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#### **Original Article**

Street Vended Juices as A Risk Factor of Microbial Diseases in District Mardan, Pakistan

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# ABSTRACT

In public spaces like streets and other outdoor areas, merchants prepare and sell foods and drinks for sale. The Food and Agriculture Organization estimates that 2.5 million individuals eat street food every day. Objective: To study the assessment of microbial contamination of juices vended in streets in District Mardan of Pakistan. Methods: 315 samples comprising juices of sugarcane, pomegranate, jaggery, plum, mango, banana and apples were aseptically collected from three Tehsils of district Mardan including Katlang, Takht Bhai and Mardan. Results: Analysis of the samples revealed that 96% of juices had high loads of bacterial pathogens such as Coliforms (96.82%), Staphylococcus (81.90%), Salmonella (64.76%). In Tehsil Katlang and Takht Bhai all collected samples were contaminated while in Mardan Tehsil 87.6% bacterial contamination was observed. Very high number of coliforms were observed in sugarcane, apple juices and Jaggery, Salmonella counts were highest 21.05x10<sup>2</sup> cfu/ml in Jagerry and Staphylococcus growth was highest in sugarcane 1.22x10<sup>3</sup> cfu/ml. **Conclusions:** It was noted that coliforms contamination is significantly higher as compared to other two bacteria indicating sewage water mixing in water used for preparation or handling of these juices. To prevent future food-borne infections, it is advised that frequent inspection of the quality of juices sold on the street be done.

# INTRODUCTION

In public spaces like streets and other outdoor areas, merchants prepare and sell foods and drinks for sale. The Food and Agriculture Organization estimates that 2.5 million individuals eat street food every day. Fruit juices are the unfermented yet fermented liquid that is extracted from the ripe portion of fresh fruits or fruits that have been kept in fresh condition through physical means or other acceptable treatments [1]. Juices are produced by separating the pulp of fresh fruits, without using heat or solvents, to produce a drinkable, untreated, unclarified, and cloudy juice [2]. Fresh juices separated from pulp are usually diluted with water because fresh juices are either too sour or too powerfully seasoned to be consumed [3]. Fruit juices are readily used by people all around the world and is considered a vital part of present-day diet as they are full of important nutrients such as vitamins, minerals and other naturally occurring chemicals. These chemicals are obtained from plants that is biologically active and are of health and remedial benefits [4]. In hot climate, vendors use local facilities to extract juice from pulp of mature fruits, dress it with ice and serve it to thirsty customer [5]. Customers prefer the fresh juices sold by street sellers because of their freshness, taste, inexpensive cost, and timely availability [6]. While the majority of cafes and restaurants sell juices in what appear to be hygienic settings, their microbiological quality is still debatable in roadside stores, parks, and bustling market places like malls and bus stops. These stores provide freshly squeezed juices made from a variety of fresh fruits, such as oranges, grapes, pomegranates, apples, pineapples, watermelons,

papayas, and carrots, that have been heavily diluted with water and ice. Gastroenteritis outbreaks brought on by dangerous E. coli bacteria persist despite routine quality control inspections and store closures. In these regions, E. coli, Salmonella, and Shigella are frequent [7-10]. Juices that have just been freshly extracted may not always be safe due to the high microbial load [11]. Environmental exposure is one method through which bacteria could enter fruits and fruit juices. Fruits that haven't been properly washed introduce microorganisms to extracts, contaminating them. Additionally, the use of unclean water for dilution, dressing with ice, lengthy storage without refrigeration, unclean surroundings-often with swarms of fruit flies and house flies-and airborne dust can all serve as causes of infection. These juices have been demonstrated to be potential reservoirs for bacteria, particularly Shigella, E. coli 0157:H7, Salmonella, and S. aureus [2, 12]. The inclusion of coliforms in fruit juices is prohibited by safe food consumption guidelines [13]. Major juice ingredients including water, sugar, natural fruit pulp, etc. may also include some microbial contamination [14]. Food-borne disease related with the use of fruit juices has been recorded in numerous locations [7, 15-18]. Salmonella was found in apple and orange juices, while E. coli 0157:H7 was found in apple juices in microbial safety investigations [19, 20]. In recent years, there has been an increase in the selling and consumption of meals on the roadside in Pakistan. Much research has been conducted on many elements of street meals and sellers in countries such as China, India, and Nigeria, but comparable studies are few in Pakistan. However, some information is accessible about Lahore's street meals [21]. As the popularity of street juices grows in KPK, it is vital to assess the quality of these beverages. The present investigation aims to assess the microbiological contamination of juices vended in streets in District Mardan.

#### METHODS

Street vended Juices were collected from district Mardan including its three tehsils Takht bhai, Katlang and Mardan (Figure 1).



#### three tehsils(B)

A total of 315 samples of juices collected including of mango, banana, pomegranate, apple, sugarcane, jaggery (sharbet) and plum juice. Equal number of samples was collected for each juice type and area for comparison (Table1).

Juice Type	Area of Sampling			
	Mardan	Takht Bhai	Katlang	
Sugarcane juice	15	15	15	
Banana juice	15	15	15	
Apple juice	15	15	15	
Pomegranate juice	15	15	15	
Jaggery juice	15	15	15	
Plum juice	15	15	15	
Mango juice	15	15	15	
Total=315	105	105	105	

Table 1: Number, Types and Area selected for sampling

Samples were collected from district Mardan during summer season. Sample collection was performed in sterile flasks and tubes, samples was store at 4°C and analysis was performed in one hour after sample collection. Fruit juices were used without any further dilution. Following media was used for the detection of bacteria: Mannitol salt agar (MSA Sigma), Eosin Methylene Blue (EMB-Sigma) and Salmonella/Shigella agar (Sigma). All three media were prepared in accordance with the manufacturer's recommendations. Inoculation of each sample was performed on each of the above-mentioned media independently using the plate spreading technique, and incubation of plate were done at 37°C in an inverted posture for 18-24 hours. The petri plates were examined after 18-24 hours of incubation for recording bacteria; colony forming units (CFU)/ml. According to Merk (1996), the color and several other characteristics of bacterial colonies on the media used were documented, and bacterial growth was detected [22].

#### RESULTS

This investigation discovered that street food and liquids were contaminated with harmful germs that cause human illness. The overall microbial contamination of juices typically eaten in Mardan area was quite high. Out of 315 samples, 302 were positive showing 96% contamination. There was a significant difference (P=0.02) in the distribution of microorganisms by region. Takht bhai and Katlang had prevalence of 100%, while Mardan tehsil had prevalence of 87.6% (Figure 2).

Figure 1: Map showing: Location of District Mardan (A) and its



**Figure 2:** Microbial contamination prevalence in street-vendor juices in several regions of Mardan District

7 kinds of juices were examined for microbial contamination among which sugarcane, banana, apple, pomegranate and mango juice were 100% contaminated, jaggery juice 93.3% while the least contaminated was Plum juice (77.8%) as shown in Table 2. Significant higher difference was noted between plum and other juices contamination (P=0.0001).

Types of Juices	Observed Samples	Positive samples	Microbial contamination (%)
Sugarcane	45	45	100
Jaggery	45	42	93.3
Plum	45	35	77.8
Banana	45	45	100
Apple	45	45	100
Pomegranate	45	45	100
Mango juice	45	45	100
Total	315	302	96

**Table 2:** Types of Juices and their microbial contamination in street vended juices in Mardan District

*Coliforms* prevalence in district Mardan was 96.82% collectively out of that *Staphylococcus* was 81.9% while that of *Salmonella* was 64.76% (Table 4). Street vended juices were highly loaded with *coliforms* having 90.5%, 100% and 100% prevalence in Mardan, Takht bhai and Katlang, respectively. Juices were least contaminated with *Staphylococcus* in Mardan (52.38%) while in Takht bhai and Katlang the food was highly contaminated having 100% and 93.33% prevalence, respectively. *Salmonella* was found in 95 samples showing 90.5% prevalence in Mardan and 91 samples showing 86.67% prevalence in Katlang while, in Takht bhai were least contamination showing only 17.14% prevalence (Table 3). There was a significant difference in overall prevalence of all the three microbes (P=0.013).

Type of	Number of s	Collective		
microbes	Mardan (n=105)	Takht Bhai (n=105)	Katlang (n=105)	Prevalence (%) (N=315)
Coliforms	95(90.5)	105 (100)	105(100)	305(96.82)
Staphylococcus	55(52.38)	105(100)	98(93.33)	258 (81.90)
Salmonella	95(90.5)	18 (17.14)	91(86.67)	204 (64.76)

N=Total number of samples tested in District Mardan n=Number of samples tested in each area(Tehsil)

**Table 3:** Microbiological assessment prevalence in juices vendedin streets at several areas of Mardan District

From the Mardan district area very high number of *Coliforms* were observed in sugarcane, apple juices and Jaggery. Mango juice had 5.01x10<sup>3</sup>, banana juice 4.72x10<sup>3</sup> Pomegranate 0.86x10<sup>3</sup> and Plum 0.155x10 cfu/ml. *Salmonella* counts varied between 0.65x10<sup>3</sup>-21.05x10<sup>3</sup> cfu/ml while absent in Plum juice. *Staphylococcus* growth was 0.49x10<sup>3</sup>-1.22x10<sup>3</sup> cfu/ml but absent in Plum juice (Table 4).

Types of Juices	Observed Samples	Positive samples	Microbial contamination (%)
Sugarcane	78.0x10 <sup>3</sup>	0.99x10 <sup>3</sup>	1.22x10 <sup>3</sup>
Jaggery	71.7x10 <sup>3</sup>	21.05x10 <sup>2</sup>	0.49x10 <sup>3</sup>
Plum	0.16x10 <sup>3</sup>	Nil	Nil
Mango	5.01x10 <sup>3</sup>	1.90x10 <sup>3</sup>	0.92x10 <sup>3</sup>
Banana	4.72x10 <sup>3</sup>	0.65x10 <sup>3</sup>	0.94x10 <sup>3</sup>
Apple	77.0x10 <sup>3</sup>	1.52x10 <sup>3</sup>	0.90x10 <sup>3</sup>
Pomegranate	0.86x10 <sup>3</sup>	Nil	Nil

**Table 4:** Mean microbial profile(cfu/ml)of juices vended in streets

 sold in Mardan District

### DISCUSSION

Microbiological assessment of juices vended, consumed and sold in Mardan District, Khyber Pakhtunkhwa, Pakistan was investigated in this study. Microbial contamination was found in 96% of the juices sold on the street. Many researches have been undertaken in many regions of the world to investigate the microbial contamination of street food and juices, with comparable results. Microbes were found in all of the beverages tested in Bangladesh, according to Khan et al., [22]. Asha et al., found microbial contamination in 100% of the juice sold on the street in Guntar, India [23]. Bello *et al.*, discovered 100% fresh juices infected with harmful microorganisms in Ogun state, South Western Nigeria [24]. Das et al., from Banglore, India, stated that 100% of the samples in their investigation were contaminated by microorganisms [25]. The high microbiological count might be attributed to a variety of circumstances, including the use of unclean water for dilution and ice manufacturing [26]. Microbial count is caused by improper cleaning of utensils, poor maintenance of premises or personal and household cleanliness, peeling of fruits ahead, shopping in busy locations and dust particles in the evening, and a lack of adequate sanitary practices. [4, 9, 27]. The current investigation found that the incidence of microbial contamination in street vended food and drink varied by location. Takht bhai had the most occurrence, followed by Katlang, while Mardan city had the lowest. Statistical analysis revealed substantial differences across all research areas. Local climate circumstances, public associated hygiene habits, and sanitary facilities all had an impact on the occurrence of

microbiological contamination in street vended juices [28]. In the current study, the majority of the samples tested positive for coliforms, Staphylococcus, and Salmonella sp. Bello et al., discovered Staphylococcus, E. coli, and Salmonella in fruit juices. This showed that sewage water was mixed with drinking water [24]. We found counts of coliforms varied between 0.16-78.0x10<sup>3</sup> cfu/ml for juices. Khan et al., found total coliforms of 210-1100 cfu/100 ml in drinks sold in streets of Dhaka [22]. Existence of coliforms in vended juices might be due to faecal contaminated water that are used for cooking [29]. Fruit juice contamination might be caused by damaged or rotten fruits used for squeezing juices, or by inadequate hygienic conditions throughout the entire process of cutting to serving. The presence of coliforms and Staphylococcus aureus may be attributed to inappropriate handling or processing with contaminated water. Contaminated water used to make ice is also a big contributor, as freezing does not kill hazardous germs. They may be able to live when the ice melts into the liquids [30]. Vendors' failure to recognize basic safety hazards contributes to an increase in microbial loads. These involve the use of crude carts and stands, the lack of flowing water for washing and dilution, long-term storage without refrigeration, and unsanitary conditions with swarming insects and airborne dust[7].

## CONCLUSIONS

Based on our findings, we believe that food sellers should be provided with adequate facilities and training. The points that needs critical control should be acknowledged, and steps be made to reduce bacterial contamination. Local governments can then implement investment, planning, mass media, and campaign rules.

### Conflicts of Interest

The authors declare no conflict of interest.

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