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Research Article

Assessment of Bacterial Load of Some Fresh and Packed Fruit Juices in Arba Minch Town, Ethiopia

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ABSTRACT

A fruit juice contains essential nutrient, mineral, antioxidant and vitamins for overall health. However, food borne illness related to fruit and fruit product is increasing and very serious problem in different part of Ethiopia is comprised Arba Minch. So, the main objective of this study was to assess the bacteriological quality of both fresh and commercially packed fruit juices available for the consumers in Arba Minch town, Southern Ethiopia. This study analysis and evaluates the bacteriological quality of some fresh and packed fruit juices available in Arba Minch town. The study was conducted from January 2017 to November 2017. A total of 120 samples was purchased from cafeteria, restaurants and supermarkets which consisted of 96 fresh juice samples 16 each of Mango, Papaya, Avocado, Orange, Apple and Mixed juice whereas from the total samples, another 24 commercially packed juices viz., Mango, pineapple, Orange and white grape were collected from supermarkets. Also, detection of pathogens and antimicrobial susceptibility testing was conducted. All fresh fruit juice samples were found to harbor TVC, TCC, FCC and TSC within the range between 5.32 ± 0.49-6.65 ± 0.31, 2.59 ± 0.42-4.87 ± 0.45, 2.00 ± 0.36-3.95 ± 0.47 and 2.08 ± 0.29-2.86 ± 0.33 log10 cfu/ml, respectively. Also, all commercially packed fruit juice samples exhibit the presence of TVC, TCC and TSC within the range of 2.26 ± 0.51-3.08 ± 0.65, 0.00 ± 0.00-0.60 ± 0.35 and 1.00 ± 0.15-1.85 ± 0.59 log10 cfu/ml, respectively with the exception of FCC in which detection was not shown. In this study the prevalence of *E. coli*, *Salmonella* and *Staphylococcus aureus* was detected for all fresh fruit juices samples of this avocado was more dominated. Antibiotic susceptibility test for *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* revealed completely resistant (100%) to a VAN and AMP. In general the study, especially exhibits the level of bacterial load found in both fresh and packed juice samples was unsatisfactory compared to gulf standards. This cause health problems and possible vehicle of foodborne outbreaks to the community. Therefore, good quality of water used; hygienic conditions related to washing of utensils, good personal and domestic hygiene during fresh fruit juice preparation can improve the bacterial quality and safety of the finished product.

Keywords: Fruit juice; Arba minch; Antibiotic resistance; Bacterial load

INTRODUCTION

Fruit juices are very common among the people of all ages everywhere in the world. Fruit juices are flesh flavor which provides health benefit and are well consumed for their nutritive value, mineral and vitamin content. However, fruit juices by their nature contain various organisms and many of these microorganisms will be harmless bacteria such as saprophytic [1]. One possible source can be damaged surfaces, such as perforations, wounds, cuts and splits that occur during growing or harvesting through which pathogenic organisms can enter fruits [2]. The disease agents spread by juice like drink not only harm large groups of people but also sometimes result in serious disability and death [1].

The practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds or prohibited from selling such items, since it is a source of their livelihood [2,3]. The total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice [1]. Many researchers believe that consumption of commercially packed juice is safe than the locally produced fresh fruit juice [4,5]. This might be the reason of using mechanized machine and also some preservatives during fruit juice processing. The juices comprise primarily water, sugar, preservatives, color, fruit pulps and other additives. But some preservatives of higher concentrations can be dangerous for our health. In spite of all these problems, enormous number of coliforms and *staphylococcus* count were identified from commercially packed fruit

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juices [5]. Food-borne disease is usually caused by certain bacteria or their toxins, which are poisonous proteins produced by these bacteria. Contamination of juices with pathogenic microorganisms has caused various illness and even some fatalities [6].

There are generalized opinions among investigators that, the most common food borne pathogenic bacteria are *Bacillus cereus, Clostridium botulinum, Escherichia coli, Shigella spp., Salmonella spp., Vibrio parahaemolyticus, Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes, Mycobacterium bovis, Listeria monocytogenes, Klebsiella spp., Enterobacter spp., Staphylococcus spp.* [2,4,7]. In Arba Minch town, there is always a great demand for both fresh and packed fruit juices as the climate remains hot and humid for most part of the year. So, the main objective of this study was designed to evaluate the bacteriological load of both fresh and commercially packed fruit juices existing in Arba Minch town for the customers.

MATERIAL AND METHODS

Description of the study area

The study was conducted in Arba Minch town from January 2017 to November 2017. Arba Minch town is the second largest town in Southern Nations, Nationalities, and Peoples Region (SNNPR) next to Hawasa and it is located in Gamo Gofa Zone of the SNNPR about 500 kilometers away from Addis Ababa. The first common name for this town was Ganta Garo. Based on the 2007 Census conducted by the CSA, this town has a total population of 74,879, of whom 39,208 are men and 35,671 women. The Arba Minch town located at an elevation of 1285 meters above sea level. Arba Minch is known as a source for fruit, including mango, banana, orange, apple, guava and pineapple.

Sampling technique and source of sample

A random sampling technique was used to take the representative of fruit juice. Locally prepared fresh fruit juices of sample were purchased from Restaurants and cafeteria from Arba Minch town. Commercially prepared packed fruit juice of sample where purchased from super market.

Collection of samples

A total of 120 samples were purchased from cafeteria, restaurants and super markets found in Arba Minch town. The samples consisted of 96 fresh juice samples 16 each of Mango (*Mangifera indica*), Papaya (*Carica papaya*), Avocado (*Persea americana*), Orange (*Citrus sinensis*), Apple (*Malus domestica*) and Mixed juice were collected from cafeteria and restaurants. From the total samples, other 24 commercially packed juices 6 each of Mango, Pinapple, Orange and white grape were collected from different super markets. The maximum types of fruit juices sold in the study area were drawn for laboratory studies. 200 ml of Fruit juice were collected in a sterile flask and transported to Arba Minch University, Microbiology laboratory by using ice box.

Sample processing method

Thirty milliliter (30 ml) of fruit juice were separately drawn and blended in 200 ml of physiological saline solutions (0.85%NaCl). The serial dilution was done for fresh fruit juice (Mango, Papaya, Avocado, Mixed juice) and packed fruit juice (Mango, orange, pineapple and white grape). The samples were homogenized and appropriate dilutions (10^{-1} up to 10^{-6}) were performed for each fresh fruit and packed juice samples. Appropriate dilutions of the sample were plated in triplicate on the solid media for bacteriological count. The actual numbers of bacteriological colony count were estimated as colony forming unit per ml (cfu/ml) by using the following formula.

Number of bacteria colony in original sample = Number of colony counted x $\mathbf{1}/\boldsymbol{df}$ x V (ml)

Where: df, Dilution factor; V, Volume in ml

Bacteriological analysis

Total viable count (TVC): Triplicate plate of nutrient agar was inoculated with 1 ml of diluted solution by using spread technique. All plates were incubated at temperature of 37°C for 24 hrs. The temperature was chosen to differentiate the mesophylls which constitute most medically important pathogenic bacteria. The colony developed on the plate was counted from incubated plate.

Total coliform count (TCC): From each samples of previously serial dilution, 1 ml was transferred on MacConkey agar (MCA). Then the plate was incubated at 37°C for 24-48 hrs. Purple red colonies surrounded by zone of precipitated bile were counted by using digital colony counter.

Fecal coliform count (FCC): Similarly, fecal coliforms count was performed using most probable number (MPN) method. Once 1 ml each of 10^{-3}, 10^{-4} and 10^{-5} dilution was inoculated into three test tubes of lactose broth (LB) with Durham's tube and incubated at 35 °C for 48 hours. Presumptive positive tubes of lactose broth were gently mixed and using inoculating loop a loop full of each positive culture was transferred to tubes of EC broth. Inoculated EC broth tubes were incubated for 48 hours at 45.5°C. Production of gas in an EC broth culture was considered as positive fecal coliform. Those tubes, which were positive in gas production within 24 hours, were used in calculation of fecal coliform.

Total *staphylococcus* count (SCC): From each samples of previously serial dilution, 1 ml was transferred in to Mannitol salt agar (MSA) and it was incubated at 37°C for 24-48 hrs, yellow and orange colonies surrounded by yellow zone due to mannitol fermentation was counted. Estimation of bacterial load was performed by Gulf standard method known as the recommended microbiological standard for fruit juices for all bacteriological analysis [8,9].

Microbial characterization

For microbial characterization, 10-15 colonies with different morphology and color were picked randomly from countable plate and were purified by repeated plating and characterized to the genus and species level using the following tests like Gram's reaction, urease test, catalase test, oxidase test, indole test, nitrate reduction, citrate test, H_2S test and VP test.

Detection of microbial pathogens

Salmonella: For detection of *Salmonella*, 25 ml juice samples were added to 200 ml buffered peptone water, vigorously shaken and the suspension was incubated at 37°C for 24 hrs for metabolic recovery and proliferation of cells. From this, 1 ml of culture was transferred into separate tubes each containing 10 ml of Selenite Cystein Broth. The broth was incubated at 37 °C for 24 hrs. After secondary enrichment, culture from enrichment broth was

separately streaked on plates of Xylose Lysine Desoxycholate (XLD) (Oxoid) medium. Pink colonies with or without black centers from selective medium was picked, purified and tested biochemically [8].

Escherichia coli: Some pathogenic bacteria such as *E. coli* were detected according to the procedures outlined by Food and Drug Administration (FDA, 2001).

Staphylococcus aureus: For detection of *S. aureus*, golden yellow colonies from Mannitol Salt Agar (MSA) during staphylococci count were picked, purified and preserved. Coagulase test was done by two ways: slide coagulase test and tube coagulase test [10].

Antimicrobial susceptibility testing

From the total of 96 fresh fruit juice samples 16 of each Avocado, Mango, Orange, Apple, Mixed juice and Papaya, around 112 bacteria isolates of *Escherichia coli*, *Salmonella* isolates and *Staphylococcus aureus* were isolated. Out of the total isolates, 12 *Escherichia coli*, 10 *Salmonella* isolates and 15 *Staphylococcus aureus* isolates were subjected to antibacterial sensitivity testing. In-vitro test was used to confirm susceptibility of isolates to chosen antimicrobial agents by means of a disc diffusion method on Mueller-Hinton Agar (Difco) [11]. Briefly, a single colony of each isolate was introduced into 2 ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface. Antibiotics impregnated discs (Ampicillin (AMP) 10 µg, Chloramphenicol (C) 30 µg, Ciprofloxacin (CIP) 5 µg, Gentamicin (CN) 10 µg, Amoxicillin (AML) 25 µg, Vancomycine (VAN) 10 µg, Norfloxacin (NOR) 10 µg, Tetracycline (TE) 30 µg, Erythromycin (ERY) 15 µg, Sulphonamides (S) 300 µg) were then placed onto the surface of the inoculated plates. Plates were incubated for 16-24 hour at 35°C. The diameters of zone of inhibition were measured to the nearest whole millimeter using the transparent rule interpreted as susceptible, intermediate and resistant.

Data analysis

After completion, data, each measurement of different variables recorded, organized and entered SPSS version 20 software and summarized in different sample descriptive statistic such as percentage, mean and finally presented by using tables.

RESULTS

The fresh fruit juice sample of total viable count (TVC), total coliform count (TCC), fecal coliform count (FCC) and total *staphylococcus* (TSC) was summarized in Table 1. Accordingly, the total viable count (TVC) of mixed juice was highest (6.65 ± 0.31 log10 cfu/ml), whereas the total viable count of papaya and mango was 6.56 ± 0.28 log10 cfu/ml and 5.88 ± 0.53 log10 cfu/ml respectively. The lowest (5.32 ± 0.49 log10 cfu/ml) TVC was observed in apple fresh fruit juice sample. Likewise the total coliform count (TCC) of papaya was highest (4.87 ± 0.45 log10 cfu/ml), whereas the total coliform count of Mango and avocado were 4.58 ± 0.43 log10 cfu/ml and 2.86 ± 0.46 log10 cfu/ml consecutively. Next to papaya the highest (4.65 ± 0.44 log10 cfu/ml) of TCC was recorded in orange fresh juice sample. Similar to TVC, the lowest (2.59 ± 0.42 log10 cfu/ml) TCC was counted in apple fresh juice sample. Regarding FCC the highest (3.95 ± 0.47 log10 cfu/ml) bacterial load was counted in avocado fresh juice sample. On the other hand, the lowest count of FCC was recorded in the case of mango (2.00 ± 0.36 log10 cfu/ml) fresh fruit juice. The total staphylococcal count (TSC) of orange fresh fruit juice was highest (2.86 ± 0.33 log10 cfu/ml) followed by mixed fresh fruit juice (2.75 ± 0.38 log10 cfu/ml) whereas the total staphylococcal counts of mango and Avocado were 2.66 ± 0.45 log10 cfu/ml and 2.59 ± 0.37 log10 cfu/ml, respectively. The lowest (2.08 ± 0.29 log10 cfu/ml) TSC was recorded in the case of apple fresh fruit juice sample.

Regarding commercially packed fruit juice samples, total viable count (TVC), total coliform count (TCC), fecal coliform count (FCC) and total *staphylococcus* (TSC) was recorded in Table 2. As shown in Table 2, the total viable count (TVC) of packed pineapple fruit juice was the highest (3.08 ± 0.65 log10 cfu/ml) followed by mango and white grape juice samples with the same result (2.96 ± 0.52 log10 cfu/ml). The lowest (2.26 ± 0.51 log10 cfu/ml) total viable count was counted in the case of orange juice sample. The

Table 1: The TVC, TCC, FCC and TSC from fresh fruit juices samples (log10 cfu/ml).

Types of Juice	Sampling area	TVC (log_{10} cfu/ml) Mean ± SD	TCC (log10 cfu/ml) Mean ± SD	FCC (log_{10} cfu/ml) Mean ± SD	TSC (log_{10} cfu/ml) Mean ± SD
Papaya	Tourist Hotel	6.56 ± 0.28	4.87 ± 0.45	2.85 ± 0.47	2.46 ± 0.36
Mango	Paradise Lodge	5.88 ± 0.53	4.58 ± 0.43	2.00 ± 0.36	2.66 ± 0.45
Avocado	Lucy Bar Hotel	5.48 ± 0.51	2.86 ± 0.46	3.95 ± 0.47	2.59 ± 0.37
Mixed juice	Lucy Bar Hotel	6.65 ± 0.31	3.91 ± 0.55	3.60 ± 0.41	2.75 ± 0.38
Orange	Tourist Hotel	5.91 ± 0.52	4.65 ± 0.44	2.90 ± 0.39	2.86 ± 0.33
Apple	Paradise Lodge	5.32 ± 0.49	2.59 ± 0.42	2.38 ± 0.35	2.08 ± 0.29

TVC, total viable count; TCC, total coliform count; FCC, fecal coliform count; TSC, total staphylococcus count; SD, standard devation.

Table 2: The TVC, TCC, FCC and TSC from commercially packed fruit juices (log10 cfu/ml).

Type of Juice	Sampling area (Super market)	TVC (log_{10} cfu/ml) Mean ± SD	TCC (log_{10} cfu/ml) Mean ± SD	FCC (log_{10} cfu/ml) Mean ± SD	TSC (log_{10} cfu/ml) Mean ± SD
Mango	Abyssinia	2.95 ± 0.52	0.60 ± 0.35	ND	1.30 ± 0.56
Orange	Abaya	2.26 ± 0.51	0.48 ± 0.67	ND	1.85 ± 0.59
Pineapple	Abyssinia	3.08 ± 0.65	0.00 ± 0.00	ND	1.00 ± 0.15
White grape	Abyssinia	2.95 ± 0.52	0.30 ± 0.25	ND	1.00 ± 0.15

TVC, total viable count; TCC, total coliform count; FCC, fecal coliform count; TSC, total staphylococcus count; ND, not detected; SD, standard devation.

total coliform count (TCC) of packed mango fruit juice sample was highest (0.60 ± 0.35 log10 cfu/ml), whereas total coliform count of packed orange and white grape fruit juice samples were 0.48 ± 0.67 log10 cfu/ml and 0.30 ± 0.25 log10 cfu/ml, consecutively. Also as shown in Table 3, fecal coliform count (FCC) was not detected in all commercially packed fruit juice samples selected for this study. The highest (1.85 ± 0.59 log10 cfu/ml) total staphylococcal count (TSC) was counted in case of packed orange fruit juice, whereas the mango was the second highest (1.30 ± 0.56 log10 cfu/ml) total *staphylococcus* count. The total *staphylococcus* count of packed pineapple and white grape fruit juice was the same (1.00 ± 0.15 log10 cfu/ml).

Microbial characterization

After different biochemical tests, it was found that, from the total of 96 fresh fruit juice sample, *E. coli* 48(50.00%), *Salmonella spp* 40(41.67%) and *Staphylococcus aureus* 65(67.71%) were identified in all fresh fruit juice samples (mango, papaya, avocado, mixed juice, orange and apple) as shown in Table 3.

Prevalence of *E. coli, Salmonella* and *S. aureus* in fresh fruit juices

Out of 96 fresh fruit juice samples, the highest percentage of *E. coli* was isolated from mango fruit juice sample which was 81.25% and also the lowest percentage was isolated from apple 4(25.00%) fresh fruit juice samples as indicated in Table 4. Regarding the incidence of *Salmonella*, all fresh fruit juice samples were positive with the highest percentage of mixed juice (62.5%) and the lowest 3(18.75%) incidence of apple fresh fruit juice samples. The prevalence of *Staphylococcus aureus* with the highest percentage of 87.5% and lowest incidence of 43.75% was isolated from avocado and mixed juice fresh fruit juice samples, respectively. In general, from the following Table 4, the prevalence of *E. coli*, *Salmonella* and *Staphylococcus aureus* was 48(50.00%), 40(41.67%) and 65(67.71%) out of 96 fresh fruit juices samples, respectively.

Table 3: Microscopic and biochemical profile of detected pathogens fresh fruit juice.

Biochemical test	*E. coli*	*Salmonella* isolate	*S. aureus*
Gram's reaction	-	-	+
Nitrate reduction	+	+	-
Urease test	+	+	+
Catalase test	+	+	+
Oxidase test	-	+	-
Indole test	+	-	-
Citrate test	-	-	-
H_2S test	-	+	-
VP test	-	-	-

+, positive; -, negative.

Antimicrobial susceptibility testing

The results of antibiotic sensitivity testing were interpreted and presented as the resistant, intermediate and susceptible of bacterial isolates to the antibiotics Table 5. All *E. coli* isolates were completely resistance (100%) to vancomycin and most isolates were moderately resistance to Ampicillin 65%, Ciprofloxacin 61%, Amoxicillin 55%, and Sulphonamides 75%. Chloramphenicol 96% and Norfloxacin 82% were moderately susceptible, whereas Gentamicin 100% is completely susceptible to *E. coli*.

In case of *Salmonella* isolates, all of them were completely resistance (100%) to Ampicillin and Vancomycin. But, they were completely susceptible (100%) chloramphenicol, Gentamicin and ciprofloxacin, and moderately susceptible to Norfloxacin (92%) and Erythromycin (79%).

Regarding *Staphylococcus aureus* all the isolates were completely resistance (100%) to Amoxicillin and vancomycin. However, they were completely susceptible (100%) to ciprofloxacin, chloramphenicol, and Gentamicin, and moderately susceptible Norfloxacin 96% and Erythromycin 88%. But all of them were intermediate (67%) to amoxicillin antibiotics (Table 5).

DISCUSSION

Fresh fruit juices are well consumed by consumer for their fresh flavor, vitamins content and nutritive value. Also in Arba Minch town, prepared fresh fruit juice is becoming more and more popular as they are usually delicious, flavor, cheapest and easily available than packed fruit juice in the study area. But many fresh fruit juice are easily contaminated during the preparation and has many hazardous effects on health of human being. Several bacteria, for example coliform, *salmonella*, *shigella* and *staphylococcus* species are representing the pathogenicity of fruit juice. After overnight incubation, distinctive morphological characteristics like pink, circular, convex colonies on MacConkey Agar and yellow colonies on Mannitol Salt Agar and biochemical tests were recorded which indicates the presence of coliforms, *Salmonella* and *Staphylococcus* species.

In the current study, all fresh fruit juice samples revealed much bacterial load than the allowed count. The bacterial load of fresh mixed juice (6.65 ± 0.31 log10 cfu/ml) and fresh apple juice (5.32 ± 0.49 log10 cfu/ml) indicates the highest and lowest total viable bacterial count, respectively Table 1. Also commercially packed fruit juice samples exhibited total viable bacterial count with the highest (3.08 ± 0.65 log10 cfu/ml) and the lowest load of (2.26 ±

Table 4: Detection of *E. coli*, *Salmonella* and *S. aureus* in fresh fruit juices in cfu/ml.

Fresh fruit juices	Number of sample	Incidence of *E. coli*	Incidence of *Salmonella*	Incidence of *S. aureus*
Papaya	16	6(37.50%)	6(37.50%)	13(81.25%)
Mango	16	13(81.25%)	4(25.00%)	12(75.00%)
Avocado	16	10(62.50%)	9(56.25%)	14(87.50%)
Mixed juice	16	8(50.00%)	10(62.50%)	7(43.75%)
Orange	16	7(43.75%)	8(50.00%)	10(62.50%)
Apple	16	4(25.00%)	3(18.75%)	9(56.25%)
Total	96	48(50.00%)	40(41.67%)	65(67.71%)

Table 5: Antibiotic susceptibility test of bacteria pathogens isolated from fresh fruit juices.

Antibiotics	*Escherichia coli*			*Salmonella* isolates			*Staphylococcus aureus*		
	R	I	S	R	I	S	R	I	S
C (30 µg)	4%	ND	96%	ND	ND	100%	ND	ND	100%
AMP (10 µg)	65%	14%	21%	100%	ND	ND	100%	ND	ND
CN (10 µg)	ND	ND	100%	ND	ND	100%	ND	ND	100%
CIP (5 µg)	61%	ND	39%	ND	ND	100%	ND	ND	100%
AML (25 µg)	55%	14%	31%	29%	71%	ND	30%	70%	ND
NOR (10 µg)	ND	18%	82%	ND	8%	92%	ND	4%	96%
VAN (10 µg)	100%	ND	ND	100%	ND	ND	100%	ND	ND
ERY (15 µg)	ND	ND	ND	ND	21%	79%	5%	5%	88%
TE (30 µg)	ND	ND	ND	68%	ND	32%	44%	22%	33%
S (300 µg)	75%	15%	10%	76%	24%	ND	63%	37%	ND

AMP, Ampicillin; C, Chloramphenicol; CIP, Ciprofloxacin; CN, Gentamicin; AML, Amoxicillin; VAN, Vancomycine; NOR, Norfloxacin; TE, Tetracycline; ERY, Erythromycin; S, Sulphonamides; ND, not detected.

0.51 log10 cfu/ml) in pineapple and orange, respectively Table 2. According to Rahman et al. [1] reported that total viable bacterial count found in most of the fresh juices were higher than the commercially packed juice, which was found to be agree with our study. The current study predicted that, commercially packed juice were more preferable for consumption than locally available fresh juice. The reason of using different safety procedures and some preservative techniques during fruit juice processing and production makes commercially packed fruit juices more preferable than fresh fruit juices. On the other hand, the sophisticated TVC of local fresh fruit juices can be due to unclean care throughout preparation and processing of the juices. The current result of viable bacteria load was in conformity to the findings of Tasnim et al. [12] which exhibits the load of viable bacteria in processed juice samples within the standard limit in the average of 10^3 cfu/ml. In the study area, fresh fruit juices are well known and consumed by the people. However, the overall quality assessment of the fresh fruit samples indicated that high bacteriological load in the study area which can expose consumers to very serious diseases.

According to Andres et al. [13] reported that presence of coliform in fruit juice is not permitted by safe food consumption standard. In the present study, except commercially packed fruit juices, all fresh fruit juices were found to be risky and not suitable for consumption for exceeding the standard limit. Presently papaya fresh fruit juices 4.87 ± 0.45 log10 cfu/ml represents the highest coliform count, whereas 2.59 ± 0.42 log10 cfu/ml was the lowest coliform count. This result is also in agreement with some previous research works [14,15]. Similarly, compared to these studies large numbers of coliforms were found in our study (Table 1). On the other hand, all commercially packed fruit juice samples were safe and favorable for consumption because of not exceeding the standard limit of coliform. The highest value of coliform count was 0.60 ± 0.35 log10 cfu/ml and the lowest value of coliform was 0.00 ± 0.00 log10 cfu/ml found in pineapple.

In the present study in all commercially packed fruit juices fecal coliform count (FCC) was not detected. However, in case of fresh fruit juices fecal coliform count was detected with the highest 3.95 ± 0.47 log10 cfu/ml and lowest 2.00 ± 0.36 log10 cfu/ml in the case of avocado and apple, respectively. Similarly, Gulzar [16] reported that the highest and lowest coliform count for fresh fruit juice samples were 8.2 x 10^6 cfu/ml and 1.53 x 10^3 cfu/ml, respectively.

The presence of staphylococci usually shows contamination and may cause human diseases through the production of toxins. The manifestation of enormous numbers of *staphylococcus* is generally an indication of poor hygiene and temperature control. The results of the current study indicates that the highest value of staphylococcal count for fresh juice sample was 2.86 ± 0.33 log10 cfu/ml found in orange and the lowest value of TSC was 2.08 ± 0.29 log10 cfu/ml found in apple. All fresh fruit juice sample showed high staphylococcal count so it is not recommended for consumption. On the contrary, in the commercially packed fruit juice samples, the highest staphylococcal count was 1.85 ± 0.59 log10 cfu/ml found in orange and the lowest value was 1.00 ± 0.15 log10 cfu/ml both in white grape and pineapple. All commercially packed fruit sample were safe for consumption as the count of staphylococcal is low in the present study. But the effective levels of toxin formation of *staphylococcus* require a large number of microorganisms approximately 10^5-10^6 cfu/ml of food [17]. A few reports have shown the prevalence of staphylococci in fruit juice samples [2,15]. Therefore, the risk problem of *staphylococcus* is not hard as the coliform.

In the current study the percentage of *E. coli* detected from mango fresh fruit juice was 13(81.25%), because the acidic nature some fresh fruit juices does not protect them from this *E. coli* pathogens. Sandeep et al. [18] has reported that *E. coli*, *Salmonella*, *Shigella* and *Staphylococcus aureus* were the potential sources of bacterial pathogens of fruit juices. Therefore, in this study only *E. coli*, *Salomonella* isolates and *Staphylococcus aureus* were analyzed. The percentage of avocado fresh fruit juice was 10(62.5%) next to mango juice. Also the prevalence of *Staphylococcus aureus* in the current study were 14(87.50%), 13(81.25%), 12(75.0%) and 10(62.5%) for avocado, papaya, mango and orange, respectively. Likewise the incidence of *Salmonella* isolates for mixed juice, avocado, orange and papaya was 10(62.5%), 9(56.25%), 8(50.0%) and 6(37.5%), respectively. The main source of these pathogens is during the preparation of juices and people, which did not careful about hand and utensil washing. The sources of contamination for the freshly squeezed street vended fruit juice can be from the fruit itself, [19] water used for dilution and extended preservation of fruit pulp and squeezed juice without refrigeration. A study by Ukwo et al. [20] revealed that fruit juices under these conditions are contaminated with *E. coli*, *Salmonella* species, *Shigella* and *Staphylococcus* species. Therefore, in this study only *E. coli*, *Salomonella* species and *Staphylococcus aureus* were analyzed.

Moreover, this study examined efficiency of 10 different common antibiotics on *Escherichia coli*, *Salmonella* isolates and *Staphylococcus aureus*. Emergence of drug resistant pathogens is one of the most serious health problems in developing countries. According to the evidence some authors, antibiotic resistance of bacterial isolates against commonly used antibiotics has been increased from time to time [21]. Sharada et al. [22] has reported that even though bacteria develop multiple resistances but their degree of resistance varies with different isolates and time. Currently the study was conducted on antibacterial sensitivity of three species of pathogenic bacteria isolates (*E. coli*, *Salmonella* and *Staphylococcus aureus*) on ten different antibiotics (Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Amoxicillin, Vancomycine, Norfloxacin, Tetracycline, Erythromycin, Sulphonamides) and the results were interpreted as resistance, intermediate and susceptible according to drug resistance chart. In the present study all *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* were completely resistant (100%) to Vancomycine. Also the current drug sensitivity pattern indicates that all the isolates of *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* were 100% susceptible to gentamicin, likewise 100% susceptibility pattern of all *Salmonella* isolates and *Staphylococcus aureus* was observed against chloramphenicol and ciprofloxacin. In agreement to the present study, Meher et al. [23] reported on susceptibility of *Salmonella* and *Staphylococcus aureus* against ciprofloxacin. Resistance of *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* isolates to specific antibiotics could possibly be due to spreading of drug resistance microbes in the environment arising from the misuse of antibiotics among the general population.

CONCLUSIONS

The overall assessment of the fresh fruit juice samples analyzed bacteriologically indicated high count and highly contaminated as showed in all analysis, total viable count, total coliform count, fecal coliform count and total *Staphylococcus* count in Table 1. These high counts, however, may pose hazard to the health of consumers especially in the current study pathogenic species like *E. coli*, *Salmonella isolates* and *Staphylococcus aureus* are present in the fresh fruit juices to be consumed by the community of the study area. This contamination is due to poor quality of water used; unhygienic conditions related to washing of utensils, poor personal and domestic hygiene during fresh fruit juice preparation. Despite of all fresh fruit juice, the bacterial load in all commercially packed fruit juice was not exceeded standard limit and safe than fresh fruit juice.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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