



Occurrence of antibiotic resistant *Escherichia coli* and *Staphylococcus aureus* in street-vended beverages accessible in an industrial zone of Bangladesh

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ARTICLE INFO

ABSTRACT

Article history:

Received 06 Sept. 2018

Received in revised form

28 Nov. 2018

Accepted 14 Dec 2018

Keywords:

Antibiogram profile;

Food safety;

Isolate;

Pathogen

The street-vended fruit beverages are considered as a source of disease causing microorganisms despite their high consumer demand and nutritional value. The study was conducted for the assessment of antibiotic resistance food-borne pathogens in street-vended fruit beverages available in an industrial zone of Bangladesh. A total of 50 samples constituting five different types of street-vended fruit beverages were collected from Gazipur city of Bangladesh. All the samples were examined for total viable microbial count followed by isolation, identification and antibiogram profile of *Escherichia coli* and *Staphylococcus aureus*. The total viable microbial count ranged from 2.3 to 8.9 log cfu/ml suggesting a significant bacterial load that exceeds the maximum permitted level of microbial load for freshly extracted fruit juices. Of the 50 samples, 29 (58.0%) were appeared as *Staphylococcus aureus* positive and 24 (48.0%) samples were contaminated with *Escherichia coli*. Antibiogram profile revealed *Staphylococcus aureus* resistance to penicillin (82.8%), amoxicillin (75.9%) and oxacillin (17.2%). The resistance to β -lactam antibiotic like oxacillin indicates the presence of highly pathogenic *Staphylococcus aureus* which is obviously a potent public health issue. In contrary, *Escherichia coli* isolates were resistant to amoxicillin (100%) and erythromycin (100%). All the isolates were found highly susceptible to ciprofloxacin and gentamicin. The antibiotic resistance pattern of the isolated organisms could complicate the treatment of food-borne illness in people. Development of holistic approach and their effective application could ensure the hygienic quality of these beverages as well as the health standard of the consumers.

Citation: Jahan M, Rahaman Sumon SMM, Md. Selim AS, Md. Rahman M. Occurrence of antibiotic resistant *Escherichia coli* and *Staphylococcus aureus* in street-vended beverages accessible in an industrial zone of Bangladesh. J food safe & hyg 2019; 5 (4): 237-247

1. Introduction

Street-vended thirst-quenching fruit/non-fruit based beverages are very popular among the people of all over the globe during summer season. There is a general believe among the consumers that these types of beverages retain unique nutritional as well as sensory attributes compared to their processed counterparts and have particular health benefits. Although these beverages are well recognized for their high nutritive value and vitamin and mineral

contents, their microbiological safety in terms of public health are always questionable. Several scientific reports from different countries such as Bangladesh, India, Pakistan, Egypt, Ethiopia, Tanzania, revealed the prevalence of pathogenic microbial contaminants in street-vended fruit beverages (SVFBs) (1-8). Fruits and water are the two major components in preparing and serving SVFBs. Fruits have been reported to contain bacterial counts up to 1.0×10^5 cfu/cm² on their surface (9,10). Consequently, improper washing of these fruits can lead to microbial hazards in SVFBs during extraction. The water available to the vendors could also be contaminated. It has been revealed in a recent review that both surface water and groundwater sources of Bangladesh are

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contaminated with different chemicals and pathogenic microorganisms such as *Escherichia coli* (*E. coli*), species of *Staphylococcus*, *Salmonella* etc. (11). Water from industrial region is also reported to contain hazardous chemicals as well as pathogenic microbes (12). Thus, 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 can also act as sources of contamination.

Food-borne diseases are now becoming a great concern involving a wide range of illnesses caused by bacterial, viral, parasitic or chemical contamination of food. Among bacteria *Clostridium botulinum*, *E. coli*, *Staphylococcus aureus* (*S.aureus*), *Listeria monocytogens*, *Bacillus cereus*, *Campylobacter jejuni* and species of *Salmonella*, *Shigella* comprise major part of food-borne pathogens (13,14). Food-borne diseases are transmitted through consumption of contaminated food or drink and affect the gastrointestinal tract. Consumption of fruit juices is also reported to cause food-borne illness (15-18). Although, coliforms are not normally the sources of serious illness but the presence of coliform bacteria in food is considered as the key indicator of the unhygienic preparation, processing and handling of food. In addition, this group of microorganism can be implicated in human infections, mainly immune compromised patients or when gastrointestinal barriers are violated, even normal 'nonpathogenic' strains of *E. coli* can cause infection (19). Besides this, *S. aureus* is considered as the third most important cause of food-borne diseases in the world among the reported food-borne pathogens (20).

Antimicrobial resistance of food-borne pathogens is an ongoing public health threat worldwide. Food has been identified as a dynamic environment for the continuing transfer of antibiotic resistance determinants between bacteria (21). In addition, resistance of these microorganisms to multi-drugs made this situation more of a concern to public health. Although there are substantial studies on antibiotic resistance profile of street-vended food-borne microorganisms in Bangladesh (22), such data on SVFBs are very limited.

With the grip of sudden and unprecedented urban growth, the population in different cities of Bangladesh has been increasing rapidly which lead to various street-vended foods more popular. There are few studies on microbiological quality of SVFBs have been carried out only in Dhaka city (4, 23, 24).

However, the other cities of Bangladesh which are paramount in terms of population and diversified industries are yet to be addressed. Gazipur is one of the largest industrial cities under the Gazipur district located in the central Bangladesh. This city is near the capital of Dhaka city with a population of 1.19 million. A substantial number of industries especially garments industries established by the government, private and international companies have been operating in this city. In addition, many higher education and research institutes are situated in this city. Consequently, large number people have been residing in this city which led to grow different types of business including street-vended foods. The street-vended food shops are generally located in the bus/railway station, busy market places, recreational places etc. Among the various types of street-vended foods, thirst-quenching fruit based beverages are very popular and in demand during summer season. Taken together, the present study was designed to evaluate the presence of antibiotic resistance food-borne pathogens in SVFBs available in a selected area of Bangladesh.

2. Materials and Methods

2.1. Study area and sampling

A total of 50 samples from five different types of SVFBs locally known as *Sharbat* were collected aseptically from ten vendors located at different places of Gazipur city, Bangladesh during summer season. About 40 ml of each sample was taken in a sterile 50-ml polystyrene conical tube (SPL, Korea) from the standard serving sold by the respective street food vendors. Chilled condition was maintained by keeping the sample tubes in icebox until transport to the laboratory for further analysis. The recipe of SVFBs was recorded while collecting the samples and presented in Table 1.

2.2. Sample preparation

The preparation of SVFB samples for respective microbial analysis was carried out according to the protocol described by Khan *et al.* (23). Since all the samples were liquid, instead of further processing 1 ml of each sample was transferred into sterilized glass test tube plugged with cork stopper containing 9 ml of 0.1% peptone water. After thorough mixing using a vortex-mixer, the sample solution was allowed to stand for approximately 5 min. Thus, the initially diluted sample was prepared.

Table 1. Five different types of SVFBs with their recipe and serving

Name of SVFBs	Recipe	Water source, serving and cleaning
Sugarcane	After cutting into 2/3 pieces, sugarcane is washed with water and syrup is extracted by pressing with a traditional hand driven (sometimes motor driven) machine. The syrup is then strained with a traditional strainer. Some vendors add lemon and spices for additional flavors, and also ice to cool the beverage.	<ul style="list-style-type: none"> • The vendors use tap water or tube well water depending on the availability. • The ice bar is collected from the nearby market. During serving, few pieces of ice are also poured into the glass. • Generally, the beverage is served in a water glass and after every serving the water is cleaned with water reserved in a plastic tank followed by rinsing with little fresh water. Disposable plastic water glass is also used by some vendors.
Lemon	Water, ice, slices of lemon juice, sugar, salt, tokma (basil seed), isabgul (psylliumhusk) are mixed in an aquarium like tank and is stirred intermittently using a plastic mug. Some vendors extract juice using a wood made lemon squeezer into water glass and then add water, crashed ice, salt, sugar and mix by shaking.	
Wood apple	After cracking, the pulp of the ripened wood apple is harvested by spoon and is kept in a stainless steel mixing bowl. The pulp is then mixed with water by hand and the released watery portion is strained with a traditional strainer. The filtrate is mixed with sugar and served with crashed ice. Some vendors used electric blender to mix the pulp with water.	
Aloevera	After careful peeling of the rind from the plant leaves and yellow layer just beneath the rind with a sharp knife, the aloe vera gel is collected into a plastic mug with the help of a spoon/fork. Then the other ingredients such as isabgul (psyllium husk), black cumin seed, water soaked tokma, horitoki, amloki powder, molasses and water extract of overnight soaked olatkombol (devil's cotton) are mixed properly with the Aloevera juice and served with ice.	
Mixed fruit	Pieces of different fruits such as apples, oranges, cherries, dates and lemons along with water, tang powder, sugar, black salt, tokma (basil seed), isabgul (psyllium husk) are added in an aquarium like tank and is stirred intermittently using a plastic mug.	

2.3. Total viable microbial load

The total viable microbial load (TVML) of each sample was enumerated using conventional standard plate count method. The plate count agar (PCA) media (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for determining plate counts of microorganisms in water by pour plate technique was used. After preparing the PCA media according to the manufacturer's procedure, the initially diluted sample was then further 10-fold serially diluted up to 10^{-8} . One milliliter of sample from each dilution was added to the sterile petri plates containing the agar media in duplicate. The sample and medium was mixed thoroughly by rotating each plate. After solidifying, plates were incubated at 37°C for 48 h. The plates having 30 to 300 bacterial colonies were selected to determine the TVML and the result was expressed as log cfu/ml.

2.4. Isolation and identification of *E. coli* and *S. aureus*

All the collected samples (50) from five different types of SVFBs were subjected to bacteriological examination for isolation and identification of *S. aureus* and *E. coli*. Isolation and identification of the bacteria was performed on the basis of culture properties, Gram staining reactions and biochemical tests as described by Quinn *et al.* (25). About 0.5 ml of each sample were inoculated into 4.5 ml of 1% peptone water broth and incubated at 37°C overnight for growth of the microorganisms. The bacterial growth was depicted by turbidity of the broth. After incubation, one loop of the cultured broth was streaked onto the different agar media plates including Mannitol Salt Agar (MSA) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and 5% Sheep blood agar for the isolation of *S. aureus*, and Eosin Methylene Blue (EMB) agar and MacConkey agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for *E. coli* isolation. The plates were then incubated at 37°C for 24 h and repeatedly sub-cultured to obtain pure culture. Among the isolates, two typical types of colony were identified as *S. aureus* and *E. coli* based on their specific cultural characteristics on selective bacteriological media. In addition, morphological characteristics of these microorganisms were identified by gram staining procedure. The picked up isolates based on the cultural characteristics were further confirmed by biochemical tests (catalase, coagulase, indole, methyl red, voges-praskauer and sugar fermentation tests). 2.5. Antibiotic susceptibility assay All the isolates were subjected to antibiotic sensitivity test by standard disc diffusion method on Muller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (26).

Sensitivity pattern of the isolates to eight different antibiotics (Penicillin, Erythromycin, Streptomycin, Amoxicillin, Oxacillin, Neomycin, Gentamicin and Ciprofloxacin) were determined. Isolates were classified into three groups according to the zone of inhibition described by CLSI standard (26): susceptible (S), intermediately susceptible (IS) and resistant (R) (Table 2).

2.6. Statistical analysis

All the data generated from the study were entered into a Microsoft spreadsheet and analyzed for the descriptive statistics.

Table 2. Disk diffusion zone diameter (mm) interpretative chart of eight commonly used antibiotics against *S. aureus* and *E. coli* (CLSI, 2018)

Antibiotics	<i>S. aureus</i>			<i>E. coli</i>		
	S	IS	R	S	IS	R
PG(10 units)	≥ 29	-	≤ 28	-	-	-
AMX(10 µg)	≥ 20	-	≤ 19	≥ 18	14-17	≤ 13
OX (1 µg)	≥ 13	11-12	≤ 10	-	-	-
CIP (5 µg)	≥ 21	16-20	≤ 15	≤ 31	21-30	≤ 20
E (15 µg)	≥ 23	14-22	≤ 13	-	-	-
S (10 µg)	≥ 21	15-20	≤ 14	≥ 15	12-14	≤ 12
Neo (30 µg)	≥ 17	13-16	≤ 12	≥ 17	13-16	≤ 12
GEN (10 µg)	≥ 15	13-14	≤ 12	≥ 15	13-14	≤ 12

PG: Penicillin G, AMX: Amoxicillin, OX: Oxacillin, CIP: Ciprofloxacin, E: Erythromycin, S: Streptomycin, Neo: Neomycin, GEN: Gentamicin, TXS: Trimethoprim-sulfamethoxazole, S: Susceptible, IS: Intermediately susceptible, R: Resistant, ND = Not Done

3. Results

As shown in Table 3, the average TVML of SVFB samples were ranged from 2.3 to 8.9 log cfu/ml. The highest average TVML was observed in sugarcane based beverage (6.6 log cfu/ml) followed by wood apple (5.9 log cfu/ml) and mixed fruit (5.4 log cfu/ml) based beverage samples. The lemon based beverage samples contained lowest average TVML (3.6 log cfu/ml).

Table 3. Total viable microbial load in SVFB samples

Name and number of SVFBs examined	Total viable microbial load (log cfu/ml)		Gulf Standards, 2000 (log cfu/ml)	
	Average	Range	Maximize load anticipated	Maximum load permitted
Sugarcane beverage (n= 10)	6.6±1.5	4.8 - 8.9		
Lemon beverage (n= 10)	3.6±1.2	2.2 - 5.6		
Wood Apple beverage (n=10)	5.9±1.0	4.5 - 7.8	3.7	4.0
Aloe vera beverage (n=10)	4.0±1.1	2.3 - 5.7		
Mixed fruit beverage (n=10)	5.5±1.1	4.1 - 7.9		

Among the five different types of SVFB, the highest percent (70%) of sugarcane based samples were positive for *S. aureus* whereas most (70%) of the Aloe vera based beverage samples were found positive for *E. coli* (Table 4). Biochemical identification confirmed 48% of the total tested samples were contaminated with *E. coli* and 58% were recognized as *S. aureus* positive (Table 4). The lowest contamination of both pathogens was identified in lemon based beverage.

As expected, colonies belong to *S. aureus* occurred β-hemolysis on blood agar media resulted golden yellow colored colonies and formed yellow colonies with yellow zones by fermenting MSA media. They were also found as Gram-positive cocci in clusters resembling bunches of grapes, catalase and coagulase tests positive. On the contrary, the isolates were identified as *E. coli* when appeared as pink colonies on MacConkey agar and produced metallic sheen on EMB agar. The isolates yielded yellow slope and butt with the production of gas in Triple Sugar Ion (TSI) agar tube and showed fermentation of all five sugars with acid and gas production. They were found as gram-negative, pink colored, large rod shaped in appearance and tested positive against indole and methyl-red tests but negative against voges-praskauer and oxidase tests.

Table 4. Frequency of isolated target bacteria from SVFB samples

Bacteria identified	Types of SVFB	Number of sample tested	Number of samples positive (%)	Total number of isolates (%)
<i>S. aureus</i>	Sugarcane beverage	10	7 (70.0%)	29 (58.0%)
	Lemon beverage	10	4 (40.0%)	
	Wood apple beverage	10	7 (70.0%)	
	Aloe vera beverage	10	6 (60.0%)	
	Mixed fruit beverage	10	5 (50.0%)	
<i>E. coli</i>	Sugarcane beverage	10	6 (60.0%)	24 (48.0%)
	Lemon beverage	10	2 (20.0%)	
	Wood apple beverage	10	5 (50.0%)	
	Aloe vera beverage	10	7 (70.0%)	
	Mixed fruit beverage	10	4 (40.0%)	

According to Table 5, the isolates belong to *S. aureus* were sensitive to erythromycin (100%), gentamicin (100%), ciprofloxacin (93.1%) and neomycin (79.3%) while the isolates were highly resistant to penicillin (82.8%) followed by amoxicillin (75.9%). About 51.7% of *S. aureus* isolates showed susceptibility to oxacillin whereas 31.0% were moderately susceptible and only 17.2% isolates were resistant. The antibiogram profile of *E. coli* isolates as shown in Table 5, demonstrated resistance against amoxicillin (100%) and erythromycin (100%) but showed susceptibility to ciprofloxacin (91.7%) and gentamicin (87.5%). The isolates were moderately susceptible to streptomycin (66.7%) and neomycin (75%).

Table 5. Susceptibility pattern of bacterial isolates from SVFBs to different antimicrobials

Antibiotics	Bacterial Isolates					
	<i>S. aureus</i> (n= 29)			<i>E. coli</i> (n=24)		
	S (%)	IS (%)	R (%)	S (%)	IS (%)	R (%)
PG(10 units)	0 (0.0)	5 (17.2)	24 (82.8)	ND	ND	ND
AMX(10 µg)	2 (6.9)	5 (17.2)	22 (75.9)	0 (0.0)	0 (0.0)	24 (100.0)
OX (1 µg)	15 (51.7)	9 (31.0)	5 (17.2)	ND	ND	ND
CIP (5 µg)	27 (93.1)	2 (6.9)	0 (0.0)	22 (91.7)	2 (8.3)	0 (0.0)
E (15 µg)	29 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (100.0)
S (10 µg)	12 (41.4)	17 (58.6)	0 (0.0)	0 (0.0)	16 (66.7)	8 (33.3)
Neo (30 µg)	23 (79.3)	4 (13.8)	2 (6.9)	0 (0.0)	18 (75.0)	6 (25.0)
GEN (10 µg)	29 (100.0)	0 (0.0)	0 (0.0)	21 (87.5)	1 (4.2)	2 (8.3)

PG: Penicillin G, AMX: Amoxicillin, OX: Oxacillin, CIP: Ciprofloxacin, E: Erythromycin, S: Streptomycin, Neo: Neomycin, GEN: Gentamicin, TXS: Trimethoprim-sulfomethoxazole, S: Susceptible, IS: Intermediately susceptible, R: Resistant, ND = Not Done

4. Discussion

The study was conducted to observe the TVML followed by isolation and identification of *E. coli* and *S. aureus* and their antibiogram profile. The TVML value, as shown in Table 3, indicates a significant bacterial load in SVFB samples tested. The highest level of bacterial count was represented by sugarcane based beverage sample whereas the lemon based beverage sample accounted the lowest number. This lower number of bacterial count may be due to acidic nature of lemon based beverage. The main reason behind the high density of bacteria in all the samples could be lack in proper hygienic practices, microbial quality of water used in diluting and dressing of the beverages, quality and preservation of fruits. The TVML observed in the SVFB samples of the present study greatly varied with the other analogous studies carried out in different parts of the globe, and a range of 2 to 9 log cfu/ml was revealed in the published reports (Table 6). It is alarming from the public health point of view that most of the SVFB samples examined in the present study or in the other studies contain high number of viable bacteria as compared to the maximum permitted level of microbial load for freshly extracted fruit juices stated in the Gulf Standards (27).

The present study also concentrated on isolating *S. aureus* and *E. coli* as these are considered as the most harmful food-borne pathogens for human. In addition, *E. coli* is also used as indicator microorganism of sanitary quality of food and water. The results of biochemical tests confirmed the tested isolates as *S. aureus* and *E. coli*; however, molecular identification of these isolates was indispensable. About half of the SVFBs samples were contaminated with food-borne pathogens either *S. aureus*, *E. coli* or both (Table 4). The possible reasons for this could be the poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils, contaminated water and ice, poor personal and domestic hygiene, peeling of fruits before hand, shop in crowded place and dust particles (28). Safe storage temperatures are rarely applied to SVFBs and prepared in open place with lack of protection against sun, dust and rain. In addition, the location of SVFB shops by the side of a busy road with heavy vehicular traffic (airborne particles) or by the side of waste disposal system or in bus/railway station or in busy market and recreational places seems to add to the contamination. Also houseflies and fruit flies due to sewage may contaminate beverages as they attract the flies (29). However, comparatively higher pathogenic contamination (93.3%) of SVFBs was reported by another study in Vidarbha, India (30).

As shown in Table 4, *S. aureus* was the most frequent isolates (58.0%) found in different SVFBs tested. Although some studies reported comparatively lower frequency (7.9 - 23.8%) of *S. aureus* (30, 31), a study in India reported that dirty clothing and contaminated hands of vendor might be attributed to 60% isolation of *S. aureus* (28). The higher frequency of isolation of *S. aureus* might be associated with improper personal hygiene and contaminated hands of vendors as this bacterium usually is related to human skin and clothing. Besides, in most cases vendors rarely worn gloves, frequently touched their skin and clothing and served without washing hands or washing their hands in common reserved water in a plastic tank.

About 48.0% of SVFBs in this study were contaminated with *E. coli* (Table 4). The presence of coliform in fruit juices is not allowed by safe food consumption standard (32). A study in Bangladesh showed that 99% street-vended juice samples had *E. coli* contamination (31) whereas another two studies in India revealed 33.3-40% contamination (28, 30). Isolation of this organism in this study is an indication of fecal contamination of the SVFBs tested because enteropathogens are known to survive on the hands for three hours or longer (33, 34).

Table 6. Global scenario of microbiological status in SVFBs

Study area	Range of total microbial load and list of major bacteria isolated	Reference
Bangladesh (Dhaka city)	3.9 to 9.0 log cfu/ml <i>E. coli</i> , <i>S. aureus</i> , <i>S. lactis</i> , <i>P. aeruginosa</i> , <i>P. vulgaris</i> , <i>M. luteus</i> , <i>E. aerogenes</i> , <i>B. cereus</i> , <i>K. pneumoniae</i> , <i>S. typhimurium</i> , Species of <i>Shigella</i> , <i>Citrobacter</i> , <i>Vibrio</i> , <i>Yersinia</i> .	(2, 4, 23, 24, 31, 41)
India (Allahabad city, Amravati city, Hyderabad city, Vidarbha, Kurukshetra, Ranchi city, Chidambaram)	5.1 to 7.4 log cfu/ml <i>S. aureus</i> , <i>B. cereus</i> , <i>Listeria</i> sp., <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. typhi</i> , <i>K. pneumoniae</i> , <i>C. freundii</i> , <i>P. agglomerans</i> , <i>L. adecarboxylata</i> , Species of <i>Proteus</i> , <i>Vibrio</i> , <i>Enterobacter</i> , <i>Shigella</i> , <i>Serratia</i>	(6, 28, 30, 42-45)
Pakistan (Multan, Peshawar city, Khyber Pakhtunkhwa, Lahore city)	2.5 to 8.0 log cfu/ml <i>S. aureus</i> , <i>B. cereus</i> , <i>M. luteus</i> , <i>E. coli</i> , Species of <i>Enterobacter</i> , <i>Salmonella</i> , <i>Klebsiella</i>	(5, 46, 47)
Egypt (Cairo)	2.0 to 6.0 log cfu/ml <i>E. coli</i> , <i>S. aureus</i> ; <i>L. monocytogenes</i> ; <i>Y. enterocolitica</i> ; <i>B. cereus</i> ; <i>S. typhimurium</i>	(1)
Ethiopia (Arba Minch, Amhara)	5.1 to 5.5 log cfu/ml <i>E. coli</i> ; <i>S. aureus</i> ; <i>B. cereus</i> ; Species of <i>Salmonella</i> , <i>Shigella</i>	(9, 48)
Nigeria (Ogun state)	4.6 to 4.8 log cfu/ml <i>S. aureus</i> ; <i>E. coli</i> ; <i>P. aeruginosa</i> , <i>B. cereus</i> , <i>B. subtilis</i> , Species of <i>Klebsiella</i> , <i>Leuconostoc</i> , <i>Enterobacter</i> , <i>Salmonella</i> , <i>Streptococcus</i> , <i>Proteus</i> , <i>Serratia</i>	(3)
Tanzania (Dar es Salaam)	2.3 to 8.5 log cfu/ml <i>E. coli</i> , <i>S. aureus</i> ; <i>P. aeruginosa</i> , <i>Salmonella</i> sp.	(7, 49)
Bangladesh (Gazipur city)	2.2 to 8.9 log cfu/ml <i>E. coli</i> , <i>S. aureus</i>	This study

B: *Bacillus*; C: *Citrobacter*; E: *Escherichia/Enterobacter*; K: *Klebsiella*; L: *Leclercia/Listeria*; M: *Micrococcus*; S: *Staphylococcus/Streptococcus/Salmonella/Shigella*;
P: *Pseudomonas/Proteus/Pantoea*; Y: *Yersinia*

Inadequate hand washing by vendors and poor knowledge about good hygiene practices (GHP) could facilitate the contamination and transmission of this pathogen via food to humans. In addition, the ice and water added during preparation and absence of good manufacturing practices were likely to provide possible sources of additional bacterial contamination (35).

The findings on antimicrobial susceptibility of all the isolated *S. aureus* are almost in agreement with the previous studies where *S. aureus* were found highly susceptible to erythromycin (71.4 - 82%), gentamicin (88.5 - 100%) and ciprofloxacin (100%) (30, 36). In contrary, their resistance to penicillin and amoxicillin indicates that *S. aureus* isolates produce beta-lactamase, an enzyme that inactivates penicillin and others closely related antibiotics (37). The antibiogram pattern of *S. aureus* isolates against oxacillin are also in agreement with Sina *et al.* (38) who also reported almost similar resistance pattern (15%) of *S. aureus* against oxacillin isolated from street food. However, some other studies revealed comparatively higher resistance (28.6-90%) to the analogous antibiotics (36, 39). This indicates some isolates of *S. aureus* from street-vended beverage have *mecA* gene, a gene responsible for resistance to beta-lactam antibiotics like methicillin, oxacillin and cephalosporins. However, the emergence of methicillin resistant *S. aureus* (MRSA) strains as staphylococcal food poisoning and nosocomial infections has become a major concern in medical practice (40).

The antibiogram profile of *E. coli* isolates is comparable with the findings revealed by Temesgen *et al.* (36). This study also reported susceptibility of *E. coli* isolates to ciprofloxacin (100%) and gentamicin (90.5%). However, susceptibility of both *S. aureus* and *E. coli* isolates to ciprofloxacin and gentamicin suggesting these antimicrobials as choice of drugs for the treatment of food-borne illness in the study area. Caution should be exercised while treating food-borne infection by *S. aureus* with methicillin group of drugs as some of the isolates developed resistant against this group.

5. Conclusion

The outcomes of the current research enable us to identify the SVFBs in the study area those are highly loaded with common food-borne pathogens in particular *E. Coli* and *S. aureus*. The substantial numbers of SVFBs contaminated with such food-borne pathogens could place consumers at high risk of food-borne infections. The antibiotic resistance pattern of the isolates could also complicate the future treatment of the food-borne illness. There are several possible ways

by which the rate of microbial contamination in SVFBs could be reduced. Some of them are mandatory food safety training of the vendors and display of the official certificate in front of the shop, marking the vending sites based on the sanitary conditions, development and implementation of adequate guidelines. In addition to this, designing of a well-structured vending cart with different compartment could also be very useful to avoid cross contamination among different beverages and also to protect from environmental contamination. Therefore, it would be highly recommended to apply a holistic approach on the entire vending procedure starts from collection of fruits, water to manufacture procedure and serving. Indeed, regular monitoring of the quality of SVFBs is required to improve the health standards of consumers.

Conflict of interest

The authors declare no conflict of interest with any organization, financial, or other regarding the material discussed in the manuscript.

Acknowledgement

The authors acknowledge the financial support from the Research Management Committee (RMC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

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