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Microbial Quality Assessment of Fresh Sugarcane Juice in Retail Shops Inside Kathmandu Valley

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Abstract

This study aims to assess the microbial quality of fresh sugarcane juice sold in retail shops inside Kathmandu Valley, as there is limited information available on the safety of popular fresh juices like sugarcane juice, which can cause food-borne illnesses when improperly prepared, despite the known health benefits of consuming fresh fruits juices. Sixty fresh sugar cane juice samples were collected from selected thirteen areas inside Kathmandu Valley. Out of 60 samples, 42 samples were contaminated with bacteria. The quality of each sample was assessed by total plate count, total coliform count, yeast and mold count, and isolation of pathogenic bacteria. The isolated bacteria were identified based on biochemical characteristics. The pH of collected samples ranged from 4.33 to 6.34, acidity from 0.01% to 0.09%, and total soluble solids from 13 to 21ºBrix. The mean bacterial count was 5.26 $\log_{10}$ CFU/ml, the coliform count was 4.67 $\log_{10}$ CFU/ml, and the yeast mold count was 4.94 $\log_{10}$ CFU/ml. *E. coli* was present in 38.1%, *Citrobacter freundii* in 47.6%, and *Salmonella* species in 14.3% of the total sample. *Vibrio cholerae* was not detected. Gentamicin, Ciprofloxacin, and Ofloxacin were effective, while Ampicillin was less effective. The study found that sugarcane juice samples from shops in Kathmandu Valley have high levels of contamination, including a high microbial load and slightly acidic pH with high total soluble solid values. The bacterial isolates exhibited varied degrees of antimicrobial susceptibilities and resistances, and the contamination was attributed to poor hygienic conditions during juice processing and handling, possibly due to the use of poor-quality water and a lack of training on food hygiene and safety. These findings suggest potential health hazards for consumers of fresh sugarcane juice.

Keywords: Foodborne diseases; fresh sugarcane juice; Kathmandu Valley; microbial quality assessment; retail shops

Introduction

Codex Alimentarius (Alimentarius, 2005) defines fruit juice as a liquid that is unfermented but has the potential to ferment. It is made from either the edible parts of fresh, healthy, and mature fruit or fruit preserved in a healthy state using appropriate techniques, including postharvest surface treatments allowed under relevant provisions. Even though pips, seeds, and peel that cannot be removed using authorized manufacturing techniques may be processed into various juices even if they are not typically included in the juice, some sections or components of pips, seeds, as well as other peel will be permitted. During the whole manufacturing process, the key physical, chemical, organoleptic, and nutritional qualities of the fruit juices from which the juice is derived are maintained. The juice may be transparent or cloudy and include recovered aromatic components (Bittisnich and Gorst, 2006).

Non-fermented and non-sparkling, fruit juice is a beverage that is made by diluting the juice, pulp, and vegetable extract of the original fruit with drinkable water, according to (Pilo et al., 2009). Fruit juices and fruit-based beverages are popular products that appeal to a diverse demographic, particularly youngsters and young adults, and represent a significant domestic and global market segment (Vasavada, 2003). Fruit juices could be chosen based on taste, product cost, and nutritional value. Fresh fruit juices are frequently consumed globally because of their nutritional value and health advantages. Fruit juices seem to be nutritious and perishable meals that might serve as a perfect substrate for harmful microbe development and proliferation (Mengistu et al., 2022). However, sugarcane juice tends to degrade rapidly even when refrigerated due to its pH, high sugar and water content, and inappropriate temperature (Ali et al., 2018).

Occasionally, production requirements for hygiene need to be properly followed. Without safety equipment and also with badly cleaned raw materials, squeezing might compromise the microbiological quality of the fresh juices. Such unsanitary processing conditions could accelerate various physicochemical changes that affect the product's constitution and pH and promote the growth of bacteria. Juices with an unusually high concentration of harmful bacteria provide a significant risk of contracting foodborne illnesses. Unpasteurized fruit juices have been linked to multiple foodborne epidemics in the previous 20 years, increasing morbidity, infections, and mortality in developing nations (Jackson and Meah, 2017, Bhunia, 2018).

The World Health Organization (WHO) report from 2017 approximations that there are about 600 million cases of foodborne infections worldwide each year, with 420,000 deaths, including 125,000 under the age of 5. Unpasteurized juices such as apple, pineapple, orange, carrot, coconut, sugarcane, lemon, mango, acai, and mixed fruit drinks were the major factors in most of these outbreaks (Raybaudi-Massilia et al., 2009, Ghenghesh et al., 2005, Bevilacqua et al., 2011).

Fresh and unpasteurized fruit juice is a favored option among people in Kathmandu Valley due to its delicious taste and nutritional benefits. Consequently, there has been a surge in the number of retail outlets offering various types of fresh fruit juices. However, the absence of a proper food safety system poses a significant threat to the long-term health, safety, and economic development of the population in Kathmandu Valley. In the summer season, microbial foodborne outbreaks such as diarrhoea, vomiting, cholera, and so on have been reported in the Kathmandu District (Maharjan et al., 2006).

In urban areas like Kathmandu, poor reporting and underreporting of foodborne disease outbreaks further aggravate this issue, making it more widespread. With the growing demand for fast food due to changes in lifestyle, the consumption of locally prepared fresh fruit juices that require microbial quality testing has increased. Hence, there is a need to establish a database on the current quality

status of fresh fruit juice to address this situation. This study aims to assess the microbial quality of fresh sugarcane juice sold in retail shops inside Kathmandu Valley, Nepal.

Methodology

Study Area

This study was conducted between April and September 2022 in the Kathmandu Valley. Thirteen locations were chosen to obtain freshly squeezed sugarcane juice, and a total of 60 fruit retail shops were selected from these locations to collect the juice.

Sample Type

Freshly squeezed sugarcane juice was taken for the assessment of its microbial quality.

Sample Analysis

The collected samples were examined in the Food Microbiology Laboratory of the National College, Kathmandu. Samples were tested for the presence of:

- Total Mesophilic bacteria
- Coliforms
- Yeasts and Molds
- *Salmonella* spp.
- *Vibrio cholerae*

Laboratory Procedure

Determination of pH of the sample:

Each sample of fruit juice was tested for pH using a pH meter inside the lab.

Determination of degree Brix of sample:

In a lab setting, a refractometer was used to determine the total soluble solids of each juice sample.

Determination of titrable acidity of the sample:

The titrable acidity of all the individual juice samples was determined by using the titration method. A 10ml aliquot was titrated with 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator. The percentage of acidity in the form of citric acid was calculated. The following relationship was used to calculate the percentage of titrable acidity.

$$Titrable\ Acidity = \frac{Vol.of\ titrate \times N\ of\ NaoH \times equivalent\ weight\ of\ citric\ acid \times volume\ made\ up \times 100}{aliquot \times Volume\ of\ sample \times 1000}$$

Serial Dilution

10 ml of the sample was pipetted with the help of a sterile pipette and mixed with 90 ml of peptone water by shaking. It was then considered as 10^{-1} dilutions. From 10^{-1} dilutions, 1 ml was transferred into a second tube containing 9 ml of peptone water and labeled 10^{-2} dilutions. Similarly, the process was repeated up to 10^{-6} dilutions, labeled as 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. After each dilution, the tube was shaken well.

Enumeration of microorganisms

Enumeration simply assumes that a single microbial cell forms a visible colony in a suitable agar medium when the diluted sample is incubated at optimum temperature.

Enumeration of total mesophilic bacteria, total coliform, total yeast, and mold

A pour plate was conducted using plate count agar (PCA) from previously produced alternative diluents, i.e., 10^{-2}, 10^{-4}, and 10^{-6}, and incubated at 37°C for 24h for the total mesophilic count. After the necessary dilutions, a pour plate on violet-red bile agar (VRBA) was done to determine the total coliform count. To achieve this, the diluted sample and agar medium were thoroughly and consistently mixed before the plate was allowed to harden. The plate was then covered with VRBA and left to dry. It was then incubated at 37°C for 24h. For complete fungal growth, 0.1 ml of the required dilutions were surface-plated on pre-poured Potato Dextrose Agar (PDA). PDA plates were then incubated for 48 hours at 28 °C.

After incubation, several culture plates were evaluated for microbial growth. Colonies were enumerated and represented as colony-forming units per mL (CFU/ml) of material. Many morphological properties of colonies were examined and documented. Typical colonies were isolated and sub-cultured on nutrient agar before being preserved at four degrees Celsius for future identification.

Identification of Bacteria (Cheesbrough, 2005)

To identify the various microorganisms via laboratory tests, the following procedure was conducted.

Detection of E. coli:

A sterile micropipette transferred 10 ml of the sample to a conical flask containing 90 ml of sterile buffered peptone water. It was followed by proper mixing and incubation at 37°C for 24h. After incubation, the culture was streaked on previously prepared and dried Eosin Methylene Blue (EMB) using an inoculating loop and incubated at 37°C for 24h. Morphological and biochemical tests further confirmed different isolated colonies with a metallic sheen.

Detection of Salmonella* spp.*:

A sterile micropipette piped a 10 ml sample into a conical flask filled with 90 ml of sterile buffered peptone water. Then it was properly mixed and incubated for at 37°C for 24h. A little shaking was applied to the incubated sample combination. Using a sterile micropipette, one ml of the sample was then moved to a tube containing 10 ml of selenite-F broth (SF), where it was incubated for 24 hours at 37 °C. Using an inoculating loop, the culture was spread on previously made and dry-dried Salmonella-Shigella agar (SS) and then cultured at 37°C for 24 hours. Using morphological and biochemical testing, various isolated colonies with or without black centers were further validated.

Detection of Vibrio cholerae:

A sterile micropipette piped a 10 ml sample into a conical flask filled with 90 ml of sterile alkaline peptone water. It was then properly mixed and incubated for at 37°C for 24h. The culture was properly incubated before being streaked with an inoculating loop on prepared and dried Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar and incubated at 37°C for 24h. Biochemical and morphological studies further confirmed several isolated yellowish colonies.

Biochemical Test

Additional biochemical tests were performed on the chosen isolates to help identify them. Both for gram-positive as well as gram-negative species, there are various sets of biochemical assays. Catalase, oxidase, oxidative/fermentative, and coagulase tests were done on gram-positive organisms, while IMVIC, TSI, citrate, and Urease tests were performed on gram-negative species.

Catalase Test

This test determines whether or not the catalase enzyme is present. The catalase enzyme in bacteria transforms hydrogen peroxide into oxygen and water. A clean glass rod was used to pick up the bacterial colony to be investigated, which was then emulsified on a slide. For this reason, a few drops of hydrogen peroxide (3%) were applied to the slide. The presence of gas bubbles showed that the catalase test was positive.

Oxidase Test

During this test, the presence of the enzyme oxidase, which catalyzes the transfer of electrons between a bacterial electron donor and the redox dye tetramethyl-p-phenylene-diamine dihydrochloride, is checked. The dye has been lightened to a rich purple hue. Oxidase paper was placed on a dry, clean glass slide and impregnated with 1% tetramethyl-p-phenylenediamine dihydrochloride for this test. The colony to be examined was picked up and smeared on the paper with a clean, sterile glass rod. A positive test result was shown by the appearance of violet within 10 seconds. However, the formation of the violet hue within 10–60 seconds and a negative response to the lack of color suggested that the test was ineffective.

Oxidative/Fermentative Test

Hugh and Leifson's semisolid media were used in this test to determine if the organisms are fermentative or oxidative. For this test, two test tubes containing the media were used. The organism samples were stabbed into both tubes with a sterile wire. Both tubes were closed with cotton and cultured at 37°C for 24h after one was filled with sterile paraffin oil to create one cm-thick column just on media. When both tubes turn yellow, the organism is oxidative and produces acid under aerobic and anaerobic conditions. However, if only the open tube goes yellow, the organism is aerobic.

Indole Production Test

This test aims to determine whether the tryptophan enzyme can break the tryptophan found in the sulfide-indole-motility media to generate indole as the byproduct of cleavage. The organism turned red when Kovac's reagent was added, indicating indole positive. Prior to autoclaving and cooling in the tubes, the SIM medium had to be prepared. With a straight wire, a small amount of organism from the subculture was extracted and stabbed into a SIM medium tube before being cultured at 37°C for 24h. After incubation, 4-5 drops of Kovac's reagent were added to the tubes containing the inoculated organisms, and the color of the liquid was checked.

Methyl Red Test

The basic principle of this test is to detect the organism's ability to produce and maintain good stable acid end products from glucose fermentation. The reagent used in this test is methyl red, and the media used is MR/VP media. After adding the reagent, the media became red after being inoculated with organisms, showing methyl red positivity. For this test, MR/VP media were prepared, inoculated with organisms with the help of a sterile inoculating loop, shaken well, and then incubated at 37°C for 24h. After incubation, the medium was thoroughly shaken, 5-6 drops of the methyl red reagent were added and thoroughly mixed, and a color change in the medium was observed.

Voges Proskauer Test

This test aims to see if the organism can ferment glucose and produce the neutral end product acetyl methyl carbinol or the reducing product 2, 3-butylene glycol. The reagent employed is Barrit's reagent, while the medium is MR/VP medium. This Barrit's reagent comprises a 3:1 mixture of 40% KOH and 5% alpha naphthol. A small amount of an organism was added to the MR/VP medium, and it was thoroughly agitated before use. After that, this medium was incubated for at 37°C for 24h. After culture, 3 ml of alpha naphthol and 1 ml of 40% KOH were added, and everything was thoroughly mixed. The media's hue changed after that, as was noticed.

Citrate Test

This test establishes whether an organism can utilize citrate as its sole carbon source. Because of the conversion of ammonium salts into ammonia and the ensuing alkalinity, the medium's indicator changes color. Simmons's citrate media is the name of the substance used in citrate testing, which also uses bromothymol blue as a pH indicator. Simmons' citrate medium tube was then taken out. After incubation, the organism was streaked over the media's incline and incubated for at 37°C for 24h. It was visible that the slant's color had changed. The citrate-positive organism has a striking blue color when viewed from a slant.

Urease Test

This test is run to determine whether or not the organisms created the Urease enzyme, which breaks down the chemical urea into carbon dioxide and ammonia. Urea agar media, which uses phenol red as an indicator, is the test medium. The urea agar media tube was removed from this location. Afterward, a small organism was removed and scattered on the media's incline using a sterile loop for inoculation before being cultured at 37°C for 24h. A change in the media's hue was noticed after incubation. When urease-positive organisms are present, the medium turns deep pink, but not when urease-negative organisms are present.

Triple Sugar Iron Agar Test

This test is used to identify which type of glucose, lactose, or sucrose the organism uses and whether or not it also produces gas and hydrogen sulfide. The medium used is triple sugar iron agar, which contains iron that reacts with hydrogen sulfide to detect its presence. It also contains 0.1% glucose, 1% lactose, as well as 1% sucrose. The triple sugar agar medium tube was removed. The organism was streaked and poked into the medium in small amounts, then incubated for at 37°C for 24h. After incubation, the media's color changed as expected.

Antibiotic Susceptibility Test

Ten antibiotic discs from seven different families were used to evaluate antibiotic susceptibility (Table 1). The discs were chosen following CLSI (Clinical & Laboratory Standards Institute) recommendations. *Salmonella*, *E. coli*, and *Citrobacter* spp. were tested against chloramphenicol (10 mg) and ampicillin (10 mcg) antibiotic discs that are available commercially (30 mcg). Modified Kirby Bauer, disk diffusion method procedures are used with Ceftazidime (30 mcg), Gentamicin (10 mcg), Ciprofloxacin (5 mcg), and Co-trimoxazole (25 mcg). Five colonies of each isolate were added to 5 ml of nutrient broth and cultured for 4 hours, and then the culture turbidity was adjusted to a McFarland standard of 0.5. The Mueller-Hinton Agar surface was then uniformly covered with the suspension after dipping the sterile cotton swab into it. The antibiotic-impregnated discs were placed over the inoculation plates, which were incubated at 37°C for 16–18 hours. After incubation, the inhibitory zone widths were determined, and susceptible, intermediate, and resistant conditions were identified using millimeter measures (Humphries et al., 2021).

Table 1: Chart for antibiotic sensitivity

Antibiotics	Strength	Diameter of zone of inhibition in mm		
	Mcg	Resistant	Intermediate	Sensitive
Ampicilin (A/S)	10/10	11	12-14	15
Cefotaxime (CTX)	30	22	23-25	26
Ceftazidime (CAZ)	30	17	18-20	21
Ciprofloxacin (CIP)	5	21	22-25	26
Norfloxacin (NX)	10	12	13-26	17
Ofloxacin (OF)	5	12	13-15	16
Gentamicin (GEN)	10	12	13-14	15
Chloramphenicol (C)	30	12	13-17	18
Nitroflurantoin (NIT)	300	14	15-16	17
Cotrimoxazole (COT)	25	10	11-15	16

Data Analysis

The laboratory test data were entered into MS Excel and cleaned. Some values were transformed to $\log_{10}$ for statistical analysis. Descriptive statistical tools such as mean, standard deviation, frequency, and percentage were used to summarize the data, and bar charts were utilized to visually present the findings.

Results and Discussion

Physiochemical Parameters

The fresh sugarcane juice samples were tested for various physicochemical parameters, which included pH, titratable acidity, and total soluble solids (measured in ºBrix). The pH of the sugarcane juice was found to be moderately acidic, with a range of 4.33 to 6.34. The total soluble solids (measured in ºBrix) ranged from 13 to 21. Additionally, the titratable acidity of the juice samples was found to range between 0.01% to 0.09%.

pH has an impact on the stability and beneficial properties of sugarcane juice (Chia et al., 2012). The juice is slightly acidic, ranging from pH 4.3 to 6.34, with a titratable acidity of 0.01% to 0.09%. The Brix value ranged from 13 to 21. Some bacteria, such as *S. typhi* and *E. coli*, are considerably more acid-resistant than others. The juice's pH and sugar content may have inhibited the growth of some organisms (Chia et al., 2012). Most of the fresh juice samples had brix levels ranging from 14º to 21ºbrix, as recommended by CODEX STAN 247:2005 for fruit juice and nectars (Bittisnich and Gorst, 2006). According to FAO, juices with more than 1.2% acid are sour, regardless of brix. Neither is classified as weak and watery of all the samples assessed, as their ºBrix is not less than 7. However, juices with less than 7º brix are categorized as weak and watery (Bates et al., 2001).

Enumeration of Microorganisms

Total Bacterial Count:

In the study area, the average total bacterial count was determined 5.26 $\log_{10}$ CFU/ml. Among the tested locations, the highest total bacterial count was observed 6.75 $\log_{10}$ CFU/ml with a standard deviation of 0.55. On the other hand, the lowest total bacterial count was found 3.77 $\log_{10}$ CFU/ml with a standard deviation of 0.12. The presence of bacteria in fruit juice is a serious concern as it can pose a threat to consumer health (Oladipo et al., 2009). Microorganisms present in fruits can be indicative of the hygienic conditions in which the product was grown, harvested, transported, stored, and processed (Bhunia, 2018, Beuchat, 1992). In Salaam City, Tanzania, the total bacterial counts in fresh sugarcane juice ranged from 1.44×10^5 to 6.0×10^5 CFU/mL (Mwambete and Mpenda, 2019). Similarly, in Dhaka, Bangladesh, street-vended fresh sugarcane juices had total viable counts within the 0 to 10^7 CFU/mL range, with significant loads of coliforms, fecal coliforms, *Vibrio*, and s*taphylococcal* counts (Afroz et al., 2019). To elaborate further, the findings of this study align with previous research that has also identified the presence of mesophilic aerobic bacteria in fresh sugarcane juices. There are several possible reasons for the prevalence of these bacteria, one of which is the storage practices of the vendors. Typically, the sugarcane is peeled and left in an open environment at room temperature before it is consumed. This environment provides an ideal setting for the growth and proliferation of mesophilic aerobic bacteria, which can result in a high bacterial load in fresh sugarcane juice.

Total Coliform Count:

The overall average total coliform count was determined 4.67 $\log_{10}$ CFU/ml with a standard deviation of 0.22. Among the tested locations, the highest total coliform count observed was 5.82 $\log_{10}$ CFU/ml with a standard deviation of 0.36. On the other hand, the lowest total coliform count was 3.72 $\log_{10}$ CFU/ml with a standard deviation of 0.12. In a related research by Alamgir et al. (2015), all samples revealed the presence of fecal coliforms between 2.51 and 0.78 $\log_{10}$ MPN/100 mL, while the coliform count ranged from 0.8×10^4 to 22.2×10^4 CFU/100 mL in the study by (Lewis et al., 2006).

The higher overall coliform count in the present study compared to other similar studies may be attributed to inadequate quality control during fresh juice preparation. Coliform bacteria in fruit juice may suggest inadequate hygiene and sanitation during preparation and handling, necessitating the use of clean and hygienic water for preparation, utensils and equipment cleaning, and machine operation to prevent contamination. Fruit juice containing coliforms, such as *E. coli* and *Citrobacter*, can cause water-borne illnesses and are therefore not safe for consumption.

Total Yeast and Mold Count:

The highest total fungal count observed in the fresh sugarcane juice was 6.16 $\log_{10}$ CFU/ml with a standard deviation of 0.68. On the other hand, the lowest total fungal count was found which was 3.7 $\log_{10}$ CFU/ml with a standard deviation of 0.11. Additionally, the average total fungal count in the study area was determined 4.94 $\log_{10}$ CFU/ml with a standard deviation of 0.45.

Contamination could be caused by mixing rotting parts with new stock, using unclean and dusty settings with insects, and delivering juice in dirty containers. These factors may account for the high levels of yeast and mold observed in this study. Fungi are the main cause of food spoilage and their presence in PDA can indicate contamination of fresh fruit juices, with the possibility of toxin production that can be harmful to human health (Samson et al., 1981).

Isolation and Identification of Pathogenic Bacteria

Samples collected from different locations contained various bacteria, including *Citrobacter freundii*, *E. coli*, and *Salmonella* spp. Among them, *Citrobacter freundii* was the most commonly found bacteria, present in 47.6% of the total samples. *E. coli* was isolated from 38.1% of the samples, whereas *Salmonella Typhimurium* was detected in the fewest samples, only 14.3%. None of the juice samples tested positive for *Vibrio cholerae* (Table 2).

During transporting, washing, rinsing, or processing by the processors, some of the bacteria discovered in this investigation may be pollutants from soil, water sources, and the outdoors or maybe a part of the natural flora (Ofor et al., 2009). *E. coli* indicates fecal contamination since it is present in the human gut. *E. coli* are naturally occurring bacteria. Urinary tract infections, gastroenteritis, and diarrhea have all been connected to certain strains of *E. coli* (Yu et al., 2006).

Because sellers do not use boiling or potable water for cleaning and juicing, *Salmonella* may have entered through contaminated water. *Salmonella* has been a growing concern to fresh foodstuffs and has caused foodborne diseases all over the world (Begum et al., 2010).

The microorganisms discovered in the current study include *Salmonella* spp. and *Escherichia coli*. The Food Safety and Standards Authority of India has said that the presence of this specific type of pathogenic bacteria might be dangerous to the public's health. Thus, these harmful microorganisms must not be present. Particularly for small children and older individuals with compromised immune systems, these bacteria may increase the risk of foodborne disease. Prospective consumers, food producers, and government agencies should be made aware of the likelihood that certain bacteria species might cause food poisoning by ingesting contaminated food. In addition to being a part of the normal flora in the human gut, *C. freundii* is also a conditionally pathogenic bacterium that may infect people with low immunity and cause a variety of ailments, such as pneumonia, meningitis, sepsis, bacteremia, urinary tract infections, and others (Jia et al., 2020).

Table 2: Frequency and percentage of different bacterial organisms detected in sugarcane juices

S.N.	Bacterial isolates	Number	Percentage (%)
1	*Citrobacter freundii*	20	47.6
2	*E. coli*	16	38.1
3	*Vibrio cholerae*	0	0
4	*Salmonella* spp.	6	14.3
5	Total	42	100.0

Antibiotic Susceptibility Test:

All 42 identified isolates from fruit juices were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion technique. The isolates were tested against ten antibiotics from seven different families: Ampicillin (AS 10/10 mcg), Ceftazidime (CAC 30/10mcg), Cefotaxime (CX 30mcg), Ciprofloxacin (CIP 5mcg), Norfloxacin (NX 10 mcg), Ofloxacin (OF 5 mcg), Chloramphenicol (C30 mcg), Co-trimoxazole (COT 25 mcg), Gentamicin (GEN10 mcg), and Nitroflurantoin (NIT 300 mcg). The results showed that Gentamicin, Ciprofloxacin, and Ofloxacin were effective against all bacterial isolates. In contrast, Ampicillin showed low efficacy against most bacterial isolates (Fig 1).

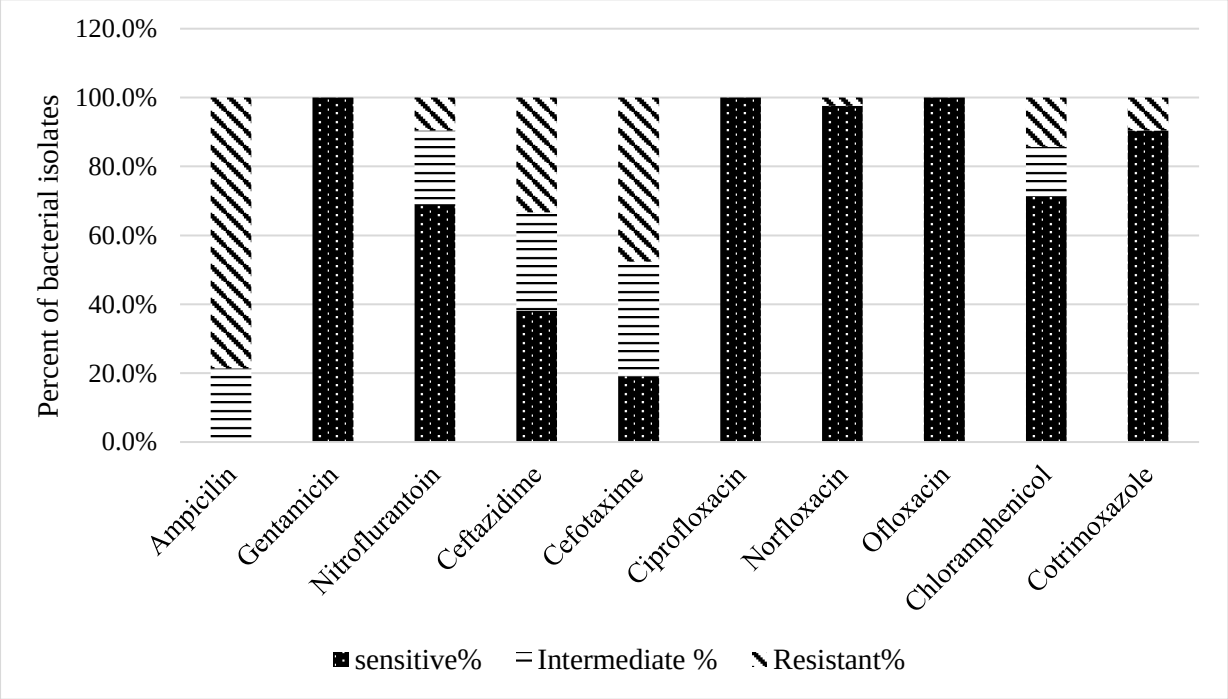

Fig 1: Antibiotic susceptibility pattern of isolated bacteria

Antibiotic Sensitivity pattern of Citrobacter freundii:

Table 3 displays the results of the antimicrobial susceptibility test conducted on 20 isolates of *Citrobacter freundii*. The table shows that 75% of the isolates were resistant, and 25% exhibited intermediate resistance to ampicillin. Regarding ceftazidime, 50% of the isolates were sensitive, 25% were intermediate, and 25% were resistant. For cefotaxime, 60% of the isolates were resistant, and 40% showed intermediate resistance. All (100%) of the bacterial isolates were sensitive to

Ciprofloxacin, Norfloxacin, Gentamicin, and Ofloxacin. For chloramphenicol, 70% of the isolates were sensitive, and 30% were intermediate. Nitrofurantoin showed 55% sensitivity and 45% intermediate resistance, while 80% of the isolates were sensitive and 20% were intermediate to Cotrimoxazole.

Most of the antibiotics were found to be effective against the isolates. Ciprofloxacin, Norfloxacin, Ofloxacin, and Gentamicin were effective against all isolates, while 75% and 25% of *Citrobacter* spp. were resistant to Ampicillin and ceftazidime, respectively. Cefotaxime resistance was found in 60% of the isolates.

According to Arens et al. (1997) the antibiotic susceptibility of *Citrobacter* spp. showed that only 2% of *Citrobacter freundii* was sensitive to Ampicilin and Cefatoxime and 80% was sensitive to Ceftazidime. Similarly, 93% were sensitive to Nitroflurantoin and Ofloxacin. Our study agrees with another study where *Citrobacter freundii* is also less sensitive to Cefatoxime and more sensitive to Ofloxacin.

Table 3: Antibiotic Sensitivity pattern of *Citrobacter freundii*

Antibiotic used	Sensitive Percent	Intermediate Percent	Resistant Percent	Total Percent
Ampicillin (AS 10/10 mcg)	0.0	25.0	75.0	100.0
Ceftazidime (CAZ 30 mcg)	50.0	25.0	25.0	100.0
Cefotaxime (CTX 30 mcg)	0.0	40.0	60.0	100.0
Ciprofloxacin (CIP 5 mcg)	100.0	0.0	0.0	100.0
Norfloxacin (NX 10 mcg)	100.0	0.0	0.0	100.0
Ofloxacin (OF 5 mcg)	100.0	0.0	0.0	100.0
Gentamicin (GEN 10 mcg)	100.0	0.0	0.0	100.0
Chloramphenicol (C 30 mcg)	70.0	30.0	0.0	100.0
Nitroflurantoin (NIT 300 mcg)	55.0	45.0	0.0	100.0
Cotrimoxazole (COT 25 mcg)	80.0	20.0	0.0	100.0

Number of isolates = 20

Antibiotic Sensitivity Pattern of E. coli

Table 4 presents the results of the antimicrobial susceptibility test conducted on 16 isolates of *E. coli.* The table shows that 75% of the isolates were resistant, and 25% showed intermediate resistance to ampicillin. For ceftazidime, 18.8% of the isolates were sensitive, 56.3% were resistant, and the remaining 25% showed intermediate resistance. All (100%) of the bacterial isolates were sensitive to Ciprofloxacin, Norfloxacin, Ofloxacin, Gentamicin, and Cotrimoxazole, respectively. For chloramphenicol, 62.5% of the isolates were sensitive, and 37.5% were resistant. Similarly, 75% of the isolates were sensitive, and 25% were resistant to Nitrofurantoin.

Geta et al. (2019) showed that most *E. coli were* sensitive to Ampicillin, contrary to this observation. Likewise, 75% of *E. coli* were resistant to Ampicillin, and 56.3% of *E. coli* were resistant to Ceftazidime. Similarly, 43.8% showed resistance to cefotaxime. 37.5% of *E. coli* isolates were resistant to chloramphenicol, 25% were resistant to Nitrofluorantoin, and 100% were sensitive to Ciprofloxacin, Norfloxacin, Ofloxacin, and Gentamicin. While the choice of antibiotics used in a similar study by (Geta et al., 2019) differed, isolates of *E. coli* showed the highest resistance (75%) to Tetracycline and the lowest resistance (50%) to Ampicillin, while all isolates were responsive to Penicillin. The differences in the concentrations of antimicrobial drugs employed, the sources of

isolates, the transmission of drug resistance, and the widespread use of antibiotics in the environment may be to blame for the variability in the values of percentage susceptibilities in this study.

Table 4: Antibiotic Sensitivity pattern of *E. coli*

Antibiotic used	Sensitive Percent	Intermediate Percent	Resistant Percent	Total Percent
Ampicillin (AS 10/10 mcg)	0.0	25.0	75.0	100.0
Ceftazidime (CAZ 30mcg)	18.8	25.0	56.3	100.0
Cefotaxime (CTX 30 mcg)	25.0	31.3	43.8	100.0
Ciprofloxacin (CIP 5 mcg)	100.0	0.0	0.0	100.0
Norfloxacin (NX 10 mcg)	100.0	0.0	0.0	100.0
Ofloxacin (OF 5 mcg)	100.0	0.0	0.0	100.0
Gentamicin (GEN 10 mcg)	100.0	0.0	0.0	100.0
Chloramphenicol (C 30 mcg)	62.5	0.0	37.5	100.0
Nitroflurantoin (NIT 300 mcg)	75.0	0.0	25.0	100.0
Cotrimoxazole (COT 25 mcg)	100.0	0.0	0.0	100.0

Number of isolates =16

Antibiotic Sensitivity Pattern of Salmonella Species

Table 5 shows the antimicrobial sensitivity pattern of Salmonella spp. Altogether 6 *Salmonella* spp. were taken for antibiotic susceptibility test. All the isolates 100% isolates were resistant to ampicillin. 50% were sensitive, and 50% were intermediate to Ceftazidime. 66.67% were sensitive and 16.67% showed resistant and intermediate patterns to Cefotaxime. 100% of bacterial isolates were sensitive toward Ciprofloxacin, Ofloxacin, Gentamicin, Chloramphenicol, Nitroflurantoin, and Cotrimoxazole. While 83.3% of isolates were sensitive and 16.7% were resistant to Norfloxacin. All *Salmonella* spp. were resistant to Ampicillin, while other isolates of *Salmonella* spp. were sensitive to other antibiotics, including Ciprofloxacin, Chloramphenicol, Ofloxacin, Cotrimoxazole, Nitroflurantoin, and Gentamicin. This study agreed with a similar study by (Kebede et al., 2016) where *Salmonella* isolates were tested for susceptibility to antibiotics: Ampicillin, amikacin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamycin, and nitrofurantoin. *Salmonella* contaminants were susceptible to all tested antibiotics. 92% of bacteria were susceptible to Gentamicin, while 85% were susceptible to Ampicillin.

Table 5: Antibiotic Sensitivity Pattern of *Salmonella Species*

Antibiotic used	**Sensitive Percent**	**Intermediate Percent**	**Resistant Percent**	**Total Percent**
Ampicillin (AS 10/10 mcg)	0.0	0.0	100.0	100.0
Ceftazidime (CAZ 30 mcg)	50.0	50.0	0.0	100.0
Cefotaxime (CTX 30 mcg)	66.7	16.7	16.7	100.0
Ciprofloxacin (CIP 5 mcg)	100.0	0.0	0.0	100.0
Norfloxacin (NX 10 mcg)	83.3	0.0	16.7	100.0
Ofloxacin (OF 5 mcg)	100.0	0.0	0.0	100.0
Gentamicin (GEN 10 mcg)	100.0	0.0	0.0	100.0
Chloramphenicol (C 30 mcg)	100.0	0.0	0.0	100.0
Nitroflurantoin (NIT 300 mcg)	100.0	0.0	0.0	100.0

Cotrimoxazole (COT 25 mcg)	100.0	0.0	0.0	100.0

Number of isolates =6

Sensitivity Pattern

The sensitive pattern of isolated bacteria is shown in Table 6. In total, 68.6% of bacteria were sensitive to the assayed antibiotics while 19.5% were resistant and the remaining 11.9% showed an intermediate pattern toward examined antibiotics.

Table 6: Sensitive Pattern in percentage

Sensitive Pattern of bacteria	Percent (%)
Sensitive	68.6
Intermediate	11.9
Resistant	19.5

Research Implication

Ensuring the safety of fresh sugarcane juice is of utmost importance. This can be achieved by following proper storage, handling, and processing practices. Retail shop vendors must be made aware of the importance of hygiene and food safety practices, such as using clean utensils, washing fruits before processing, and storing juice at the correct temperatures.

To address the microbial quality status of fresh fruit juice, it is important for the government to establish a database. Additionally, regular monitoring and testing of fresh sugarcane juice and other fruit juices by the government should be conducted to ensure compliance with national and international food safety standards. This will help prevent the spread of foodborne illnesses and improve public health.

Conclusion

The study concludes that fresh sugarcane juice samples from the retail shops in Kathmandu Valley have high levels of contamination, with slightly acidic pH and high total soluble solid values. The high microbial load found in the freshly squeezed sugarcane juice samples exceeds the specifications set for fruit juices in some parts of the world, and varied degrees of antimicrobial susceptibilities and resistances were observed on bacterial isolates. These findings reflect poor hygienic conditions during juice processing or handling, potentially due to the use of poor-quality water and the lack of training on food hygiene and safety. As a result, the study highlights potential health hazards for consumers of fresh sugarcane juice.

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