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Isolation, Identification and Antibiotic Susceptibility of Pathogenic Microorganisms from Fresh Fruit Juices

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ABSTRACT

Fruit juices have antioxidant properties. Sugar, minerals, phenolic, and vitamins are the main components of fresh fruit juices. However, when fresh juices are processed and harvested into vended forms for sale, hygienic procedures are neglected. Due to unhygienic conditions, foodborne illnesses occur among people. The present study aimed to isolate, identify, and evaluate the microbiological quality of the different fruit juices produced from the local streets and canteens of Faisalabad. A total of 120 fresh fruit samples were analyzed for the presence of bacterial pathogens. The highest percentage of bacteria in juices was *Escherichia coli* (23.8%), *Staphylococcus aureus* (9.7%), *Klebsiella* spp. (13.6%), *Salmonella* spp. (8%), *Proteus* spp. (8%), *Pseudomonas aeruginosa* (23.4%) and *Shigella* spp. (13.1%). The highest bacterial count was observed in sugar cane juice (5.75 ± 1.50 log CFU/mL) and the lowest in lemon juice (4.09 ± 3.54 log CFU/mL). The contamination is mainly due to unhygienic conditions as well as poor quality of water used for dilution related to washing of utensils, domestic hygiene, peeling of fruits much earlier before processing, poor personal and contaminated water, and ice, in shops located in crowded places. Antibiotic-resistant bacteria cause serious bacterial infections. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli* were resistant to piperacilin, chloramphenicol, gentamycin, and tobramycin. There should be regular monitoring of street vended fresh juices to check the quality of juices regularly. This would help to avoid any future pathogenic infection. These findings demonstrated that the fresh juice sample's quality was unsatisfactory and inappropriate to consume. Consequently, these findings will help local consumers become more knowledgeable in addition to the researchers. It is recommended that street juice vendors adopt proper hygiene protocols and get appropriate training on food safety, sanitation, and microbial quality.

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INTRODUCTION

Juices are very delicious, nutritive, non-alcoholic, and stimulating (Tiencheu *et al.*, 2021). People like to drink from all over the world (Leon *et al.*, 2023). Most of the minerals like phosphorus, calcium, and sodium (Doharey *et al.*, 2021; DP *et al.*, 2023) and vitamins (especially vitamin C1) are present in fruit juices (Yahia *et al.*, 2019; Amusat *et al.*, 2024). Coloring, pulps, sugar, water, and preservatives are the main ingredients of processed juices (Dasenaki and Thomaidis, 2019; Akter *et al.*, 2024). People may use these juices in processed or natural forms (Basar *et al.*, 2007; Amusat *et al.*, 2024). According to the World Health Organization, fruit juice is classified as a source of free sugar *(*Ruxton *et al*., 2021*)*. Fruit juices are most commonly used on different occasions. They are an important source of immediate energy production because fruits are rich in different antioxidants, acids (ascorbic acid, etc.), and phenolics (Arfaoui, 2021). Unpasteurized juices are prepared by street vendors and are more attractive to consumers due to their fresh taste (Shanta *et al*., 2021). They are used for their medicinal value, nutritional value, and refreshing nature (Akter *et al*., 2024). Vegetables and fruit juices have a great effect on improving the blood lipid levels of hyper-cholesterolemia patients and have detoxification of the human body (Iqbal *et al*., 2015; Fabjanowicz *et al*., 2024). Food-borne diseases are the main problem caused by contaminated food (Kumari *et al*., 2023). They have many health and economic impacts (Lima Tribst *et al*., 2009; Mandha *et al*., 2023). Microorganisms interact with food. Even fresh juice contains a lot of microorganisms (Pierre and Sivasubramani, 2015; Starek *et al*., 2019). Juices have different pollution sources, such as dust, birds, open sanitation systems, water, treatment, rotten fruits, air, insect pests, soil (the main source of spoilage microorganisms), etc. (Lima Tribst *et al*., 2009; Siddique *et al*., 2018; Berihu *et al*., 2024). *Salmonella* spp. *Cryptosporidium* and *Escherichia coli* O157:H7 are the main pathogens associated with the outbreak of consumption of unpasteurized fruit juice (Ketema *et al*., 2008; Bulti and Melkam, 2018; Pius *et al*., 2021; Neggazi *et al*., 2024). Contaminated food affects the gastrointestinal tract (GIT), the most common foodborne disease in humans (Nagaraja *et al*., 2014; INEC, 2019; Tenea *et al*., 2023). Bloody diarrhea, diarrhea, and fever are major outbreak symptoms of *Salmonellosis* (He *et al*., 2023). Violent vomiting and nausea are caused by the intake of *Staphylococcus aureus* (Abdallah *et al*., 2024). Hemolytic uremic syndrome (HUS), characterized by fatal acute renal failure in children thrombocytopenia, microangiopathic hemolytic anemia, acute renal failure; and hemorrhagic colitis are symptoms of non-bloody diarrhea and is caused by an enterohemorrhagic serotype (E. coliO157:H7) bacteria (Fiorentino *et al*., 2023). The parasite *Cyclospora cayetanensis* pathogen causes diarrhea anorexia, nausea, abdominal cramping, fever, and weight loss in humans (Salomão, 2018).

The presence of antibacterial compounds, the availability of nutrients, pH, competitive microbial communities, water activity, and redox potential are the key factors affecting the deterioration of juice (Javed *et al*., 2023). They affect texture, development of flavor, color changes, and carbon dioxide production. In the past two decades, many countries have documented many food-borne diseases because Salmonella and E. coli survive the acidic environment (<4.5) in unpasteurized juice (Aneja *et al*., 2014; Kaddumukasa *et al*., 2017; Neggazi *et al*., 2024).

It has been observed that *Clostridium perfrigens*, *Bacillus cereus,* and *Staphylococcus aureus* caused food poisoning by producing toxins in juice (Ahmed *et al*., 2018; Worku and Hailu, 2023). Sweeteners, water, preservatives, and spices are the original qualities of the fruit. These should be free of pathogenic microorganisms. European regulations restrict the use of hazardous chemicals because they contain radioactive substances or strains of mycotoxins and heavy metals. The sanitation process eliminates 99% of microorganisms from contact points and equipment surfaces to ensure the safety of the final product; therefore, it should be carefully monitored (Aadil *et al*., 2019). Unpasteurized juice can carry *E. coli* O157:H7 and Salmonella (Silva, 2023). Chlorinated water is used to wash the peel to remove surface microorganisms and disinfect those (Aadil *et al*., 2019).

Juice is widely known for its nutritional value and is an important part of the diet (Farah *et al*., 2023). There were no quality and safety measures to minimize contamination in the juice. Due to improper handling, and contaminated raw materials and equipment, fruit juices were easily invaded by bacterial pathogens (Rahman *et al*., 2011; Jagessar and Craig, 2024). The spoilage and biological deterioration of juice are affected by some physical and chemical parameters as well as pH, storage, color, ascorbic acid, temperature, chemical composition, and total soluble solids (TSS). The economic loss of the processor is the deterioration of the physical and chemical parameters caused by the consumer's rejection of the product (Kaddumukasa *et al*., 2019). By storing food under refrigeration (0-4°C), spore germination can be prevented and the toxins that may be produced can reach potentially dangerous levels (Alim *et al*., 2023). 6 to 7 is the optimal pH value for the growth of mesophilic bacteria. Consumers in schools, shops, roadside stalls, and even travelers, offices, and universities can suffer from food-borne illnesses from contaminated juice (Worku and Hailu, 2023). In the juice processing process, unsanitary technology will increase a large number of microorganisms in fresh juice, thereby increasing the health hazards to consumers (Kaddumukasa *et al*., 2019). Most organisms are resistant to antibiotics, and they can cause problems with treatment procedures Mycobacterium tuberculosis (old pathogen) is an MDR (multi-drug resistant) pathogen found in both industrialized and developing countries (Davies and Davies, 2010; Mancuso *et al*., 2023). In the era of antibiotics, human pathogens (*Staphylococcus aureus*) showed resistance to methicillin and penicillin (Alghamdia *et al*., 2023). This is the adaptive evolution of bacteria in the era of antibiotics (Pantosti *et al*., 2007; Hutchings *et al*., 2019). We should explore new options to choose new antibiotics, because sometimes an antibiotic that is effective today may not be effective against microorganisms in the future (Sarkar *et al*., 2003; Uddin *et al*., 2021; Cook and Wright, 2022; Thakare *et al*., 2020). The main objectives of this study were to investigate microbiological examination, identification, and characterization of bacteria from fresh fruit juices prepared in different streets of Faisalabad.

MATERIAL AND METHODS

Collection of samples

The most commonly consumed fresh fruit juices in Faisalabad such as orange juice (*Citrus reticulata* Blanco), Banana juice (*Musa acuminate*), Pomegranate juice (*Punica granatum*), Apple juice (*Malus domestica*), Sugar cane juice (*Saccharum officinarum*), Pineapple juice (*Ananas comosus*), Lemon juice (*Citrus limon*) and Grapefruit juice (Citrus × paradise) were selected for the microbiological study. A total of 120 fresh fruit juice samples were collected from the local streets and canteens of Faisalabad. Samples were collected in sterile falcon tubes stored at 4°C and analyzed within an hour after procurement.

Table 1. Sources of collected samples.

Name of sample	No. of samples	Location
Orange	10	Hospital canteen
Pineapple	15	Street Corner shop
Lemon	20	Hospital canteen
Sugar cane	15	Juice corner shop
Grapefruit	10	Street shop
Pomegranate	10	Street corner
Banana	15	Roadside
Apple	25	Juice corner
	Total = 120	

Measurement of pH

The pH of juice samples was measured using a pH meter (Hanna Instruments Inc., Woonsocket, RI) (Tarafdar and Kaur, 2021; Tian *et al*., 2018).

Isolation of pathogenic bacteria

All samples were serially diluted with sterilized buffered peptone water (0.1%). 1 mL of homogenized juice sample was diluted ten times by using 9 ml sterile distilled water and each sample was further diluted up to 10-5 (10-1, 10-2, 10-3, 10-4, 10-5 respectively). 1 mL of each diluted sample was inoculated on a nutrient agar plate by a glass spreader. The Agar plate method was used for the enumeration of bacterial colonies. Salmonella- Shigella agar, Mannitol salt agar, MacConkey agar, Pseudomonas agar (Cetrimide Agar), nutrient agar, and EMB (Eosin Methylin agar) media were used to purify pathogenic bacteria. The total viable count was done by using plate count agar after incubation at 35°C for 24 hours. *Staphylococcus aureus* was cultured on MSA agar, *Salmonella typhimurium* on *P. aeruginosa* and *Protius* spp. were on Pseudomonas agar (Cetrimide Agar), *Salmonella typhi* on Salmonella Shigella agar, *Klebsiella pneumonia* on nutrient agar, *E. coli* was cultured on MacConkey agar and EMB (Eosin Methylin agar) (Kader *et al*., 2014, Pierre and Sivasubramani, 2015; Pius *et al*., 2021).

Bacterial load determination and identification

Bacterial load was determined by counting the colonies on Agar plates and the mean TVC count was estimated as CFU/mL. For identification of bacteria, the 24-hours-old culture of isolates was observed under a microscope by gram stain method (Coico, 2006) and further biochemical tests were performed for the identification of pathogens such as oxidase test, Simon citrate, Indole test, urease test, MR-VP test, catalase test, and methods described in "Bergey's Manual of Systematic Bacteriology" (Buchanan and Gibbons, 1949; Parte *et al*. 2012). Further identification of bacteria was performed based on methods described in "Compendium of methods for the microbiological examination of foods". Further identification of bacteria was performed based on methods described in Bergey´s Manual for Determinative Bacteriology (Bergey, 1994).

Antibiotic susceptibility testing (Antibiogram)

Antibiotic sensitivity testing was performed using the Kirby-Bauer disk diffusion method on Mueller Hinton agar (MHA) as per CLSI 2015 guidelines (Bruno, 2019; Weinstein *et al*., 2018). After that fresh isolates (24-hours-old culture) were used for the testing procedure. Pre-cultured antibiotics were placed over the media and then incubated for 24 hours at 37̊ C. After the incubation period plates were observed to determine the zone of inhibition indicating the susceptibility against the antibiotic. 11 different antibiotics of different concentrations were tested against bacterial isolates. Tetracycline (TE) 10µg, Chloramphenicol (C) 30µg, Cefuroxime (CXM) 30µg, Ciprofloxacin (CIP) 5µg, Cefepime (FEP) 30µg, Doxycycline (DO) 30µg, Piperacilin (TZP) 110µg, Tobramycin (TOB) 30µg, Gentamycine (CN) 10µg, Cefotaxime (CTX) 30µg and Cefixime (CFM) 5µg antibiotics were used against all isolates. After incubation of 24 hours diameters of the zone of inhibition were measured by using the transparent rule in mm (Lucky *et al*., 2016).

RESULTS AND DISCUSSION

Data collection

After data collection, data was organized, analyzed, and summarized. Mean and SD values were determined using ANOVA. Statistical analysis was done using SPSS v. 20.0. Fresh fruit juices have high nutritive values, fresh flavor, and vitamin content (Faizi, 2022; Pinto *et al*., 2022). Consumers like to consume fresh juices. Fresh fruit juices provide affordable sources of nutrients to consumers, because of their low price, availability at the right time, and taste (Tenea *et al*., 2023). However, these street juices are frequently associated with many bacterial diseases such as diarrhea due to their serving practices and improper handling (Odewale *et al*., 2023; Worku and Hailu, 2023). Contamination in fresh juices is the main issue for consumer's health (Wedajo *et al*., 2019). This study aimed to isolate and detect pathogenic microorganisms from fresh fruit juices causing serious illnesses in humans.

Isolation of bacteria from fresh fruit juices

In this study, pathogenic microorganisms were isolated and identified which showed a high occurrence of microbial load in fresh fruit juices. They consist of many pathogens such as *P. aeruginosa*, *E. coli*, *S aureus*, *Protius* spp., *Shigella* spp., *S. typhimurium*, *K. pneumonia* shown in (Table 2). A total of 120 fruit juice samples were collected from different street vendors, hospitals canteens, and shops, and then tested. The present study found pH values of different fresh fruit juices that are shown in (Table 2). Low pH inhibits the growth of bacteria *(*Naeem *et al*., 2012*)*. Most juices showed positive results for bacterial infections. *Escherichia coli* was detected in 6 of the analyzed samples (23.8%), *Proteus* spp. (8%), *Shigella* spp. (13.1%), *Pseudomonas aeruginosa* (23.4%), *S. typhimurium* (8%), *Staphylococcus aureus* (9.7%), and *Klebsiella pneumonia* (13.6%). The findings of this research are similar to Jagessar and Craig, (2024), who reported that based on bacterial loads, the tested fruit juice samples were deemed unsafe for human consumption. The pH of the samples, though low and acidic, still promoted *E. coli* and *S. aureus* bacterial growth.

Table 2. Number of bacteria and pH values of fresh fruit juices.

Juices	pH	No of bacteria	Isolates
Sugar cane	5	23	*Proteus* spp. (5), *Staphylococcus aureus* (5), *Salmonella typhimurium* (8)and *Shigella* spp. (5)
Pomegranate	3.5	30	*Staphylococcus aureus* (5), *Pseudomonas aeruginosa* (5), *Klebsiella pneumonia* (15), *Escherichia coli* (5)
Grapefruit	3.7	23	*Escherichia coli* (10), *Salmonella typhimurium* (5)and *Shigella*spp. (8)
Apple	3.2	23	*Escherichia coli* (8), *Pseudomonas aeruginosa* (15)
Banana	3.2	25	*Escherichia coli* (10),*Proteus* spp. (8), *Staphylococcus aureus* (7)
Orange	3	37	*Klebsiella pneumonia* (7), *Staphylococcus aureus* (6), *Salmonella typhimurium* (6), *Shigella* spp. (18)
Pineapple	4.2	49	*Klebsiella pneumonia* (10), *Escherichia coli* (8), *Pseudomonas aeruginosa* (25), *Proteus* spp. (6)
Lemon	2.4	25	*Pseudomonas aeruginosa* (10), *Escherichia coli* (15)

Table 3. No of bacteria and frequency of bacteria in fresh fruit juices.

Type of juices	Total isolates	*P. aeruginosa*	*Proteus* spp.	*S. aureus*	*E. coli*	*S. typhimurium*	*Klebsiella* spp.	*Shigella* spp.
Orange	37(15.7%)	0	0	6(16.2)	0	6(16.2%)	7(2.9%)	18(7.6%)
Apple	23(9.7%)	15(65.2%)	0	0	8(34.7%)	0	0	0
Banana	25(10.6%)	0	8(32%)	7(28%)	10(40%)	0	0	0
Grapefruit	23(9.7%)	0	0	0	10(43.4%)	5(21.7%)	0	8(34.7%)
Pomegranate	30(12.7%)	5(16.6%)	0	5(16.6%)	5(16.6%)	0	15(50%)	0
Lemon	25(10.6%)	10(40%)	0	0	15(60%)	0	0	0
Sugar cane	23(9.7%)	0	5(21.7%)	5(21.7%)	0	8(34.7%)	0	5(21.7%)
Pineapple	49(20.8%)	25(51%)	6(12.2%)	0	8(16.32%)	0	10(20.4%)	0
Total	235(100%)	55(23.4%)	19(8%)	23(9.7%)	56(23.8%)	19(8%)	32(13.6%)	31(13.1%)

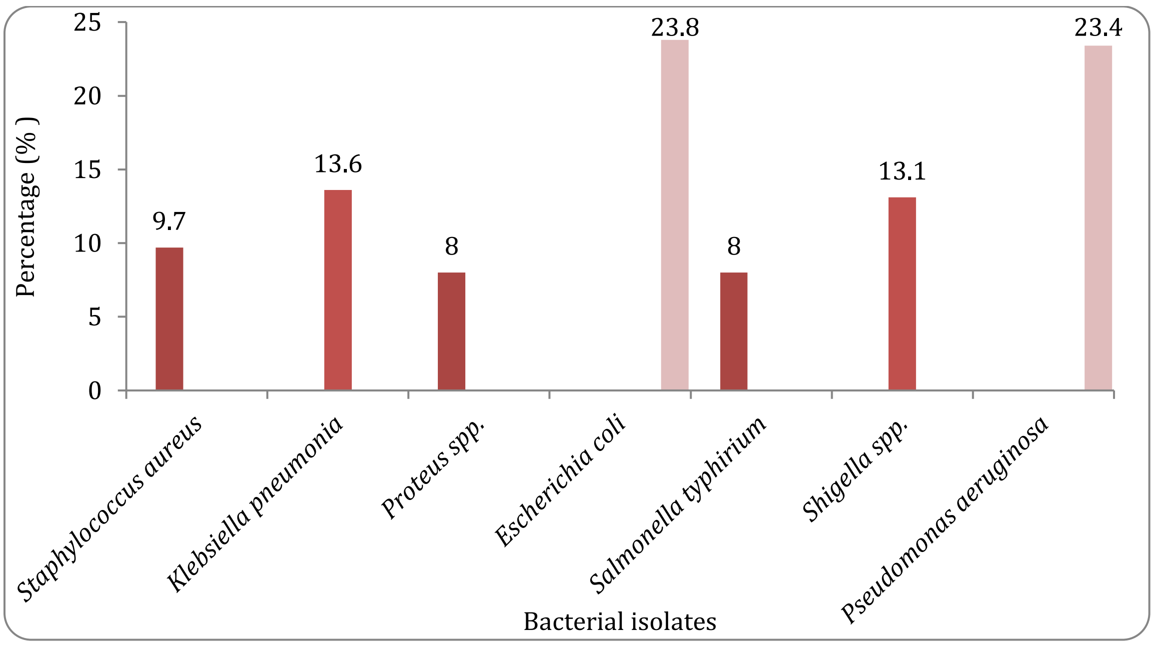

Figure 1. Prevalence of pathogenic bacteria in fruit juice samples.

Prevalence of pathogenic microorganisms

In this study, the prevalence and high microbial load of pathogenic bacteria were observed in fresh fruit juices (Figure 1). That could be associated with the processing of juices, preservation without refrigeration, proper washing of fruits, poor handling, and unhygienic conditions. A number of research studies have also reported that the high prevalence of bacteria in juices was due to poor sanitary conditions (Kebede *et al*., 2018; Diana *et al*., 2012; Odewale *et al*., 2023; Kumari *et al*., 2023).

Almost all fresh juices showed a much higher viable bacterial count than the permitted count > 1 × 104CFU/mL (Table 4). The highest bacterial count (5.75 ± 1.50 log CFU/mL) for fresh fruit juice samples was found in sugar cane, collected from the roadside and the lowest count was (4.09 ± 3.54 log CFU/mL) found in lemon juice collected from the street corner. Variations in TVC indicated that almost all fresh fruit juices were contaminated due to unhygienic maintenance during the preparation of juices (Standard, 2000; Worku and Hailu, 2023). It was observed that the prevalence of *Staphylococcus aureus* was high in bananas, *Escherichia coli* was in lemon, *Shigella* spp. in orange, *P. aeruginosa* in pineapple, *Proteus* spp. was in bananas, *Klebsiella* spp. was in pomegranate and *S. typhimurium* was higher in sugar cane, collected into streets, shops, and roadsides (Pierre and Sivasubramani, 2015). In this study Mean TVC ± SD of TVC (CFU/mL) ranged from (1.25 × 104 ± 3.54 CFU/mL) to (5.75 × 105 ± 1.50 CFU/mL). TVC value in banana juice was (5.03 × 105 ± 4.30 CFU/mL), in Orange juice (4.79 × 105 ± 3.11CFU/mL), in Apple juice (4.075 × 105 ± 5.55 CFU/mL), in Pineapple juice (3.59 × 105 ± 4.02 CFU/mL), in Grapefruit juice (4.37 × 105± 3.99 CFU/mL) and Pomegranate juice mean TVC was (3.79 × 105 ± 2.43CFU/mL).

Table 4. TVC values of fresh fruit juices.

Sample	Maximum	Minimum	Mean TVC ± SD
Sugar cane	8×10^5	5×10^5	$5.75 \times 10^5 \pm 1.50 \times 10^5$
Apple	8×10^5	1.5×10^4	$4.075 \times 10^5 \pm 5.55 \times 10^5$
Pineapple	8×10^5	1×10^4	$3.59 \times 10^5 \pm 4.02 \times 10^5$
Banana	8×10^5	1×10^4	$5.03 \times 10^5 \pm 4.30 \times 10^5$
Lemon	1.5×10^4	1×10^4	$1.25 \times 10^4 \pm 3.54 \times 10^3$
Orange	7×10^5	1.8×10^4	$4.79 \times 10^5 \pm 3.11 \times 10^5$
Pomegranate	5×10^5	1.5×10^4	$3.79 \times 10^5 \pm 2.43 \times 10^5$
Grapefruit	8×10^5	1×10^4	$4.37 \times 10^5 \pm 3.99 \times 10^5$

Figure 2. TVC of fresh fruit juices.

Identification tests

All isolates were identified according to their biochemical, physiology, and morphological characteristics. *S. aureus* Gram-positive cocci (GPC) was 19(8%) in the juices sample. Gram-negative rods (GNR) were 216 (92%) in the fresh juices sample. All isolates were positive for different biochemical tests (Table 5). Overall, 235 different bacterial isolates were identified in 120 through biochemical tests. Among these, 56 (23.8%) *E. coli* and 55 (23.4%) *P. aeruginosa* were high in fresh juices, 19(8%) *Proteus* spp., 23 (9.7%) *S. aureus*, 19 (8%) *S. typhimurium*, 32 (13.6%) *Klebsiella* spp., 31 (13.1%) and *Shigella* spp. were also identified in fresh fruit juices (Table3). In the present study, the *Escherichia coli* count was higher as compared to other isolates. These results are consistent with the study conducted by Jagessar and Craig, 2014, reported that *E. coli* was heavily contaminated different unpasteurized fruit juices sold at a local market in Guyana. Similarly, (Mandal and Mandal, 2018; Rao, 2015; Lucky *et al*., 2016; Odewale *et al*., 2023) reported various kinds of potential human pathogenic bacteria from different types of street vended fruit juices.

Table 5. Biochemical tests of different bacterial colonies.

Bacteria	MR test	VP test	Catalase test	Oxidase test	Citrate test	Urease test	Indole test
K. pneumonia	-	+	+	-	+	+	-
Proteus spp.	+	_	+	_	+	+	_
P. aeruginosa	_	_	+	+	+	_	_
S. aureus	+	+	+	_	+	+	_
Shigella spp.	+	_	+	_	_	_	+
E. coli	+	_	+	_	+	_	+
S. typhimurium	+	_	+	_	_	_	_

Antibiotic resistance pattern

The presence of Enterobacteriaceae and *E. coli* in food samples indicates fecal contamination of these sources (Odewale *et al*., 2023). This could be explained by poor processing practices, inadequate hand washing, and the use of contaminated water (Orji *et al*., 2016; Snyder and Worobo, 2018; Rubaratuka *et al*., 2023). The presence of *S. aureus* is a very good indicator of less personal hygiene because they are widely distributed on the surface of human skin, nasal secretions, and eyes and in nature (Tambekar *et al*., 2007; Opeolu *et al*., 2010; Ogodo *et al*.2016). The trend of antibiotic resistance highly increases because isolated bacteria have multiple drug resistance abilities, and due to misuse of antibiotics, which is an extremely public health concern, many outbreaks occur worldwide (Onyeneto and Okpalla, 2013; Mandal and Mandal, 2018).

In this study, isolates were tested against different antibiotics on Muller-Hinton-Agar by the disc diffusion method. The results of the antibiotic sensitivity test were interpreted and are presented as the resistant and susceptible percentages of bacterial isolates to the antibiotics. S. aureus was found resistant to all drugs except tobramycin, chloramphenicol, ciprofloxacin, doxycycline, cefotaxime, cefepime, and tetracycline depicted in (Table 6). These findings are under earlier study in which *Staphylococcus aureus* (gram-positive bacteria) is resistant to multiple antibiotics; daptomycin, vancomycin, methicillin, and penicillin (Pantosti *et al*., 2007). These findings are under earlier study in which Staphylococcus aureus is resistant to multiple antibiotics (Ezeh *et al*., 2023).

Enteropathogenic, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus*, *Shigella* spp., and *S. typhimurium*, were found to be multidrug-resistant bacteria in street food samples (Nur *et al*., 2021). In this study, *S. typhimurium* and Pseudomonas were found resistant to tetracycline, gentamycin, ciprofloxacin, cefixime, cefotaxime, piperacilin, and doxycycline. In the case of E. coli, all drugs were effective except gentamycin, tobramycin, cefotaxime, piperacilin, and tetracycline. The susceptibility pattern of *Shigella* spp. towards different drugs showed that all drugs were effective except for ciprofloxacin, piperacilin, and gentamycin. *Proteus* spp., and *K. pneumonia* were resistant to tetracycline, chiloramphenicol, cefuroxime, ciprofloxacin, cefotaxime, gentamycin, tobramycin, and piperacilin.

Pseudomonas aeruginosa, *Staphylococcus aureus*, and E. coli were resistant to piperacilin, gentamycin, chloramphenicol, and tobramycin. In case of susceptibility, *E. coli* was 100% susceptible to doxycycline, *Staphylococcus aureus* was 100% susceptible to doxycycline and tobramycin, and *Pseudomonas aeruginosa* was 100% susceptible to the cefotaxime. The result of this study is in line with the report of Odewale *et al*., (2023) where different bacterial isolates from different fruit juices showed antibiotic resistance patterns against multiple antibiotics.

Table 6. Antibiotics susceptibility and resistant pattern of bacterial isolates from juices.

Isolates Antibiotics	*Pseudomonas* Spp. N=55		*Klebsiella* spp. N=32		*Staphylococcus* spp. N=23		*S. typhimurium.* N=19		*E. coli* N=56		*Shigella* spp. N=31		*Protius* spp. N=19	
	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%
CN (10µg)	10	90	90	10	100	0	55	45	50	50	70	30	40	60
TE (10µg)	20	80	80	20	25	75	50	50	75	25	35	65	75	25
C (30µg)	ND	ND	10	90	100	0	25	75	30	70	10	90	70	30
CXM (30µg)	40	60	15	85	75	25	ND	ND	35	65	40	60	70	30
CIP (5µg)	30	70	80	20	25	75	50	50	10	90	50	50	65	35
FEP (30µg)	25	75	40	60	25	75	45	55	20	80	30	70	10	90
DO (30µg)	75	25	20	80	0	100	30	70	0	100	30	70	ND	ND
TZP(110µg)	100	0	70	30	70	30	80	20	50	50	75	25	20	80
TOB (30µg)	10	90	40	60	0	100	45	55	100	0	45	55	40	60
CFM (5µg)	100	0	15	85	75	25	85	15	25	75	40	60	10	90
CTX (30 µg)	0	100	ND	ND	25	75	50	50	60	40	20	80	50	50

Note: ND, not determined.

Overall results obtained in this study indicated that fresh fruit juices sold by street vendors are highly contaminated with pathogens. The high prevalence of bacteria in juice samples is an alarming signal to the consumer's health. Some hygienic regulations should be issued for street vendors to take some precautionary measures during the processing of fresh fruit juice.

CONCLUSION

In conclusion, seven (7) species of bacteria were found in the tested samples of banana, Orange, Apple, Pineapple, Grapefruit juice, Sugar cane, Lemon, and Pomegranate juices. It was revealed that Sugar cane juice had the overall greatest number of Colony Forming Units while Lemon juice had the lowest number of Colony Forming Units. The greatest microbial diversity was found in Sugar cane, Orange, and Pineapple juice with five (5) species, followed by Lemon and Apple juice with two (2) species. The most frequent bacteria isolated was *Escherichia coli* which was likely because of its ability to survive even in low pH. Human health is at risk from this pathogen. It is recommended that there is an urgent need for authorities to enforce the rules regarding vending practices and regularly monitor street vending locations to make sure the proper procedures are followed at all times and during the fresh juice production process. In this regard, it is important to routinely design and provide relevant short course training to vendors and customers on food safety, microbiological quality, sanitation, and sanitary procedures related to the processing of fresh juices.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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