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## Human bacteria in street-selling fruit juices in Chandrapur city (M.S.), India

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### ABSTRACT

Consumption of bacterial contaminated street selling fruit juices have adverse effect on human health. A study based on standard technology aims to test the quality and safety of freshly squeezed juices in the Chandrapur city, Maharashtra. The objective of this study was to evaluate the quality of fruit juices. The presence of faecal and non-faecal coliform indicates quality of fruit juices. The samples were collected from 5 crowded areas in Chandrapur city where fruit juices have high demands. The study showed that fruit juices sold on street are poor in quality due to presence of bacteria such as *Escherichia coli*, *Streptococcus*, *Salmonella typhi* etc. It is observed that fruit juices sold in city e.g. Janata college, Main bus stop, Anchaleshwar gate, Sardarpatel mahavidyalaya and Gandhi market are contaminated and unhealthy for human consumption. The overall study concluded that contamination is mainly due to the unhygienic conditions associated with utensils, poor quality of fruits, industrialization, waste disposal, heavy traffic, crowding and poor quality of water used for dilution. The consumption of contaminated fruit juices are unhealthy to human and take immediate action by Municipal Corporation. It was put forward that regular monitoring of quality of street selling fruit juices to avoid outbreaks of pathogen in the future.

**Keywords:** Fruit juices, Chandrapur, food pathogen, coliform, gram staining.

### INTRODUCTION

Fruit juices are high importance due to rich source of vitamins and minerals. Fruit juices are common beverages and sold at public places and roadside shops. It is ready to eat and becomes a public health threat due to poor quality of water, handling and cleaning. In India, many cases of food borne illness are reported by the consumption of fruit juices (Sandeep M. *et al.*, 2001). Inappropriate washing of utensil and fruits increase the possibility of contamination. In addition, use of poor quality of water and ice for dilution, unhygienic surroundings, poor quality of fruits, prolonged preservation, swarming fruit flies and houseflies, vehicle traffic and dust also act as a source of infection. The numbers of bacteria present in fruits are high and counts up to  $1.0 \times 10^5$  CFU/cm<sup>2</sup> (Harrigan, 1998). The study on fruit juices sold at Visakhapatnam showed *E. coli* (27.7%), *Shigella* (16.6%), *Salmonella* (38.8%) and *Streptococcus faecalis* 6.2% (Joy E. Lewis *et al.*, 2006). Contaminated fruit juices shown potential source of bacterial pathogen notably *E. coli* O157:H7, *Salmonella*, *Shigella* and *Streptococcus aureus* (Buchanan *et al.*, 1999; Ryu *et al.*, 1998; Sandeep *et al.*, 2001). Fruit juices sold by roadside vendors are contaminated as compared to stored juices sold at shops of company brand (Sharma, 2013). While the infectious dose for these bacteria

contained in fruit juices is not yet fixed, depend on standard provided for drinking water (ISI standards, 1973; ICMR, 1975; WHO, 1984), the bacterial number requires causing infection are low with reference to *coliform* and *streptococci*. Chandrapur (Lat. 19.950°N; Long. 79.297°E) is fort city and located in central India in the eastern part of Maharashtra state. The city are located in tropical reagon, due to hot and dry climate there is always a great demand for fruit juices and fresh vegetables. The consumption of fruit juices has both positive and negative effects on consumers. The consumption of good quality of fruit juices help to enhance immune power and protect from dehydration. The hot wheather continues for a greater part of the year (February to July) increasing high demands of fruit juices in city. Although most cafes and restaurants provide juices under hygienic conditions, in the market, near colleges and roadside shops their microbiological quality remains questionable. In this shops, juices extracted by squeezing from variety of fruits including oranges, mangoes, grapes, pineapple, sugarcane are served after dilution with water and ice. However, in the absence of good manufacturing practices the nutrient richness of fruit juices are good medium for bacterial growth and leads to food borne illness. In city, many cases of food borne illness are reported. In view of the high demand for fruit juices in many areas in the city a rapid review of street selling fruit juices was undertaken in view to assess their safety for human consumption and possible source of bacterial contamination.

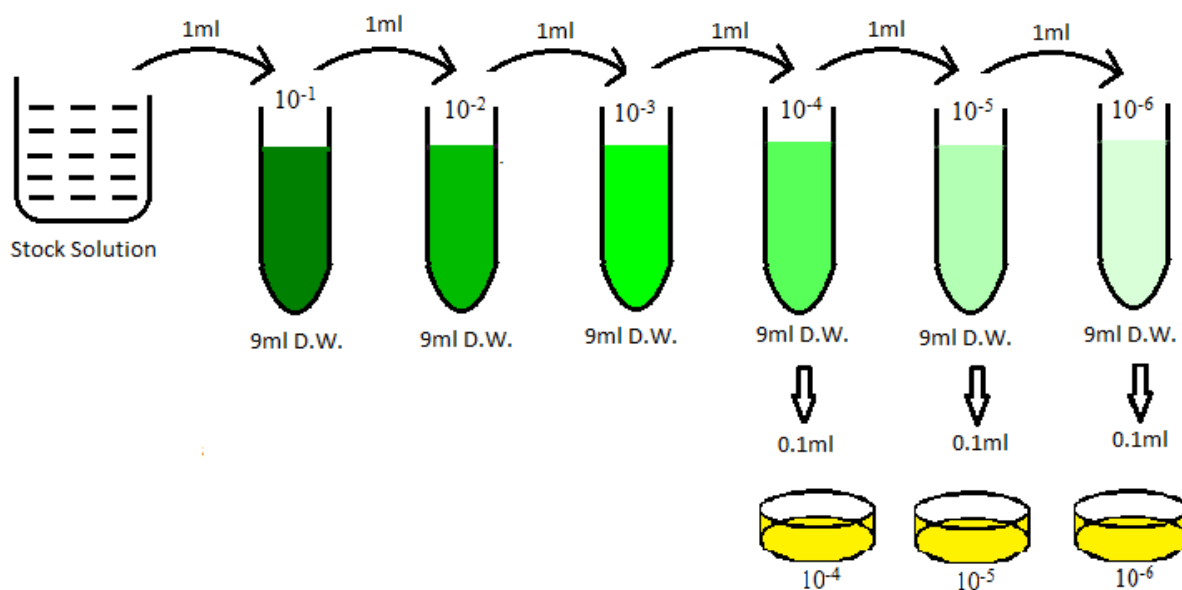
## MATERIALS AND METHODS

### Sample Collection

During the study, 5 locations in the city were chosen for collection of samples. These area are: Area 1 Janata College, Area 2 Main bus stop, Area 3 Anchaleshwar gate, Area 4 Sardar Patel Mahavidyalya, and Area 5 Gandhi market. Total 5 juice sample were collected from different local street shop. 4 varieties of fruit juices namely pineapple, sugarcane, mango and orange were chosen based on the consumer demand. All samples were collected in sterile containers kept at 4°C and analysed within an hour after collecting.

### Serial Dilution

1ml of juice sample was added into 9ml of sterile distilled water to prepare stock solution. Then the test tubes were labelled as  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ . After that 1ml from the stock solution transferred into first tube which was  $10^{-1}$  and shaken well in order to get equal distribution of microorganism. And then, 1ml from first test tube i.e.  $10^{-1}$  was transferred into second test tube  $10^{-2}$  and again shaken. The procedure was repeated to complete serial dilution up to  $10^{-6}$ .



### Spread Plate Method

After completion of serial dilution, three petri plates containing Nutrient agar are prepared and labelled as a  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ . After that 0.1ml from test tube  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  were transferred to respective plates and spread the sample with the help of spreader. All the plates were incubated at 37°C for 24-48 hours. Finally, the plates showing colonies were counted and noted down for further study.

### Gram staining

Gram staining was done by using standard procedure given by Christen Gram to differentiate between gram positive and gram negative bacteria. The purple colour single colony indicated bacteria were gram positive whereas pink colour indicated gram negative bacteria.

### Motility test

The motility test for isolates are performed by the Hanging drop method.

### Biochemical analysis

To confirm and characterize the isolates several biochemical tests were carried out. Most of the methods were done according to the microbiological laboratory manual (Cappuccino & Sherman, 2005). The biochemical tests performed were IMViC test (Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test), Triple sugar iron test, oxidase and catalase test.

### Bacterial Identification

Identification and confirmation of bacteria were made using the growth in selective media such as Nutrient agar, MaConkey agar (M298, Himedia) and Eosin methylene blue agar (M317, Himedia) for *E. coli*157:H7, Baird Parker agar (M043, Himedia) for *Staphylococci*, *Salmonella-Shigella* (M108D, Himedia) agar for *Salmonella* and *Shigella* species.

### Result and Discussion

A total of 15 samples from 5 locations were examined. A summarized account of result obtained for the microbiological analysis of the juices is given in tables 1, 2 and 3. Overall all examination of samples showed contamination by pathogenic bacteria.

Table 1. Viable count of colony present in different fruit juice sample

Sr. No	Sample	No. of bacterial colony	CFU/ml
1	Janata College	150	$6 \times 10^4$
2	Main bus stop	180	$7.2 \times 10^5$
3	Anchaleshwar gate	160	$6.4 \times 10^5$
4	Sardar Patel Mahavidyalya	190	$7.6 \times 10^5$
5	Gandhi market	220	$8.8 \times 10^5$

Table 2. Morphological Characteristics of bacterial colonies, gram reaction and motility

Bacterial Isolates	Colour on Nutrient agar	Configuration	Margin	Elevation	Gram reaction	Shape of isolates	Motility
1	White	Circular	Irregular	Flat	Positive	Rods in chain	Motile
2	Mucoid	Circular	Entire	Slightly raised	Negative	Rod	Motile
3	White	Circular	Irregular	Flat	Positive	Rods in chain	Motile
4	Yellow	Circular	Entire	Convex	Positive	Cocci	Non-Motile
5	White	Circular	Irregular	Flat	Positive	Rods in chain	Motile
6	Off white	Irregular	Undulate	Flat	Positive	Cocci	Motile
7	Mucoid	Circular	Entire	Slightly raised	Negative	Rod	Motile
8	Yellow	Circular	Entire	Convex	Positive	Cocci	Non-Motile
9	Off white	Irregular	Undulate	Flat	Positive	Cocci	Motile
10	Mucoid	Circular	Entire	Slightly raised	Negative	Rod	Motile
11	Off white	Circular	Entire	Flat	Negative	Cocci	Non-Motile
12	Off white	Irregular	Undulate	Raised	Negative	Long single rod	Non-Motile
13	Off white	Circular	Entire	Flat	Negative	Cocci	Non-Motile
14	Off white	Irregular	Undulate	Raised	Negative	Long single rod	Non-Motile

Table 3. Biochemical Characteristics of bacterial isolates

Bacterial Isolates	Catalase	Oxidase	Indole	Methyl red	Vogesproskauer	Citrate utilization test	Glucose	Lactose	Sucrose	Organism Interpretation
BI-1	-	-	+	-	+	-	-	-	-	<i>Bacillus spp.</i>
BI-2	+	-	+	+	-	+	-	+	+	<i>Escherichia coli</i>
BI-3	-	-	+	-	+	-	-	-	-	<i>Bacillus spp.</i>
BI-4	+	-	+/-	-	-	-	-	+	+	<i>Staphylococcus spp.</i>
BI-5	-	-	+	-	+	-	-	-	-	<i>Bacillus spp.</i>
BI-6	+	+	+	+	-	+	-	-	-	<i>Salmonella spp.</i>
BI-7	+	-	+	+	-	+	-	+	+	<i>Escherichia coli</i>
BI-8	+	-	+/-	-	-	-	-	+	+	<i>Staphylococcus spp.</i>
BI-9	+	+	+	+	-	+	-	-	-	<i>Salmonella spp.</i>
BI-10	+	-	+	+	-	+	-	+	+	<i>Escherichia coli</i>
BI-11	+	-	-	+	-	+	-	-	-	<i>Shigella spp.</i>
BI-12	+	-	-	-	+	+	-	+	+	<i>Klebsiella spp.</i>
BI-13	+	-	-	+	-	+	-	-	-	<i>Shigella spp.</i>
BI-14	+	-	-	-	+	+	-	+	+	<i>Klebsiella spp.</i>

After biochemical tests, it was found that, 15 fruit juice sample were contaminated by pathogenic bacteria. *Escherichia coli* and *Bacillus spp.* were found in 10 different juice samples,

*Staphylococcus spp.* were found in 8 different fruit juice samples, *Salmonella spp.* and *Shigella spp.* were found in 6 different sample, *Klebsiella spp.* in 5 different samples. *Vibrio cholera* was not encountered in any one of the sample. The examination of fruit juices also showed that pathogenic bacterial counts were significantly high in sugarcane, pineapple and mango. The high ambient temperature reduce shelf life fruit juice and seems to favour the growth of bacteria. The sample collected from Area 2 Main bus stop, Area 5 Gandhi market and Area 3 Anchaleshwar gateshows high contamination as compare to Area 1 Janata College and Area 4 Sardar Patel Mahavidyalya. It is observed that, the high contamination of fruit juices is because of heavy vehicular traffic, high crowd and unhygienic location of shops.

Overall, the result of study showed that all the fruit juices sold on street were contaminated by pathogenic bacteria. It is concluded that contamination is mainly due to unhygienic condition, poor quality of water used for dilution, lack of sanitization, heavy traffic and industrialization. The presence of pathogenic *E. coli O157:H7*, *Salmonella spp.* and *shigella spp.* in juice samples showed that consumption of fruit juices are unhealthy to humans and needs immediate action. It was put forward that regular monitoring of quality of street selling fruit juices to avoid outbreaks of pathogen in the future.

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