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Incidence of *Salmonella typhi* in fruit juices sold in Allahabad city and their antibiotic susceptibility pattern

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Abstract
Continuous rise in the number of outbreaks of food borne multi drug resistant (MDR) typhoid in India is an escalating problem and is linked to fresh fruit juices. MDR isolates of *Salmonella typhi* are on rise and are becoming a challenge for timely and appropriate treatment. In present work eighty fruit juice samples (40 each of pomegranate and orange) were collected from different locations of Allahabad City. The samples were analyzed for the presence of *Salmonella typhi*. The isolated *Salmonella typhi* were subjected to ten commercial antibiotic employing disc diffusion assay. Total 22 isolates of *Salmonella typhi* were collected from juice samples and incidence of *Salmonella typhi* from these samples was found to be 25% ($16.9x10^4$cfu/ml) in case of orange and 30% ($6.08x10^4$cfu/ml) in pomegranate juice. Among the ten commercial antibiotics tested, ofloxacin and chloramphenicol showed 100% sensitivity against *Salmonella typhi* isolates followed by co-trimoxazole, gatifloxacin and ciprofloxacin with a sensitivity of 95.45%. The least sensitivity (18.18%) shown by ampicillin and intermediate sensitivity was observed in cefixime (36.36%), nalidixic acid (68.18%), ceftriaxone (72.73%) and norfloxacin (90.91%). It was observed that out of total 22 isolates only one was found to be multi drug resistant. Appropriate administration of hygienic measures such as checking the quality of water used for the dilution as well as prevailing unhygienic condition related to washing of utensils and maintenance of the premises may reduce risk.

Keywords: Fruit juices, unprocessed, *Salmonella typhi*, multi drug resistance pattern

Introduction
Fruit juices are well recognized for their nutritive values, minerals and vitamins contents. In many countries they are common man's beverages and are sold at all public places and roadside shops. However in view of their ready consumption, quick methods of cleaning, handling and extraction, they could often prove to be a public health threat. There are reports of food borne illness associated with the consumption of fruit juice at several places in India and elsewhere (Parish, 1997; Canada, 2000; Sandeep *et al*., 2001) [25, 27]. Considering its nutritive value and palatability fruit juices are highly recommendable. However, bearing in mind the method of extraction, an inevitable question arises over safety. There are reports of food borne illness associated with the consumption of fruit juices at several places in India (Parish, 1997) [28]. Most fruits contain bacterial counts upto 1.0x105cfu/cm^2 on their surface (Harrigan, 1998) [14]. Improper washing of fruit add these bacteria to extracts leading to contamination. Juices have shown to b potential source of bacterial pathogens (Sandep *et al*. 2001). Bryan (1977) [27, 4] reported that many microorganisms will enter the fruit juice at the time of extraction and cause contamination. Source of contamination may vary. It is mainly due to poor quality of water used for dilution, improper washing of fruits add these bacteria to extracts, prevailing unhygienic conditions, related to washing of utensils, maintenance of the premises and location by the side of a busy road or by the side of the waste disposal system. During transportation to the market or the processing plant mechanical damage may increase susceptibility to decay and growth of microorganisms may take place. The processing units of the juices are likely primary causes of high microbial load. There is no justification for processed ready to eat food being contaminated with these organisms and their presence even in small numbers results in such foods being of unacceptable quality or potentially hazardous (Schmidt *et al*., 1997) [28]. There are several reports that suggest that fruit juices have shown to be potential source of bacterial pathogens, notably E. coli 0157:H7, Salmonella spp., Shigella spp. and *Staphylococcus aureus* (Ryu and Beuchat 1998; Uljas and Igham 1998; Buchmann *et al*., 1999; Sandeep *et al.*, 2001) [26, 32, 27]. The incidence of Salmonella is also reported by (Lewis *et al*., 2006) [21] in grape, mango and orange juices. Typhoid fever continue to be a global health problem with an estimated 12 to 33 million cases occurring worldwide each year.

The disease is endemic in many developing countries particularly the Indian subcontinent, south and Central America (Miller and Pegues, 2000) [24]. In India, *Salmonella enterica serovar typhi* drug resistance has been reported since 1960 following the first outbreak of multidrug resistant *Salmonella enterica serovar typhi* in Calicut. Since then multi drug resistant *Salmonella enterica serovar typhi* have appeared throughout the world, especially in South America, the Indian subcontinent, Africa and South East Asia (Gautam *et al*. 2002) [10]. Chloramphenicol resistant *Salmonella enterica serovar typhi* causing an outbreak has earlier been reported from Chandigarh (Kapil *et al*., 1994) [17]. Drug resistance to in Salmonella is of considerable importance to both clinicians and the microbiologists and poses a major problem for public health authorities. Resistance to commonly used antibiotics such as chloramphenicol, ampicillin and co trimoxazole has been reported from different parts of India (Gautam *et al*., 2002) [10]. There is always a great demand for fresh unprocessed fruit juices, especially in tropical countries like India were hot weather continues for a greater part of year increasing the need for this commodity. However, the microbiological quality remains questionable. Contamination of these food products makes them unacceptable for human consumption has become a global health problem. Despite periodic quality control checks outbreaks of Salmonellosis through consumption of the fruit juice is not uncommon. Therefore, regular monitoring is required both of the processed and unprocessed fruit juice to assess its safety for human consumption and as a possible source of MDR – Salmonella typhi. The Multi drug resistant and antibiotic susceptible strains of *Salmonella typhi* can survive in endemic areas along with epidemiologically independent pathogens, and are not in competition for continued persistence and transmission (Achla *et al*., 2004) [2]. Therefore the present study was undertaken to study the incidence of MDR *Salmonella typhi* in fruit juices sold in Allahabad City.

Materials and Methods

1. **Study Samples:** For the present study 40 samples each of unprocessed orange and pomegranate juice (Total 80) were collected from different locations of Allahabad city.
2. **Sample Collection:** Ten ml samples of unprocessed orange and pomegranate juice were collected in sterile glass bottles from four different localities of Allahabad City. The collected samples from each location were then taken to the laboratory for analysis. The samples were stored at 4 ^{0}C if required.
3. **Isolation of *Salmonella typhi* from unprocessed fruit juice:** The isolation of *Salmonella typhi* from fruit juices was done as per the procedure given by Wells and Butterfield (1997) [34]. For the one ml of juice was mixed with 9 ml of Ringer's solution and serial dilution was made up to 10^{-4}. The sample (10^{-4}) was plated with Salmonella – Shigella Agar media using pour plate technique. The plates were incubated at 37 ^{0}C for 24 – 48 hours along with media control and were observed for the colonies.
4. **Identification of the isolates:** The identification of the isolates was done on the basis of cultural, morphological and biochemical tests as given in the Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1984) [15]. For biochemical identification sugar fermentation, indole production, Methyl Red test, Voges – Proskauer test, Citrate Utilization test, Nitrate reduction test, triple sugar iron test, catalase test and Motility test were performed.
5. **Antibiotic Susceptibility Test:** Antibiotic resistance of *Salmonella typhi* isolates was assessed by disc diffusion assay technique as described by Achla *et al.*, (2005) [1]. The commercial antibiotics discs viz. Ampicillin, Ciprofloxacin, Ceftriaxone, Chloramphenicol, Cotrimoxazole, Cefixime, Norfloxacin, nalidixic acid, Gatifloxacin and Ofloxacin were placed on Nutrient Agar plates previously seeded with 18 hours broth culture of the *Salmonella typhi* isolates. The plates were incubated at 37 ^{0}C for 48 hours, after which zone of inhibition was examined (Bauer *et al*., 1966) [3] and was compared with CLSI standard.
6. **Statistical Analysis:** The data obtained from the present study were analyzed using two way analysis of variance (ANOVA), Z test and t test and the significance was tested against 5% F value and interpreted accordingly.

Results and discussion

1. Total incidence of *Salmonella typhi* in fruit juice samples: In the present study, total eighty (80) samples of orange (40) and pomegranate (40) were analysed for the presence of *Salmonella typhi*. Out of the 40 orange samples, 10 (25%) were found to be positive for the presence of *Salmonella typhi* and out of 40 pomegranate samples, 12 (30%) were found to be positive (Table 1, Figure 1).

Table 1: Total incidence of *Salmonella typhi* in fruit juices

Sample Type	Total Number of Samples	Total Number of Positive Samples	% Incidence
Orange	40	10	25
Pomegranate	40	12	30

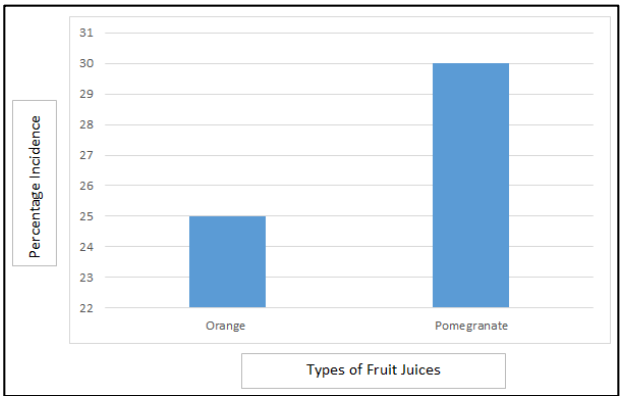

Fig. 1: Total incidence of *Salmonella typhi* in fruit juices

Statistically the difference was found to be non significant. several previous studies also indicate presence of *Salmonella typhi* in fruit juice samples (Golden *et al*., 1993; Wells and Butterfield, 1997; Lewis *et al*., 2006) [11, 34, 21] where they reported almost same incidence of *Salmonella typhi* in different juice samples. According to these studies main reason for the presence of *Salmonella typhi* in fruit juice could be improper handling and washing of the utensils, use of contaminated water and over exposure of the fruit samples in an open area which can enhance the incidence of pathogens in the fruit juice. Further, a slightly higher incidence of *Salmonella typhi* in pomegranate juice could be due to the process of juice extraction in which the seeds of pomegranate are already taken out and kept in the open condition, whereas orange juice is peeled just at the time of juice extraction.

2. Incidence of *Salmonella typhi* in different localities of Allahabad city: The samples of fruit juices were collected from different localities of Allahabad city. On the basis of the incidence of *Salmonella typhi* in different zones of Allahabad city, maximum incidence was found to be in east zone of Allahabad in the case of pomegranate (40%) followed by west zone (36.36%) than south zone (33.33%) and minimum incidence was found to be in north zone (10%). In orange juices maximum incidence (30%) was again found in east zone followed by south zone (27.27%) than west zone (22.22%) and minimum incidence was observed in north zone (20.00%). On comparing the incidence of *Salmonella typhi* in different fruit juice samples it was found that pomegranate samples were more contaminated with *Salmonella typhi* in comparison to orange juice samples. However, on analysis the data statistically, the difference in the incidence of *Salmonella typhi* in different fruit juices and different locations in Allahabad city was found to be non significant

Table 2: Incidence of *Salmonella typhi* in different localities of Allahabad city

Locations	Orange			Pomegranate		
	Total number of samples	Total number of +ve samples	% incidence	Total number of samples	Total number of +ve samples	% incidence
North	10	02	20.00	10	01	10.00
South	11	03	27.27	09	03	33.33
East	10	03	30.00	10	04	40.00
West	09	02	22.22	11	04	36.36

Lewis *et al.* (2006) [21] also reported that in the road side shops and recreational areas (Beaches and Parks) and busy market places (Shopping malls and bus stations etc.) the microbiological quality of fruit juices remains questionable. In both the fruit juice samples, maximum incidence was observed in east zone (Bahrana, Allahpur and Rambagh) where always heavy traffic of vehicles on road is observed. The shops situated in this zone are heavily exposed in the open air, which can allow entrance of microorganisms in fruit juices. There are several reports that suggests that pathogenic bacteria can also be transmit from contaminated water in fruit juices (Fernandes *et al*. 2000; Levine *et al*., 1991; Mazounie *et al*., 2000; Korhonen *et al*., 1996; Wang and Doyle 1998) [20, 23, 18, 33]. Since the Salmonella is water borne pathogens, it may be assumed that water used for the washing and other purposes by the handlers is contaminated with this pathogens. Similar environmental conditions were found in the other zones of Allahabad city, which support non significant difference in the incidence of *Salmonella typhi* in all the zones.

3. Viable count of *Salmonella typhi* in fruit juices sold in Allahabad City: The samples were also analysed for total viable count of *Salmonella typhi*. From the study it was revealed that orange juice samples were showing higher cell concentration ranging from $1.0x10^4$ to $42.0x10^4$ with an average of $16.9x10^4$ in comparison to pomegranate samples which were ranging from $2.0x10^4$ to $15.0x10^4$ with an average of $6.08x10^4$. On statistical analysis it was observed that the difference in cell count was significant (Table 3).

Sample type	Total number of samples	Viable count of *S. typhi* in fruit juices (10^4)		
		Minimum	Maximum	Average
Orange	40	01	42	16.9
Pomegranate	40	02	15	6.08

The presence of *Salmonella typhi* in fruit juice samples was already reported by many workers. Generally the fruit are normally supplied at moderate temperature i.e. 10 – 15 ^{0}C, pH varies between 3.8 – 7.6 depending on the type of the fruit. These conditions may favor the multiplication of bacteria in juices. Further for consumption raw juices are normally diluted at 1:0.1 – 1.0 ratio with water. For example orange juice has 1:1 water, pineapple and grsape 1:0.5 and 0.3 and mango 1:0.1 and pomegranate is diluted with milk (1:0.5). Lewis *et al*. (2006) [21] concluded that the addition of milk in pomegranate juice can be responsible for its lower Salmonella count in comparison of orange juice. This statement is also supported by the present finding where again lower Salmonella count is observed in pomegranate juice.

4. Antibiotic susceptibility pattern of *Salmonella typhi* isolates: all the studied *Salmonella typhi* isolates were subjected for antibiotic sensitivity pattern. From the study it was observed that out of 22 *Salmonella typhi* isolates, all the isolates were found to be sensitive for chloramphenicol and ofloxacin with percentage sensitivity of 100%. For ciprofloxacin, co-trimoxazole and gatifloxacin, isolates showed same percentage sensitivity i.e. 95.45% (Figure 2).

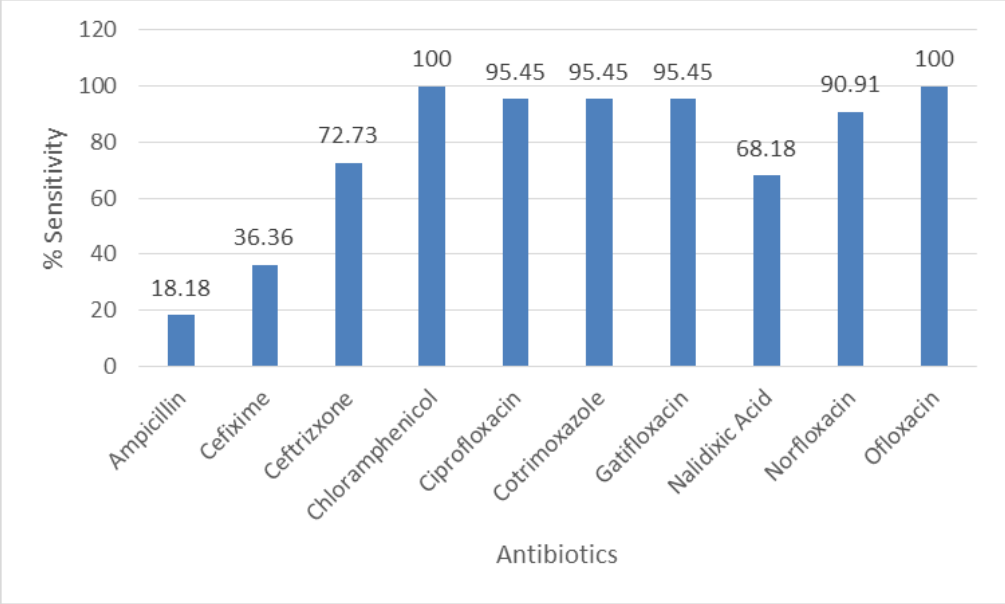

Fig. 2: Percentage sensitivity pattern of *Salmonella typhi* isolates

However, in India, by the mid 1990s, reports begin to appear of treatment failure with ciprofloxacin followed by reports from 2001 onwards of rising MICs of ciprofloxacin of *S. typhi* isolates. In 2008 there are reports of high level ciprofloxacin resistant *Salmonella enterica* from many centres in India (Daga *et al*., 1994; Jesudesan *et al*., 1996; Harish *et al*., 2004; Harish *et al*., 2006; Dutta *et al*., 2008) [7, 16, 12, 13, 8]. Further, isolates showed 90.91% sensitivity towards norfloxacin, 72.73% for ceftriaxone, 68.18% for nalidixic acid, 36.36% for cefixime and minimum sensitivity was observed against ampicillin i.e. 18.18%. Further from the study it was also observed that out of 22 isolated *Salmonella typhi* isolates only one was found to be multi drug resistant organism because it showed resistance towards three antibiotics viz. ampicillin, cefixime and nalidixic acid. MDR Salmonella serovar typhi isolates commonly harbor a plasmid of incompatibility group HI1. A 365-bp region of RepHI1A region was detected in MDR strains of Salmonella serovar typhi isolated in India (Shanahan *et al*., 1998) [29]. oriT is located within the Tra1 region of the plasmid and contains the nic site, which is one of many genes required for conjugative transfer of IncHI1 plasmid (Lawley *et al*., 2002) [19]. However other isolates showed sufficient sensitivity against studied antibiotics. Drug resistant *Salmonella typhi* has been reported in India since 1960 and outbreaks of these strains occur at intervals in various parts of india (Sridhar *et al*., 1983). Achla *et al*., (2005) [30, 1] reported that sporadic outbreaks of typhoid fever are associated with heterogenous isolates of *Salmonella typhi* and they observed shifts among the three population of *Salmonella typhi*. Like the present study, the authors also reported 100% sensitivity against chloramphenicol and ofloxacin. There are several reports that suggest that change in the levels of antimicrobial resistance after selection pressure of the drug reflect increased exposure of bacteria to antimicrobial compounds over the past several decades and resistance may immerged in vivo during treatment (Shanaham *et al*., 1998; Chomal and Deodhar., 2000; Threlfall *et al*., 2001; Madhulika *et al*., 2004) [29, 6, 31, 12].

Conclusion

The contamination of juice samples could be due to poor quality of water used for the dilution as well as prevailing unhygienic condition related to washing of utensils and maintenance of the premises. The locations by the side of the busy road with heavy vehicular traffic (air borne particle) or by the side of waste disposal system should be avoided for establishing a street vended juice shop. The emergence of multi drug resistant *Salmonella typhi* is becoming a great threat to modern chemotherapeutics and has gained considerable attention. Lack of sanitary condition in street vended juice shops and the occurrence of pathogenic *E. coli* 0157:H7, *Shigella* and *S. typhimurium* is alarming and an immediate action by the suitable agency must be taken. Regular monitoring of the quality of fruit juice for human consumption must be introduced to avoid any future pathogens outbreaks. Further investigation will be required to develop efficient methods for controlling the MDRST outbreaks in tropical countries such as India.

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