Incidence of *E. coli* O157: H7 in Fresh Fruit Juices of Street Vendors from Different Areas of Lahore City, Pakistan

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**ABSTRACT**

Pakistan is among one of the countries where food and water borne illnesses due to *Escherichia coli* O157:H7 contamination are frequent. Present study was undertaken for detection of the possible sources of *E. coli* O157:H7 in street vended fresh fruit juices sold in streets and along the road sides of Lahore city. About 162 fresh juice samples of sugar cane, plum, tamarind, peach, lemonade and slush that were taken from different localities of Lahore and analyzed for the presence of *E. coli* O157:H7. Sugar cane juice samples from Wahdat colony and Ichrah were found 13% more contaminated with *E. coli* O157:H7 than the samples of other areas. While for tamarind, Railway station and Gulshan Ravi and for plum juices Railway station and Moon market had 41% more contamination of *E. coli* O157:H7 as compared to others. In case of peach Ichrah samples had shown highest counts (53%). For slush, Railway station samples revealed countless contamination. Whereas for lemonade juices about 50% of all the areas studied had 13% more contamination as compared to others. Our studies suggest that ignorance of hygienic practicing, unpasteurized fruit juices and use of fecal contaminated water in juice making is the potential candidate for *E. coli* O157:H7 contamination. Efforts should be taken to develop awareness and to improve the handling practices along with maintaining hygienic conditions to avoid food borne illnesses. These preventive measures can reduce the degree of harmful pathogen contamination to fresh juices sold at roadside outlets and hence helps in preventing outbreaks of infective diseases as well as improve the general health conditions of people.

**Key words:** Fruit Juices, Street vendors, Contamination, *E. coli* O157:H7, microbes

**INTRODUCTION**

*Escherichia coli* O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a causative agent of Hemorrhagic colitis an acute food borne disease (CDC, 1996; CDC, 1997; Karch et al., 2005; Carney et al., 2006). *Escherichia coli* O157:H7 serotype was first reported in USA in 1975 from a foodborne outbreak (Riley et al., 1983; Wachsmuth et al., 1991). Ever since it has become the most important serotype in foodborne illnesses (Karmali, 1989) and is associated with many disease outbreaks throughout the UK, USA, Canada, Japan, Sweden and many more (Michino et al., 1999; Willshaw et al., 2001; Woodward et al., 2002; Cagney et al., 2004; Carroll et al., 2005; Sartz et al., 2006; Uhlich et al., 2008). Cattle are asymptomatic carriers of the organism as a result carcasses may become contaminated with the pathogen following slaughter (Chapman et al., 2001). Outbreaks have also been reported to be associated with water supplies contaminated with bovine faeces. Person-to-person transmission has also been reported at nurseries (Al-Jader et al., 1999). Undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks, however *E. coli* O157:H7 outbreaks have implicated alfalfa sprouts, unpasteurized fruit juices such as apple juice, cider, dry-cured salami, lettuce, game meat, and cheese curds (Zhao et al., 1993; Cody et al., 1999; Hilborn et al., 1999, 2000; Michino et al., 1999). Fruit juices and nectars in colloquial terms are food products of great nutritional value, rich in vitamins, mineral salts, simple sugars and phytochemicals which are easily assimilated by humans. In many countries around the world especially developing one's there is a trend of unpasteurized fresh fruit juice consumption that are sold along road side shops and by street vendors that has been related to many food and water borne illnesses due to *E. coli* O157:H7 presence (Swerdlow et al., 1992; Luna-Gierke et al., 2014; Sodha et al., 2014). This infectious agent has become common, distressing and sometimes life-threatening problem for millions of people around the world (Mosupye & Holy, 2000; Muinde & Kuria, 2005; Lewis et al., 2006; Chumber et al., 2007; Ghosh et al., 2007). Countries having hot summers where temperature reaches upto 50 °F, people in order to gain respite from the rising mercury tend to
consume fresh juices. To fulfill their thirst, several roadside stalls spring up to cater the emergent demand. In view of the unsatisfactory sanitary and hygienic conditions that prevail in the vicinity of these stalls there is an increase in outbreaks of infective diseases (Abraham et al., 1997; Bhaskar et al., 2004; Subbannayya et al., 2007).

Fruits are constantly contaminated by airborne microorganisms both externally and internally during growth and harvesting (Laidler et al., 2013; Wright et al., 2013; Ahmeda et al., 2014). However main sources of contamination in fresh fruit juices are unwashed instruments and utensils, germ-infested water used for dilution purpose prolonged preservation without low temperature maintenance, polluted surroundings often with swarming houseflies and fruit flies and airborne dust. Such unpasteurized fresh juices have shown to be potential sources of bacterial pathogens notably E. coli O157:H7, species of Salmonella, Shigella and Staphylococcus aureus (Lewis et al., 2006). Improperly prepared fresh fruits and vegetable juices are recognized as an incipient cause of food borne illness (Mudgil et al., 2004).

E. coli O157:H7 has the ability to survive in food, soil, water and manure (Lynn et al., 1998; Hancock et al., 1998, 1998a). It also forms bio-films and therefore is protected from the effect of biocides and shows their persistence in water distributary system (Daly et al., 1998). Numerous outbreaks of E. coli O157:H7 infection due to consumption of unpasteurized fresh fruit juices have been reported (Besser et al., 1993; Griffin et al., 1994; Cody et al., 1999; Hilborn et al., 2000). In 1997, unpasteurized fresh apple cider was responsible for an E. coli O157:H7 outbreak in Connecticut and New York, USA (CDC, 1996-1997). E. coli O157:H7 survival in variety of juices is because of its ability to survive at low pH (Miller et al., 1994; Artz & Killham 2002; Vojdani et al., 2008). Street vendors serve a great part in fulfilling this demand as the fruit juices they supply are quite economical. Unhygienic practices offered in making these fruit juices and hence their microbial quality are questionable. Their unawareness towards concept of contamination is borne to food borne illnesses. In view of all these circumstances incidence of E. coli O157:H7 in different street vended fresh juices was undertaken.

MATERIALS AND METHODS

Sampling: Total 162 samples of variety of fresh fruit juices (Sugar cane, Plum, Tamarind, Peach, Lemonade and fruit Slushes) were taken from different localities of Lahore city. Samples were kept at 4°C before being processed and within shortest time period microbial quality regarding E. coli O157:H7 was analyzed in Health Microbiology Lab of Lahore College for Women University Lahore (LCWU), Pakistan.

Enrichment Media for E. coli O157:H7: Enrichment media used for the isolation of E.coli O157:H7 was Tryptone Soya broth (Lab M) supplemented with vancomycin, cefixime, ceftulodon according to manufacturer’s details. Juice sample was added into broth which was incubated at 37°C for 24 hours.

Selective Media for Isolation of E. coli O157:H7: Sorbitol MacConkey agar (Lab M) was used as a selective media for the isolation of E. coli O157:H7 from fresh fruit juice samples (Sugar cane, Plum, Tamarind, Peach, Lemonade and Slush) according to manufacturer’s details. The inoculated plates were incubated at 37°C for 24 hours. Colony forming units (CFU/ml) of E. coli O157:H7 was calculated. All samples were preceded in triplicates.

Statistical Analysis: For statistical analysis, Duncan Multiple range test was applied using SPSS version 13.0 at significance level 0.005. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Street vended fruit juices are well appreciated by the consumers because of their taste, low price, and ease in availability (Ohikpehai, 2003). In spite of potential benefits offered by the fruit juices, concerns over their safety and quality have been raised; as freshly prepared juices have not been processed through standard steps to eliminate or minimize the microbial content (Mahale et al., 2008).

In the present study, incidence of E. coli O157:H7 contamination in different fresh fruit juice samples of Sugar cane, Plum, Tamarind, Peach, Lemonade and Slush were taken from most popular market places of Lahore city, i.e., Ichhra, Railway
Station, Shadman, Garhi Shahu, Wahdat Colony, Neelam Block, Gulshan Ravi and Faisal Town. In case of sugar cane, *E. coli* O157:H7 count ranged from $6.87 \times 10^3 \pm 0.94 \times 10^3$ CFU/ml to uncountable. Among these countless values were recorded for Ichhra, Wahdat Colony, Railway Station, Neelam Block, Garhi Shahu, Moon Market, Gulshan Ravi and Faisal Town while countable value was recorded for Shadman (Fig., 1, Table I). Plum juice samples were analyzed, the contamination of *E. coli* O157:H7 ranged from $3.17 \times 10^3 \pm 0.49 \times 10^3$ (CFU/ml) from Ichhra to uncountable from Railway station and Moon market, respectively. Overall Railway Station and Moon Market each had the highest count among all the areas studied (Fig., 1, Table I).

For tamarind juice, count ranged from $9.0 \times 10^3 \pm 0.35 \times 10^3$ (CFU/ml) from Wahdat colony to uncountable from Railway Station and Gulshan Ravi, respectively (Fig., 1; Table I). Peach fruit juice samples had *E. coli* O157:H7 count that ranged from $13.67 \times 10^3 \pm 0.57 \times 10^3$ (CFU/ml) from Faisal town to uncountable from Ichhra (Fig., 1, Table I). *E. coli* O157:H7 count for Lemonade was enumerated that ranged from $12.2 \times 10^3 \pm 0.8685 \times 10^3$ (CFU/ml) from Garhi shahu to countless from all the other areas (Ichhra, Wahdat Colony, Railway Station, Neelam Block, Shadman, Moon Market, Gulshan Ravi and Faisal Town) as shown in Fig., 1 and Table I. Counts for *E. coli* O157:H7 for slushes was recorded from $2.77 \times 10^3 \pm 0.54 \times 10^3$ (CFU/ml) from Garhi Shahu to $75.0 \times 10^3 \pm 0.96 \times 10^3$ (CFU/ml) from Railway Station. Railway station, Neelam Block, Moon Market and Gulshan Ravi showed significantly high counts (Fig., 1; Table I). Regarding lemonade, Railway Station had significantly more count as compared to other areas (Fig., 1; Table 1).

Global tropical fruit consumption per capita has increased 33% over the last 20 years. Food borne illnesses because of the consumption of the fruit juices are reported in India and other places (Mosupye & Holy, 2000; Muinde & Kuria, 2005; Lewis et al., 2006; Chumber et al., 2007; Ghosh et al., 2007). In the present study, 162 juice samples of sugarcane, plum, tamarind, peach, lemonade and slush were analyzed for *E. coli* O157: H7 contamination. It was observed that more contamination was seen in lemonade juice for *E. coli* O157: H7 that could be due to addition of contaminated water for dilution purpose and pathogen tolerance towards acidity of juice (Miller, 1994; Zhao et al., 1993; Conner & Katriola, 1995). Thus water plays a major role in the contamination of lemonade. *E. coli* O157:H7 persist in drinking
troughs and manure heaps for months or years thus facilitating prolong contamination of the locality and re-infection of animals (Bolton et al., 1999; Lejeune et al., 2001a-b; Guan & Holley, 2003).

Overall, it is concluded that among all the areas studied Railway Station showed significantly highest contamination in all the juice samples analyzed. The main reason behind this might be due to poor quality of water used for dilution, prevailing unhygienic conditions and practices related to washing of hands, utensils, maintenance of the premises and site of juice stall location by waste disposal system and overcrowding (Barro, 2006; Tambekar et al., 2008, 2009 and 2011). The occurrence of pathogenic bacteria in fruit juices is alarming enough for an immediate action by the responsible agency. It is suggested that regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen out breaks. Therefore, microbial analysis of commercially sold fruit juices should be done and regulation in the issuance of permit to produce and sell these products should be under strict quality control to reduce and mitigate exposure to harmful microbes deleterious to consumers' health.

Table I: Colony forming unit per ml of *E. coli* O157: H7 from fresh fruit juices of street vendors from different localities of Lahore city.

<table>
<thead>
<tr>
<th>Study area</th>
<th>Sugar cane juice</th>
<th>Plum juice</th>
<th>Tamarind juice</th>
<th>Peach juice</th>
<th>Lemonade juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ichhra</td>
<td>Uncountable</td>
<td>3.17×10²±</td>
<td>46.33×10²±</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Wahdat colony</td>
<td>Uncountable</td>
<td>13.1×10²±</td>
<td>9.0×10²±</td>
<td>41.13×10³±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Railway station</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>93.67×10³±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Neelam block</td>
<td>Uncountable</td>
<td>36.63×10²±</td>
<td>35.6×10²±</td>
<td>79.27×10³±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Shadman</td>
<td>6.87×10³±</td>
<td>15.53×10²±</td>
<td>60.0×10²±</td>
<td>78.97×10³±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Garhi shahu</td>
<td>Uncountable</td>
<td>22.47×10²±</td>
<td>14.33×10²±</td>
<td>44.8×10²±</td>
<td>12.2×10³±</td>
</tr>
<tr>
<td>Moon market</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>14.32×10²±</td>
<td>66.1×10²±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Gulshan ravi</td>
<td>Uncountable</td>
<td>45.67×10²±</td>
<td>Uncountable</td>
<td>28.97×10³±</td>
<td>Uncountable</td>
</tr>
<tr>
<td>Faisal town</td>
<td>Uncountable</td>
<td>85.43×10³±</td>
<td>51.83×10³±</td>
<td>13.67×10³±</td>
<td>Uncountable</td>
</tr>
</tbody>
</table>

Values shown are Mean ± S.E.M. Superscripts in parenthesis represent statistical difference (p≤ 0.005) between values that represent differences among different places at each fresh juice.
REFERENCES
Guan, T.Y. & Holley, R.A., 2003. Pathogen survival in swine manure environments and


