safety. About 70% participants had no waste disposal facility. Remarkably, 69% of those surveyed had no knowledge on food poisoning and living microbes on palmer sides of human. Almost 70% of interviewees were unaware that the surrounding environment can contribute to pollution.

Table 1. Knowledge of street vendors on food safety and hygiene (N=71)

Variable	Co-variable	n (Percentage)
Knowledge on food poisoning?	Yes	22 (30.99%)
	No	49 (69.01%)
Knows that dirty hands carry microorganism?	Yes	22 (30.99%)
	No	49 (69.01%)
Knows washing hands with soap removes microorganism?	Yes	45 (63.38%)
	No	26 (36.62%)
Knows surrounding environment can increase contamination?	Yes	21 (29.58%)
	No	50 (70.42%)

Table 2. Practice of street vendors on food safety and hygiene (N=71)

Variable	Co-variable	n (Percentage)
Any drain around the cart?	Yes	40 (56.34%)
	No	31 (43.66%)
Wear any hand gloves?	Yes	18 (25.35%)
	No	53 (74.65%)
Wear any hair net?	Yes	11 (15.49%)
	No	60 (84.51%)
Wear any mask?	Yes	19 (26.76%)
	No	52 (73.24 %)
Wash hands between handling and money swap?	Yes	14 (19.72%)
	No	55 (77.46%)
	Assistance	2 (2.82%)
Juice storage container has lid?	Yes	33 (46.48%)
	No	38 (53.52%)
Source of raw water	Boiled water	35 (49.30%)
	Municipal water	36 (50.70%)
Surrounding area free from contamination?	Yes	15 (21.13%)
	No	46 (64.79%)
	May be	10 (14.08%)
Raw & prepared juice kept apart?	Yes	49 (69.01%)
	No	21 (29.58)
	Non responsive	1 (1.41%)
Cover raw ingredients with anything?	Yes	21 (29.58%)
	No	50 (70.42%)
Utensils being washed after every purchase?	Yes	50 (70.42%)
	No	18 (25.35%)
	Disposable plastic	3 (2.82%)
Source of ice	Fish market	14 (19.72%)
	Homemade	9 (12.68%)
	Middlemen	47 (66.20%)
Proper WMS available?	Yes	19 (26.76%)
	No	52 (73.24%)

Association between food safety and hygiene practice and microbial load

Some of the safety and hygiene procedures were found to have a significant link with TCC and TPC (Table 3 and 4). The microbiological contamination was higher when hand gloves were not used (mean: 13.67 vs. 30.71 and 7466.33 vs. 38182.14, respectively), and there was a significant correlation between this and the hygiene practice (p=0.0095 and 0.0160 for TCC and TPC, respectively). Despite the fact that bacteria can pass through gloves to reach food, there was less transmission when gloves were used as a barrier (Montville et al., 2001). The participants were found to be reluctant to wear face masks, which is comparable to a study conducted in Universiti Kebangsaan Malaysia, despite the fact that face masks are a personal protection equipment to ensure safe food handling (Sani and Siow, 2014). High difference between the mean values of covering face with mask and not wearing mask was observed for TPC (mean: 6659.60 vs. 36403.33) and significant association was found with the practice (p=0.0135 and 0.0298 for TCC and TPC respectively). The practice of washing hands during food handling and transaction of money was found to have significant association with TCC (p= 0.0218). People in Bangladesh are not used to cleansing their hands after handling money, and many are unaware that they might be infected with a severe sickness caused by pathogenic bacteria (Angelakis et al.,

2014). Mean values for not washing hands to washing hands (36453.57 vs. 11499.67 for TPC and 30.21 vs. 14.83 for TCC) after every purchase and transaction of money also inferred to the similar repercussion. Approximately 80% of old two taka notes were found to be contaminated with total coliforms and only 16% of notes were found to be contaminated with fecal coliforms (Hosen et al., 2006). Source of raw water used in the preparation of juice had direct association with TCC (p=0.0473) and mean values of boiled to municipal water for TCC also indicated the same (17.14 vs. 30.15). Municipal water exiting the water treatment facility of Chittagong Water Supply and Sewerage Authority (CWASA) contained no total or faecal coliform (Zuthi et al., 2009). Coliforms may have infiltrated the water supplied to the customers in the form of juice either through the distribution system or by the poor practice of hygiene and improper handling by the street vendors. An analysis of the frequencies of the indicator bacteria in water samples from various regions of each municipal water system was conducted where various pollution indicator bacteria were detected, for example- total coliforms (TC), fecal coliforms (FC) etcetera (Clark et al., 1982). Another immensely significant correlation was found between ice and microbial load (p=0.0084 & 0.0059 for TPC and TCC respectively). A similar finding demonstrated that the bulk of ice used in juice preparation was supplied by intermediaries which was contaminated with coliform and the main source of water for ice was bore water (Reddi et al., 2015). Mean values of the source of ice confirmed the correlation between types of ice and microbial contamination in street vended juice for TCC (homemade=11.40 and middleman=30.33) and TPC (homemade= 2699.60, middleman= 37723.33). TCC and TPC were found to have a significant association with types of juice prepared in the street (p=0.0059 for TCC and p=0.0051 for TPC). Remarkable difference was observed between the mean values of sugarcane and lemon juice for both TCC and TPC (sugarcane=33.80, lemon=17.40 and sugarcane=44875.00, lemon=13059.80, respectively). From figure 1 and figure 2, it was observed that lemon juice had less bacterial load than sugarcane juice and the reason might be the acidic environment of lemon juice which is unfavorable for microorganism. Contaminated surrounding area, unwrapped raw materials and poor waste management system had remarkable association with the TCC (p=0.0064, 0.0177 and 0.0079, respectively) and the mean values of contaminated to clean surroundings, wrapped to uncovered raw materials and poor to good waste management facilities established the association (30.93 vs. 13.17, 8.33 vs. 28.65, 30.20 vs. 11.80, respectively). Uncovered juices attract flies from contaminated surroundings. Genres like *Enterobacter, Escherichia, Klebsiella, Proteu*s are considered to be vital microbes carried by blowfly and housefly microbiomes (Junqueira et al., 2017). Since, raw juices were observed contaminated by flies and insects, keeping the raw materials and prepared juices in close contact might have elevated the chance of cross-contamination in prepared juice. The finding in the study also implicated the significant association between TCC and cross-contamination (mean values for juices kept apart to juices kept in close contact=14.80 and 29.20 respectively, p=0.0461). Another crucial association was observed in both TPC and TCC with washing utensils after every purchase. The mean values for TPC and TCC (5939.60 vs. 36643.33 and 12.40 vs. 30.00, respectively) and p values indicated the same (p=0.0242 for TPC and p=0.0118 for TCC). Cleaning utensils with either soap water or hot water was considered to reduce the number of microorganisms and improve the safety (Bonfoh et al., 2006). Specific food safety knowledge about the preparation of street vended fruit juices, such as understanding of food poisoning, unwashed hands as carriers of germs, and the effect of soap water on bacteria, has proven significant associations with food borne pathogens and quality of juice. According to a study conducted in Hyderabad, India, there was a significant gap found between knowledge and practice of the street vendors, hence higher microbiological load in the fruit juices (Reddi et al., 2015). Based on the findings of this study, it can be inferred that the vendors lacked information about food safety and hygiene resulting in poor practices and a high microbial load. The recommended microbiological standards for fruit juices: Fruit juices should be prohibited if they contain toxins, high microbiological

concentration and agricultural pesticide remnants (Ahmed et al., 2018). Microbiological standards for juice and water samples are mentioned in the Table 5 and 6, respectively.

Table 3. Association of food safety knowledge with microbial contamination (n=20)

Variable	Co-Variable	TPC			TCC		
		Mean (cfu/ml)	SEM	p value	Mean (cfu/ml)	SEM	p value
Knows that dirty hands carry microorganism?	Yes	14049.71	7377.20	0.07	16.86	3.95	0.04
	No	37000.00	7749.89		30.31	3.88	
Knows washing hands with soap remove microorganism?	Yes	10766.33	5787.35	0.04	14.67	3.25	0.02
	No	36767.86	7488.99		30.29	3.70	
Knows surrounding environment can increase contamination?	Yes	1674.50	389.65	0.02	10.00	2.12	0.009*
	No	35790.63	6559.18		29.50	3.26	

Table 4. Association of food safety practice with microbial contamination (n=20)

Variable	Co-Variable	TPC			TCC		
		Mean (cfu/ml)	SEM	p value	Mean (cfu/ml)	SEM	p value
Any drain around the cart?	Yes	35790.63	6559.18	0.02	29.50	3.26	0.01*
	No	1674.50	389.65		10.00	2.12	
Wear any hand gloves?	Yes	7466.33	4845.93	0.01	13.67	2.87	0.01*
	No	38182.14	7180.86		30.71	3.60	
Wear any hair net?	Yes	8021.14	4110.38	0.01*	14.43	2.54	0.01*
	No	40246.15	7432.28		31.62	3.77	
Wear any mask?	Yes	6659.60	4994.23	0.02	12.60	3.08	0.01
	No	36403.33	6981.39		29.93	3.46	
Wash hands between handling and money swap?	Yes	11499.67	6306.17	0.06	14.83	3.36	0.02
	No	36453.57	7502.18		30.21	3.70	
Source of raw water	Boiled water	14371.14	7339.81	0.07	17.14	3.96	0.04
	Municipal water	36826.92	7801.52		30.15	3.92	
Juice Type	Sugarcane	44875.00	8756.14	0.01*	33.80	4.52	0.01*
	Lemon	13059.80	4819.48		17.40	2.67	
Surrounding area free from contamination?	Yes	6416.33	4085.02	0.01	13.17	2.57	0.01*
	No	38632.14	7106.79		30.93	3.56	
Raw &prepared juice kept apart?	Yes	10099.60	8430.50	0.07	14.80	5.07	0.04
	No	35256.67	6988.78		29.2	3.48	
Cover raw ingredients with anything?	Yes	1386.00	370.40	0.06	8.33	1.86	0.01
	No	33834.71	6464.28		28.65	3.18	
Utensils being washed after every purchase?	Yes	5939.60	4275.77	0.02	12.40	2.91	0.01
	No	36643.33	6952.55		30.00	3.45	
Source of ice	Homemade	2699.60	1068.61	0.01*	11.40	2.16	0.01*
	Middlemen	37723.33	6700.74		30.33	3.37	
Availability of proper Waste Management System	Yes	3379.60	1731.61	0.01	11.80	2.44	0.01*
	No	37496.67	6770.71		30.20	3.41	

*highly significant

Table 5. Microbiological limits for fruit juices according to FSSAI are listed below (Food Safety Standards Authority of India, 2011)

Microorganisms	**Maximum limit**
Total bacterial count	Not more than 50
Total coliform count	Absent in 100 ml

Table 6. The recommended limits of microbiological loads in the water by Department of Public Health Engineering (DPHE), Bangladesh and WHO are listed below (DPHE, 2018)

Parameters	DPHE standard	WHO guideline
Coliform (faecal)	0 cfu (N/100 ml)	0
Coliform (total)	0 cfu (N/100 ml)	0

4. CONCLUSION

Roadside juices are very well-liked by the general public due to their low cost and wide availability. However, the overall results of the current study showed that the juices were prepared and served by food vendors in unhygienic conditions, who had limited awareness of food safety and hygiene practices, which resulted in a greater microbial load than the permitted maximum. To ensure food safety and lower the risk of food borne disease, targeted food safety training and regular supervision are essential. As part of the study's future prospects, precise and verified tests using PCR and biochemical tests should be carried out for certain organisms.

Figure 1. Distribution of TPC in street vended fruit juices in ten zones of Chattogram city

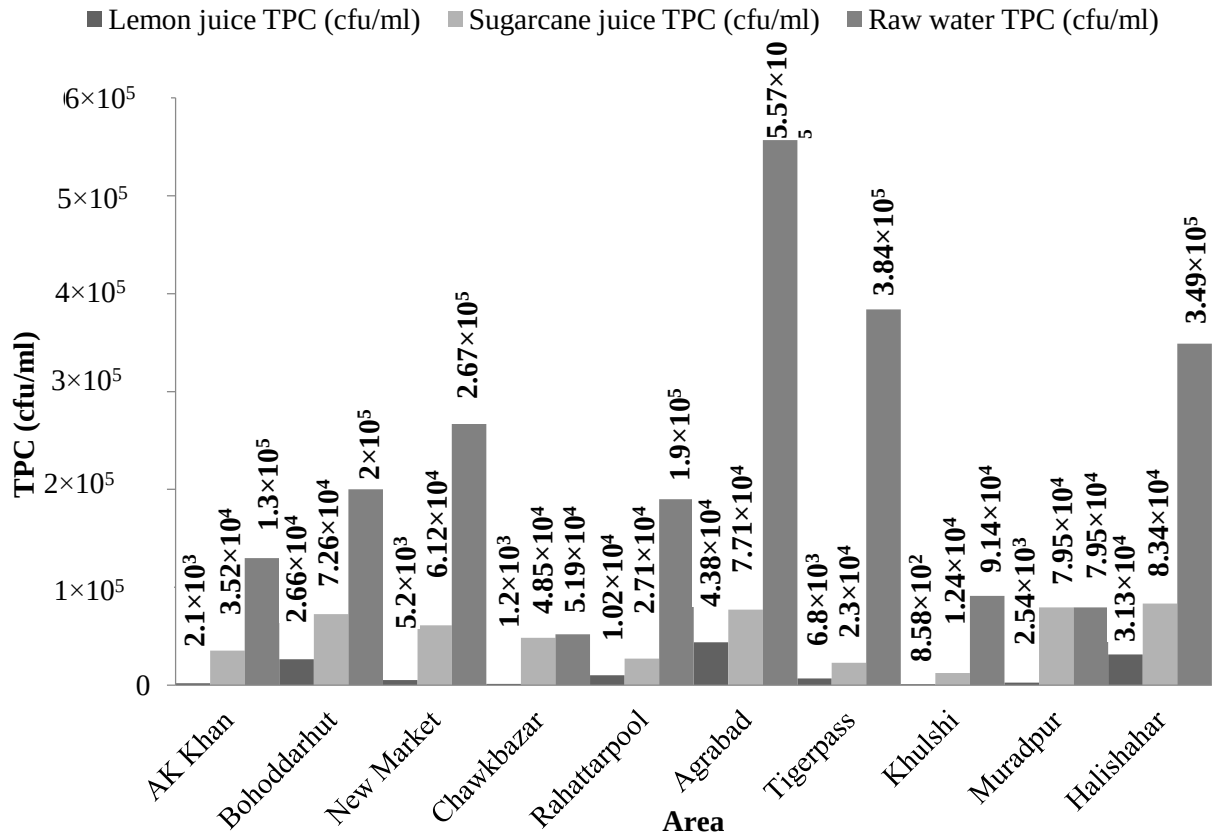
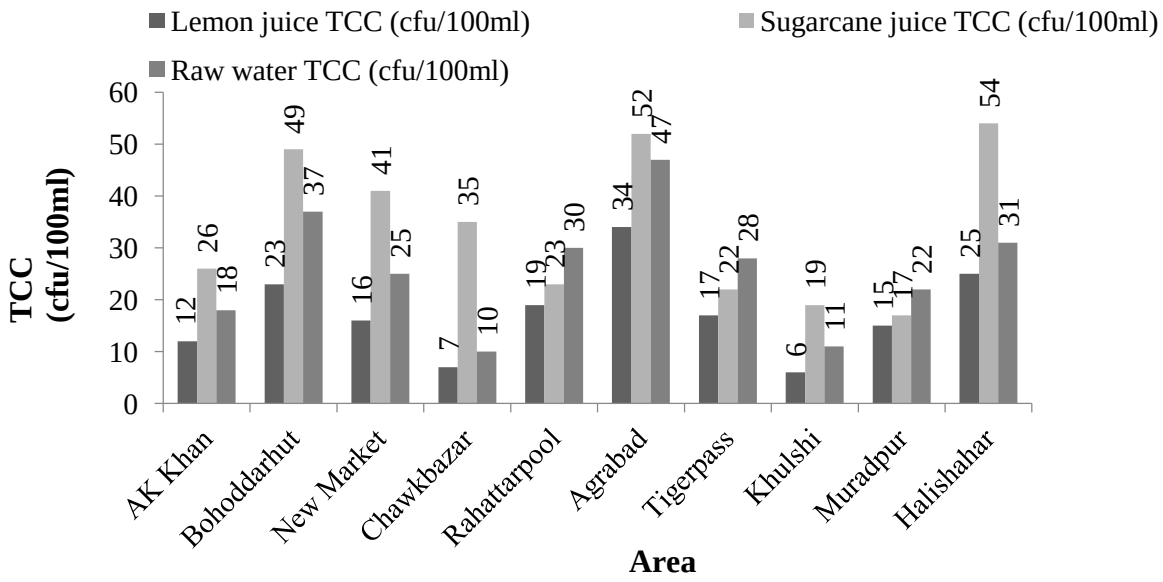


Figure 2. Distribution of TCC in street vended fruit juices in ten zones of Chattogram city



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