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Characterization of Some Pathogenic Bacteria Associated with Fresh Juices Sold in the Central Bus Station at Khartoum State

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Abstract: ***Fruits and fruit juice has been traditionally assumed as low risk foods. Recently, fruits as well as fruit juices have been acknowledge as "emergency vehicles" for foodborne illness due to bacterial pathogens. The increase in the consumption of fruit juices possibly contribute to the increase in the number of repeated foodborne diseases outbreaks linked to the fruit juices. In Sudan these products are produced under unhygienic conditions, therefore this study aimed to investigate the presence of some pathogenic bacteria associated with these products sold by vendors and hawkers. A total of 30 samples of fresh fruit juices of mango, guava, lemon, orange, fruit pulps including aradeib (Tamarindus indica), tabeldi (Adansonia digitata), gudeim (Grewia tenax), Shaeer or barely powder (Hordeum vulgare) and calyx of karkade (Hibiscus sabdariffa) were collected randomly from juice stalls, cafeterias and hawkers in the central bus station at Khartoum. The pathogenic bacteria investigated were Staphylococcus sp., Salmonella sp. and E. coli using appropriate selective media. Staphylococci count was in the range of log 3-log 7. Salmonella was detected in all examined samples. The count of Escherichia coli ranged from 0.00 to 1100MPN/ml and eight samples (26.7%) were free from E. coli. The Staphylococcal isolates were identified as Staphylococcus aureus (55%), Staphylococcus epidermidis (35%) and Staphylococcus saprophyticus (10%) while Salmonella isolates were identified as Salmonella typhi (60%), Salmonella paratyphi (26.7%) and Salmonella orizonae (13.3%). These results revealed that the counts of these bacteria are higher than the permissible level recorded by Gulf Stan, Sudanese Standards and Metrology Organization (SSMO), and Codex Stan. The prevalence of these pathogens in fresh fruit juices render them to be unsuitable for human consumption and considered as a source of serious food outbreaks. To produce high quality juice, it is necessary to follow hygienic and safety conditions during processing, displaying and serving.***

Keywords: Sudan, fresh fruit juices, microbiology, pathogenic microorganisms, *Staphylococcus*, *Salmonella*, *E. coli*, hawkers and venders

1. Introduction

Fresh fruit juices are popular non-alcoholic drinks consumed by many people throughout the world. They are highly nutritious food that provides the human body with vitamins, minerals and other essential nutrients [1]. Due to their high acidic constituents and natural flavor, many people prefer to drink them and they considered these juices are free from any type of microorganisms. Generally, these products are not always safe due to their high microbial load [2].

Fresh juices will be contaminated at any point of the long procedure of processing as peeling, slicing, soaking, extraction, mixing, handling, serving and displaying. During these steps many spoilage and pathogenic microorganisms will be introduced into these products. As recorded by many researchers' fresh juices are noticeably harbor different pathogenic microorganisms as *E. coli* 0157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* [3]. [4] reported that the existence of *E. coli*, *Salmonella*, *Staphylococcus aureus* in fruit juices are implicated in or responsible about different foodborne outbreaks. Non-typhoidal salmonellosis outbreaks due to the consumption of fresh fruit juices have been referred to the faecal contamination and poor processing practices [5].

Many types of bacteria; such as *E. coli* and *Salmonella* will grow and survive under adverse conditions. [6] stated that *Salmonella* spp, *E. coli* O157:H7 and *Listeria monoctogenes* grew at pH ranging from 3.5 to 3.6 at 5^{o}C for 72 hours. Another study revealed that *Salmonella* ser. *enteritidis* grew in apple, pear and melon juice at pH of 4.0, 4.2 and 5.9 respectively at 35^{o}C for 24 hours [7]. *Salmonella* and faecal coliforms were not detected in unpasteurized and refrigerated orange juice stored at 4, 8 and 12^{o}C for 72 hours [8]. However, many pathogens can grow in acidic medium and cause many foodborne diseases [9].

The presence of pathogenic bacteria that caused food outbreaks related to the consumption of fresh fruit juices in Sudan has been reported. [10] found that the most dominant bacteria isolated from fresh vended juices sold in local market at Khartoum State were *E.coli spp* (33%), *Enterococcus faecalis* (19%), *Salmonella typhy* (15%), *Salmonella paratyphi* A (5%), *Staphylococcus aureus* (8%) and *Klebsiella* 5(4%). Also she recorded that the most dominant bacteria in guava juices was *Salmonella typhi* which comprises 11.6%, in mango juice was *E. coli* (10.8%) and mixed culture (30%) isolated from other juice samples. These pathogenic bacteria are the causative agent of serious foodborne diseases [11].

Therefore the present research aimed to detect, isolate and identify the possible presence of the faecal coliforms and some pathogenic microorganisms in fresh fruit juices and fruit pulps sold in the central bus station (Jackson Bus Station) at Khartoum State.

2. Materials and Methods

2.1. Study area

Jackson yard or square is an area located south west Khartoum, the capital city of Sudan. This yard is used as a central station for buses coming from different Neighborhoods in Khartoum state. Several shops, cafeteria, restaurants and stalls are located there for selling fresh juices, bottled water, soft drink and sandwich beside hawkers who vended different types of food including fresh juices, fresh vegetables and fruits.

2.2 Samples collection

A total of thirty fresh juice samples were randomly collected and purchased from juices dealers at Jackson bus station. These samples were prepared from different fresh fruits (mango, orange, lemon, guava, and mixed fruits), pulp of ardeeb (*Tamarindus indica*), tabeldi (*Adansonia digitata*), gudeim (*Grewia tenas*), calyx of karkade (*Hibiscus sebggrifia*) and Shaeer or barely powder (*Hordeum vulgare*). All collected samples were kept in sterile insulated iced containers and were immediately transported to the laboratory for microbiological investigation.

2.3. pH determination

The pH of fresh juice samples was determined by homogenizing 10 ml of the juice of each sample with 90 ml of distilled water [12] using PYE model 211-pH meter (HANNA- pH 211- Portugal).

2.4. Microbiological parameters investigated

2.4.1. Preparation of serial dilution

Thirty ml of each sample were aseptically poured into sterile conical flask containing 270 ml of sterile peptone water and mixed well to obtain homogenous solution. Then ten-fold dilution was carried out as described by [13].

2.4.2. Staphylococci enumeration

From suitable dilutions of each sample, 0.1 ml was drawn aseptically and spread onto the surface of dried Baird-Parker agar medium using sterile glass rode. Then all plates were incubated at 37^{o}C for 2 days. *Staphylococcus* sp. colonies were counted using colony counter (Quebec Colony Counter) and the results were expressed as cfu/ml for each sample. An extra-confirmatory coagulase test was carried out to differentiate the positive coagulase *Staphylococcus aureus* and negative Staphylococci species using tube coagulase test containing rabbit plasma. Clotting of plasma indicated the positive results [14].

2.4.3. *Salmonella* detection

Methods of [15]-[13] were used for *Salmonella* detection. Twenty five mls of each sample were drawn, poured aseptically and mixed well with 250 ml of sterile Nutrient Broth (NB) and incubated at 37^{o}C for 24 hours. Ten ml of the enriched mixture were drawn aseptically into 100 ml Selenite Broth, and then incubated at 37^{o}C for 24 hours. A loop full from the incubated broth was streaked onto the surface of dried Bismuth Sulphite Agar medium. Plates were then incubated at 37^{o}C for 24 hours. Extra confirmatory tests were conducted onto Triple Sugar Iron and Kligler Iron slant Agar media respectively and then incubated at 37^{o}C for 24 hours.

2.4.4. Fecal coliforms determination

For *E. coli* determination the Most Probable Number (MPN) method was used. MacConkey and Brilliant Green Bile Lactose Broth (BGB) were used for presumptive and confirmatory tests respectively for faecal coliforms presence [16]. The positive tubes from Brilliant Green Bile Lactose Broth (BGB) medium were sub-cultured into *Escherichia coli* (EC) broth medium for faecal colifroms presence and incubated at 44.5^{o}C for 2 days. The Most Probable Number (MPN) for faecal colifroms was reported using the MPN table [16]. Extra confirmatory tests were conducted. A loop full from the positive tubes of EC medium was streaked onto Eosin Methelene Blue agar medium (EMB) and incubated at 37^{o}C for 48 hours. Colonies with green metallic sheen revealed the positive result for *Escherichia coli*. Extra confirmatory tests of *E coli* were carried out by the IMVEC test [13].

2.4.5. Purification and identification of the microbial isolates

Predominant bacterial isolates were selected and sub-cultured onto nutrient agar medium. The purified isolates were then kept in a refrigerator for further tests. Identification of bacterial isolates were carried out by the conventional methods which was based on the cultural, morphological and biochemical tests [17]-[14]-[13].

3. Results and Discussion

Results recorded in Table 1 represent the pH of the collected fresh juice samples obtained from karkade, barley powder, fruits pulp of ardeeb, tabeldi and gudeim. The pH of the samples ranged between 2.36 and 5.61. Tamarind showed the lowest pH values (2.36-2.40), while barely powder showed the highest value ranging from 5.20 to 5.61. [18] found that the pH values of Tamarind leather samples treated with different concentrations of sucrose and dried by different drying system ranged from 2.36 to 2.88.

With regards to the pH of the juices obtained from fresh fruits, lemon juice samples reported the lowest pH ranged from 2.12 to 2.50, while the other samples had a pH in the range of 3.25-4.71(Table 2). The pH values of the fresh fruit juices in this work are lower than that obtained by [19] who found that the mean pH values of juices prepared from orange, sweet lemon and carrot were 4.34, 5.08 and 5.89 respectively and similar to that recorded by [20] who found that the pH of pineapple and sweet lime juices were acidic (< 3). Another study conducted by [21] found that the lemon juice samples had a pH of 2.3.

Generally, the faecal coliforms (*E. coli*) counts of the investigated samples recorded a high count ranged from 0.00 to > 1100 MPN/ml. Guava juice sample obtained from cafeteria 5 and cocktail juice sample (stall 11) had a high count of >1100MPN/ml followed by mango juice (stall 10), fruit cocktail juice (stall12) and baobab juice (stall 13) which reported 1100MPN/ ml. *E. coli* was detected in 73.3% of the analyzed samples, while 26.7% of the samples were devoid from *E. coli* particularly those with low pH (2.38-3.25) and guava of pH 4.0 (Table. 1, 2). However, these values were higher than those mentioned by [22] who found that coliforms and *E. coli* was present in 99% of the fresh juice samples sold in Dhaka in Bangladesh. *E. coli* incidence in the collected juice samples may be attributed to the use of contaminated water, cross contamination from surfaces, utensils and surrounding area (Plate 1) or from infected juice dealers [23]. [24] attributed the high contamination of cocoa drinks in Kumasi to the unhygienic sanitation and production practices as hand mixing of cocoa powder with sugar and non-potable water collected from streams nearby when municipal water is not available. The absence of *E. coli* in some fruit juice samples in this study might be attributed to the availability of clean water, good sanitation and juice preparation practices or may be due to the low pH which it suppress the growth of this bacterium. Fruit juices contain different organic acids as tartaric acid which are toxic and affect the viability of *E. coli* by affecting their purine bases thus resulting in the denature of the essential enzymes in this bacterium [25]-[18].

Results obtained showed the ability of *E. coli* to grow in the acidic medium of such juices and this indicated their capability to survive in low pH. Pathogens' surviving in acidic juices is referred to their ability to regulate their internal pH and maintain it at neutral pH due to the combination of the passive and active homeostasis force [26]. Adaptation of enteric bacteria in very low acid medium is attributed to the enzymes induction which leads to the raising of internal pH and activates enzymes that devoted to the protection and repair of proteins and DNA [27] Many researches documented that many pathogenic microorganisms can survive in fruit juices with low pH medium [28]. Fresh fruit juices are characterized by the low acidic pH where many pathogenic bacteria as *Salmonella* and *E. coli* will survive and adapt themselves to grow in this medium. This explains how the infection or foodborne illness caused due to the consumption of fresh fruit juices [29].

Table 1: Values of pH, counts of E. coli and presence of Salmonella sp. in fresh fruit juices prepared from hibiscus calyx, barely powder and different fruits pulp

E. coli **MPN/ml**	***Salmonella*** **sp. cfu/ml**	**pH**	**Sample Sources**	**Juice Types**	**Sample No**
0.00	+	3.00	Hawker 1	Calyx of *Hibiscus* (Karkade)	1
0.00	+	2.90	Hawker 2	Calyx of *Hibiscus* (Karkade)	2
0.00	+	3.12	Hawker 3	Calyx of *Hibiscus* (Karkade)	3
11	+	3.10	Hawker 4	Calyx of *Hibiscus* (Karkade)	4
0.00	+	5.61	Hawker 5	Barley powder (Sheer)	5
15	+	5.20	Hawker 6	Barley powder (Sheer)	6
0.00	+	2.40	Hawker 7	Tamarind (Ardeeb pulp)	7
0.00	+	2.38	Hawker 8	Tamarind (Ardeeb pulp) seeds	8
40	+	3.95	Cafeteria 6	*Grewia tenax* (Gudeim pulp)	9
3.3	+	3.78	Cafeteria 7	*Grewia tenax* (Gudeim pulp)	10
3.3	+	4.25	Cafeteria 8	Baobab (Tabeldi pulp)	11
1100	+	3.33	Stall13	Baobab (Tabeldi pulp)	12
280	+	2.36	Stall14	Tamarind (Ardeeb pulp)	13

Legend:
(+): detected or present

Table 2: Values of pH, counts of E. coli and presence of Salmonella sp. in fresh fruit juice samples collected from different sources

E. coli **MPN/ml**	***Salmonella*** **sp. cfu/ml**	**pH**	**Sample Sources**	**Juice Types**	**Sample No**
0.00	+	3.25	Cafeteria 1	Mango (Fresh fruits)	1
15.0	+	4.30	Cafeteria 2	Mango (Fresh fruits)	2
40	+	3.90	Cafeteria 3	Orange (Fresh fruits)	3
4.6	+	2.40	Cafeteria 4	Lemon (Fresh fruits)	4
>1100	+	4.10	Cafeteria 5	Guava (Fresh fruits)	5
0.00	+	4.00	Stall1	Guava (Fresh fruits)	6
43	+	4.38	Stall 2	Guava (Fresh fruits)	7
1.00	+	3.33	Stall3	Orange (fresh fruits)	8
9	+	3.55	Stall4	Orange (fresh fruits)	9
3.3	+	3.52	Stall 5	Orange(fresh fruits)	10
43	+	2.10	Stall6	Lemon (fresh fruits)	11
15	+	2.32	Stall7	Lemon (fresh fruits)	12
210	+	2.25	Stall8	Lemon(fresh fruits)	13
150	+	4.71	Stall9	Mango (fresh fruits)	14

1100	+	3.90	Stall10	Mango(fresh fruits)	15
>1100	+	4.40	Stall 11	Fruit cocktail	16
1100	+	4.37	Stall12	Fruits cocktail	17

Legend:
(+): detected or present

Plate 1: The surrounding environment near some juice stalls in the central bus station at Khartoum State

Salmonella was detected in all samples studied (Table 1 and 2). [30] detected *Salmonella typhi* in one juice sample sold in Nagpur city in India. These findings in this study are not in accordance with that obtained by [31] who found that all ready-to-eat foods used for breakfast and fresh fruit juices sold in Tirumala in India were free from *Salmonella* sp., *Shigella* and *Vibrio cholera*. [32] found that some packaged fruit juices sold in Port Hacourt Metropolis, Nigeria did not reported any growth of *Salmonella*, *Shigella* and *Vibrio* species.

Many studies confirmed the presence of *Salmonella* in some fruit juices as apple [33] Another study claimed that *Salmonella* found in 50% of the fresh fruit and vegetables juice samples in Nagpur city in India as recorded by [20]. A microbiological survey of freshly squeezed juices obtained from retail business across Victoria did not show any *Salmonella* presence [34] The prevalence of this bacterium may be due to fecal contamination or due to the use of contaminated water or from food handlers [35]. [22] suggested that *Salmonella* sp. may have gained entry through contaminated water with animal and human feces because some vendors do not use boiled and potable water for washing and juicing process.

The Staphylococcal counts ranged from log 3.0 to log 7.0 cfu/ml for all juice samples obtained from hibiscus, barely powder, pulp of some Sudanese traditional fruits and those obtained from fresh fruits (Fig. 1 and 2). Tamarind juice samples exhibited low counts ranging from log 3.1 to log 3.3cfu/ml, while the highest counts ranged from log 6 to 6.7cfu/ml was reported for barely juice samples. With respect to the juices obtained from fresh fruits, results revealed that lemon juice samples showed counts ranged from log 3 to log 3.3cfu/ml. The cocktail juice samples recorded the highest counts (log 6.8- log 7cfu/ml) as compared to the other samples. These findings are similar to those obtained by [36] who found that the mean count of this bacterium in fresh juices in Dhaka city was log 3.85cfu/ml. Another study was conducted by [37] who found that the mean *staphylococcal* counts ranged from log 4.0cfu/ml as in grape juice and to log 4.54cfu/ml as in avocado juice sold in Ogun State South West Nigeria. Also these findings in this research are in accordance with that reported by [1] who stated that the staphylococcal count ranged between log 2.41 and log 7.08cfu/ml for fresh juices available in Jessore city. The count values of *Staphylococcus* sp. of all juices samples were not within the permissible level that was established by the [38] where juice samples should be free from *Staphylococcus*. The presence of this bacterium in the investigated juice samples may be referred to the cross-contamination during preparation, processing and handling. Humans are considered as the principal source of these organisms. Staphylococci are found in the nose, throat and skin of up to 60% of healthy humans [39] pointed that the presence of *staphylococcus aureus* in juices and other foods may be attributed to the hair falling from juice makers. Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in the developing countries [40]. In addition, the displaying of fresh juices in the open air without proper cooling beside the crowded roads is considered another source of contamination.

Staphylococcal isolates (20 isolates) were identified by conventional methods using different biochemical tests (Table 3). These isolates were identified as *Staphylococcus aureus* (55%), *Staphylococcus.*

Table 3: Biochemical tests of ***Staphylococcus sp.*** obtained from juice samples from different sources

Species	Acid from tested sugars 1 2 3 4 5 6 7 8 9 10										Haemolysis	Coagulase	Urease	Arginine hydrolysis	Nitrate reduction	VP	O/F	Acid from glucose	Oxidase	Catalase	Anaerobic growth	Growth in air	Motility	Endo spore staining	Gram staining	Shape	Isolates Code	Isolates No No
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	LJ	1
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	GJ	2
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	MJ	3
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	HCJ	4
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	OJ	5
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	Cock J	6
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	GuJ	7
St. saprophyticus	-	-	-	-	+	+	+	-	+		-	-	+	-	+	+	F	+	-	+	+	+	-	-	+	COC CI	MJ	8
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	BJ	9
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	LJ	10

Legend:
(d) Delayed reaction. (+) Positive reaction 1-10:Sugars tested (OJ): Orange juice. (GuJ) Gudiem juice.
(w) Weak reaction. (-) Negative reaction. 1-Lactose. 4- Fructose. 7-Xylose. 10-Mannose. (GJ) Guava juice. (BP) Baobab pulp juice
(F) Fermentative. (St) *Staphylococcus* 2-Maltose. 5- Sucrose. 8-Cellobiose. (LJ):Lemon juice. (HCJ): Hibiscus calyx juice.
LJ(+) Positive reaction (VP) Voges-Proskauer 3-Mannitol. 6-Trehalose. 9-Raffinose. (MJ): Mango Juice. (Cock J): Cocktail juice.
(O/F)Oxidation fermentation test

Con. Table 3: Biochemical tests of *Staphylococcus sp.* obtained from juice samples from different sources.

Species	Acid from tested sugars 1 2 3 4 5 6 7 8 9 10										Haemolysis	Coagulase	Urease	Arginine hydrolysis	Nitrate reduction	VP	O/F	Acid from glucose	Oxidase	Catalase	Anaerobic growth	Growth in air	Motility	Endo spore staining	Gram staining	Shape	Isolates Code	Isolates No
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	GJ	11
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	LJ	12
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	OJ	13
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	TP	14
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	OJ	15
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	Cock J	16
St.	-	-	-	-	+	+	+	-	+		-	-	+	-	+	+	F	+	-	+	+	+	-	-	+	COC	HCJ	17

saprophyticus																									CI		
St. aureus	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	GJ	18
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	GuJ	19
St. epidermidis	+	-	-	-	-	+	+	-	+	d	-	-	+	+	+	+	F	+	-	+	+	+	-	-	+	COC CI	MJ	20

Legend:
(d) Delayed reaction. (+) Positive reaction 1-10: Sugars tested
(w) Weak reaction. (-) Negative reaction. 1-Lactose. 4- Fructose. 7-Xylose. 10-Mannose. (MJ): Mango Juice. (Cock J): Cocktail juice. (TP) Tamarind pulp juice.
(F) Fermentative. (St) *Staphylococcus* 2-Maltose. 5- Sucrose. 8-Cellobiose. (GJ) Guava juice. (OJ): Orange juice. (GuJ) Gudiem juice.
(+) Positive reaction. (VP) Voges-Proskauer 3-Mannitol. 6-Trehalose. 9-Raffinose. (LJ): Lemon juice. (HCJ): Hibiscus calyx juice. (O/F) Oxidation fermentation test.

epidermidis (35%) and *Staphylococcus saprophyticus* comprised 10%. As it can be seen from the results, *Staphylococcus aureus* found in most samples and its presence may reveal the cross-contamination from juices maker and servers, indicating poor personal hygiene dealing with handling, displaying and selling of this product by vendors and hawkers. Food handlers are considered the main source of this bacterium which introduce into juices [11]. [41] reported that the *Staphylococcus aureus* is considered as the third most important cause of disease globally amongst the reported food-borne illnesses.

With respect to the *Salmonella* identification different biochemical tests were used (Table 4) and the *Salmonella* percentage occurrence is presented in Fig. 3. The isolates were identified as *Salmonella typhi* (60%), *Salmonella paratyphi* (26.7%) and *Salmonella orizonae* (13.3%). The presence of *Salmonella typhi*, *Salmonella paratyphi*, *E. coli* and *Streptococcus feacalis* indicated the human origin contamination and also faecal contamination from other sources [42]. Many types of bacteria are normal microflora of many soils as *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, while *Salmonella*, *Shigella*, *E. coli* and *Campylobacter* are normally present in human and animal intestinal tract. These microorganisms will contaminate raw fruits and vegetables through faeces, sewage, untreated surface water or water used for irrigation [43].

Results showed that all fresh fruit samples harbor high levels of different enteropathogenic bacteria as *Salmonella* sp, *E. coli* and *Staphylococcus* sp. due to poor knowledge and awareness about food hygiene and safety among juice dealers and vendors. Other sources of contamination as water, ice, raw materials and processing play an important role in introducing pathogens in juices. The above mentioned bacteria are considered as public health threats. As it can be seen from the results all fresh fruit juices did not meet the standard that establish by public health authorities. Health education and training regarding good manufacturing practices (GMP) and good hygienic practices (GHP), implementation of standard hygienic practices, regular monitoring and applying of regulations are essential and recommended for food vendors and juice servers to avoid any food poisoning. The prevalence of spoilage and pathogenic microorganisms in fresh fruit juices for immediate usage will not be eliminated but it can be reduced by good handling, following sanitary hygienic practices and proper holding temperature during preparation, serving and displaying.

Table 4: Identification of Salmonella isolated from collected fresh juice samples obtained from juice dealers at Jackson bus station

Species	Acid from tested sugars							Indole	Arginine hhdrolysis	Uraese	VP	Gelatin hydrolysis	Citrate utilization	O/F	Acid from glucose	Oxidase	Catalase	Growth in air	Motility	Endo -spore staining	Gram staining	Shape	Isolates Code	Isolates No
	7	6	5	4	3	2	1																	
S. typhi	-	+	+	-	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	HC	1
S. typhi	-	+	+	-	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	HC	2
S. paratyphi A	-	-	+	+	-	-	-	+	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	TP	3
S. arizonae	-	-	+	+	+	-	-	-	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	BP	4
S. typhi	-	+	+	-	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	BP	5
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	OJ	6
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	GuJ	7
S. arizone	-	-	+	+	+	-	-	+	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	LJ	8

Legend:
(d)Delayed reaction. (VP) Voges-Proskauer 1-7: Sugars tested (HC) Hibiscus calyx. (GuJ) Gudiem juice.
(F) Fermentative. 1-Arabinose. 2-Lactose. (TP) Tamarind pulp juice. (LJ) Lemon juice.
(+) Positive reaction. 3-Sucrose. 6-Mannitol. (BP) Baobab pulp juice. (MJ) Mango juice.
(-) Negative reaction. 4-Adonitol 5-Dulcitol. 7-Inositol. (OJ) Orange juice. (GJ) Guava juice. (O/F)Oxidation fermentation test

Con. Table 4: Identification of Salmonella isolated from collected fresh juice samples obtained from juice dealers at Jackson bus station

Species	Acid from tested sugars							Indole	Arginine hhdrolysis	Uraese	VP	Gelatin hydrolysis	Citrate utilization	O/F	Acid from glucose	Oxidase	Catalase	Growth in air	Motility	Endo -spore staining	Gram staining	Shape	Isolates Code	Isolates No
	7	6	5	4	3	2	1																	
S. paratyphi A	-	-	+	+	-	-	-	+	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	MJ	9
S. paratyphi A	-	-	+	+	-	-	-	+	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	CockJ	10
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	GJ	11
S. paratyphi A	-	-	+	+	-	-	-	+	+	-	-	-	+	F	+	-	+	+	+	-	-	Rod	TP	12
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	OJ	13
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	LJ	14
S. typhi	-	+	+	+	-	-	-	-	+	-	-	-	-	F	+	-	+	+	+	-	-	Rod	OJ	15

Legend:
(d)Delayed reaction. 1-7: Sugars tested (HC) Hibiscus calyx. (GuJ) Gudiem juice.
(F) Fermentative. 1-Arabinose. 2-Lactose. (TP) Tamarind pulp juice. (LJ) Lemon juice.
(+) Positive reaction. 3-Sucrose. 6-Mannitol. (BP) Baobab pulp juice. (MJ) Mango juice.
(-) Negative reaction. 4-Adonitol 5-Dulcitol. 7-Inositol. (OJ) Orange juice. (GJ) Guava juice.
(O/F)Oxidation fermentation test (VP) Voges-Proskauer

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