

Original Research Article

**Microbial analysis of Vended Fruit Juices and Sauces Consumed in 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 ( $1.0 \times 10^4$  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.

**1. INTRODUCTION**

Fruit juice is a popular soft drink made from the pulp of several fresh fruit varieties [1]. 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 [2]. 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 [2,3]. 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 [4]. Unfortunately, consuming such tainted fruit and vegetable juices has been linked to a number of reports of significant morbidity and mortality each year worldwide [5,6]. 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 [7]. 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 [8, 9]. 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 [10]. 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 [11, 12, 13]. Antibiotic-resistant bacteria have been found in apple and orange juice samples in a number of investigations [14, 15 16, 17]. 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.

## 2. MATERIAL AND METHODS

### 2.1. 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.



**Fig 1. Sample collection site.**

### 2.2. 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  $10^{-4}$  [18]. 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  $37^{\circ}\text{C}$ . 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 [18, 19].

### **2.3. 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<sup>-2</sup> and 10<sup>-4</sup> 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.

### **2.4. 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<sup>-2</sup> and 10<sup>-4</sup> 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 [20].

### **2.5. 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 [19].

### **2.6. 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 against ten popular antibacterial medications Table 1 [21]. 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 diameter of zones of inhibition's widths were measured following incubation and classified as sensitive, intermediate, and resistant Table 2.

**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

## 2.7. 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.

## 3. 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  $2.19 \times 10^4$  CFU/ml (Orange juice 4) to  $4.48 \times 10^6$  CFU/ml (Sugarcane juice 5) and from  $2.70 \times 10^5$  CFU/ml (Tamarind sauce 3) to  $3.26 \times 10^6$  CFU/ml (Tomato sauce 1) in various fruit juices, while Total viable bacterial count ranged from  $2.18 \times 10^4$  CFU/ml (Orange juice 4) to  $9.69 \times 10^8$  CFU/ml (Lemon juice 4) in vended fruit juice samples and in various sauces it was ranged from  $1.02 \times 10^5$  CFU/ml (Tamarind sauce 1) to  $2.84 \times 10^8$  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) [22]. 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  $1.0 \times 10^4$  CFU/mL [23]. 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 [24]. Furthermore, a higher TVBC value could suggest that the fresh juices were made in an unsanitary condition [23].

Twenty-two out of thirty-nine samples had coliform; the highest count was  $4.21 \times 10^3$  CFU/mL in lemon juice 5, while the lowest count was  $1.00 \times 10^2$  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) [25]. 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 [4, 26, 27]. 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 [28].

**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 <sup>8</sup>	3.26×10 <sup>6</sup>	1.80×10 <sup>3</sup>	2.40×10 <sup>4</sup>	3.00×10 <sup>4</sup>	5.00×10 <sup>3</sup>	1.00×10 <sup>2</sup>
2	Tomato sauce 2	1.32×10 <sup>8</sup>	2.16×10 <sup>6</sup>	1.10×10 <sup>3</sup>	2.11×10 <sup>4</sup>	2.99×10 <sup>4</sup>	4.21×10 <sup>3</sup>	0.91×10 <sup>2</sup>
3	Tomato sauce 3	1.71×10 <sup>8</sup>	3.01×10 <sup>6</sup>	1.20×10 <sup>3</sup>	2.45×10 <sup>4</sup>	3.10×10 <sup>4</sup>	3.20×10 <sup>3</sup>	1.02×10 <sup>2</sup>
4	Mustard sauce 1	1.82×10 <sup>6</sup>	4.11×10 <sup>5</sup>	1.29×10 <sup>2</sup>	2.95×10 <sup>3</sup>	3.19×10 <sup>3</sup>	3.29×10 <sup>2</sup>	0.62×10 <sup>2</sup>
5	Mustard sauce 2	2.12×10 <sup>6</sup>	3.17×10 <sup>5</sup>	1.99×10 <sup>2</sup>	2.35×10 <sup>3</sup>	3.17×10 <sup>3</sup>	3.20×10 <sup>2</sup>	0.65×10 <sup>2</sup>
6	Mustard sauce 3	2.22×10 <sup>6</sup>	3.12×10 <sup>5</sup>	1.92×10 <sup>2</sup>	2.25×10 <sup>3</sup>	3.13×10 <sup>3</sup>	3.23×10 <sup>2</sup>	0.66×10 <sup>2</sup>
7	Tamarind sauce 1	1.02×10 <sup>5</sup>	3.21×10 <sup>5</sup>	1.19×10 <sup>2</sup>	2.15×10 <sup>3</sup>	2.39×10 <sup>3</sup>	3.19×10 <sup>2</sup>	0.42×10 <sup>2</sup>
8	Tamarind sauce 2	1.12×10 <sup>5</sup>	2.71×10 <sup>5</sup>	1.29×10 <sup>2</sup>	2.35×10 <sup>3</sup>	2.19×10 <sup>3</sup>	2.29×10 <sup>2</sup>	0.43×10 <sup>2</sup>
9	Tamarind sauce 3	1.17×10 <sup>5</sup>	2.70×10 <sup>5</sup>	1.21×10 <sup>2</sup>	2.15×10 <sup>3</sup>	2.13×10 <sup>3</sup>	2.25×10 <sup>2</sup>	0.40×10 <sup>2</sup>
10	Orange juice 1	3.84×10 <sup>4</sup>	3.26×10 <sup>4</sup>	0	0	3.00×10 <sup>2</sup>	5.00×10 <sup>2</sup>	0
11	Orange juice 2	4.14×10 <sup>4</sup>	4.16×10 <sup>4</sup>	0	0	2.90×10 <sup>2</sup>	4.00×10 <sup>2</sup>	0
12	Orange juice 3	4.10×10 <sup>4</sup>	3.16×10 <sup>4</sup>	0	0	2.91×10 <sup>2</sup>	3.99×10 <sup>2</sup>	0
13	Orange juice 4	2.18×10 <sup>4</sup>	2.19×10 <sup>4</sup>	0	0	2.15×10 <sup>2</sup>	2.19×10 <sup>2</sup>	0
14	Orange juice 5	6.12×10 <sup>4</sup>	3.17×10 <sup>4</sup>	1.11×10 <sup>2</sup>	1.15×10 <sup>2</sup>	2.35×10 <sup>3</sup>	2.13×10 <sup>3</sup>	0
15	Lemon juice 1	2.84×10 <sup>8</sup>	3.26×10 <sup>6</sup>	0	4.76×10 <sup>5</sup>	2.52×10 <sup>5</sup>	0	0
16	Lemon juice 2	4.04×10 <sup>8</sup>	4.30×10 <sup>6</sup>	0	9.00×10 <sup>3</sup>	3.00×10 <sup>4</sup>	0	1.00×10 <sup>2</sup>
17	Lemon juice 3	2.60×10 <sup>8</sup>	2.70×10 <sup>6</sup>	0	2.10×10 <sup>4</sup>	3.30×10 <sup>5</sup>	0	0
18	Lemon juice 4	9.69×10 <sup>8</sup>	2.80×10 <sup>6</sup>	1.80×10 <sup>3</sup>	0	2.00×10 <sup>5</sup>	0	0
19	Lemon juice 5	1.96×10 <sup>8</sup>	3.30×10 <sup>5</sup>	4.21×10 <sup>3</sup>	1.40×10 <sup>4</sup>	6.00×10 <sup>4</sup>	5.00×10 <sup>3</sup>	3.00×10 <sup>2</sup>
20	Pineapple juice 1	1.00×10 <sup>8</sup>	6.40×10 <sup>5</sup>	1.00×10 <sup>2</sup>	2.00×10 <sup>4</sup>	0	7.20×10 <sup>3</sup>	0
21	Pineapple juice 2	2.00×10 <sup>8</sup>	5.60×10 <sup>5</sup>	0	3.20×10 <sup>4</sup>	0	8.92×10 <sup>3</sup>	1.10×10 <sup>2</sup>
22	Pineapple juice 3	2.00×10 <sup>8</sup>	2.70×10 <sup>5</sup>	2.00×10 <sup>2</sup>	1.00×10 <sup>4</sup>	0	9.52×10 <sup>3</sup>	2.00×10 <sup>2</sup>
23	Pineapple juice 4	1.33×10 <sup>6</sup>	1.28×10 <sup>5</sup>	0	1.10×10 <sup>5</sup>	0	6.00×10 <sup>3</sup>	4.50×10 <sup>3</sup>
24	Pineapple juice 5	3.60×10 <sup>8</sup>	2.08×10 <sup>6</sup>	2.00×10 <sup>2</sup>	8.80×10 <sup>4</sup>	3.24×10 <sup>5</sup>	5.84×10 <sup>3</sup>	0
25	Papaya juice 1	1.02×10 <sup>8</sup>	2.50×10 <sup>5</sup>	0	2.00×10 <sup>3</sup>	2.36×10 <sup>5</sup>	9.67×10 <sup>3</sup>	0
26	Papaya juice 2	5.32×10 <sup>8</sup>	1.96×10 <sup>6</sup>	3.00×10 <sup>3</sup>	3.20×10 <sup>5</sup>	4.21×10 <sup>5</sup>	6.20×10 <sup>3</sup>	1.00×10 <sup>2</sup>
27	Papaya juice 3	1.04×10 <sup>8</sup>	1.12×10 <sup>6</sup>	1.00×10 <sup>2</sup>	5.80×10 <sup>4</sup>	0	6.28×10 <sup>4</sup>	1.85×10 <sup>3</sup>
28	Papaya juice 4	6.30×10 <sup>7</sup>	2.90×10 <sup>5</sup>	0	1.30×10 <sup>4</sup>	0	7.20×10 <sup>2</sup>	9.00×10 <sup>2</sup>
29	Papaya juice 5	5.10×10 <sup>6</sup>	0	0	0	1.00×10 <sup>4</sup>	0	0
30	Apple juice 1	2.44×10 <sup>8</sup>	3.26×10 <sup>6</sup>	0	4.26×10 <sup>5</sup>	2.52×10 <sup>5</sup>	0	0
31	Apple juice 2	4.14×10 <sup>8</sup>	4.30×10 <sup>6</sup>	0	5.00×10 <sup>3</sup>	2.00×10 <sup>4</sup>	0	1.00×10 <sup>2</sup>
32	Apple juice 3	2.30×10 <sup>8</sup>	2.70×10 <sup>6</sup>	0	2.11×10 <sup>4</sup>	3.33×10 <sup>5</sup>	0	0
33	Apple juice 4	5.60×10 <sup>8</sup>	2.80×10 <sup>6</sup>	1.80×10 <sup>3</sup>	0	2.01×10 <sup>5</sup>	0	0
34	Apple juice 5	1.66×10 <sup>8</sup>	3.10×10 <sup>5</sup>	3.21×10 <sup>3</sup>	1.40×10 <sup>4</sup>	6.00×10 <sup>4</sup>	5.00×10 <sup>3</sup>	3.00×10 <sup>2</sup>
35	Sugarcane juice 1	6.44×10 <sup>8</sup>	3.20×10 <sup>6</sup>	1.56×10 <sup>2</sup>	0	3.00×10 <sup>4</sup>	0	1.00×10 <sup>3</sup>
36	Sugarcane juice 2	3.72×10 <sup>8</sup>	2.00×10 <sup>6</sup>	0	0	2.00×10 <sup>4</sup>	4.00×10 <sup>3</sup>	6.10×10 <sup>2</sup>
37	Sugarcane juice 3	1.20×10 <sup>8</sup>	2.88×10 <sup>6</sup>	3.00×10 <sup>2</sup>	2.40×10 <sup>4</sup>	1.00×10 <sup>4</sup>	7.60×10 <sup>2</sup>	2.00×10 <sup>2</sup>
38	Sugarcane juice 4	2.92×10 <sup>8</sup>	2.88×10 <sup>6</sup>	2.00×10 <sup>2</sup>	4.00×10 <sup>4</sup>	1.00×10 <sup>5</sup>	7.32×10 <sup>3</sup>	0
39	Sugarcane juice 5	8.40×10 <sup>8</sup>	4.48×10 <sup>6</sup>	0	1.50×10 <sup>4</sup>	3.40×10 <sup>5</sup>	6.60×10 <sup>3</sup>	1.00×10 <sup>2</sup>

Antibiotic-resistant microorganisms can contaminate food. Among the biggest threats to public health is this one [10]. These days, one of the biggest worldwide issues is the emergence of antibiotic resistance in harmful bacteria, which eventually complicates the therapeutic process [29]. 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 microbs	TSI				MIU		MR test	VP test	Citrate test	Oxidase test
	Slant	Butt	Gas	H <sub>2</sub> 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) [30, 31, 32]. 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 [33].

**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 spp.</i>		<i>Klebsiella spp.</i>		<i>Vibrio spp.</i>		<i>Salmonella spp.</i>	
	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 [34]. 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) [35]. 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) [36]. 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 [37, 38]. 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 [39]. 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.

#### 4. 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.

#### **Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

#### **REFERENCES**

- [1] Ahmed, T., Das, K.K. and Uddin, M.M. (2018). The Microbiological Quality of Commercial Fruit Juices-Current perspectives. *Bangladesh Journal of Microbiology*, 35(2), 128-133. <https://doi.org/10.3329/bjm.v35i2.42643>
- [2] Amarowicz, R., Carle, R., Dongowski, G., Durazzo, A., Galensa, R., Kammerer, D., Maiani, G. and Piskula, M.K. (2009). Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. *Molecular Nutrition Food Research*, 53(2), 151–183. <https://doi.org/10.1002/mnfr.200700486>
- [3] Liu, R.H. (2013). Health-Promoting Components of Fruit and vegetables in the Diet. *Advances in Nutrition*, 4(3), 384– 392. <https://doi.org/10.3945/an.112.003517>

- [4] Kechero, F.K., Baye, K., Tefera, A.T. and Tessema, T.S. (2019). Bacteriological quality of commonly consumed fruit juices and vegetable salads sold in some fruit juice houses in Addis Ababa, Ethiopia. *Journal of Food Safety*, 39(1), e12563. <https://doi.org/10.1111/jfs.12563>
- [5] Sharma, N., Singh, K., Toor, D., Pai, S.S., Chakraborty, R. and Khan, K.M. (2020). Antibiotic Resistance in Microbes from Street Fruit Drinks and Hygiene Behavior of the Vendors in Delhi, India. *International Journal of Environmental Research and Public Health*, 17(13), 4829. <https://doi.org/10.3390/ijerph17134829>
- [6] Callejón, R.M., Rodriguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C. and Troncoso, A.M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes. *Foodborne Pathogens and Disease*, 12(1), 32–38. <https://doi.org/10.1089/fpd.2014.1821>
- [7] Ahmed, M.S.U., Nasreen, T., Feroza, B. and Parveen, S. (2009). Microbiological Quality of Local Market Vended Freshly Squeezed Fruit Juices in Dhaka City, Bangladesh. *Bangladesh Journal of Scientific and Industrial Research*, 44(4), 421–424. <https://doi.org/10.3329/bjsir.v44i4.4591>
- [8] Aneja, K.R., Dhiman, R., Aggarwal, N.K., Kumar, V. and Kaur, M. (2014). Microbes associated with freshly prepared juices of citrus and carrots. *International Journal of Food Science*, 2014, 408085. <https://doi.org/10.1155/2014/408085>
- [9] Verma, A. and Gaur, S. (2017). Microbiological Analysis of Street Vended Sugarcane Juice in Noida City, India. *International Journal of Pharma and Bio Sciences*, 8(3), 496–499. <https://doi.org/10.22376/ijpbs.2017.8.3.b496-499>
- [10] Tabassum, S. and Uddin, M.A. (2016). Microbiological Analysis of Different Flavoured Milk Samples Collected from Various Areas of Dhaka city, Bangladesh. *Bangladesh Journal of Microbiology*, 33 (1 and 2), 11-15. <https://doi.org/10.3329/bjm.v33i1.39597>
- [11] Islam, R., & Mithun, M. H. (2024). Probiotics in aquaculture: A pathway to safer and healthier fish farming. *Archives of Agriculture and Environmental Science*, 9(4), 847-857. <https://doi.org/10.26832/24566632.2024.0904030>

- [12] Mithun , M. H., Kar , A., Prome, S. M., Jahan, I., Akter, A., & Hasan, S. E. (2024). A Comprehensive Review on Cell Death. *Journal of Knowledge Learning and Science Technology* ISSN: 2959-6386 (online), 2(3), 170-188. <https://doi.org/10.60087/jklst.vol2.n3.p188>
- [13] Molton, J.S. (2013). The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clinical Infectious Diseases*, 56(9), 1310-1318. <https://doi.org/10.1093/cid/cit020>
- [14] Sarker, M.A.R., Haque, M.M., Rifa, R.A., Ema, F.A. Islam, M.A. and Khatun, M.M. (2018). Isolation and identification of bacteria from fresh guava (*Psidium guajava*) sold at local markets in Mymensingh and their antibiogram profile. *Veterinary World*, 11(8), 1145-1149. <https://doi.org/10.14202/vetworld.2018.1145-1149>
- [15] Mandal, S. (2018). Multiple Antibiotic Resistance Indices of Potential Pathogenic Bacteria Isolated from Street Vended Fruit and Sugarcane Juices, Malda Town, India. *Acta Scientific Pharmaceutical Sciences*, 2(10), 89–94.
- [16] Tadesse, H.A., Gidey, N.B., Workelule, K., Hailu, H., Gidey, S., Bsrat, A. and Taddele, H. (2018). Antimicrobial Resistance Profile of *E. coli* Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. *Veterinary. Medicine International*, 2018, 8903142. <https://doi.org/10.1155/2018/8903142>
- [17] Kebede, H., Hadush, H., Gebrecherkos, T. and Chaithanya, K.K. (2018). Public health risks and bacterial safety of fruit juices prepared in Axum town, north Ethiopia. *Journal of Pharmacy Research*, 12(4), 509-515.
- [18] American Public Health Association (APHA). (1998). Standard methods for the examination of water and wastewater. Washington, D.C., USA: American Public Health Association.
- [19] Cappuccino, J.G. and Sherman, N. (2014). Microbiology. A laboratory manual. London, United Kingdom: Pearson Education.
- [20] Alfrad, E.B. (2007). Bensons microbiological applications. New York, USA: McGraw-Hill Book Company.

- [21] Bauer, A., Kirby, J.C. and Sherris, M.T. (1966). Antibiotic susceptibility testing by standardized single disc method. *American Journal Clinical Pathology*, 45(4), 493-496. [https://doi.org/10.1093/ajcp/45.4\\_ts.493](https://doi.org/10.1093/ajcp/45.4_ts.493)
- [22] Noor, R., Uddin, M.A., Haque, M.A., Munshi, S.K., Acharjee, M. and Rahman, M.M. (2013). Microbiological study of vendor and packed fruit juices locally available in Dhaka city, Bangladesh. *International Food Research Journal*, 20(2), 1011-1015.
- [23] Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates. (2000). Microbiological criteria for foodstuffs-Part I. Gulf Standard No. 1016/2000. U.A.E. Standard No. 1016/2002. Riyadh, Saudia Arabia: ESMA United Arab Emirates
- [24] Asghar, U., Nadeem, M., Nelofer, R., Mazhar, S., Syed, Q. and Irfan, M. (2018). Microbiological Assessment of Fresh Juices Vended in Different Areas of Lahore City. *Electronic Journal of Biology*, 14(4), 106-110.
- [25] Food and Drug Administration (FDA). (2013). Revised guidelines for the assessment of microbiological quality of processed food. FDA circular, No: 2013-010. Philippines: Philippines Department of Health.
- [26] Islam, S., Ahmed, R., Rahman, M.R., Hasan, M.M., Islam, M.S. and Khalil, M.M.R. (2014). Microbial load, antimicrobial sensitivity and plasmid profiles of *Vibrio cholerae* in fruit juice. *International Journal of Natural and Social Sciences*, 1(2), 82-90.
- [27] Uddin, M.A., Jabin, T., Siam, E.A., Afreen, M.T., Uddin, S.S. and Sayeda, U. (2020). Microbiological analysis of different categories of food items in Dhaka city, Bangladesh. *Food Research*, 4(4), 1351 – 1356. [https://doi.org/10.26656/fr.2017.4\(4\).099](https://doi.org/10.26656/fr.2017.4(4).099)
- [28] Malik, Y., Omar, B.J. and Singh, A. (2020). Bacteriological analysis of street-vended fruit juices available in Rishikesh, Uttarakhand. *Journal of Family Medicine and Primary Care*, 9(2), 938-942. [https://doi.org/10.4103/jfmpe.jfmpe\\_818\\_19](https://doi.org/10.4103/jfmpe.jfmpe_818_19)
- [29] Uddin, M.A., Hasan, M., Haque, M.U. and Noor, R. (2011). Isolation and Identification of Pathogenic *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus* spp. in Raw Milk Samples Collected from Different Areas of Dhaka City, Bangladesh. *Stamford Journal of Microbiology*, 1(1), 19-23. <https://doi.org/10.3329/sjm.v1i1.9098>

- [30] Haryani, Y., Noorzaleha, A.S., Fatimah, A.B., Noorjahan, B.A., Patrick, G.B., Shamsinar, A.T., Laila, R.A., Son, R. (2007). Incidence of *Klebsiella pneumonia* in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD– PCR analysis. *Food Control*, 18(7), 847-53.
- [31] Fuentes, F.A., Hazen, T.C., Lopez-Torres, A.J., Rechani, P. (1985). *Klebsiella pneumoniae* in orange juice concentrate. *Applied and Environmental Microbiology*, 49(6), 1527-29.
- [32] Ghenghesh, K.S., Belhaj, K., El-Amin, W.B., El- Nefathi, S.E., Zalmum, A. (2005). Microbiological quality of fruit juices sold in Tripoli–Libya. *Food Control*, 16(10), 855-8.
- [33] Lewis, J.E., Thompson, P., Rao, B.V., Kalavati, C., Rajanna, B. (2006). Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. *International Journal of Food Safety*, 8(1):35-8.
- [34] Subbannayya, K., Bhat, G.K., Shetty, S., Junu, V.G. (2007). How safe is sugarcane juice? *Indian Journal of Medical Microbiology*, 25(1), 73-4.
- [35] Tambekar, D.H., Shirsat, S.D., Suradkar, S.B., Rajankar, P.N., Banginwar, Y.S. (2007). Prevention of transmission of infectious disease: Studies on hand hygiene in health-care among students. *Continental Journal of Biomedical Sciences*, 1(6):e10.
- [36] Bagde, N.I., Tumane, P.M. (2011). Studies on microbial flora of fruit juices and cold drinks. *Asiatic Journal of Biotechnological Resources*, 2(4):454-60.
- [37] Tambekar, D.H., Murhekar, S.M., Dhanorkar, D.V., Gulhane, P.B., Dudhane, M.N. (2009). Quality and safety of street vended fruit juices: A case study of Amravati City. *Journal of Applied Biosciences*, 14, 782-787.
- [38] Titarmare, A., Dabholkar, P., Godbole, S. (2009). Bacteriological analysis of street vended fresh fruit and vegetable juices in Nagpur City, India. *International Journal of Food Safety*, 11, 1-3.
- [39] VGDHS. (2005). (Victorian Government Department of Human Services, Food Safety Unit). Microbiological survey of freshly squeezed juices from retail businesses across Victoria, Melbourne, Australia.