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Research Article

Estimates and burden of foodborne pathogens in RTE beverages in relation to vending practices

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Abstract

Objectives: Growing trend of street-vended food in underdeveloped countries offers low-cost food to many sections of population. Although it provides job opportunities to many urban dwellers, several health hazards are associated with this business. The present study investigates the burden of foodborne pathogens in Ready-To-Eat (RTE) beverages in relation to vending practices among street vendors of Rawalpindi City, Pakistan according to standardized methods and protocols.
Materials and Methods: Six densely populated locations of Rawalpindi city were selected. Commonly consumed sugar cane juice (SCJ) and tamarind prune (dried plums) drink (TPD) (locally called as Imli Alu Bukhara sherbet) from five vendors from each location were chosen in summer season where the temperature reaches above 40°C. Mean and the standard deviation were obtained by univariate and bivariate analyses. Association between the study variables was assessed through cross-tabulations, chi-square, and correlation tests.
Results: All the samples were found unsatisfactory in comparison to guidelines of aerobic plate count. Total coliform was observed in 86.7 per cent of SCJ and 70.0 per cent of TPD samples. Fourteen samples of SCJ exceeded the limit of >1100 MPN/ml value, whereas samples of TPD exceeded this limit for *Escherichia coli*. All of SCJ and 93.3 per cent of TPD samples depicted the presence of S*almonella aureus*. S*almonella* spp. were found significantly high in 73.3 per cent samples of SCJ and 23.3 per cent samples of TPD.
Conclusions: The incidence of high bioloads attributes towards a potential reservoir of foodborne pathogens due to unhygienic vending practices.

Key words: Ready-To-Eat beverages; foodborne pathogens; street vendors; vending practices.

Introduction

Fruits are a fabulous source of vital nutrients including minerals, vitamins, saccharides, phytochemicals, and a wide range of antioxidants. Besides normal consumption, their juices and drinks are recognized as natural, nutritional, refreshing, recreational, traditional, and therapeutic beverages against multiple health issues. They are important for their fighting role in cancer, stabilizing blood sugar levels, helping in weight loss, reducing fevers, clearing the kidneys, preventing tooth decay, and a host of other health benefits (Iqtedar and Yasin, 2014; Jha et al., 2016; Nair and Gayatri, 2016). Freshly extracted juices are also known as instant energy boosters, encompass the live enzymes and nutrients, and easily absorbed by the body for quick nourishment (Aneja et al., 2014). Consumers usually prefer the freshly squeezed juices due to their 'fresh flavor' attributes, so they

are simply prepared by mechanical means and finally consumed in the form of unfermented, cloudy, unprocessed juice, ready to drink or consumption (Nonga et al., 2014). It is stated that around 50 per cent of the population prefers to consume street-vended foods, which signifies that this segment of population is at risk (Rana and Ahirrao, 2016). Safety of fruit juices heavily depends on fruit composition itself, its variety, origin, harvesting conditions (Braide et al., 2012), extracting instruments, utensils, water content, storage conditions (Chuku and Akani, 2015), vending site, and the surrounding environment (Iqtedar and Yasin, 2014; Ahmed et al., 2017). They should be free from microorganisms, as bacteria, fungi, and viruses are reported to be the potential pathogenic organisms to cause food-borne diseases due to the presence of sufficient nutrients, water activity, and pH of fruit juices which facilitate the microbial growth (Aneja et al.,, 2014; Afreen et al., 2016). In addition to these, bacteria and fungi can also be invaded through damaged surface of the fruits like cuts, wounds, punctures, and splits that took place during fruit maturing, harvesting, and processing and thus stay alive during processing and shorten the shelf life of fruit as well as life threatening for the consumer (US Food and Drug Administration, 2008; Olaniyi, 2013; Jha et al., 2016). Various pathogenic organisms such as *Escherichia coli*, coliforms, enterococci, *Salmonella* spp., and *Vibrio cholera* have been reported in juices by several workers (Yadav et al., 2011; Aneja et al., 2014; Jha et al., 2016) and hence become a challenge for food safety officials (Valdramidis and Koutsoumanis, 2016).

The current study was carried out to investigate the microbial flora along with their risk factors in freshly squeezed juices and conventional drinks openly sold in the streets of Rawalpindi, the fourth largest densely populated city of Pakistan. This city covers the market places only for food items at large, often coined as street foods. Vending stalls are located almost everywhere, on roadways, near to taxi stands, bus and train stations, construction sites, schools, hospitals, and office premises. Therefore, it is very important to learn the dynamics of this exponentially growing business and its potential implications on the society. So limited information about the safety of street beverages is documented in Rawalpindi even there is huge consumption in the city. Provision of this information will be useful for understanding the food-related safety challenges in general and for the regulatory agencies in order to take appropriate steps to tackle the worsening situation.

Materials and Methods

Sample collection

Six densely populated locations (Pir Wadhai, Faizabad, Raja Bazaar, Commercial, Saddar, and Bakra Mandi coded as PW-A, FA-B, RB-C, CA-D, SD-E, and BM-F, respectively) of Rawalpindi city were selected. Commonly consumed sugar cane juice (SCJ) and tamarind prune (dried plums) drink (TPD) (locally called as *Imli Alu Bukhara sherbet*) from five vendors from each location were chosen in summer season where the temperature reaches above 40°C (108°F). Before collection of samples, the chosen vendors were interviewed for handling practices based on the type of vending, washing of utensils, water source, serving and covering of food items, hand washing habits, and other risk variables through a pretested structured questionnaire adapted as reported in the literature previously (Abdalla et al., 2009; Mensah et al., 2012; Adjrah et al., 2013). Along with self-administered questionnaire, 60 samples of the so-called juices were collected in a sterile container and were analysed on the same day as per standard laboratory techniques.

Microbial analysis

For microbial analysis, 20 ml of fresh juice was diluted with 180 ml of peptone media (Oxoid, Basingstoke, UK) and homogenized for 2 min (10^{-1} dilution). Subsequently, the first dilution was filtered through sterile filter paper (Whatman No. 42) to remove the solid particles. After filtration, the filtrate was serially diluted into the peptone solution. For inoculation, 0.1 ml sample of the appropriate dilutions was spread on selective and differential agar plates and incubated at 37°C for 24–48 h. Triplicate agar plates with colonies were counted and the ranges and mean counts were reported as cfu/ml and later converted to $\log_{10}$ cfu/ml. Finally, counts were compared with the microbiological guidelines for Ready-To-Eat (RTE) foods (Center for Food Safety, Food and Environmental Hygiene Department, 2014) and are presented in Table 1.

Nutrient agar (Oxoid, Basingstoke, UK), violet red bile, and salt mannitol agars were used for enumeration of aerobic plate count (APC), total coliform count (TCC), and S*almonella aureus*, respectively. *Salmonella* was detected though pre-enrichment in lactose broth (Oxoid, Basingstoke, UK) then the pre-enriched culture was inoculated in tetra-thionate broth and xylose lysine desoxycholate agar. Enumeration of *E. coli* was expressed through MPN following APHA recommendations (American Public Health Association (APHA), 1992). Three serial dilutions (1.0, 0.1, and 0.01 ml) were inoculated on MacConkey broth (MC-Oxoid, Basingstoke, UK) containing inverted Durham tube and incubated at 37°C. The presence of 10 per cent or more gas displacement in the Durham tube was considered to be the positive test. Positive tubes with gas and acid production were streaked on eosin methylene blue agar. Confirmation of thermo-tolerant *E. coli* was patterned on lactose broth, brilliant green lactose bile broth, and tryptone water and incubated at 44°C for 24 to 48 h. Yeast and moulds were also noticed through potato dextrose agar. Confirmatory tests were done through API 20E biochemical kit (bioMerieux, Maret 1'Etoile, France) for *E. coli*, staphylase test kit (Oxoid, Basingstoke, UK) for typical *S. aureus*, and Oxoid Salmonella Test Kit (DR1108A) for endorsement of suspected colonies of *Salmonella* as per manufacturer's instructions along with the gram staining of each microbe.

Statistical analysis

The results of the study were compiled on Statistical Package for Social Sciences software, version 20.0 (SPSS Incorporated, Atlanta, IL). Mean and the standard deviation were obtained by univariate and bivariate analyses. Association between the study variables was assessed through cross-tabulations, chi-square, and correlation tests.

Results

Microbial estimates in beverages

Findings showed that APC ranged from $1.5e^5$ to $5.8e^8$ log cfu/ml in beverages with the mean concentration for SCJ ($4.6e^7 \pm 9.1e^7$)

Table 1. Guidelines for determination of microbial quality in RTE beverages

Indicators	Microbiological quality levels	
	Satisfactory	Unsatisfactory
APC*	5.0×10^3–4.0×10^4 (cfu/ml)	$>4.0 \times 10^4$ (cfu/ml)
TCC*	10–100 (cfu/ml)	>100 (cfu/ml)
Escherichia coli	<100 MPN/ml	>100 MPN/ml
Staphylococcus aureus	<100 (cfu/ml)	>100 (cfu/ml)
Salmonella spp.	not detected in 25 ml	detected in 25 ml
Yeast and moulds	2–3 (cfu/ml)	>3 (cfu/ml)

*APC = aerobic plate counts; TCC = total coliform count.

and $4.2e^7 \pm 1.1e^8$ for TPD. SCJ showed the higher levels prominently from CA-D than TPD at $P = 0.002$ and PW-A location exposed the greater count for TPD. In comparison to standard guidelines, all of the samples were unsatisfactory as they had exceeded the recommended limits. Exceeded levels of TCC were observed in 26 (86.7 per cent) of SCJ samples and 21 (70.0 per cent) of TPD in comparison to the recommended standard of $>10^2$ cfu/ml; however, the non-significant difference ($P = 0.117$) was observed between the type of beverages. The overall mean concentration was $2.0e^7 \pm 4.6e^7$ varying between $1.9e^5$ and $1.8e^8$ cfu/ml in SCJ and $4.7e^6 \pm 8.4e^6$ ranging between $1.5e^5$ and $3.5e^7$ cfu/ml in TPD, respectively (Table 2). The maximum mean concentration of $7.3e^7 \pm 9.3e^7$ was witnessed in SCJ samples obtained from CA-D with the minimum concentration ($3.7e^5 \pm 1.9e^5$) in PW-A (Table 3).

In the case of TPD, a highest mean load of total coliform ($1.6e^7 \pm 0.0$) was found in one sample of FA-B and remarkably rest of the samples were satisfactory. The status of *E. coli* contamination in RTE beverages is depicted in Figure 1. SCJ samples were more contaminated than the TPD samples. Fourteen samples of SCJ exceeded the limit of >1100 MPN/ml, whereas four samples of TPD exceeded this limit. Eight samples (26.7 per cent) of SCJ and 18 (60 per cent) samples of TPD were found satisfactory at $P = 0.009$ according to the Hong Kong (Center for Food Safety, Food and Environmental Hygiene Department, 2014) with 30 per cent from FA-B, 40 per cent from RB-C, 70 per cent from CA-D, and 60 per cent each from SD-E and BM-F (Figure 2).

In the present investigation, beverages also showed the prevalence of *S. aureus*. All of the SCJ 30 (100 per cent) and 28 (93.3 per cent) of the TPD showed the presence of this pathogen (Table 2). Highest mean concentration of $1.7e^8 \pm 1.7e^8$ in SCJ was observed in FA-B with the least mean concentration of $9.2e^5 \pm 1.4e^6$ in PW-A. Like SCJ samples, the TPD from the same location showed the highest count ($4.1e^7 \pm 2.7e^7$) (Table 3) and similarly least mean concentration was observed in PW-A. However, non-significant ($P = 0.150$) difference was observed between both types of samples.

Pathogenic S*almonella* spp. were found significantly ($P = 0.000$) in 22 (73.3 per cent) samples of SCJ and 7 (23.3 per cent) samples of TPD (Table 2). All samples from PW-A showed the satisfaction level followed by 7 (70 per cent) from FA-B, 5 (50 per cent) from BM-F, 4 (40 per cent) from RB-C, and 3 (30 per cent) and 2 (20 per cent) from SD-E and CA-D, respectively (Figure 2).

Furthermore, 29 samples of SCJ and 30 samples of TPD were found unsatisfactory at $P = 0.313$ (Figure 2) with yeast and moulds. Only one sample of SCJ from SD-E was found satisfactory. Highest mean concentration $8.5e^8 \pm 9.9e^8$ was observed in SCJ samples from FA-B and lowest ($1.8e^5 \pm 1.5e^5$) was observed in PW-A.

Risk factor assessment for microbial contamination of RTE beverages

Table 4 reflects that aerobic count was significantly correlated with risk factors such as vending site ($P = 0.004$, with maximum mean concentration of $5.9e^7 \pm 1.3e^8$ on stalls near to footpaths), washing of utensils ($P = 0.021$, all of them were washing the utensil in bucket, with the maximum concentration of $4.4e^7 \pm 1.0e^8$), covering of food ($P = 0.003$, maximum concentration of $5.2e^7 \pm 1.2e^8$, where majority were not covering their food items properly, served food with bare hands ($P = 0.014$, with mean concentration of $2.9e^7 \pm 8.5e^7$ in the category of 'YES', and habit of hand washing ($P = 0.010$).

Types of beverages, pH, vending site, washing of utensils, and hand washing habits were the risk factors responsible for the TCC significantly at $P = 0.029$, 0.041, 0.005, 0.011, and 0.032, respectively. However, other risk variables showed the non-significant correlation, but more than 50 per cent samples were contaminated with TCC in each category and the highest mean concentration of $5.2e^7 \pm 8.6e^7$ was observed in the category of washing hands after touching each food item.

Risk factors like type of juice or drink, pH, vending site, washing of utensils, source of water, and hand washing habit showed the significant correlation at P values of 0.007, 0.021, 0.032, 0.021, and 0.039, respectively, with *E. coli*; however, in each further category of major risk contributors, samples with varied percentage were found unsatisfactory with the highest mean concentration of $5.5e^2 \pm 7.8e^2$ in the category of washing hands after touching each food item again. In the present study, type of juice, vending site, source of water, covering of food, food serving with bare hands, and washing utensils also showed the correlation with *S. aureus*, *Salmonella spp.*, and yeast and moulds with varying levels for the microflora count.

Table 2. Microbial contamination in RTE beverages

Food item	Mean ±SD	Range	Satisfactory *n* (%)	Unsatisfactory *n* (%)	*P*
Aerobic plate count (log cfu/ml)					
Sugar cane juice	4.6e7 ± 9.1e7	1.9e5–3.8e8	0 (0.0)	30 (100)	0.002
Tamarind prune drink	4.2e7 ± 1.1e8	1.5e5–5.8e8	0 (0.0)	30 (100)	
Total coliform count (log cfu/ml)					
Sugar cane juice	2.0e7 ± 4.6e7	1.9e5–1.8e8	04 (13.3)	26 (86.7)	0.117
Tamarind prune drink	4.7e6 ± 8.4e6	1.5e5–3.5e7	09 (30.0)	21 (70.0)	
Escherichia coli (log MPN/ml)					
Sugar cane juice	3.0e2 ± 4.3e2	3.6e0–1.1e3	8 (26.7)	22 (73.3)	0.009
Tamarind prune drink	1.5e2 ± 3.0e2	3.0e0–1.1e3	18 (60.0)	12 (40.0)	
Staphylococcus aureus (log cfu/mL)					
Sugar cane juice	2.0e7 ± 4.6e7	3.0e4–4.0e8	0 (0.0)	30 (100)	0.150
Tamarind prune drink	4.7e6 ± 8.4e6	1.6e5–9.0e7	02 (6.66)	28 (93.3)	
Yeast and moulds (log cfu/ml)					
Sugar cane juice	2.2e8 ± 4.9e8	1.8e4–2.5e9	01 (3.0)	29 (97.0)	0.313
Tamarind prune drink	6.8e7 ± 1.5e8	1.2e5–6.3e8	0 (0.0)	30(100)	
Salmonella spp.					
Sugar cane juice	–	–	8(26.7)	22(73.3)	0.000
Tamarind prune drink	–	–	23(76.7)	7 (23.3)	

Table 3. Log mean (log cfu/ml) concentration for microflora in RTE beverages

Food items	APC	TCC	*Staphylococcus aureus*	Yeast and moulds
Pir Wadhai				
Sugar cane juice	2.8e5 ± 6.2e4	3.7e5 ± 1.9e5	9.2e5 ± 1.4e6	1.8e5 ± 1.5e5
Tamarind prune drink	6.8e5 ± 9.8e5	3.9e5 ± 3.3e5	5.9e5 ± 7.9e5	2.6e5 ± 8.1e4
Faizabad				
Sugar cane juice	1.5e8 ± 1.1e8	2.0e7 ± 0.0	1.7e8 ± 1.7e8	8.5e8 ± 9.9e8
Tamarind prune drink	1.7e8 ± 2.3e8	1.6e7 ± 0.0	4.1e7 ± 2.7e7	2.3e8 ± 2.7e8
Raja Bazaar				
Sugar cane juice	7.9e6 ± 4.6e6	1.3e7 ± 2.2e6	2.4e7 ± 2.7e7	4.3e7 ± 4.4e7
Tamarind prune drink	1.3e7 ± 1.3e7	5.4e6 ± 7.8e6	6.8e6 ± 1.1e7	1.7e7 ± 1.8e7
Commercial				
Sugar cane juice	8.9e7 ± 1.6e8	7.3e7 ± 9.3e7	9.6e6 ± 1.4e7	8.7e7 ± 1.4e8
Tamarind prune drink	6.0e7 ± 6.9e7	1.3e7 ± 1.9e7	1.5e7 ± 1.3e7	1.0e8 ± 1.7e8
Saddar				
Sugar cane juice	2.5e7 ± 6.4e6	1.2e7 ± 5.9e6	1.5e7 ± 1.3e7	3.4e8 ± 3.7e7
Tamarind prune drink	5.6e6 ± 4.6e6	1.4e6 ± 9.5e5	9.0e6 ± 1.2e7	5.4e7 ± 8.8e7
Bakra Mandi				
Sugar cane juice	7.0e6 ± 1.3e7	4.4e6 ± 6.0e6	2.0e6 ± 1.7e6	3.4e6 ± 1.7e6
Tamarind prune drink	2.2e6 ± 9.6e5	2.9e6 ± 7.7e5	1.7e6 ± 1.6e6	3.4e6 ± 1.8e6

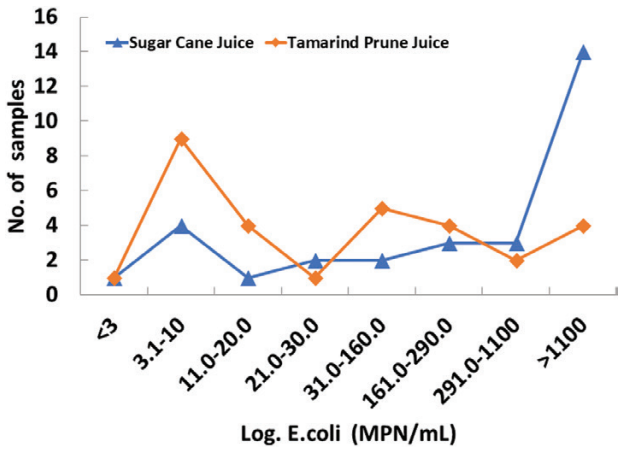

Figure 1. *Escherichia coli* count of locally prepared RTE beverages.

Table 5 also depicts the correlation of microflora with other risk factors without the calculation of their mean concentrations. Timings of the beverage's preparation were closely correlated with the occurrence of APC, TCC, *E. coli*, and *Salmonella* spp. at $P = 0.013$, 0.028, 0.007, and 0.034, respectively. Practice of handling at ground level was significantly related to the presence of APC, TCC, and *S. aureus* at $P = 0.048$, 0.047, and 0.002, respectively. Significant P values of less than 0.05 were also observed in the use of clean water for preparation factor in relation to APC, TCC, *S. aureus*, and *Salmonella* spp. The presence of storage facilities on vending site and waste management were also closely correlated with the occurrence of APC, TCC, and *Salmonella* spp. at varying levels of correlation. Other factors showed the non-significant correlation, but the samples are not satisfactory due to the presence of one or the other pathogen in RTE beverages.

Discussion

Regardless of beneficial effects of fruit beverages like their taste, flavour, nutritive composition, and energizing impact, their quality and safety concerns have been raised all over the world and are in reality they are the most contaminated ones. Freshly squeezed fruit juices and conventional drinks have little or partially processing steps for the reduction of pathogens if contamination occurs. In the present study, all of the samples had exceeded the level of APC at significance or difference level of $P = 0.002$. The findings were concurrent with other studies done in India (25×10^4 CFU/ml and 20×10^4 CFU/ml from two locations) (Divyashree et al., 2014; Kiranmai et al., 2016); Lahore, Pakistan (>4 $\log_{10}$ CFU/ml) (Iqbal et al., 2015); and Peshawar, Pakistan (4×10^2–3×10^7 CFU/ml), specifically in SCJ (Abid and Ali, 2008). It is a known fact that viable count is more in fresh juices than the processed ones just because of mishandling practices during preparation (Nair and Gayatri, 2016). TCC, a basic indicator of bacterial contamination of water supply, was detected in more than 70 per cent samples, reflected that the source of water itself is questionable. Majority of the vendors used the tap water supplied by water and sanitation agency and hence mainly depend on the readily available source and not the particular choice of vendors themselves. Furthermore, all of the vendors practiced washing their utensils in buckets, so contaminated water and repeated use of same water itself is a source of pathogens that can be transferred to utensils and causes cross-contamination. A review done by Nabeela et al. (2014) highlighted that a total of 7000 water samples from all over Pakistan revealed that the presence of 71 and 58 per cent samples contaminated with total coliforms and faecal coliforms, respectively, and account for 20 to 40 per cent of all diseases in the country. Making a comparison, it can be established that people who do not have the access to potable water, how the food consumed by them is safe for their health. In addition to water quality, the use of commercial ice in beverages further enhances the microbial load. It is confirmed that microbial load in ice samples exceeded the standard threshold collected from different manufacturing units and retail outlets (Nair and Gayatri, 2016; Rao et al.,, 2016).

Vendors always used to make small pieces of ice from the big one in a big rubber tube tied at both ends by means of a wooden stick. This practice is usually done on the dusty roadside and they pulled the ice cubes directly into the utensils. So the addition of roadside dust by dirty rubber bag may be a source of microbial contamination. Therefore, in the present study, mostly all of the samples

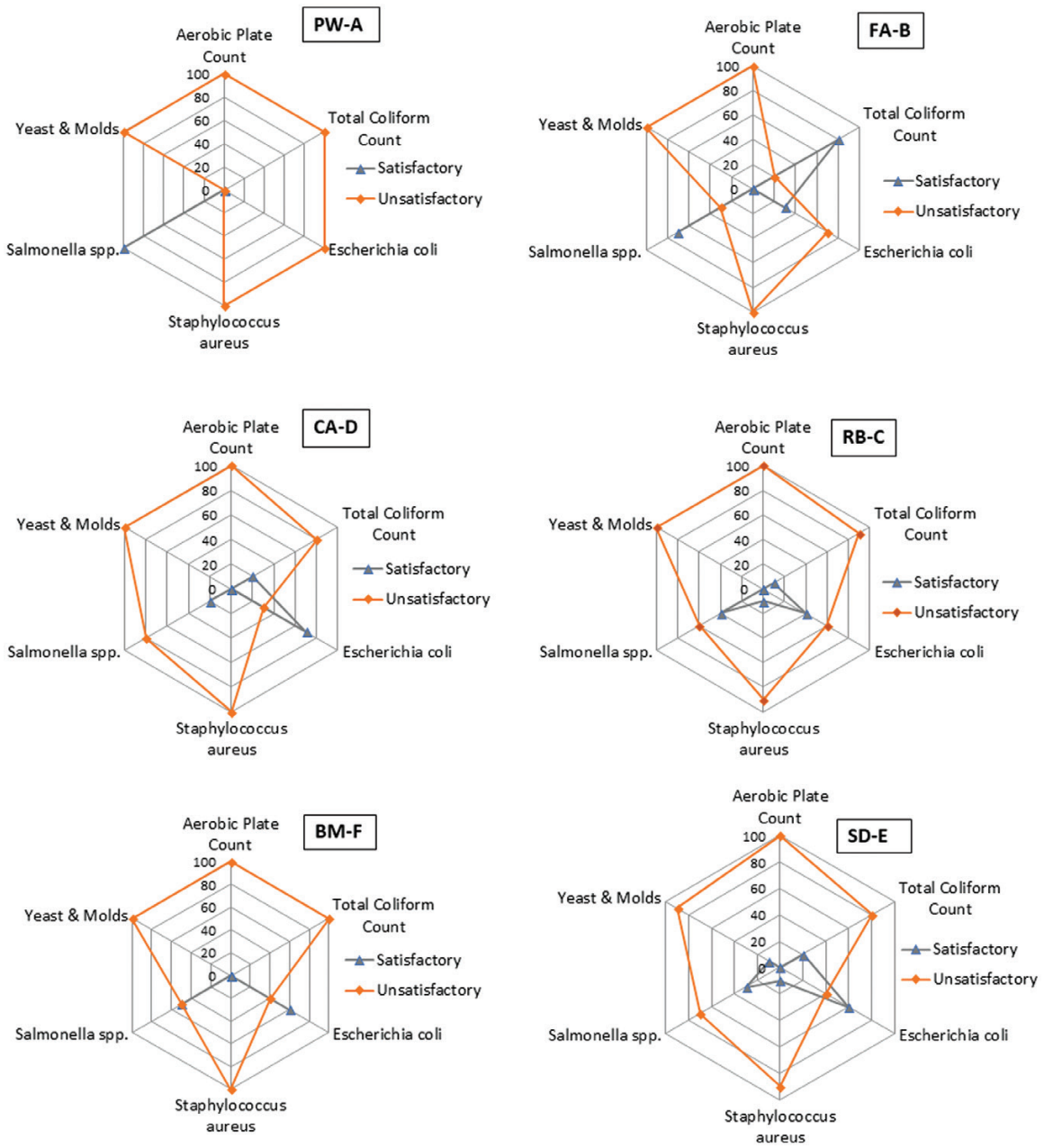

Figure 2. Percentage distribution of microflora to the final microbiological quality of RTE beverages.

were unsatisfactory. These exhibited the presence of one or more pathogens similar to the study done in India where 100 per cent of the samples showed contamination (Kiranmai et al., 2016; Rao et al., 2016). Another reason is that Rawalpindi is one of the oldest cities of Pakistan, and not very much attention has been given to the drainage sytem. Stalls are located near the open drainage which may account for positive culture of TCC and *E. coli*. It is a known fact that when the number of storage hours increases, there will be more chances of microbial growth due to sufficient nutrients, water activity, pH of the fruit itself, and mostly the bacteria counting 1×10^5 cfu/cm^2 present on the fruit surface (Nair and Gayatri, 2016). In terms of *E. coli*, all of the samples from PW-A were found unsatisfactory and so can be synchronized with those of a similar study (Batool et al., 2013) that shows 50 per cent of the SCJ showed contamination of *E. coli*. Similarly, in the other cities of Pakistan and 80% of juices samples assessed in India had this pathogen (Kiranmai et al., 2016).

TPD also showed the high prevalence (41 per cent contamination) obtained from Lahore city of Pakistan (Iqtedar and Yasin, 2014). Results related to *S. aureus* were also in accordance with the previous study done in Lahore where mean *Staphylococcal* count was found to be 5.45 ± 1.06 $\log_{10}$ CFU/ml in all fruit juices, which is greater than the permissible limits (Iqbal et al., 2015). In India, 19 out of 20 samples exhibited the presence of *Staphylococci* (Kader et al., 2014). More than 70 per cent of the TPD and around 25 per cent samples were free from *Salmonella* with the highest percentage from PW-A in this study but showed the presence of *E. coli* and hence can be proposed that the level of contamination depends on the consumption rate. This location is known to be the principal station

Table 4. Risk factor assessment for microbial contamination in RTE beverages

Risk factors assessed	Category	APC			TCC			*E. coli*			*S. aureus*			Salmonella			Y & M		
		Log CFU/ml (M ± SD)	UN *n* (%)	Test statistics	Log CFU/ml (M ± SD)	UN *n* (%)	Test statistics	LogMPN/ml (M ± SD)	UN *n* (%)	Test statistics	Log CFU/ml (M ± SD)	UN *n* (%)	Test statistics	S *n* (%)	UN n (%)	Test statistics	Log CFU/ml (M ± SD)	UN n (%)	Test statistics
Juice	SCJ	4.6E7 ± 9.1E7	30(100)	*r* = 0.818 *P* = 0.000*	2.0E7 ± 4.6E7	26(86.7)	*r* = 0.924 *P* = 0.029*	3.3E2 ± 4.5E2	3 (60)	*r* = 0.204 *P* = 0.007*	2.0E7 ± 4.6E7	30(100)	*r* = 0.910 *P* = 0.015*	8(26.7)	22(73.3)	*r* = 0.500 *P* = 0.000*	2.2E8 ± 4.9E8	29(97)	*r* = 0.111 *P* = 0.066
	TPD	4.2E7 ± 1.1E8	30(100)		4.7E6 ± 8.4E6	21(70)		1.9E2 ± 3.4E2	31(56.3)		4.7E6 ± 8.4E6	28(93.3)		23(76.7)	7(23.3)		6.8E7 ± 1.5E8	30(100)	
pH	≤3.5	1.4E8 ± 2.5E8	5(100)	*r* = 0.063 *P* = 0.632	1.4E6 ± 1.4E6	3(60)	*r* = 0.174 *P* = 0.041*	3.3E2 ± 4.5E2	3 (60)	*r* = 0.897 *P* = 0.021*	2.4E7 ± 4.0E7	5(100)	*r* = 0.242 *P* = 0.667	5(100)	0(0.0)	*r* = 0.650 *P* = 0.000*	8.1E7 ± 1.6E8	5(100)	*r* = 0.981 *P* = 0.017*
	>3.5	3.5E7 ± 7.3E7	55(100)		1.4E6 ± 3.6E7	44(80)		1.9E2 ± 3.4E2	31(56.3)		2.6E7 ± 7.0E7	53(96.3)		26(47.3)	29(52.7)		1.5E8 ± 3.8E8	54(98)	
Vending site	Dhaba	5.1E6 ± 7.6E6	5(100)	*r* = 0.501 *P* = 0.004*	5.2E6 ± 5.7E6	5(100)	*r* = 0.976 *P* = 0.005*	2.1E1 ± 1.3E1	4 (80)	*r* = 0.617 *P* = 0.032*	1.5E7 ± 2.9E7	5(100)	*r* = 0.751 *P* = 0.043*	1(20)	4(80)	*r* = 0.091 *P* = 0.492	7.1E7 ± 1.4E8	5(100)	*r* = 0915 *P* = 0.014*
	Footpath	5.9E7 ± 1.3E8	36(100)		1.8E7 ± 4.3E7	30(64)		2.2E2 ± 3.8E2	22 (61)		3.4E7 ± 8.2E7	36(100)		15(42)	21(58)		1.8E8 ± 4.5E8	35(97)	
	Mobile	2.9E7 ± 4.6E7	19(100)		4.5E6 ± 5.8E6	12(100)		3.5E2 ± 4.9E2	8 (42)		1.0E7 ± 1.3E7	17(90)		15(80)	4(21)		8.1E7 ± 1.7E8	19(100)	
Utensils washing	In bucket	4.4E7 ± 1.0E8	60(100)	*r* = 0.912 *P* = 0.021*	1.3E7 ± 3.5E7	47(78)	*r* = 0.840 *P* = 0.011*	2.1E2 ± 3.6E2	36 (56.6)	*r* = 0.716 *P* = 0.021*	2.6E7 ± 6.6E7	58(97)	*r* = 0.063 *P* = 0.632	31(52)	29(48)	*r* = 0.144 *P* = 0.049*	1.4E8 ± 3.7E8	59(98)	*r* = 0.751 *P* = 0.043*
Source of water	Tap water	4.7E7 ± 1.1E8	53(100)	*r* = 0.101 *P* = 0.441	1.4E7 ± 3.8E7	41(77)	*r* = 0.074 *P* = 0.619	2.2E2 ± 3.7E2	30 (56.6)	*r* = 0. 911 *P* = 0.046*	2.1E7 ± 4.5E7	51(96)	*r* = 0.919 *P* = 0.013*	27(51)	26(49)	*r* = 0.763 *P* = 0.040*	1.4E8 ± 3.8E8	52(98)	*r* = 0.945 *P* = 0.009*
	Boring	1.6E7 ± 2.0E7	7(100)		6.6E6 ± 6.3E6	6(86)		6.2E1 ± 7.7E1	4 (57.1)		6.1E7 ± 1.5E8	7(100)		4(57)	3(43)		1.5E8 ± 2.5E8	7(100)	
Food covering	No	5.2E7 ± 1.2E8	42(100)	*r* = 0.841 *P* = 0.003*	1.6E7 ± 4.1E7	33(77)	*r* = 0.133 *P* = 0.374	2.6E2 ± 4.0E2	27 (79.4)	*r* = 0.184 *P* = 0.256	3.2E7 ± 7.7E7	41(98)	*r* = 0.951 *P* = 0.025*	17(41)	25(59)	*r* = 0.342 *P* = 0.007*	1.6E8 ± 4.2E8	41(98)	*r* = 0.099 *P* = 0.453
	Yes	2.6E7 ± 4.5E7	18(100)		6.3E6 ± 9.9E6	14(78)		1.3E2 ± 2.7E2	7 (20.5)		1.0E7 ± 1.2E7	17(94.4)		14(78)	4(22)		8.6E7 ± 1.7E8	18(100)	
Served with bare hands	No	3.8E7 ± 4.6E7	4(100)	*r* = 0. 913 *P* = 0.014*	1.1E6 ± 1.1E6	2(50)	*r* = 0.074 *P* = 0.620	4.7E2 ± 5.7E2	2 (5.88)	*r* = 0.209 *P* = 0.195	1.5E7 ± 1.7E7	4(100)	*r* = 0.742 *P* = 0.044*	4(100)	0(0.0)	*r* = 0.258 *P* = 0.046*	9.9E6 ± 1.2E7	4(100)	*r* = 0.098 *P* = 0.462
	Yes	2.9E7 ± 8.5E7	56(100)		1.4E7 ± 3.6E7	45(80)		1.8E2 ± 3.3E2	32 (94.1)		2.6E7 ± 6.8E7	54(97)		27(48)	29(52)		1.5E8 ± 3.7E8	55(98)	
Hand washing	I	5.6E7 ± 1.4E8	18(100)	*r* = 0.904 *P* = 0.010*	5.6E6 ± 6.2E6	15(83)	*r* = 0.829 *P* = 0.032*	1.9E2 ± 3.0E2	11(32.3)	*r* = 0.813 *P* = 0.039*	2.7E7 ± 5.4E7	18(100)	*r* = 0.064 *P* = 0.632	9(50)	9(50)	*r* = 0.099 *P* = 0.454	2.1E8 ± 5.9E8	18(100)	*r* = 0.107 *P* = 0.421
	II	7.0E7 ± 1.3E8	5(100)		5.2E7 ± 8.6E7	4(80)		5.5E2 ± 7.8E2	4 (11.7)		5.1E7 ± 1.0E8	5(100)		1(20)	4(80)		5.4E7 ± 7.8E7	5(100)	
	III.	3.5E7 ± 7.2E7	37(100)		1.2E7 ± 3.2E7	28(75)		2.0E2 ± 3.6E2	19 (55.8)		2.1E7 ± 6.7E7	35(95)		21(57)	16(43)		1.2E8 ± 2.1E8	36(97)	

I = before preparation of food; II = after touching each food item; III = before preparation food + after using toilet.
*Correlation is significant at the 0.01 level (2-tailed).

Table 5. ·Risk factor assessment for microbial contamination in RTE beverages

Risk factors assessed	Category	APC		TCC		*E. coli*		*S. aureus*		Salmonella		Yeast and mould	
Timings of food preparation	In the morning, during the sale, morning + during the sale, at previous night + morning	$r = 0.920$	$P = 0.013$*	$r = 0.617$	$P = 0.028$*	$r = 0.967$	$P = 0.007$*	$r = 0.132$	$P = 0.324$	$r = 0.275$	$P = 0.034$*	$r = 0.163$	$P = 0.217$
Food handled at ground level	No, yes	$r = 0.815$	$P = 0.048$*	$r = 0.752$	$P = 0.047$*	$r = 0.698$	$P = 0.063$	$r = 0.715$	$P = 0.002$*	$r = 0.236$	$P = 0.070$	$r = 0.018$	$P = 0.890$
Presence of flies on food	No, yes	$r = 0.740$	$P = 0.028$*	$r = 0.088$	$P = 0.555$	$r = 0.208$	$P = 0.197$	$r = 0.114$	$P = 0.394$	$r = 0.211$	$P = 0.105$	$r = 0.095$	$P = 0.476$
Washing of food items before preparation	No, yes	$r = 0.133$	$P = 0.312$	$r = 0.170$	$P = 0.252$	$r = 0.907$	$P = 0.040$*	$r = 0.117$	$P = 0.184$	$r = 0.499$	$P = 0.000$*	$r = 0.167$	$P = 0.206$
Use of clean water for food preparation	No, yes	$r = 0.658$	$P = 0.050$*	$r = 0.298$	$P = 0.042$*	$r = 0.164$	$P = 0.311$	$r = 0.830$	$P = 0.029$*	$r = 0.350$	$P = 0.006$*	$r = 0.084$	$P = 0.528$
Type of utensils used for serving of food	Paper bags, polythene bags, stainless steel, plastic, disposable glass	$r = 0.204$	$P = 0.117$	$r = 0.114$	$P = 0.445$	$r = 0.085$	$P = 0.600$	$r = 0.112$	$P = 0.404$	$r = 0.207$	$P = 0.112$	$r = 0.034$	$P = 0.800$
Presence of food storage facilities	No, yes	$r = 0.703$	$P = 0.050$*	$r = 0.204$	$P = 0.169$	$r = 0.626$	$P = 0.027$*	$r = 0.166$	$P = 0.212$	$r = 0.412$	$P = 0.001$*	$r = 0.199$	$P = 0.131$
Type of food storage facilities	Ice box, deep freezer, refrigerator	$r = 0.753$	$P = 0.003$*	$r = 0.191$	$P = 0.479$	$r = 0.071$	$P = 0.753$	$r = 0.153$	$P = 0.485$	$r = 0.275$	$P = 0.183$		
Duration of food storage	Not stored, 1, 2, >2 days	$r = 0.016$	$P = 0.901$	$r = 0.900$	$P = 0.009$*	$r = 0.277$	$P = 0.083$	$r = 0.872$	$P = 0.007$*	$r = 0.344$	$P = 0.007$*	$r = 0.148$	$P = 0.262$
Use of leftover food	Thrown away, used at home, reuse after heating, completely used, distributed to people, no answer	$r = 0.618$	$P = 0.066$	$r = 0.199$	$P = 0.424$	$r = 0.908$	$P = 0.049$*	$r = 0.803$	$P = 0.033$*	$r = 0.146$	$P = 0.264$	$r = 0.171$	$P = 0.194$
Use of covering (gloves, head)	No, yes	$r = 0.088$	$P = 0.502$	$r = 0.088$	$P = 0.559$	$r = 0.070$	$P = 0.669$	$r = 0.273$	$P = 0.038$*	$r = 0.095$	$P = 0.472$	$r = 0.175$	$P = 0.184$
Use of Apron	No, yes	$r = 0.133$	$P = 0.334$	$r = 0.094$	$P = 0.549$	$r = 0.163$	$P = 0.351$	$r = 0.259$	$P = 0.051$	$r = 0.098$	$P = 0.477$	$r = 0.151$	$P = 0.277$
Types of apron	Cotton, PVC aprons	$r = 0.339$	$P = 0.143$	$r = 0.243$	$P = 0.403$	$r = 0.233$	$P = 0.491$	$r = 0.471$	$P = 0.042$*	$r = 0.492$	$P = 0.027$*	$r = 0.143$	$P = 0.200$
Washing of all coverings	Daily, after 1 day, after 2 days, weekly	$r = 0.218$	$P = 0.275$	$r = 0.181$	$P = 0.446$	$r = 0.357$	$P = 0.145$			$r = 0.196$	$P = 0.328$	$r = 0.125$	$P = 0.535$
Management of waste	Roadside, waterhole	$r = 0.988$	$P = 0.002$*	$r = 0.763$	$P = 0.045$*	$r = 0.136$	$P = 0.402$	$r = 0.163$	$P = 0.222$	$r = 0.966$	$P = 0.006$*	$r = 0.048$	$P = 0.721$

*Correlation is significant at the 0.01 level (2-tailed).

for the inter-state transport system. A large number of people travel daily within and outside the city from this station, so these beverages are readily consumed by the people, hence contributing in less multiplication of microbes. Although other locations are also considered the hub of business and residential area, the transport station at this place gives uniqueness to this place and mostly the passengers rely on these readily available beverages during the hot summer season. Vendors also have the habit of keeping the sugar cane uncovered and unpeeled which attract the houseflies due to sweet odour; however, in terms of TPD, vendors usually used to prepare this drink in large amount much before the time of consumption that appeals the houseflies. In addition to this, around 60 per cent of the vendors throw the waste on roadside which is significantly associated with APC, TCC, and *Salmonella*. It has been reported that samples may be contaminated when the distance between the stall and waste bin is less than 2 m (Kiranmai et al., 2016), so the chances of contamination may be increased due to flies and rodents on roadside garbage near the vending stalls and act as a vector for inviting contamination. However, in the present study, houseflies were present at all stalls even those who covered their stalls exhibited the non-significant correlation ($P \leq 0.05$) with the microflora, but the samples were found unsatisfactory from all locations for one or the other reason.

The presence of yeast and moulds in more than 90 per cent samples indicates that fungal contamination occurs due to improper storage facilities and it may harbour in their storage spaces at high temperature. Sixty-one per cent of the fungal contamination was observed in juices with the reason of using the same water for washing utensils and improper storage (Kiranmai et al., 2016). Majority of vendors were not using gloves, head coverings, and apron which indicates the negligence, ignorance, or failure of implementation of food safety codes. The same has also been reported in other studies (Cortese et al., 2016; Kiranmai et al., 2016; Rao et al., 2016) that most of the people who are educated and aware of these did not use the coverings. Wearing gloves is one of the safe methods to prevent the transmission, but it is not recommended as a substitute of hand washing. Conover and Gibson (2016) reported that wearing gloves is a false sense of security and food handlers wash their hands less frequently or less often after high-risk tasks. In the present study, vendors were also in practice to wash their hands before preparation of food, after touching each food item, before preparation, and after using toilet with the percentages of 30, 8.3, and 61.6, respectively. In these three categories, all of the samples were contaminated at $P = 0.010$. Hand washing is a primary way to reduce the spread of pathogens and help prevent the infectious diseases. Food safety code describes that food workers should wash hands immediately before handling the raw or cooked food, clean utensils and equipments, single-use articles, unwrapped single-service even after using the rest room, after touching bare human body parts, and before putting on gloves (United States Food and Drug Administration, 2013). Despite very efforts and attention by the food industry, the compliance of proper hand washing often fails (Conover and Gibson, 2016). In this study, majority of the vendors reported that they washed their hands before preparation and after using toilet, but contamination of the samples indicated that either they did not wash their hands properly or due to some other reason samples were not satisfactory. So, there are multiple factors and lack of encouragement of basic food safety codes by these roadside vendors that add these microbes to beverages and consequently cause contamination. Furthermore, the combination of industrial waste into the drinking water streams and the application of fertilizers and additives are also heuristically extending throughout the country.

Conclusion

The estimates of *E. coli*, coliforms, *Salmonella*, and *S. aureus* like pathogens in beverages showed the clear indication towards the risk of foodborne diseases for regular consumers. The estimation of high microflora directs an alarming situation for government and local health authorities that they should take concrete initiatives by providing the proper infrastructure, safe water supply, and eradicate the other accountable factors from food preparation till its consumption. The consumption and selling of these beverages cannot be stopped at any cost due to the source of livelihood for vendors and nutritious for the consumers. There should be a framework of licensing system under the management of local health authorities with the awareness program on food safety and hygiene and ways to see the contamination in street-vended beverages.

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Conflict of interest statement

None declared.

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