MICROBIOLOGICAL ASSESSMENT OF STREET VENDED FRESH FRUIT JUICES AVAILABLE IN THE KARACHI CITY

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ABSTRACT

The present work has been undertaken to report the microbiological quality of fresh fruit juices available from street vendors in Karachi. Altogether nine (9) types of fresh fruit juices were analyzed for total viable count (TVC), total coliform (TCC), total fecal coliform count (TFC). The highest bacterial load ($1.2x10^9$cfu/ml) for fresh fruit juice sample was found in banana and the lowest load was ($1.77x10^4$ cfu/mL) found in a lemon juice. All samples of fruit juices showed the presence of coliform and fecal coliform ranging from 7 to ≥2400 MPN/100mL. Five (5) bacterial isolates were identified as *Escherichia coli, Staphylococcus* sp., *Pseudomonas* sp., *Streptococci* sp., *Salmonella* sp. Fungal isolates were identified as *Aspergillus* spp., *Rhizopus* sp., *Penicillium* sp. and *Mucor* sp. The highest fungal load was found in banana, apple and watermelon and lowest in orange juice.

Key words: Fruit juice, Street vendors, Karachi, Coliforms.

INTRODUCTION

Fresh Juices belong to a class of beverages which are non-alcoholic aqueous liquid extracted from one or more fruits and vegetables. Fresh juices consumed under hygienic conditions supply vitamins, minerals and fiber to the body and provide resistance to the body through inhibition of many diseases like congestive heart failure (CHF), urinary tract infection and breast cancer in women (Saenz and Sepulveda, 2001; Hyson, 2011). However, mal-manufacturing practices during their production provides ideal environment for the growth and multiplication of microorganisms as these juices fulfill their nutritional requirements (Ketema *et al*., 2001). The organisms enter in the fruits and vegetables through the abraded surfaces mostly during the harvesting and pot harvesting storage. Juices prepared from contaminated raw materials and under unhygienic conditions also contribute heavy microbial load. (Victorian Government Department of Human Services 2005; Oliveira *et al*., 2006; Nicolas *et al*., 2007). Owing to their increasing demand these juices are now recognized as an emerging cause of food borne diseases (Parish, 1997). Predominant pathogenic microbial flora found in the juices includes *E. coli*, species of *Salmonella*, *Shigella*, and *S. aureus* (Buchaman *et al*., 1999; Sandeep *et al*., 2001; Barro *et al*., 2006).

Karachi the most industrialized and populous city of Pakistan remains hot and humid for most part of the year, therefore, the demand for the street vended fresh juices is increasing day by day. They normally sold in the city under unhygienic condition and are popular among the general population due to their affordability at low cost. The people consume these juices in order to quench their thirst. Considering the importance, the present work has been undertaken to report the microbiological quality of freshly pressed or squeezed juices from street vendors.

MATERIALS AND METHODS

Collection of Samples

During the study conducted from January to September, 2014, seasonal juice samples were collected from different local markets of Karachi. These were located in Gulshan-e-Iqbal (S-1), Saddar (S-2), Quaidabad (S-3), Korangi (S-4), Sakhi Hassan (S-5). Samples were obtained from at least 3 shops in each zone. Nine varieties of fruit juices namely Mango, Banana, Sugarcane, Apple, Orange, Carrot, Watermelon, Dates and Lemon were elected. Botanically sugarcane is stem while carrot is root. Sterile glass containers were utilized for the collection of samples, which were maintained at 4ºC in an ice box during its carriage to the laboratory.

Enumeration of Microorganism

250 ml of each sample was extracted in vendors own utensil, transferred into sterile glass container was assessed by Most probable Number Techniques (MPN) using following parameters: Total Coliforms count (TCC) and Total fecal coliforms (TFC). The samples were processed in laminar flow hood using sterilized culture media. The sterility of media was checked prior to use. The TC was estimated using lactose broth (Merck, Germany) of single and double strength. The positive lactose tubes were used for the determination of FC using EC broth (Merck, Germany). These analyses were performed in accordance with the method described in Standard Methods for the

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Examination of Water and Wastewater (APHA, 2005). From the juice sample, positive lactose broth tubes a loop-full of the medium was also streaked on Mack Conkeys and EMB (Merck) agar plates for the characterization of Gram negative bacteria . After streaking, the plates were incubated at 37^{o} C in an incubator.

For Total Viable Count (TVC) portion of juices were diluted as 1:10 using sterile phosphate buffer which were subsequently diluted with the same as needed and then enumerated for total viable count using nutrient agar. Pour plate method and spread plate method were used for bacterial and fungal isolation expressed as colony forming units (cfu) per ml respectively. In all the cases counts were made up to 72 hours. Total coliforms were detected by MPN procedure according to standard method (APHA, 2005). Presence of fecal coliforms were determined using Brilliant green lactose bile (BGLB) broth (44.50ºC for 48 h), followed by confirmation of gas positive tubes using Eosin methylene blue (EMB) agar. Inoculated plates were incubated at requisite time-temperature combinations (FDA, 2001). In all cases for confirmation of the pathogens typical colonies were identified on the basis of cultural, microscopic analysis and biochemical characteristics (Buchanan and Gibson, 1984).

Isolation of fungal species

Fungal species were isolated by transferring 1.0 ml of the juice in a sterilized petri plate followed by the addition of 10-15 ml of molten Sabouraud 4 % dextrose agar (Merck) containing 200 mg/l streptomycin. The Petri plate was left for the solidification of agar. The plates were incubated at room temperature for a week. When the fungal spores appeared the species were characterized by morphological and microscopic examination by using the standard manuals (Ellis 1971; 1976; Barnett and Hunter 1972; Nelson *et al*., 1983).

Identification of Microorganism

For presumptive identification of microorganism isolates were selected for major biochemical tests such as Indole, Methyl-Red (MR), Voges-Proskaure (VP), Citrate Utilization, Triple Sugar Iron (TSI), Catalase test and Oxidase tests. The tests were carried out following the standard methods. Bacterial isolates were then identified according to the "Bergey's Manual of Determinative Bacteriology" (Bergey *et al.,* 1994).

RESULTS AND DISCUSSION

Altogether nine (9) types of fresh juices were analyzed for total viable count (TVC), total coliform (TCC), total fecal coliform count (TFC). Bacterial and fungal species were also isolated and identified during the study.

Total Viable Count (TVC)

The total viable bacterial count of all samples is presented in Table 1. All types of fruits juices showed significant values of total viable count. The highest bacterial load ($1.2x10^9$cfu/ml)($\log_{10}$ 5.56) for fresh fruit juice sample was found in a banana sold in Saddar and the lowest load was (150 cfu/mL) ($\log_{10}$ 2.18) found in an apple juice of Sakhi Hassan area. All samples showed higher bacterial viable count than the standard maximum permitted bacterial load i.e. $1.0x10^4$ (Gulf Standards, 2000).

Total Coliform Count (TCC)

Table 1 shows the mean coliform count that was observed in all fruit juices samples. The samples showed the presence of total coliform count ranging from 7 ($\log_{10}$ 0.84) (Gulshan-e-Iqbal) to ≥2400 ($\log_{10}$ 3.38)MPN/100mL (Saddar, Korangi and Quaidabad). the lowest count was observed in dates juice while highest coliform count was observed in banana and sugarcane juices. The presence of *E. coli* (43 to ≥2400 MPN/100mL) has been reported in various types of street vended juices in Dhaka, Bangladesh (Ahmed *et al.,* 2009).

Total Fecal Coliform Count (TFC)

The recommended allowable limit for Total fecal coliform count for any per ml of juice consumed is zero (Gulf Standards, 2000). The mean results of TFC are given in Table 1 which showed that the juices prepared in the study areas do not met the standards followed for the fresh fruit juices. All sample showed the presence of fecal coliform ranging from 3 ($\log_{10}$ 0.477) (Gulshan-e-Iqbal) to ≥2400 ($\log_{10}$ 3.38) MPN/100mL (Saddar and Quaidabad). Apple, mango, orange and dates juices showed low TFC while sugarcane juice showed high TFC value.

Identification of Microorganism

Distinctive pink, circular, convex colonies were observed on MacConkey Agar after 24 hours incubation, which was primarily considered as coliforms. Under microscope these Isolates were observed as Gram negative, single, short

rods which signify the characteristics of coliforms. Based on the biochemical characteristics, isolates were confirmed as *Escherichia coli, Staphylococcus* sp., *Pseudomonas* sp., *Streptococci* sp., *Salmonella* sp. (Table 2).

Table 1. Bacteriological analysis of street vended fruit juices available in Karachi.

Sample	TVC (log_{10} CFU/mL)	TCC (log_{10} MPN/100mL)	TFC (log_{10} MPN/100mL)
Mango	4.56±1.677	2.64±0.318	2.36±0.662
Banana	5.57±1.917	2.35±0.752	2.51±0.582
Sugarcane	5.13±1.779	2.51±0.633	2.51±0.784
Apple	4.95±1.206	2.63±0.621	1.40±0.806
Orange	4.72±1.148	1.77±0.400	1.25±0.571
Carrot	5.42±1.056	1.92±0.465	1.98±0.443
Water Melon	5.09±1.321	1.96±0.320	1.37±0.400
Dates	5.43±0.961	1.74±0.555	1.60±0.636
Lemon	3.42±0.951	2.17±0.575	1.69±0.671

Data represent means ±standard deviations of 15 measurements

Table 2. Microorganism isolated from freshly squeezed juices sold in street vended stalls in Karachi.

Sample	**Bacterial Isolate**
Mango	*Escherichia coli, Staphylococcus aureus, Streptococci* sp.
Banana	*E.coli, S. aureus, Streptococci* sp.
Sugarcane	*E.coli, S. aureus, Pseudomonas sp. , Streptococci* sp.
Apple	*E.coli, S. aureus, Streptococci* sp.
Orange	*S. areus, Salmonella* sp., *Streptococci* sp.
Carrot	*S. aureus, Pseudomonas* sp., *Streptococci* sp.
Water melon	*S. aureus, Salmonella* sp., *Streptococci* sp.
Dates	*E.coli, S. aureus, Streptococci* sp.
Lemon	*S. aureus*

Estimation of Fungal Species

Mean values of fungal species found in fruit juice samples are presented in Table 3. Based on the cultural and morphological characteristics of most documented keys in fungal identification, fungi isolates were identified as *Aspergillus* sp., *Rhizopus* sp., *Penicillum* sp. and *Mucor* sp. The fungus *Aspergillus* was found to be multifarious in comparison to others fungi. It is the major toxogenic fungi (D'Mello and Macdonald, 1997) that produces aflatoxins which are mycotoxins produced as teratogenic, carcinogenic and mutagenic secondary metabolites (Frisvad *et al*., 2005). The highest fungal load was found in Banana, apple and watermelon juices which were sold in Saddar, Quaidabad and Korangi and lowest fungal load was observed in orange juice available in Sakhi Hassan . The significant presence of *A. flavus* and *A. niger* in the juice samples provides clear evidence of airborne fungal contamination is the most susceptible in Juices (especially in milk shakes) and these species are identical to the airborne fungal flora of Karachi which have been reported by Rao *et al*., (2009).

The presence of high degree of microbial load owes to the fact that there is high pollution load in these temporary markets of Karachi. Not even the fruits used are appropriately washed; even the utensils used are occluded with dust and dirt. Peel present on the fruits provides natural protective covering against micro-flora and insects but the fruits used by these vendors are mostly bruised and of low quality with improper storage which lead to the growth of pathogenic micro-organisms. In addition flies add on extra dirt, dust and germs thus further increasing the microbial load of these juices. Higher levels of TVC in these juices reflect poor agriculture practices and hygiene codes like post harvest washing with contaminated water. Use of rotten fruits may be another factor

causing growth of micro-organisms which cause devastating effects on human health. Additionally environmental factors like garbage, sewage spills further worsen the health conditions caused by the consumption of these juices. These environmental factors cause diseases like diarrhea, cholera and various GI disorders. Use of diluents like ice and water used during preparation of these juices serves as another source of contamination. High temperature and pH value also poses its effects on microbial growth. Therefore, it is necessary that the juices which are sold on road side must be periodically checked and monitored in food laboratories in order to ensure human safety.

Table 3. Fungal analysis (log $_{10}$ CFU/values) of street vended fruit juices available in Karachi.

Samples	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus candidus*	*Aspergillus fumigates*	*Fusarium* sp.	*Rhizopus* sp.	*Penicilium* sp.	*Mucor* sp.
Mango	2.5 ±0.021	---	---	1.2±0.025	---	---	---	---
Banana	1.7±0.033	1.1±0.011	2.1±0.027	3.7±0.049	---	---	0.3±0.022	
Sugarcane	3.7±0.041	---	---	2.5±0.037	1.3±0.014	---	---	
Apple	0.5±0.015	0.4±0.023	1.7±0.021	0.8±0.018	2.5±0.016	0.5±0.044	---	0.2±0.008
Orange	0.1±0.028	---	---	0.5±0.016	---	---	---	---
Carrot	3.5±0.022	---	---	---	1.7±0.028	0.3±0.051	---	---
Water melon	3.7±0.001	0.2±0.008	1.8±0.036	0.2±0.029	---	0.2±0.039	0.1±0.014	0.3±0.021
Dates	2.6±0.018	1.6±0.04	1.2±0.018	---	1.1±0.031	---	---	---
Lemon	1.2±0.030	1.2±0.005	0.4±0.0016	---	---	---	---	---

Data represent means ±standard deviations of 15 measurements

CONCLUSION

In the light of the present study it can be concluded that these un-bottled street vended juices are not safe for human consumption as they give rise to many food borne infections which would lead to poor health conditions among the population. It is therefore, necessary that the government must implement awareness programs among these street vendors as well as general public along with the surveillance system which would monitor food safety according to the International Health Standards.

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