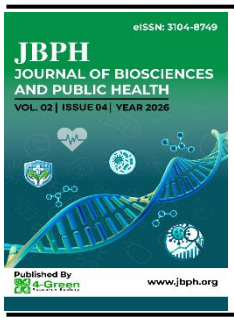

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Original Article

High Microbial Load and Antibiotic-Resistant Pathogens in Street-Vended Sugarcane Juice: A Public Health Perspective

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ABSTRACT

Background: Street-vended sugarcane juice is a widely consumed beverage in Bangladesh because of its refreshing taste and nutritional value. However, inadequate hygienic practices during extraction, handling, and vending may lead to bacterial contamination, increasing the risk of foodborne diseases and the dissemination of antimicrobial-resistant bacteria. This study aimed to assess the bacterial contamination and antibiotic resistance patterns of bacteria isolated from street-vended sugarcane juice collected from selected locations in Gopalganj, Bangladesh. **Methods**: Four freshly extracted sugarcane juice samples were collected from distinct street vendors. Total viable bacterial counts were determined by the spread-plate method on plate-count agar. Isolates were purified and presumptively identified by colony morphology on selective media, Gram staining, and a panel of biochemical tests. Antimicrobial susceptibility was assessed by the Kirby–Bauer disc-diffusion method against ten commonly used antibiotics following CLSI guidelines. **Results**: Microbiological analysis revealed substantial bacterial contamination in all samples, with TVC values ranging from 3.2×10^6 to 4.2×10^6 CFU/mL. *Staphylococcus* spp. and *Klebsiella* spp. were detected in 75% of the samples, while *Enterobacter* spp. and *Escherichia coli* were identified in 50% of the samples. Antibiotic susceptibility testing showed that all bacteria were resistant to amoxicillin, ampicillin, erythromycin, and metronidazole. In contrast, high susceptibility to gentamicin and co-trimoxazole, and intermediate susceptibility patterns were observed for tetracycline, cefotaxime, doxycycline, and ciprofloxacin. **Conclusion**: The findings indicate that street-vended sugarcane juice in the study area is contaminated with potentially pathogenic bacteria exhibiting multidrug resistance, posing a significant public health concern. Strengthening food hygiene practices, ensuring vendor education, and implementing routine microbiological surveillance are essential to improve the microbiological safety of street-vended beverages and reduce the risk of foodborne infections.

1. INTRODUCTION

Fruits are an essential component of a healthy diet due to their rich content of vitamins, minerals, dietary fiber, carbohydrates, and phytonutrients. These bioactive compounds have been associated with numerous health benefits, particularly enhanced immune function and protection against various diseases. One of the most common ways to consume as freshly prepared juice [1]. Freshly prepared fruit juices are often act as "rapid energy boosters" because of rapidly absorbed into the bloodstream, providing an immediate energy [2]. The demand for street-vended fruit juices has increased considerably among urban populations due to their affordability, unique taste, variety, and convenience [3]. To maximize customer access, most vendors set-up their stalls in high-traffic public areas. However, the absence of basic sanitary facilities, including access to clean running water, waste disposal systems, and hygienic toilet facilities, raises serious concerns regarding the hygienic conditions of food preparation areas and the personal hygiene practices of vendors [3,4]. Furthermore, the increasing prevalence of multidrug-resistant food-borne bacteria has exacerbated this public health challenge, contributing to outbreaks linked to contaminated fruit juice consumption [5]. Sugarcane (*Saccharum officinarum* L.) is a biennial herbaceous plant belonging to the family *Poaceae* and is primarily cultivated for its sucrose-rich stalks. In Bangladesh, sugarcane is grown on approximately 0.18 million hectares of land, with an average annual production of about 7.3 million tons. Of the total sugarcane produced, approximately 32.36% is used for sugar production, 52.69% for molasses production, and 14.39% for seed and juice production [6]. Sugarcane juice is a nutritious and refreshing natural beverage containing essential vitamins, minerals, simple carbohydrates are readily digestible by the human body [7]. Its popularity is largely attributed to its pleasant taste and excellent thirst-quenching properties.

Despite its nutritional value, sugarcane juice is highly perishable because of its favorable physicochemical characteristics for microbial growth, including high moisture content, elevated sugar concentration, high water activity, and a near-neutral pH [8]. In Bangladesh, a considerable number of vendors are involved in the extraction and sale of fresh sugarcane juice. However poor sanitation of the sugarcane stalks, extraction equipment, water used during processing, and food-contact surfaces can lead to microbial contamination [9]. Street-vended fruit juices have been found to harbor a variety of bacterial pathogens, including *E. coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., and *S. aureus* [10, 11]. Similarly, grape, sweet lime, pineapple, and sapota juices have been reported to contain pathogenic microorganisms such as *E. coli*, *Salmonella* spp., *Shigella* spp., and *S. aureus*. Moreover, street-vended fruit juices have tested positive for *Staphylococcus* spp. and fecal coliform bacteria [12].

Consumption of contaminated sugarcane juice poses a significant public health risk by increasing the incidence of foodborne diseases, particularly among nutritionally vulnerable populations. Common bacterial contaminants such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Staphylococcus aureus* have been associated with Food poisoning, urinary tract infections, Lower respiratory tract infections, gallbladder infections, middle ear infections, and healthcare-associated bacteremia [13]. In addition, *E. coli* is a major etiological agent of diarrhea, pyogenic infections, septicemia, and other potentially life-threatening conditions [14]. The detection of *Pseudomonas* spp., *Staphylococcus* spp. and *Escherichia coli* is particularly alarming because these microorganisms can produce enterotoxins that may cause foodborne illnesses in freshly prepared sugarcane juice is a matter of concern [15, 16].

Despite the popularity of sugarcane juice and the documented hygiene deficits of street-vending practices, data specifically from southern Bangladesh remain scarce. Gopalganj, a growing urban center, hosts numerous juice vendors whose products are consumed daily by students, workers, and the general public. Systematic assessment of bacterial load, species composition, and resistance profiles in this locality is therefore warranted.

Therefore, the present study aimed to- quantify total viable bacteria in street-vended sugarcane juice collected from four sites in university areas; isolate and phenotypically identify the bacterial genera, and determine the antimicrobial susceptibility patterns of the recovered isolates. The findings are expected to inform local food-safety interventions. Also, can contribute to the broader understanding of street-food-associated antimicrobial resistance and risk of foodborne disease outbreaks.

2. MATERIALS AND METHODS

2.1. Sample collection

The study was conducted during April-May 2024 in the Gopalganj city area in Bangladesh. A total of four street-vended sugarcane juice samples were collected from different vendors. Four freshly extracted sugarcane juice samples (approximately 20 mL each) were aseptically collected from different street vendors at four locations in the city; GSTU Campus (**BM 1**), Nobinbag (**BM 2**), Bangabandhu College More (**BM 3**), and Chourangi (**BM 4**). Samples were transferred into sterile Falcon tubes, immediately placed in an icebox, transported to the laboratory, and stored at 4°C. All microbiological analyses were completed within 24 h of collection.

2.2. Sample preparation

Each sugarcane juice sample was serially diluted using sterile phosphate-buffered saline (PBS). Briefly, 1 mL of each sample was aseptically transferred into 9 mL of sterile PBS to obtain a 10^{-1} dilution. Subsequent serial tenfold dilutions were prepared as required for microbiological analysis.

2.3. Total viable count (TVC)

The total viable bacterial count was determined using the spread plate technique. Briefly, 50 µL aliquots from appropriate serial dilutions were spread evenly onto Plate Count Agar (PCA) plates using a sterile spreader. The inoculated plates were incubated at 37°C for 24 hours. After incubation, plates containing 30–300 colonies were selected for enumeration, and the bacterial load was expressed as colony-forming units per milliliter (CFU/mL) of the sample.

2.4. Isolation, purification, and phenotypic identification

Bacteriological analysis was performed following standard microbiological procedures for the isolation of aerobic bacteria. Each sugarcane juice sample was first enriched in Nutrient Broth and incubated overnight at 37°C. The enriched cultures were subsequently streaked onto Nutrient Agar plates using the streak plate technique to obtain isolated colonies. Pure cultures were obtained through repeated sub-culturing until morphologically homogeneous colonies were observed. The purified isolates were then inoculated onto selective and differential media, including Mannitol Salt Agar (MSA),

MacConkey Agar, Eosin Methylene Blue (EMB) Agar, and Salmonella–Shigella (SS) Agar, and incubated at 37°C for 24 hours to facilitate bacterial identification. The isolated bacteria were identified according to the standard procedures recommended by the International Commission on Microbiological Specifications for Foods (ICMSF). Identification was based on colony morphology, Gram staining, and a series of biochemical tests included motility, indole production, oxidase, citrate utilization, coagulase, and catalase tests. The overall laboratory procedure illustrated in **Figure 1.**

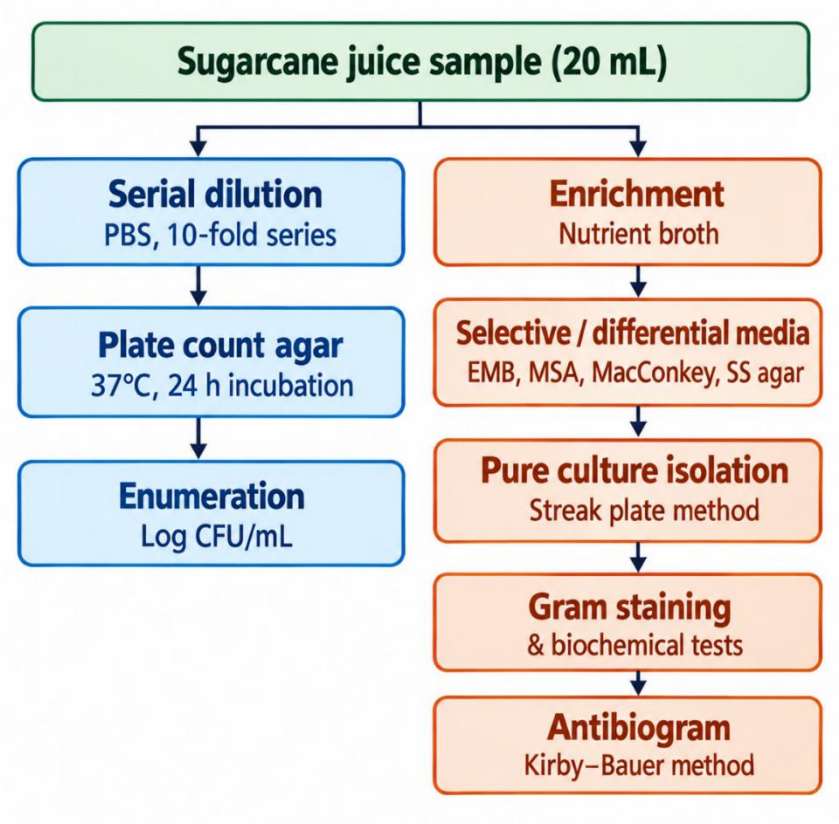

Figure 1. Laboratory workflow for microbiological analysis of street- vended sugarcane juice samples.

2.5. Antibiogram study

The antimicrobial susceptibility of the isolated bacterial strains was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Difco) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Commercially available antibiotic discs were placed on the inoculated agar surface, and the plates were incubated at 37°C for 18–24 hours. After incubation, the diameters of the inhibition zones around each antibiotic disc were measured in millimeters. The bacterial isolates were classified as susceptible, intermediate, or resistant according to the CLSI interpretative criteria.

2.6. Quality control

All media and reagents were prepared according to manufacturers' instructions and sterility-checked before use. Positive and negative controls were included in biochemical and susceptibility testing where appropriate. Results were recorded and tabulated for descriptive analysis; no inferential statistics were applied owing to the limited sample size.

3. RESULTS

3.1. Total viable count (TVC) results for the microbial load

Table 1 presents TVC of street-vended sugarcane juice samples collected from different vendors. Among the samples analyzed, the sample (BM 1) collected from the Gopalganj Science and Technology University (GSTU) campus exhibited the highest bacterial load, with a TVC of 4.2×10^6 CFU/mL followed by sample BM4 (3.8×10^6).

Table 1. Microbial load by total viable count.

Samples	No. of colonies	Dilution Factor	Volume of culture plate (mL)	Colony Forming Unit (CFU/mL)
BM 1	210	10^3	0.05	4.2×10^6
BM 2	180	10^3	0.05	3.6×10^6
BM 3	160	10^3	0.05	3.2×10^6
BM 4	190	10^3	0.05	3.8×10^6

Note. BM 1 = GSTU Campus; BM 2 = Nobinbag; BM 3 = Bangabandhu College More; BM 4 = Chourangi.

3.2. Isolation and cultural characteristics

Figure 2 illustrating the morphological examination on selective media identified distinct bacterial growth patterns across the 12 purified isolates.

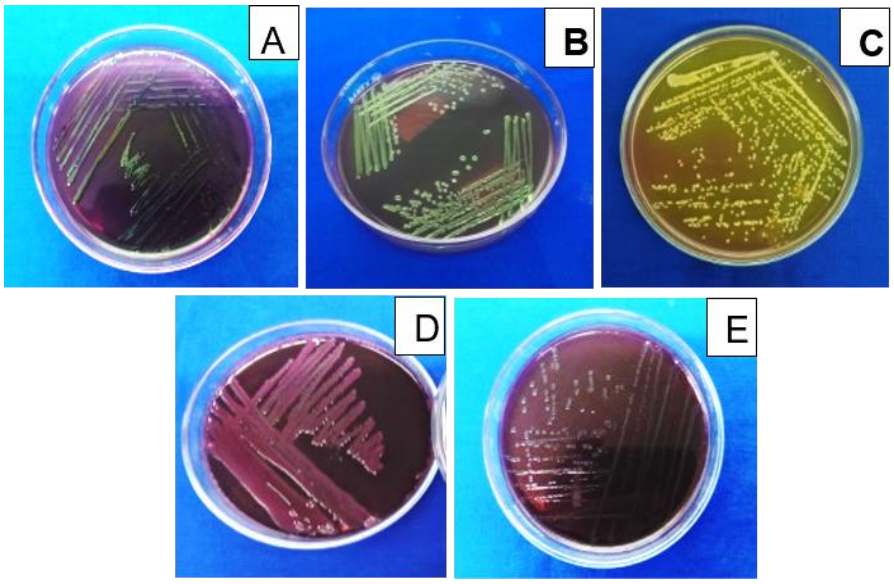

Figure 2. Bacterial growth patterns observed on street-vended sugarcane juice samples collected from different vendors after cultivation using the spread-plate technique. Here, (A) *Citrobacter* spp., (B) *Escherichia coli* (C) *Staphylococcus* spp. (D) *Klebsiella* spp. (E) *Enterobacter* spp.

A total of 12 bacterial isolates belonging to six presumptive groups were recovered from four samples of street-vended sugarcane juice (**Table 2**). *Staphylococcus* spp. and *Klebsiella* spp. were the most frequently detected organisms, each present in 75% (3/4) of the samples (3 isolates each). *Escherichia* coli and *Enterobacter* spp. were isolated from 50% (2/4) of the samples (2 isolates each), while *Citrobacter* spp. and *Salmonella* spp. were each recovered from a single sample (25%; 1 isolate each). Colony morphologies on the respective selective/differential media were consistent with the presumptive identifications of these genera.

Table 2. Results of isolation of bacteria from street-vended sugarcane juice.

Presumptive Bacterial Group	Selective/Differential Media	Observed Colony Morphology	Sample Prevalence (N=4)	Total Isolates (N=12)
***Staphylococcus* spp.**	Mannitol Salt Agar	Medium yellowish colonies	75% (3/4)	3
***Klebsiella* spp.**	MacConkey Agar	Large, mucoid, bright pink lactose-fermenting colonies	75% (3/4)	3
Escherichia coli	EMB Agar	Greenish-black colonies with metallic sheen	50% (2/4)	2
***Enterobacter* spp.**	MacConkey Agar	Pink mucoid colonies (smaller than *Klebsiella*)	50% (2/4)	2
***Citrobacter* spp.**	MacConkey Agar	Light pink colonies (late lactose fermenter at 48 h)	25% (1/4)	1
***Salmonella* spp.**	SS Agar	Tiny, non-lactose fermenting colonies with black centers	25% (1/4)	1

Note: EMB= Eosin Methylene Blue; **SS=** Salmonella–Shigella. (Representative agar plate photographs for culture characterization were not captured during primary laboratory testing).

3.3 Biochemical characterizations

Biochemical characterization of the 12 isolates (**Table 3**) confirmed the presumptive identifications obtained from selective media. Three isolates were identified as *Staphylococcus* spp. (Gram-positive, catalase-positive, coagulase-positive), three as *Klebsiella* spp. (Gram-negative, VP-positive, urease-positive, non-motile), two as *Enterobacter* spp., two as *Escherichia* coli (indole-positive, MR-positive), and one each as *Citrobacter* spp. and *Salmonell*a spp. (H_2S-positive on TSI). All Gram-negative isolates were oxidase-negative and catalase-positive, with TSI reactions consistent with their respective genera.

Table 3. Biochemical test results.

Sample Code	Isolate ID	Gram Stain	MR	VP	Oxidase	Catalase	Motility	Indol	Urease	Citrate	TSI Slant	TSI Butt	TSI Gas	TSI (H_2S gas)	Coagulase	Presumptive Identification
BM 1	1A	−	−	+	−	+	−	−	+	+	Y	Y	+	−	N/A	*Klebsiella* spp.
	1B	−	−	+	−	+	+	−	−	+	Y	Y	+	−	N/A	*Enterobacter* spp.
	1C	−	+	−	−	+	+	+	−	−	Y	Y	+	−	N/A	*Escherichia coli*
	1AA	+	+	+	−	+	−	−	+	+	Y	Y	+	−	+	*Staphylococcus* spp.
BM 2	2A	−	−	+	−	+	−	−	+	+	Y	Y	+	−	N/A	*Klebsiella* spp.
	2B	−	+	−	−	+	+	−	−	+	R	Y	+	+	N/A	*Salmonella* spp.
BM 3	2AA	+	+	+	−	+	−	−	+	+	Y	Y	+	−	+	*Staphylococcus* spp.
	3A	−	+	−	−	+	+	−	+	+	Y	Y	+	+	N/A	*Citrobacter* spp.
	3B	−	−	+	−	+	−	−	+	+	Y	Y	+	−	N/A	*Klebsiella* spp.
BM 4	4A	−	−	+	−	+	+	−	−	+	Y	Y	+	−	N/A	*Enterobacter* spp.
	4B	−	+	−	−	+	+	+	−	−	Y	Y	+	−	N/A	*Escherichia coli*
	4AA	+	+	+	−	+	−	−	+	+	Y	Y	+	−	+	*Staphylococcus* spp.

Note: MR= Methyl Red; **VP=** Voges–Proskauer; **TSI**=Triple Sugar Iron (Y = Yellow/Acidic, R = Red/Alkaline); **N/A:** Not Applicable

In **Figure 3**, *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. tested positive in the methyl red (MR) test, whereas *Klebsiella* spp. tested negative. All isolates were positive for the catalase test, as evidenced by gas bubble formation. Except for *Staphylococcus* spp., all isolates tested negative in the oxidase test, showing no color change. The Voges-Proskauer (VP) test yielded positive results for *Salmonella* spp. and *Klebsiella* spp., whereas *E. coli* and *Staphylococcus* spp. showed negative results.

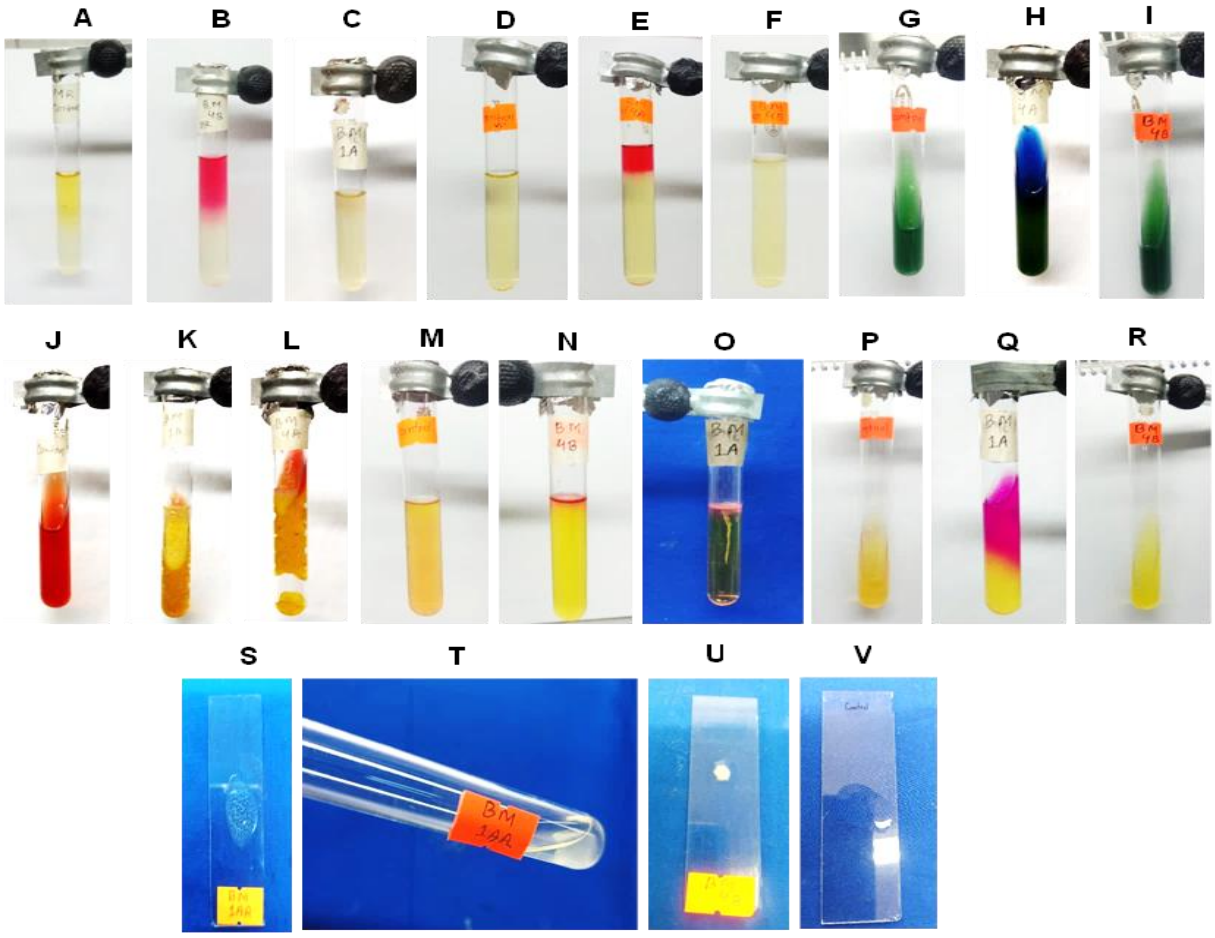

Figure 3: Biochemical test results: A= MR control, B=MR positive=MR negative, D=VP control, E= VP positive, F= VP negative, G= citrate control, H= Citrate positive, I= Citrate negative, J= TSI control K, L = TSI yellow slant and butt and gas positive, M=Motility and Indole control, N = Motility and Indole positive, O= Motility and Indole negative, P= Urease control, Q= Urease positive, R= Urease negative, S, T= Coagulase positive, U= Catalase positive, V= Catalase negative.

3.4. Antimicrobial susceptibility profiles

Figure 4 shows representative antibiotic susceptibility testing results on Mueller-Hinton agar using the disc diffusion method. Clear zones of inhibition surrounding antibiotic discs indicate susceptibility of the tested isolates. (A) *Enterobacter* spp.; (B) *Staphylococcus* spp.; (C) *Klebsiella* spp.

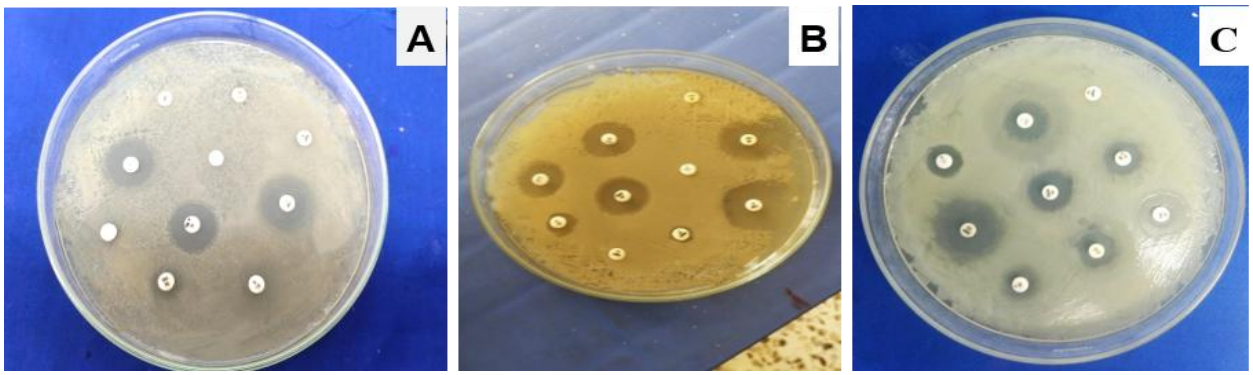

Figure 4. Representative images of antibiotic sensitivity test. Different antibiotic discs were placed on Muller-Hinton agar media and cultured for bacterial growth. A= *Enterobacter* spp., B= *Staphylococcus* spp., C= *Klebsiella* spp.

The average results of the antibiotic susceptibility assay are presented in (**Table 4**). Co-trimoxazole and Gentamicin were found to be effective against all six bacterial isolates. *Enterobacter* spp. and *Salmonella* spp. were resistant to Doxycycline and Tetracycline. Ciprofloxacin was resistant *Staphylococcus* spp. and intermediate for *Enterobacter* spp. Furthermore, all isolates exhibited resistance to Amoxicillin, Erythromycin, Ampicillin, and Metronidazole.

Table 4. Antimicrobial susceptibility profiles of bacterial isolates from sugarcane juice samples.

Antibiotic	***Staphylococcus* spp.**	***Klebsiella* spp.**	***Enterobacter* spp.**	***E. coli***	***Salmonella* spp.**	***Citrobacter* spp.**
Ampicillin	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Tetracycline	23 (S)	19 (I)	0 (R)	24 (S)	10 (R)	23 (S)
Gentamicin	26 (S)	26 (S)	30 (S)	25 (S)	24 (S)	27 (S)
Erythromycin	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Ciprofloxacin	11 (R)	21 (S)	16 (I)	29 (S)	22 (S)	21 (S)
Amoxicillin	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Doxycycline	20 (S)	15 (I)	9 (R)	20 (S)	16 (I)	22 (S)
Metronidazole	0 (R)	9 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Co-trimoxazole	28 (S)	30 (S)	28 (S)	28 (S)	29 (S)	30 (S)
Cefotaxime	10 (R)	24 (S)	10 (R)	0 (R)	17 (I)	16 (I)

Note: Values represent the calculated zones of inhibition (mm). **I** = intermediate, **S** = sensitive, and **R** = resistant, indicating the responses of the bacterial isolates to the respective antibiotics [17].

4. DISCUSSION

The contamination profile observed street-vended sugarcane juice in Gopalganj contaminated by various types of bacteria. The more alarming finding is the presence of fecal coliforms in all samples, as they are known to only survive briefly in the environment outside the gut, and their presence suggests a contamination source that is there more recently, or at least present at the time of sampling, as opposed to historical or incidental contamination [18,19]. The "elevated" microbial counts (3.2×10^6–4.2×10^6 CFU/mL) are not just "higher" than normal relative to any particular pathogen, directly associated with symptomatic foodborne illness as a class, stand-alone from each pathogen's virulence [20]. Added to this, the identified genera like *Klebsiella*, *E. coli* and *Salmonella* are well recognized causes of gastroenteritis, urinary tract infection and invasive bloodstream infection, respectively, and S. *aureus* includes a pathogen that can cause intoxication without being viable at the time of ingestion. Other studies also have been documented in street foods sold near educational institutions and in retail meat samples from local markets in Bangladesh, underscoring inadequate hygienic practices and antimicrobial resistance circulating in the informal food sector [21-23].

Beyond the elevated microbial load, the antimicrobial-resistance patterns observed are of particular concern. This pattern of high resistance to ampicillin, erythromycin, amoxicillin and metronidazole and high susceptibility to gentamicin, co-trimoxazole and ciprofloxacin is alarming for public health. This resistance pattern is likely attributable to the widespread availability of these antibiotics at low cost, their frequent sale without prescription, and their common use as first-line agents in both human and veterinary healthcare sectors in Bangladesh. In this context, the resistance profile of street-vended juice isolates is an indirect marker of the local antimicrobial consumption profile, already reported in many countries like India, Nigeria, and Ethiopia [13, 24, 25].

A question, the results do not answer, is whether sugarcane juice is a passive reservoir of resistance that is already present in the environment or an active source for resistance gene exchange? The characteristics of sugarcane juice high nutrient content, warm temperature, frequent human handling offer a potential scenario for horizontal transfer between co-occurring genera, which would allow the transfer of resistance determinants regardless of the resistance status of any individual plant's species [26, 27]. Then contaminated juice is not only a reflection of the antimicrobial resistance issue, it also can be an amplifier as it introduces resistant microbes and mobile resistance elements into a broader human population via an unregulated, high turnover food product [28]. The present study cannot confirm whether gene transfer is occurring; future work using plasmid profiling or whole-genome sequencing would be needed to answer this question.

The findings must be interpreted in light of several limitations. The sample size was small (n = 4), restricting statistical power and generalizability. Bacterial identification relied solely on cultural and biochemical criteria and therefore remains presumptive; molecular confirmation was not performed. Minimum inhibitory concentration (MIC) assays were not performed, and the observed inhibition-zone diameters were not correlated with specific antimicrobial resistance genes. Consequently, the study could not determine the underlying molecular mechanisms responsible for the observed resistance phenotypes. Despite these constraints, the consistent recovery of high bacterial loads and resistant isolates across all sampling sites provides clear evidence of inadequate hygienic practices and a tangible public-health risk. Strengthening

vendor education on basic food-hygiene principles, ensuring access to potable water, enforcing equipment sanitation, and implementing periodic microbiological surveillance are essential measures to reduce consumer exposure

5. CONCLUSIONS

This study demonstrates that street-vended sugarcane juice sold in Gopalganj, Bangladesh, is frequently contaminated with potentially pathogenic bacteria, with microbial loads exceeding the permissible limits established for fruit juices in many countries. The bacterial isolates also exhibited diverse antibiotic resistance patterns, including resistance to commonly used antimicrobial agents, raising concerns about the potential dissemination of antimicrobial-resistant bacteria through ready-to-consume beverages. These findings suggest that the juice is often prepared, handled, and marketed under inadequate hygienic and sanitary conditions, likely due to the use of contaminated water, poor equipment sanitation, and insufficient knowledge of food safety practices among vendors. From a public health perspective, the consumption of contaminated sugarcane juice may increase the risk of foodborne illness and contribute to the spread of antimicrobial resistance within the community. Strengthening food safety regulations, improving vendor hygiene training, ensuring access to safe water, and implementing regular microbiological surveillance are essential to minimize these risks and protect consumer health.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial or personal interests that could have appeared to influence the work reported in this paper.

ETHICAL CONSIDERATION

This study did not involve human participants or animals; therefore, ethical approval was not required.

AUTHOR CONTRIBUTIONS

Md. Sarafat Ali: Conceptualization, methodology, formal analysis, writing original draft, review & editing. **B M Tanjim Abid**: Investigation, formal analysis, review & editing. All authors have read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available in the article. Further details may be obtained from the corresponding author upon request.

DECLARATION OF AI USE

The AI tools were not used to generate scientific content, interpret data, or draw conclusions. All literature selection, data interpretation, and final manuscript preparation were performed entirely by the authors, who accept full responsibility for the accuracy and integrity of the work.

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