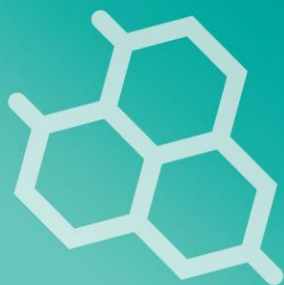


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## BACTERIOLOGICAL QUALITY OF COMMONLY VENDED STREET FOODS AND ANTIMICROBIAL RESISTANCE PATTERNS OF THE BACTERIAL ISOLATES

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**BACTERIOLOGICAL QUALITY OF COMMONLY VENDED STREET FOODS AND  
ANTIMICROBIAL RESISTANCE PATTERNS OF THE BACTERIAL ISOLATES**

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**ABSTRACT**

Food safety problems are particularly becoming an increasingly serious threat to public health in developing countries. The aim of this study was to assess the bacterial load, isolate, identify, and characterize bacterial isolates among commonly vended street foods in Dinajpur City of Bangladesh. Antimicrobial susceptibility of isolates was also performed using commonly used antibiotics. A total of 64 street food samples from four different food items were aseptically collected, analyzed and bacteria were counted by standard plate count method. Ten grams of each food sample was transferred in to 90 ml of buffered peptone water and homogenized. The homogenates were serially diluted and a volume of 0.1 ml dilution was spread on solid media and incubated at 35-37 °C for 24 h. The study revealed that 39 (61%) of the food samples had pathogenic bacterial contamination. Three different bacterial species including *Escherichia coli* (18.75%), *Klebsiella* spp. (6.25%) and *Staphylococcus* spp. (35.93%) were isolated. The Total Viable Count (TVC) in singara ranging from 2.0 to 2.9 CFU/g, in sugar cane juice ranging from 3.1 to 3.8 CFU/ml, in jilapi ranging from 2.1 to 3.6 CFU/g and in chola ranging from 2.7 to 3.5 CFU/g. The antibiotic susceptibility testing was done for isolated species using the Kirby-Bauer disk diffusion method. Antibiogram study of the isolated organisms revealed that isolated *E. coli* were found to be resistant to doxycycline, ampicillin, neomycin, cefixime, norfloxacin, levofloxacin, and azithromycin. *Staphylococcus* spp. isolates were found to be resistant to methicillin, ampicillin, amoxicillin, and penicillin. And *Klebsiella* spp. isolates were found to be resistant to amoxicillin, cloxacillin, cefixime, and imipenem. Ciprofloxacin was found to be the most effective antimicrobial against all isolates. This study confirmed considerable rate of contamination in street vended foods in Dinajpur City. The identified foodborne bacteria and antibiotic resistant isolates could pose a public health problem in that locality. Therefore, regular inspection, health education and training of

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■ vendors on food handling and safety practices are recommended.

## 1. INTRODUCTION

Street vended food is defined according to Food and Agriculture Organization (FAO) as ‘ready to eat foods and beverages prepared and sold by vendors especially in streets and similar public places for immediate consumption without further processing or preparation (FAO, 1989). Though the street vended food plays an important role both economically and socially in meeting food demands in urban people (Cress-Williams, 2001), microbiologically contaminated street food is considered a global problem, which is liable to be a significant contributor to the transmission of food borne diseases (WHO, 1999; Mamun et al., 2013). Food borne diseases causes high morbidity mainly in developing countries (Ram et al., 2007; Saha et al., 2009); due to poor hygienic condition during food preparation and the lack of awareness about food safety (WHO, 1999; Kibret & Tadesse,(2013). Now a days everyone like street vended foods but low-income people eat the most. The consumption of street foods is also common in countries where unemployment is high, salaries and work opportunities are low and social programs are limited (Oladipo and Adejumobi, 2010). There are around 128 varieties of street foods in Bangladesh. Singara, Jilapi, Sugarcane juice, Chola are the most popular street foods in Bangladesh. Street foods are playing a vital role in meeting the food requirements of many inhabitants and being appreciated by consumers for their affordability, accessibility, variety and unique organoleptic properties. Currently the incidence of food borne illness involving a broad range of diseases caused by pathogenic microorganisms is rising worldwide and becomes a public health concern. And foodborne illness is an important challenge to public health and cause a significant economic problem in many countries (Andargie, 2009). In developed countries, an estimated one third of the population is affected by foodborne diseases each year. However foodborne diseases are common and leading causes of illness in developing countries because of the prevailing poor hygienic and sanitation conditions or practices, weak food safety and regulatory systems, lack of resources and lack of education. In response to these problems, health and other concerned organizations are increasing their effort to improve the quality and safety of foods and to prevent foodborne disease (Heaton and Jones, 2008). Thus, it is essential to provide the overall evidence on the prevalence of microorganisms of public health concern in street foods. In Bangladesh, handmade street vended foods like Singara, Sugarcane juice, Jilapi and Chola etc. are too popular. A report revealed that approximately 62-78 % of the shops in Bangladesh situated by the street side and sell such kind of products, in which 58-66% shops are located by the sides of open drains, sewerage, manholes and dustbins. Where 94% of served water from the municipal tap water (Faruque et al., 2010), that is frequently claimed as contaminated (Parveen et al., 2008). Sometimes the vendors store the drinking water in lidless plastic drums where the water comes into contact with open air indicating further possible contamination of water (Fareque et al., 2010; Khairuzzaman et al., 2014). All the circumstances addressed above are likely to occur cross contamination of street vended foods. Street vended foods have been considered to be reservoir of antibiotic resistant bacteria, which constitute direct threat to human health but the potential microbial risk of street foods remain largely unexplored. Street vended foods are now a day’s serious public health concern. The lack of hygiene in the preparation of street vended food increases the risk of disease outbreak worldwide. A major risk factor is street food vendor’s lack of knowledge about the causes of food borne disease. Poor hygiene, a lack of access to potable water and waste disposal and unsanitary environmental conditions, such as

proximity to sewers and garbage dumps, all contribute to the public health risks associated with street foods. Bacterial agents in foods are the leading cause of serious and fatal food borne illness. Among the thousands of different bacterial species, *Staphylococcus*, *E. coli*, *Klebsiella* spp, *Salmonella*, *Clostridium*, *Vibrio*, *Campylobacter*, *Bacillus* and *Enterobacteriaceae* cause more than 90% of food poisoning illness.

Although there is a growing demand for street food products, information on the bacteriological profile, bacterial load and antimicrobial susceptibility patterns of bacterial isolates from street foods in Dinajpur City are lacking. So, the present study was undertaken to determine the microbiological quality of some commonly consumed street vended foods surrounding the street of different areas at Dinajpur City of Bangladesh.

## 2. MATERIALS AND METHODS

### 2.1. Selection of study area

The current study was conducted in four different vending sites (Boro math, Suihari, lilimor, and Maldhapotti) of Dinajpur town, Northern Bangladesh where there are a high number of vendors and customers as well as an abundance of the types of street foods sold there.

### 2.2. Study period and the laboratory

The study was conducted from July, 2022 to December, 2022, in the Bacteriology Laboratory of the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh.

### 2.3. Sample size and collection of food samples

The study included a total number of sixty four ready to eat street vended food samples which were collected from different street food vendors located at Dinajpur city of Bangladesh. The selected food items were Singara, Sugarcane Juice, Jilapi and Chola which were collected from the selected study areas for this study (Table 1) (Figure 1). Approximately 100 g of each food sample was collected aseptically in buffered peptone water (BPW) using sterile plastic zip lock bags from the original closed container and moved (using box that contain ice pack) to the Department of Microbiology in HSTU. They were analyzed within 24 hours of sampling.

**Table 1: Summary of the street food samples collected from different places of Dinajpur city**

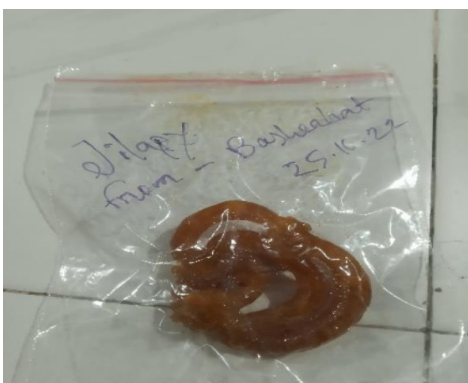
Serial number	Street food items	Vending places	Number	Total number
01	Singara	Lili mor, Suihari, Maldhopatti, Boro math.	16	64
02	Sugarcane juice	Lili mor, Suihari, Maldhopatti, Boro math.	16	
03	Jilapi	Lili mor, Suihari, Maldhopatti, Boro math.	16	
04	Chola	Lili mor, Suihari, Maldhopatti, Boro math.	16	

### 2.4. Preparation of samples

Using digital balance, a quantity of 10 g food sample was taken aseptically and transferred carefully into a sterile pastel containing 90 ml of 0.1% BPW. The measured 10 g samples were homogenized in a measured volume of 90 ml BPW and shaken vigorously using a vortex to dislodge adhered bacteria (Kiiyuki, 2003). The homogenate sample gave a 1:10 dilution, from which further dilution was made by adding 1 ml of homogenate into 9 ml of BPW. Serial dilutions of  $10^{-2}$  and up to  $10^{-6}$  were made using test tubes and then inoculated into the appropriate culture media which were prepared according to the manufacturer's instruction.



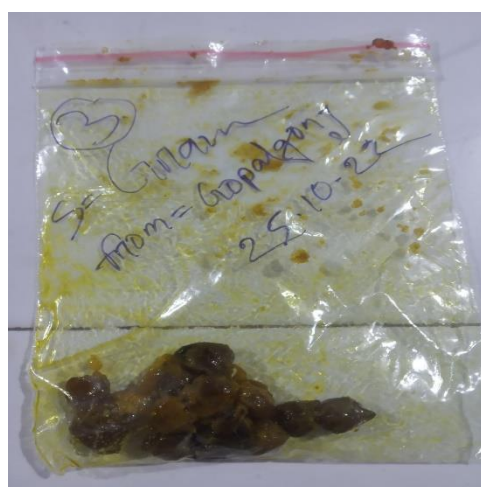
**Singara**



**Jilapi**



**Sugarcane juice**



**Chola**

**Figure 1:** Experimental samples of street vended foods (Singara, Jilapi, Sugarcane juice and Chola) tested in this study

### 2.5. Enumeration of total viable count (TVC)

Exactly, 100  $\mu$ l of 10-fold dilution from each sample was inoculated and spread aseptically onto a previously prepared plate count agar (Hi-Media, India) using a micropipette for each dilution for the determination of total viable count. The diluted samples were spread as quickly as possible on the surface of the plate using an L-shaped sterile glass spreader. The plates were then incubated for 24–48 h at 30–35 °C. The colonies were counted from plates containing more than 30 and less than 300 microbial colonies (Eromo et al., 2016; Nemo et al., 2017). The average number of colonies in particular dilution was multiplied by the dilution factor to obtain total viable count. The results of the total bacterial count were expressed as the number of colony forming units (CFU) per gram of food samples (Salamandane et al., 2021; Authority, 2009)

### 2.6. Isolation of bacteria

Bacteriological analyses were performed using standard method for aerobic bacteria (Brown, 2005). Each sample of collected street foods such as Singara, Sugarcane Juice, Jilapi and Chola were inoculated separately in nutrient broth (NB) to promote bacterial growth. Each group of these media was incubated at 37°C for overnight. The colonies on primary cultures were repeatedly sub-cultured

by streak plate method (Cheesbrough, 1985) until the pure culture with homogenous colonies were obtained. Different bacteriological culture media such as Nutrient agar, Eosin Methylene Blue (EMB) agar, Staphylococcus Agar N. 110 and Mannitol Salt Agar (MSA) were used for sub-culturing and incubated at 37°C for 24 to 48 h for growth. After incubation at 37°C, the plates were observed for any kind of growth on the culture media.

### 2.7. Identification of bacteria

The cultural examinations of collected street food (Singara, Sugarcane Juice, Jilapi and Chola) samples for bacteriological study were performed according to the standard method (ICMSF, 1986). Identification of bacteria was performed on the bases of colony morphology, Gram's staining reaction and biochemical tests. Biochemical tests, such as Oxidase, Catalase, Methyl Red (MR), Voges-Proskauer (VP), Urease test, Triple Sugar Iron (TSI), Iodole test, and Citrate utilization tests were performed as per the standard methods (Cheesbrough, 1985).

### 2.8. Antimicrobial susceptibility testing

The antimicrobial susceptibility of bacterial isolates was tested using the Kirby–Bauer disk diffusion method (Kirby, 2009) on Mueller Hinton agar (Hi-Media) media following the recommendations of the Clinical and Laboratory Standards Institute (Wayne, 2020). Morphologically identical 3–5 pure colonies of isolated bacteria from the overnight incubated nutrient agar media were suspended in nutrient broth by using sterile wire loop with reference to 0.5 McFarland standards and incubated at 37 °C for up to 4 h. Then by using sterile cotton swab, the broth was uniformly inoculated into Mueller Hinton agar media. After 3–5 minutes of inoculation, selected antibiotic discs were applied to the surface of the medium by considering the size of the plate. Antibiotic discs were selected for isolated foodborne pathogens as per the CLSI 2020 guideline and by considering the availability and frequency of prescription for the treatment of bacterial infections in human diseases, particularly foodborne diseases. The antibiotics tested in this study were ampicillin (25 µg), amoxicillin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 U), tetracycline (30 µg), vancomycin (30 µg), streptomycin (10 µg), azithromycin (30 µg), erythromycin (15 µg), neomycin (30 µg), ceftriaxone (5 µg), cloxacillin (5 µg), cefixime (5 µg), cotrimoxazole (25 µg), imipenem (10 µg), levofloxacin (5 µg), cefotaxime (30 µg), norfloxacin (10 µg), and doxycycline (30 µg). After overnight incubation at 37 °C, the zone of inhibition of growth around each disk was measured in millimeters and interpreted in accordance with standards as sensitive, intermediate, or resistant (Wayne, 2020).

## 3. RESULTS

### 3.1. Prevalence of bacteria isolated from street vended food samples

Three different genera of bacteria such as *Staphylococcus* spp., *Escherichia coli*, and *Klebsiella* spp. were successfully isolated from different street food (Singara, Sugarcane Juice, Jilapi and Chola) samples. In case of singara, six (37.50 %) were positive for *Staphylococcus* spp. and three (18.75 %) were positive for *E. coli*. In case of sugarcane juice, 5 (31.25%) were positive for *Staphylococcus* spp., and four (25 %) were positive for *Klebsiella* spp. In case of jilapi, 7 (43.75 %) were positive for *Staphylococcus* spp. and 5 (31.25%) were positive for *Escherichia coli*. In chola, five (31.25%) were positive for *Staphylococcus* spp. and four (25%) were found positive for *E. coli*. Out of 64 tested food samples, prevalence of *Staphylococcus* spp. was found to be the highest (43.75%) in jilapi and lowest but same (31.25%) in sugarcane juice and in chola. Prevalence of *E. coli* was the highest (31.25%) in jilapi and the lowest prevalence (18.75%) was in singara samples. *Klebsiella* spp. was isolated from sugarcane juice only and the prevalence was 25%. The overall bacterial prevalence was 61 % out of

64 examined street food samples in this study (Table 2).

**Table 2: Prevalence of the isolated bacteria in different types of street vended foods**

Street food items	Number	Isolated bacteria	Number of isolates	Prevalence
Singara	16	<i>Staphylococcus</i> spp.	6	37.50%
		<i>E. coli</i>	3	18.75%
Sugarcane juice	16	<i>Staphylococcus</i> spp.	5	31.25%
		<i>Klebsiella</i> spp.	4	25%
Jilapi	16	<i>Staphylococcus</i> spp.	7	43.75%
		<i>E. coli</i>	5	31.25%
Chola	16	<i>Staphylococcus</i> spp.	5	31.25%
		<i>E. coli</i>	4	25%
Total no. of tested food samples	64		39	61% (Overall prevalence)

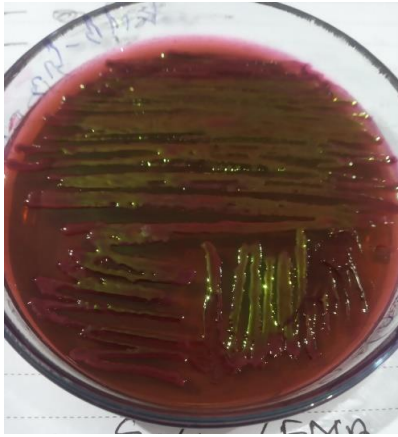
### 3.2. Cultural characteristics

Cultural characteristics of each type of bacterial isolates were studied for the determination of size, shape and colony morphology on different bacteriological culture media. Pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media (Table 3) (Figure 2, 3, 4 & 5).

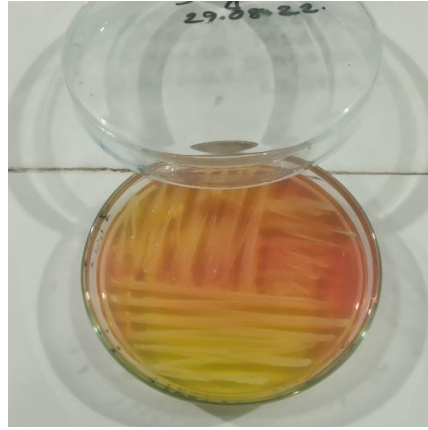
**Table 3: Cultural characteristics of the bacterial isolates from street vended foods**

Name of the culture media	Cultural properties	Identified organisms
NA	Large mucoid, white colony	<i>E. coli</i>
MAC	Produced large mucoid, rose-pink colonies	
EMB	Mucoid colony and metallic green sheen appearance	
NA	Large mucoid, white colony	<i>Klebsiella</i> spp.
MAC	Produced large mucoid, rose-pink colony	
EMB	Mucoid no metallic sheen with transmitted light, grey brown and pink color with clear edge	
NA	Large Mucoid Colony	<i>Staphylococcus</i> spp.
MSA	Yellow color colony	
Staphylococcus Agar No. 110	White color colony	
Blood agar	White color colony with beta hemolysis	

**Legends:** NA= Nutrient Agar, MAC= MacConkey Agar, MSA: Mannitol Salt Agar, EMB= Eosin Methylene Blue.



**Figure 2:** *E. coli* produced green Metallic sheen colonies on EMB agar



**Figure 3:** *Staphylococcus* spp. produced yellow colonies on Mannitol salt agar



**Figure 4:** *Staphylococcus* spp. produced White colonies on Staphylococcus agar No. 110



**Figure 5:** *Klebsiella* spp. produced pink color mucoid colonies on MacConkey agar

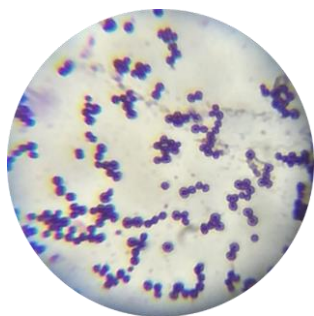
### 3.3. Staining characteristics

The staining characteristics of the isolated bacteria were determined according to Gram's staining technique (Table 4) (Figure 6, 7 & 8). Isolated *E. coli* revealed gram negative, pink colored, short rod shaped organisms arranged singly, paired or in short chain form; Other hand Gram positive *Staphylococcus* spp. bacteria revealed violet color, small round shaped appearance, arranged in grape like clusters when observed under microscope (100 X objective) and *Klebsiella* spp. isolates revealed gram negative, small rod shaped organisms arranged singly, paired or in short chain form.

**Table 4: Gram staining properties of the bacteria isolated from street vended foods**

Staining	Morphology characteristics	Result	Identified organisms
Gram staining and viewed under Microscope	Single, paired or in short chain, pink color colony appearance	(-) ve	<i>E. coli</i>
Gram staining and viewed under Microscope	Violet color colony with round shaped and grape like appearance.	(+) ve	<i>Staphylococcus</i> spp.
Gram stain and viewed under Microscope	Pink color, small rod-shaped organism arranged in single, paired or short chain	(-) ve	<i>Klebsiella</i> spp.

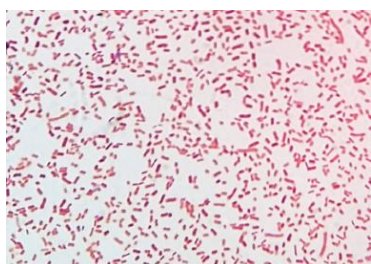




**Figure 6:** Microscopic photograph of *Staphylococcus* spp. revealed round, grape-like clusters and violet color bacteria under 100X objective



**Figure 7:** Microscopic photograph of *E. coli* revealed Gram negative short, rod shaped bacteria arranged singly, paired or in short chain under 100X objective



**Figure 8:** Microscopic photograph of *Klebsiella* spp. revealed Gram negative pink color, small rod shaped bacteria paired or in short chain arrangements under 100X objective

### 3.4. Biochemical characteristics

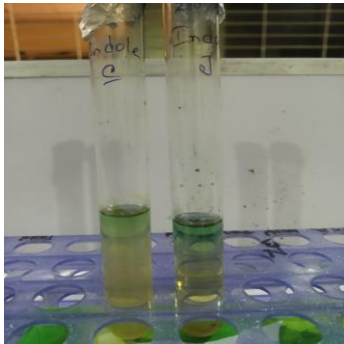
Isolated bacteria were *E. coli*, *Staphylococcus* spp., and *Klebsiella* spp. The identified isolates were characterized by using different biochemical tests (MR, VP, TSI, MIU, Indole, Catalase, Oxidase and Citrate utilization tests etc.) (Table 5). Isolated *E. coli* and *Staphylococcus* spp. were positive and *Klebsiella* spp. was negative for methyl red test. All isolates were positive for catalase test with gas bubble formation. All isolates were negative for oxidase test with no colour change. *E. coli* and *Staphylococcus* spp. isolates were negative and *Klebsiella* spp. was found positive for voges-proskauer test. Indole test was also positive (presence of a cherry red colored ring on the surface of the media) for *E. coli* whereas negative (absence of a cherry red colored ring on the surface of the media) for *Staphylococcus* spp. and *Klebsiella* spp. (Figure 9, 10 &11). Simmons citrate agar test was negative (no color change of the medium) for *E. coli* and *Staphylococcus* spp. whereas positive (formation of Prussian blue color on the slant) for *Klebsiella* spp. MIU test were positive (Diffuse, hazy growth, slightly opaque media) for *E. coli*, *Staphylococcus* spp. and negative (no color change)

for *Klebsiella* spp. TSI agar slant reactions showed yellow color slant and butt for *E. coli* and *Staphylococcus* spp. whereas red color slant and yellow butt for the isolated *Klebsiella* spp.

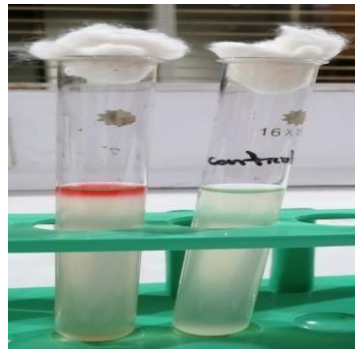
**Table 5: Biochemical characteristics of the bacterial isolates**

SI No	Cata	Oxi	Ind	Cit	MR	VP	MIU			TSI	Identified organisms
1	+	-	+	-	+	-	+	+	-	Yellow slant, Yellow butt with H <sub>2</sub> S	<i>E. coli</i>
2	+	-	-	-	+	-	+	-	+	Yellow Slant, Yellow Butt, Without H <sub>2</sub> S	<i>Staphylococcus</i> spp.
3	+	-	-	+	-	+	-	-	+	Red slant, Yellow butt with H <sub>2</sub> S	<i>Klebsiella</i> spp.

**Legends:** Cata=Catalase, Oxi=Oxidase, Ind=Indole, Cit=Citrate Utilization, MR=Methyl Red, VP=Voges Proskauer, MIU=Motility Indole Urease, TSI=Triple Sugar Iron, (+) =Positive, (-) =Negative



**Figure 9:** Negative Indole test showed By *Staphylococcus* spp. (Absence of a Cherry red color ring)



**Figure 10:** Positive Indole test showed by *E. coli* (Presence of a cherry red color ring)



**Figure 11:** Negative Indole test showed by *Klebsiella* spp. (Absence of a cherry red color ring)

### 3.5. Antimicrobial susceptibility profiles of the isolated bacteria

All the three isolated bacteria (*E. coli*, *Staphylococcus* spp. and *Klebsiella* spp.) were subjected to antibiotic susceptibility testing. The results are presented in table 6, 7 and 8.

**Table 6: Antimicrobial profile against *E. coli***

Organism	Name of antibiotics	Zone of inhibition (mm)	Interpretation
<i>E. coli</i>	Streptomycin	14mm	S
	Doxycycline	00 mm	R
	Ampicillin	00mm	R
	Neomycin	00 mm	R
	Cefixime	00mm	R
	Norfloxacin	00 mm	R
	Levofloxacin	00mm	R
	Ciprofloxacin	16mm	S
	Azithromycin	00mm	R

**Legends:** R= Resistance, S= Sensitive and I= Intermediate.

**Table 7: Antimicrobial profile against *Staphylococcus* spp.**

Organism	Name of antibiotics	Zone of inhibition	Interpretation
<i>Staphylococcus</i> spp.	Ciprofloxacin	27mm	S
	Methicillin	00mm	R
	Ampicillin	00mm	R
	Amoxicillin	00mm	R
	Tetracycline	20mm	S
	Ceftriaxone	18mm	I
	Penicillin	00mm	R
	Erythromycin	14mm	I
	Gentamicin	14mm	I

**Legends:** I= Intermediate, S= Sensitive and R= Resistant.

**Table 8: Antibiotic profile against for *Klebsiella* spp.**

Organism	Name of antibiotics	Zone of Inhibition	Interpretation
<i>Klebsiella</i> spp.	Gentamicin	13mm	I
	Amoxicillin	00mm	R
	Ceftriaxone	31mm	S
	Cloxacillin	00mm	R
	Cefixime	00mm	R
	Co-Trimoxazole	25mm	S
	Ciprofloxacin	38mm	S
	Imipenem	00mm	R
	Levofloxacin	32mm	S
	Tetracycline	15mm	S
	Cefotaxime	23mm	S
	Chloramphenicol	22mm	S
	Norfloxacin	32mm	S

**Legends:** R= Resistant, I= Intermediate and S= Sensitive.

### 3.6. Bacterial load of the tested street food samples by Total Viable Count (TVC)

The TVC of street food samples are presented in table 9. TVC in singara ranged from  $2.0 \times 10^3$  to  $2.9 \times 10^4$  CFU/g, in sugarcane juice ranged from  $3.1 \times 10^3$  to  $3.8 \times 10^4$  CFU/ml, in jilapi ranged from  $2.1 \times 10^3$  to  $3.6 \times 10^4$  CFU/g and in chola ranged from  $2.7 \times 10^3$  to  $3.5 \times 10^4$  CFU/g. The highest numbers of bacterial colonies were observed in sugarcane juice sample ( $3.8 \times 10^4$  CFU/ml) and the lowest numbers were in singara sample ( $2.0 \times 10^3$  CFU/g) (Table 9).

**Table 9: Total Viable Count among commonly vended street foods at selected vending sites in different locations at Dinajpur City**

Sample No.	Singara (CFU/g)		Sugarcane juice (CFU/ml)		Jilapi (CFU/g)		Chola (CFU/g)	
	1	$2.0 \times 10^3$	$1.9 \times 10^4$	$3.4 \times 10^3$	$2.9 \times 10^4$	TFTC	TFTC	TFTC
2	TFTC	TFTC	TFTC	TFTC	$4.5 \times 10^3$	$3.6 \times 10^4$	$2.7 \times 10^3$	$1.5 \times 10^4$
3	TFTC	TFTC	$4.2 \times 10^3$	$3.8 \times 10^4$	TFTC	TFTC	TFTC	TFTC
4	$2.8 \times 10^3$	$1.7 \times 10^4$	TFTC	TFTC	TFTC	TFTC	$4.0 \times 10^3$	$3.1 \times 10^4$
5	TFTC	TFTC	TFTC	TFTC	$3.2 \times 10^3$	$2.2 \times 10^4$	TFTC	TFTC
6	$3.5 \times 10^3$	$2.9 \times 10^4$	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC
7	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC
8	TFTC	TFTC	$3.1 \times 10^3$	$2.6 \times 10^4$	TFTC	TFTC	$4.2 \times 10^3$	$3.5 \times 10^4$
9	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC
10	$2.7 \times 10^3$	$1.6 \times 10^4$	TFTC	TFTC	TFTC	TFTC	TFTC	TFTC
11	TFTC	TFTC	TFTC	TFTC	$2.5 \times 10^3$	$1.5 \times 10^4$	TFTC	TFTC
12	TFTC	TFTC	TFTC	TFTC	$3.7 \times 10^3$	$2.6 \times 10^4$	$3.5 \times 10^3$	$2.0 \times 10^4$
13	TFTC	TFTC	$4.4 \times 10^3$	$3.2 \times 10^4$	TFTC	TFTC	TFTC	TFTC
14	$4.7 \times 10^3$	$2.5 \times 10^4$	TFTC	TFTC	$2.1 \times 10^3$	$1.4 \times 10^4$	TFTC	TFTC
15	TFTC	TFTC	$4.7 \times 10^3$	$2.5 \times 10^4$	TFTC	TFTC	$3.2 \times 10^3$	$2.3 \times 10^4$

**Legends:** TFTC= Too few to count, THTC= Too high to count, CFU=Colony forming unit

## 4. DISCUSSIONS

The control of foodborne pathogens is an essential measure in preventing the appearance and spread

of foodborne diseases in the population (Woh et al. 2017). Most food hawkers in Bangladesh are unaware of food safety regulations and usually have no training. They prepare and store food without adhering to hygienic standards, exposing them to contamination from various sources such as raw foodstuffs, water, knives, utensils, flies, vendors' bare hands, and occasional food handling by consumers (Bhuiyan, 2012; Nicolas et al. 2007). Unhygienic conditions, such as sewage, an inefficient waste dumping system, and an insufficient water supply attract flies, contaminating food (Tambekar et al. 2009). Our study shows results that are in line with the above comments. Street food vending has become an important public health issue owing to the potential to cause food borne diseases. Vendors who lack an adequate understanding of the basic food safety issues mostly display foods sold on the streets (Rane, 2011). Street vended foods are sold by a hawker or vendors in a street or public places, such as market or fairs in Bangladesh. It is often sold a potable food stall, food cart or food truck. Street vended foods are more delicious and popular. It is available everywhere in Bangladesh. The study was conducted to analyze the bacteriological quality of street vended foods including Singara, Jilapi, Sugarcane juice and Chola that were collected from four selected different places in Dinajpur city. A total of 64 Ready to Eat Street foods were examined in this study. Three different genera of bacteria were isolated and identified from the tested street vended foods samples. The isolated bacteria were *E. coli*, *Staphylococcus* spp. and *Klebsiella* spp. Out of 64 tested food samples, total 39 samples had bacterial contamination. Some of these pathogens cause gastroenteritis and diarrhoea especially *E. coli*, which is a major cause of travellers and childhood diarrhoea. *Klebsiella* spp. and *Staphylococcus* spp. are considered opportunistic pathogens but produce significant endotoxin that can mediate fatal infection. The TVC in Singara ranged from  $2.0 \times 10^3$  to  $2.9 \times 10^4$  CFU/g, in Sugarcane juice ranged from  $3.1 \times 10^3$  to  $3.8 \times 10^4$  CFU/ml, in Jilapi ranged from  $2.1 \times 10^3$  to  $3.6 \times 10^4$  CFU/g, and in Chola ranged from  $2.7 \times 10^3$  to  $3.5 \times 10^4$  CFU/g. These results were almost similar to the findings of previous researchers (Tania et al. 2023). In this study, the isolated organisms were characterized by morphological examination by using Gram's staining techniques. Isolated *E. coli* revealed gram negative single, paired or in short chain form, *Klebsiella* spp. revealed pink color short rod shaped single or paired arrangement or short chain, and *Staphylococcus* spp. isolates showed violet color, small round shape, grape like arrangement. Similar observations were reported by previous researchers (Khalif et al. 2018; Paul et al. 2018). The isolates of *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus* spp. were also identified by different biochemical tests such as Catalase, Indole, MR, VP, MIU, Oxidase, Citrate test etc. Similar observations were reported by Khalif et al. (2018). The results of antibiotic sensitivity test revealed that all of the isolated *Escherichia coli* was found resistant to Doxycycline, Ampicillin, Neomycin, Cefixime, Norfloxacin, Levofloxacin and Azithromycin whereas the isolate was sensitive to Ciprofloxacin and Streptomycin. *Staphylococcus* spp. isolates were found sensitive to Ciprofloxacin and Tetracycline; *Klebsiella* spp. isolates were found sensitive to Co-Trim oxazole, Ciprofloxacin, Levofloxacin, Tetracycline, Cefotaxime, Chloramphenicol, ceftriaxone and Norfloxacin. According to the microbiological standard of foods in Bangladesh, aerobic plate counts ranging from  $10^1$  to  $10^2$  CFU/g can be said to be safe,  $10^2$  to  $< 10^4$  CFU/g acceptable,  $10^4$  to  $< 10^5$  CFU/g not acceptable. The current study found that the most commonly vended popular street foods in different places of Dinajpur City were heavily contaminated with foodborne pathogens resistant to multiple antibiotics. Consuming these contaminated foods may result in foodborne infection and intoxication, making treatment with common antibiotics more difficult.

Our epidemiological data provided valuable baseline information suited for use of public health organizations and consumers. Public health organizations should be concerned since microorganisms causing food borne illness and food spoilages. Thus, reduction of contamination is an achievable policy objective. Therefore, this study suggests appropriate measures to be taken for controlling the dissemination of resistant genes containing pathogens by avoiding discriminate use of antibiotics without prescriptions for treatments and improving sanitation and hygiene standards for street vended foods with good handling procedures and practices for food safety. This study has certain limitations. First a relatively small sample size and secondly a limited geographical area selected for sample collection accounting for it and hence the results may not be generalized to other sites. Future studies in connection with the present research work might be isolation and characterization of further bacterial pathogens from street vended foods covering more significant number of samples from a wide selected geographical area will provide more comprehensive information on contamination of street vended foods. Detection of antibiotic resistance genes by molecular microbiological techniques. Detection of virulence factors from the isolated bacteria. Development of effective vaccines.

### 5. CONCLUSIONS

Three different genera of bacteria such as *Staphylococcus* spp., *E. coli* and *Klebsiella* spp. were successfully isolated and identified from the tested food samples. Out of 64 examined food samples, total 39 samples were found positive culturally. The results of this study showed that, the majority of street-vended food items in the selected study areas were contaminated with one or more different pathogenic bacteria. The study indicated that contaminated street vended foods with multi drug resistant bacteria collected from street food serving local small scale food vendors that present health risk to consumers. Also, there is a high chance that the multi-drug resistant genes from the bacterium could horizontally transfer to another *Enterobacteriaceae* family with the help of plasmid and they are transferring antimicrobial resistance leading to difficulties in selecting appropriate antibiotic therapeutic treatments. Hence appropriate measures should be taken for controlling the dissemination of these resistant genes by planning and following proper antibiotic stewardship regimes in the community. In addition, knowledge about proper sanitation, personal hygiene, uses of clean water, cleaning of utensils and containers for storage food items is very much essential, along with spreading awareness among the local street vended foods and handlers towards proper hygienic practices. This study's insights and future extensions also call for harmonizing food safety practice and training for street vended food establishments on a continuous basis with oversights from local municipalities regulating these food service enterprises. Ambulant vending style, proximity of vending areas to waste drainage, exposure of street foods to dust, sunlight, and winds, and an inappropriate way of street food transportation were found to be significant for the unsatisfactory bacteriological quality of street-vended foods. As a result, comprehensive health education and training for street food vendors, regular inspections of vending area environmental sanitation, and regular antibiotic resistance testing for foodborne bacteria are required. The study, on the other hand, provides helpful baseline data for public health specialists in the management of human infections caused by foodborne disease in the surveyed area. However, more research into the molecular characterization of foodborne bacterial pathogens is required.

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#### **Conflict of interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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