

**MICROBIAL PROFILING AND ANTIBIOGRAM OF BACTERIA ISOLATED FROM
VENDED FRUIT JUICES AND SAUCES CONSUMED IN CAFES AND
RESTAURANTS IN NARSINGDI CITY, BANGLADESH**

ABSTRACT

Fresh fruit juices from cafes and restaurants are a popular beverage in Narsingdi city of Bangladesh during summer season. Food-borne outbreaks may arise from them since harmful germs from the unsanitary surroundings of the food preparation area easily infect them. The purpose of the current study was to identify the microbiological characteristics of various fruit juices and sauces consumed in different cafes and restaurants in Narsingdi City, Bangladesh. The detection of total viable bacterial load, total fungi count, coliforms, and some other pathogenic bacteria was checked in a total of thirty-nine juice and sauce samples, specifically of six categories of juices (orange, lemon, pineapple, sugarcane, papaya, and apple) and three categories of sauces (tomato sauce, mustard sauce, and tamarind sauce). Total viable bacteria in these samples ranged from 10^4 to 10^8 CFU/mL. Sixty percent of the samples had total coliforms (including *Escherichia coli* and *Klebsiella* spp.), all of which alarmingly exceeded the conventional bacteriological limits (1.0×10^4 CFU/mL) advised for fruit juices. *Vibrio* spp., *Salmonella* spp., and *Staphylococcus* spp. were identified as harmful microorganisms. Standard biochemical, microscopic, and cultural assays were used to identify each of these bacterial isolates. Sulfomethoxazole-trimethoprim, Ciprofloxacin, Tetracycline, Nalidixic Acid, Gentamicin, Ceftriazone, Ampicillin, and Netilmicin were among the drugs against which the isolates showed variable levels of drug resistance. The microbiological evaluation of these well-known ready-to-drink goods is therefore constantly required; otherwise, they could pose health risks.

Keywords: Microbial profiling; Antibioqram; Fresh fruit juices; Sauces; Cafes and Restaurants; Narsingdi; Bangladesh.

INTRODUCTION

Fruit juice is a popular soft drink made from the pulp of several fresh fruit varieties (**Ahmed et al., 2018**). In Bangladesh, millions of people drink fresh juices from street cafes and restaurants during summer seasons, making them a popular beverage. Juices of orange, lemon, pineapple, sugarcane, papaya, and apple are some of the most commonly consumed juices. These are valued not just for their vitamins, minerals, and nutritional content but for their pleasant flavor and fragrance. The fruit provides the juice's inherent flavor. Numerous components included in fruit juices are beneficial for preserving good health. Fruits generally contain flavonoid glycosides, dietary fiber, calcium, vitamin C, carotenoids, carotene, phenolic acids, amino acids, aromatic compounds, polyphenols, potassium, vitamin D, a little amount of salt, and fat, despite the variety in their constituents (**Amarowicz et al., 2009**). These fruit juice constituents help to create collagen, cartilage, blood vessels, and muscles and have been shown to help prevent heart disease, certain types of cancer, diabetes, cataracts, Alzheimer's disease, and asthma (**Amarowicz et al., 2009; Liu, 2013**). Fruit juices have become very popular all over the world due to their health benefits as well as the sensation of increased energy they provide when consumed. Because vendors require relatively little initial investments, there has been a notable increase in the consumption of both restaurant-based foods and beverages, particularly in low and medium-income countries (LMIC). According to **Sharma et al. (2020)**, the cost of these foods remain within the price range of those people with lower socioeconomic status. Unfortunately, consuming such tainted fruit and vegetable juices has been linked to a number of reports of significant morbidity and mortality each year worldwide (**Callejón et al., 2015; Kechero et al., 2019**). The majority of Bangladesh's urban areas offer vendor-based fruit juices and beverages. Tea, coffee, fruit juices, sherbets, and a variety of carbonated soft drinks are consumed here on the streets or at the roadside cafes and restaurants. Many people of various ages and financial levels use these freshly squeezed juices, especially during the summer (**Ahmed et al., 2009**). However, there is a risk of increased microbiological contamination of the fruit juices due to the vendors' inadequate training on food safety and hygiene procedures. Both Gram-positive and Gram-negative bacteria, including *Salmonella enterica serovar Typhi*, *Escherichia coli*, *Pseudomonas species*, *Staphylococcus aureus*, and *Vibrio cholerae*, are commonly found in street juice. Many diseases, including typhoid fever, food poisoning, gastroenteritis, enteric fever, and diarrhea, are caused by these pathogens and can result in life-threatening circumstances. These cases are seen all over the world (**Aneja et al., 2014;**

Verma and Gaur, 2017). Recent years have seen a significant increase in the prevalence of medication failure brought on by antibiotic resistance, which poses serious risks to public health in poor nations like Bangladesh (Tabassum and Uddin, 2016). The natural transfer of drug-resistance genes along with non-prescription antibiotics has already been identified as a contributing factor to this type of drug resistance (Molton, 2013). Antibiotic-resistant bacteria have been found in apple and orange juice samples in a number of investigations (Tadesse *et al.*, 2018; Sarker *et al.*, 2018; Mandal, 2018; Kebede *et al.*, 2018). In considering these facts, the current study first evaluated the microbiological quality of these refreshing drinks that are sold in the city of Narsingdi, Bangladesh. It next outlined the pattern of antibiotic resistance of the bacteria that were identified from these samples.

MATERIAL AND METHODS

Collection and Processing of Samples

Total 30 fruit (orange, lemon, pineapple, sugarcane, papaya & apple) juice samples, and 3 tomato sauce, 3 mustard sauce, 3 tamarind sauce, were collected specially from different cafes and restaurants of Narsingdi city including Narsingdi Sadar, Velanogor, Baniachol, Saheprotap and Satirpara. Samples were collected during the period of January 2020 to December 2020. Sample were collected in a sterile pot with aseptic condition and delivered to the lab as soon as possible using the FDA's recommended methodology (2013) to the BCSIR Microbiology Lab, Dhaka.



Figure 1. Sample collection site.

Estimation of Total Number of Viable Fungi and Bacteria

The total viable count using nutrient agar was calculated using the spread plate method. For counting bacteria and fungus, this medium is special and nonselective. In accordance with the American Public Health Association's (APHA) sample dilution requirements, the samples were diluted ten times up to 10^{-4} (APHA, 1998). 0.1 mL of each sample from the dilutions 10^{-2} and 10^{-4} were put over the nutritional agar (NA) in order to count the total viable bacterial count (TVBC). For twenty-four hours, the NA plates were incubated at 37°C . 0.1 mL of each of the 10^{-2} and 10^{-4} dilutions of all samples were distributed over the MacConkey agar and incubated for 24 hours at 37°C in order to estimate the total coliforms (APHA, 1998; Cappuccino and Sherman, 2014).

Estimation of *Staphylococcus* spp., *Klebsiella* spp., and *E. coli*

The MacConkey agar and Eosin Methylene Blue agar plates were utilized to estimate the levels of total and fecal coliform. 100 μl of each sample was plated from the 10^{-2} and 10^{-4} dilutions, and they were incubated for 24 hours at 37°C . The identical process was used on fresh Mannitol Salt Agar (MSA) to determine the total number of *Staphylococcus* species.

Estimation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.

The enrichment process was used to isolate and quantify *Shigella*, *Vibrio*, and *Salmonella* species. In order to enhance *Salmonella*, *Shigella*, and *Vibrio* spp., 1 mL of samples were placed into 9 mL of selenite cysteine broth (SCB) and alkaline peptone water (APW), and the mixture was then incubated at 37°C for 4-6 hours. To isolate *Salmonella*, *Shigella* and *Vibrio* species, respectively, an aliquot of 0.1 mL of each sample from the 10^{-2} and 10^{-4} dilutions was distributed onto *Salmonella-Shigella* (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar. Within 24 to 48 hours of incubation at 37°C , typical colonies began to emerge (Alfrad, 2007).

Biochemical Identification

Finally, a number of biochemical analyses were carried out to verify each isolate's identity. In accordance with conventional protocols, a number of biochemical assays, including the Methyl Red-Voges-Proskauer (MR-VP) test, the Citrate test, the Indole test, the Urea test, and the Triple Sugar Iron (TSI) test, were conducted to identify bacterial isolates (Cappuccino and Sherman, 2014).

Antibiotic Susceptibility Test

Using antibiotic discs (Neo-Sensitabs, Rosco, Denmark) and Mueller-Hinton Agar (Difco, Detroit, MI), isolates were examined using the disc diffusion assay (Bauer *et al.*, 1966) against ten popular antibacterial medications. In short, two milliliters of Mueller-Hinton broth were mixed with one colony of each isolate, incubated for four hours, and the culture turbidity was then corrected to a McFarland standard of 0.5. The whole surface of the agar was covered with sterile cotton swabs that had been dipped into the solutions. The zones of inhibition's widths were measured following incubation and classified as sensitive, intermediate, and resistant.

Table 1: Antibiotic discs used in this study

Sl. No.	Antibiotic	Letter Code	Quantity	Source
1	Sulfomethoxazole-trimethoprim	SXT	30 µg	Oxoid Ltd, UK
2	Ciprofloxacin	CIP	5 µg	Oxoid Ltd, UK
3	Tetracycline	TET	25 µg	Oxoid Ltd, UK
4	Nalidixic acid	NA	30 µg	Oxoid Ltd, UK
5	Gentamicin	GEN	30 µg	Oxoid Ltd, UK
6	Ceftriazone	CEF	10 µg	Oxoid Ltd, UK
7	Ampicillin	AMP	30 µg	Oxoid Ltd, UK
8	Netilmicin	NET	30 µg	Oxoid Ltd, UK

Table 2: Zone size measured for specific antimicrobial agent

Sl. No.	Antibiotics	Sensitivity (mm)	Intermediate(mm)	Resistance(mm)
1	Sulfomethoxazole-trimethoprim	>18	14-17	<13
2	Ciprofloxacin	>18	13-17	<12
3	Tetracycline	>23	16-20	<22
4	Nalidixic acid	>21	18-20	<17
5	Gentamicin	>21	16-20	<15
6	Ceftriazone	>16	14-15	<13
7	Ampicillin	>19	16-18	<15
8	Netilmicin	>15	13-14	<12

STATISTICAL ANALYSIS

SPSS version 16 (the statistical package for social sciences) was used to analyze the data. The significance of the differences was examined using the Chi-square or Fisher's exact test. Statistical significance was defined as a p-value 0.05.

RESULTS AND DISCUSSION

The goal of the current study was to identify the handling procedures and bacterial quality that make unpasteurized fruit juices susceptible to contamination in the Narsingdi city of Bangladesh. It was found that all 30 fruit juices and 09 different sauce samples were highly contaminated with a wide variety of bacteria.

Total Viable Bacteria and Fungi count

To identify microorganisms, a series of biochemical tests were conducted; the outcomes are shown in Table 4. The results of the three independent runs of the biochemical tests were determined to be repeatable. There is only one representative data displayed. The total fungal count ranged from 2.19×10^4 CFU/ml (Orange juice 4) to 4.48×10^6 CFU/ml (Sugarcane juice 5) and from 2.70×10^5 CFU/ml (Tamarind sauce 3) to 3.26×10^6 CFU/ml (Tomato sauce 1) in various fruit juices, while Total viable bacterial count ranged from 2.18×10^4 CFU/ml (Orange juice 4) to 9.69×10^8 CFU/ml (Lemon juice 4) in vended fruit juice samples and in various sauces it was ranged from 1.02×10^5 CFU/ml (Tamarind sauce 1) to 2.84×10^8 CFU/ml (Tomato sauce 1), indicating the presence of both bacteria and fungi species in significant amounts (Table 3). This result was almost identical with the study of **Noor *et al.* (2013)**. In the case of total viable bacteria, the Gulf Standard No. 1016/2000 (**Emirates Authority for Standardization and Metrology (ESMA), United Arab Emirates, 2000**) states that the maximum bacterial load allowed is 1.0×10^4 CFU/mL. It was clear from Table 3 that the majority of fruit juices and sauces across all nine categories did not satisfy this requirement. According to Gulf Standard No. 1016/2000, **Asghar *et al.* (2018)** showed that 20% of samples had less TVBC of maximum bacterial load and that almost 80% of fresh vended fruit juice samples had a higher value. Furthermore, a higher TVBC value could suggest that the fresh juices were made in an unsanitary condition (**Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000**).

Twenty-two out of thirty-nine samples had coliform; the highest count was 4.21×10^3 CFU/mL in lemon juice 5, while the lowest count was 1.00×10^2 CFU/mL in pineapple juice 1 (Table 3). The presence of coliform bacteria, particularly *E. coli* and *Klebsiella* spp., in these samples suggested that fecal contamination might be present. *Salmonella* spp. were found in 23 samples and *Vibrio* spp. in 29 samples (Table 3), all of which were alarmingly beyond the **FDA's suggested standard limit (2013)**. **Islam *et al.* (2014)** in Dhaka, Bangladesh, and **Sharma *et al.* (2020)** in India also

found a significant load of these bacteria. *E. Coli*, *Salmonella*, *Klebsiella*, and *Vibrio* species have the ability to cause foodborne illnesses, hence their presence in several samples was a sign of impending health risks (Uddin *et al.*, 2020). The primary cause of these problems may have been the overall lack of knowledge among street vendors on food rules, good hygiene practices (GHP), and the causes of diarrheal illnesses. All of these factors increase the risk of food contamination in cafes and restaurants. In addition, the vendors lack necessary auxiliary services including adequate and high-quality water supply and facilities for disposing of trash (Malik *et al.*, 2020).

Table 3: Microbiological assessment of different types of fruit juice samples

Sl No	Sample name	TVBC (CFU/mL)	TFC (CFU/mL)	<i>E. coli</i> (CFU/mL)	<i>Klebsiella</i> spp (CFU/mL)	<i>Staphylococcus</i> spp (CFU/mL)	<i>Vibrio</i> spp (CFU/mL)	<i>Salmonella</i> spp (CFU/mL)
1	Tomato sauce 1	2.84×10 ⁸	3.26×10 ⁶	1.80×10 ³	2.40×10 ⁴	3.00×10 ⁴	5.00×10 ³	1.00×10 ²
2	Tomato sauce 2	1.32×10 ⁸	2.16×10 ⁶	1.10×10 ³	2.11×10 ⁴	2.99×10 ⁴	4.21×10 ³	0.91×10 ²
3	Tomato sauce 3	1.71×10 ⁸	3.01×10 ⁶	1.20×10 ³	2.45×10 ⁴	3.10×10 ⁴	3.20×10 ³	1.02×10 ²
4	Mustard sauce 1	1.82×10 ⁶	4.11×10 ⁵	1.29×10 ²	2.95×10 ³	3.19×10 ³	3.29×10 ²	0.62×10 ²
5	Mustard sauce 2	2.12×10 ⁶	3.17×10 ⁵	1.99×10 ²	2.35×10 ³	3.17×10 ³	3.20×10 ²	0.65×10 ²
6	Mustard sauce 3	2.22×10 ⁶	3.12×10 ⁵	1.92×10 ²	2.25×10 ³	3.13×10 ³	3.23×10 ²	0.66×10 ²
7	Tamarind sauce 1	1.02×10 ⁵	3.21×10 ⁵	1.19×10 ²	2.15×10 ³	2.39×10 ³	3.19×10 ²	0.42×10 ²
8	Tamarind sauce 2	1.12×10 ⁵	2.71×10 ⁵	1.29×10 ²	2.35×10 ³	2.19×10 ³	2.29×10 ²	0.43×10 ²
9	Tamarind sauce 3	1.17×10 ⁵	2.70×10 ⁵	1.21×10 ²	2.15×10 ³	2.13×10 ³	2.25×10 ²	0.40×10 ²
10	Orange juice 1	3.84×10 ⁴	3.26×10 ⁴	0	0	3.00×10 ²	5.00×10 ²	0
11	Orange juice 2	4.14×10 ⁴	4.16×10 ⁴	0	0	2.90×10 ²	4.00×10 ²	0
12	Orange juice 3	4.10×10 ⁴	3.16×10 ⁴	0	0	2.91×10 ²	3.99×10 ²	0
13	Orange juice 4	2.18×10 ⁴	2.19×10 ⁴	0	0	2.15×10 ²	2.19×10 ²	0
14	Orange juice 5	6.12×10 ⁴	3.17×10 ⁴	1.11×10 ²	1.15×10 ²	2.35×10 ³	2.13×10 ³	0
15	Lemon juice 1	2.84×10 ⁸	3.26×10 ⁶	0	4.76×10 ⁵	2.52×10 ⁵	0	0
16	Lemon juice 2	4.04×10 ⁸	4.30×10 ⁶	0	9.00×10 ³	3.00×10 ⁴	0	1.00×10 ²
17	Lemon juice 3	2.60×10 ⁸	2.70×10 ⁶	0	2.10×10 ⁴	3.30×10 ⁵	0	0
18	Lemon juice 4	9.69×10 ⁸	2.80×10 ⁶	1.80×10 ³	0	2.00×10 ⁵	0	0
19	Lemon juice 5	1.96×10 ⁸	3.30×10 ⁵	4.21×10 ³	1.40×10 ⁴	6.00×10 ⁴	5.00×10 ³	3.00×10 ²
20	Pineapple juice 1	1.00×10 ⁸	6.40×10 ⁵	1.00×10 ²	2.00×10 ⁴	0	7.20×10 ³	0
21	Pineapple juice 2	2.00×10 ⁸	5.60×10 ⁵	0	3.20×10 ⁴	0	8.92×10 ³	1.10×10 ²
22	Pineapple juice 3	2.00×10 ⁸	2.70×10 ⁵	2.00×10 ²	1.00×10 ⁴	0	9.52×10 ³	2.00×10 ²
23	Pineapple juice 4	1.33×10 ⁶	1.28×10 ⁵	0	1.10×10 ⁵	0	6.00×10 ³	4.50×10 ³
24	Pineapple juice 5	3.60×10 ⁸	2.08×10 ⁶	2.00×10 ²	8.80×10 ⁴	3.24×10 ⁵	5.84×10 ³	0
25	Papaya juice 1	1.02×10 ⁸	2.50×10 ⁵	0	2.00×10 ³	2.36×10 ⁵	9.67×10 ³	0
26	Papaya juice 2	5.32×10 ⁸	1.96×10 ⁶	3.00×10 ³	3.20×10 ⁵	4.21×10 ⁵	6.20×10 ³	1.00×10 ²
27	Papaya juice 3	1.04×10 ⁸	1.12×10 ⁶	1.00×10 ²	5.80×10 ⁴	0	6.28×10 ⁴	1.85×10 ³
28	Papaya juice 4	6.30×10 ⁷	2.90×10 ⁵	0	1.30×10 ⁴	0	7.20×10 ²	9.00×10 ²
29	Papaya juice 5	5.10×10 ⁶	0	0	0	1.00×10 ⁴	0	0
30	Apple juice 1	2.44×10 ⁸	3.26×10 ⁶	0	4.26×10 ⁵	2.52×10 ⁵	0	0
31	Apple juice 2	4.14×10 ⁸	4.30×10 ⁶	0	5.00×10 ³	2.00×10 ⁴	0	1.00×10 ²
32	Apple juice 3	2.30×10 ⁸	2.70×10 ⁶	0	2.11×10 ⁴	3.33×10 ⁵	0	0
33	Apple juice 4	5.60×10 ⁸	2.80×10 ⁶	1.80×10 ³	0	2.01×10 ⁵	0	0
34	Apple juice 5	1.66×10 ⁸	3.10×10 ⁵	3.21×10 ³	1.40×10 ⁴	6.00×10 ⁴	5.00×10 ³	3.00×10 ²
35	Sugarcane juice 1	6.44×10 ⁸	3.20×10 ⁶	1.56×10 ²	0	3.00×10 ⁴	0	1.00×10 ³
36	Sugarcane juice 2	3.72×10 ⁸	2.00×10 ⁶	0	0	2.00×10 ⁴	4.00×10 ³	6.10×10 ²
37	Sugarcane juice 3	1.20×10 ⁸	2.88×10 ⁶	3.00×10 ²	2.40×10 ⁴	1.00×10 ⁴	7.60×10 ²	2.00×10 ²
38	Sugarcane juice 4	2.92×10 ⁸	2.88×10 ⁶	2.00×10 ²	4.00×10 ⁴	1.00×10 ⁵	7.32×10 ³	0

39	Sugarcane juice 5	8.40×10^8	4.48×10^6	0	1.50×10^4	3.40×10^5	6.60×10^4	1.00×10^2
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Antibiotic-resistant microorganisms can contaminate food. Among the biggest threats to public health is this one (Tabassum and Uddin, 2016). These days, one of the biggest worldwide issues is the emergence of antibiotic resistance in harmful bacteria, which eventually complicates the therapeutic process (Uddin *et al.*, 2011). To determine the antibiogram profile of the presumed identified bacterial isolates toward a few commonly recommended antibiotics, the Kirby-Bauer disk diffusion test was used in this investigation.

Table 4: Biochemical results of *Salmonella* spp., *Klebsiella* spp., *E. coli*, *Staphylococcus* spp. and *Vibrio* spp. according to the Gulf Standard No. 1016/2000 (Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000)

Identified microbes	TSI				MIU		MR test	VP test	Citrate test	Oxidase test
	Slant	Butt	Gas	H ₂ S	Motility	Indole				
<i>E. coli</i>	Y	Y	+	-	+	-	+	-	-	-
<i>Salmonella</i> spp.	R	Y	-	+	+	-	+	-	-	-
<i>Klebsiella</i> spp.	Y	Y	+	-	-	-	-	-	+	-
<i>Vibrio</i> spp.	Y	Y	-	-	+	+	+	-	+	+
<i>Staphylococcus</i> spp.	Y	R	+	+	+	-	+	-	+	-

The isolated bacteria' antibiogram test results were displayed in Table 5. *Salmonella* species exhibited the highest levels of resistance to gentamicin and nalidixic acid, 96% and 69%, respectively. Ciprofloxacin (72%), ampicillin (63%), and ceftriaxone (31%), followed by these antibiotics (Table 5). Another member, *Klebsiella* spp., was shown to be less sensitive to Ciprofloxacin and Sulfomethoxazole-trimethoprim (8% and 20%, respectively), while the highest susceptibility was reported to be 93% to Nalidixic acid. *E. coli*, the most potent fecal coliform, exhibited a considerable degree of sensitivity to sulfomethoxazole-trimethoprim (51%). Furthermore, it was discovered that Ampicillin was less efficient against *E. coli* since it exhibited a 92% resistance to the antibiotic. Tetracycline and Gentamicin resistance in *Vibrio* spp. was 71% and 62%, respectively. According to Table 5, the most resistant strains of pathogenic *Staphylococcus* species were to Netilmicin (92%) and Ampicillin (81%).

The discovery of *Klebsiella* spp. in fruit juice is concerning and consistent with the earlier findings of Haryani *et al.* (2007), who discovered *Klebsiella* spp. in Malaysian street food. Along with other harmful proliferation, *Klebsiella* spp. were also found in fruit juice by Fuentes *et al.* (1985) and Ghenghesh *et al.* (2005). In addition to posing major health risks, the presence of *Klebsiella*

spp. can cause cross-contamination with other street food. According to **Lewis et al. (2006)**, the use of crude stands and carts, inadequate refrigeration, insufficient fruit washing, and an unsanitary surrounding environment may all contribute to the high microbial load found in foods sold on the street.

Table 5: Antibiotic resistance and sensitivity pattern (%) of *E. coli.*, *Staphylococcus* spp., *Klebsiella* spp., *Vibrio* spp. and *Salmonella* spp. collected from different fruit juices and sauces in the Narsingdi city.

Antimicrobial agents	<i>E. coli</i>		<i>Staphylococcus</i> spp.		<i>Klebsiella</i> spp.		<i>Vibrio</i> spp.		<i>Salmonella</i> spp.	
	Res. (%)	Sen. (%)	Res. (%)	Sen. (%)	Res. (%)	Sen. (%)	Res. (%)	Sen. (%)	Res. (%)	Sen. (%)
SXT	47	51			92	8				
CIP			30	70	80	20			72	15
TET							71	15		
NA					7	93			96	4
GEN	20	80	25	75			62	30	69	31
CEF									31	60
AMP	92	5	81	19	70	30			63	25
NET			92	8						

Note: SXT=Sulfomethoxazole- trimethoprim, CIP=Ciprofloxacin, TET=Tetracycline, NA =Nalidixic acid, GEN=Gentamicin, CEF= Ceftriazone, AMP= Ampicillin, NET =Netilmicin; Res= Resistant, Sen= sensitive.

The high *E. coli* microbial load found in this study is almost identical to that found in the study of **Subbannayya et al. (2007)** of juices sold on Indian street vendors. The primary source of *E. coli* infection may be tainted water used for juice diluting or dishwashing. *E. coli* and other coliform bacteria may be present because of unsanitary processing methods and insufficient hand washing by food workers, according to **Tambekar et al. (2007)**. Additionally, the lack of *Salmonella* spp. and *E. coli* in other juice samples may be because of the high quality of the drinkable water used to make the juice and the sparing use of tainted animal dung during the fruit's growth stages. The present investigation revealed that nearly every juice sample from every location was tainted with *Staphylococcus* species. This was comparable to earlier research conducted in India by **Bagde and Tumane (2011)**. Fruit juices containing *Staphylococcus* species may be the result of food handlers' contaminated hands and unclean clothing, which ultimately points to poor hygiene during food preparation (**Tambekar et al. 2009; Titarmare et al. 2009**). A limited number of harmful bacteria were examined in this exploratory examination. However, it was discovered that the cutting board, knives, spoons, glass, and jugs were not regularly cleaned during the juice sample collection process, raising the possibility of cross-contamination. If utensils or equipment used in juice

processing are regularly cleaned with water and detergents, cross contamination can be prevented (VGDHS, 2005). One may argue that the findings of this study could be helpful in implementing a proper HACCP strategy with excellent GMP procedures and in monitoring the microbiological quality of various fruit juices to prevent any future outbreaks of food-borne disease.

CONCLUSION

It was shown that the current study could identify the microbiological characteristics of fresh fruit juice that was gathered from sellers at various significant sites in Narsingdi city, Bangladesh. The findings showed that the local fruit juice samples had a significant microbial load. The majority of the samples had an elevated microbiological load and were unsafe for food, according to FDA recommendations (2013) and Gulf Standard No. 1016/2000 (Emirates Authority for Standardization and Metrology (ESMA), United Arab Emirates, 2000). Furthermore, fruit juice samples have been found to contain strains of *E. Coli*, *Salmonella*, *Klebsiella*, *Staphylococcus*, and *Vibrio* spp. that are resistant to several commonly used antibiotics, including Sulfomethoxazole-trimethoprim (30 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Nalidixic Acid (30 µg), Gentamicin (10 µg), Ceftriazone (30 µg), Ampicillin (10 µg), and Netilmicin (30 µg). As a result, it is crucial that all relevant governmental and non-governmental organizations take preventative action as soon as possible and come up with a determined plan to create a microbiological safety outline for making fruit juices that are both healthy and free of pathogens.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable

Data availability: Data will be made available on request

Additional information: No additional information is available for this paper.

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