ISOLATION AND CHARACTERIZATION OF FAECAL COLIFORMS IN STREET VENDED FRUIT JUICES AND ITS SAFETY EVALUATION: A CASE STUDY OF BELLARY CITY, INDIA.

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Summary

The present study was undertaken for detection, isolation, characterization of the possible sources of faecal coliforms in street vended fresh fruit juices sold along the road sides of Bellary city, India and assessed its safety for human consumption. The samples of orange fruit juices were collected from the four different public hot spot areas of the city and subjected them for Most Probable Number [MPN] test to estimate the coliform population in both water and food (juice) samples. The study was further extended for the isolation of coliforms by a selective solid media and then identified the presence of different types of Enterobacteria by using rapid biochemical tests. Based on the MPN studies, juice sample-1 was found to be most contaminated with a count of 1, 40,000 coliforms/ 100 ml, sample -3 with a count of 1,10,000 coliforms/ 100 ml, sample-2 was 1,500 coliforms/100ml. The least count of only 400 coliforms/100ml was observed in sample-4. Where as, the water samples-1, 2 and 3 were also found to be totally contaminated with faecal coliforms with a count of 1,100 microbes per 100ml. But sample-4 contains only 28 microbes/ 100 ml. The colony characteristics, microscopic examination as well as rapid biochemical studies showed the presence of four different pathogenic coliforms in all four food and water samples. They are *Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogens* and *Escherichia coli.* The MPN analysis showed high levels of contamination in first three fruit juices as well as water samples. This would be possible because of the poor quality of water used in juice preparation. Moreover, water is one of the major sources of sewage contamination.

Key Words: Street vended fruit juices, Faecal coliforms, Bacterial contamination.

Introduction

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In many tropical countries they have become common man's beverages and are sold at all public places along roadside shops (1). In India, especially in the metropolitan and other cities a huge section of the population of all income and age groups consume fresh pressed and squeezed juices, most of these juices are sold by street vendors (2). Most fruits contain bacterial counts up to 1.0 X 10^5 cm^2 on their surfaces. Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notable *E. coli* 0157:H7, species of *Salmonella, Shigella* and *Staphylococcus aureus* (1). The presence of coliforms on the surface of vegetables is indicative of fecal contamination (3). Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illness (2). Food borne disease outbreaks from enteropathogenic bacteria, such as *Salmonella, Vibrio cholerae, Vibrio parahaemolyticus* and *Staphylococcus aureus* are common cases of food borne infection throught the world (4). Foods and beverages prepared and sold by street vendors have contributed to transmission of cholera and enteric diseases in Latin America. Cholera transmission was associated with consumption of street-vended beverages in Peru, Thailand (5-6), Ecuador (7) and Guatemala (8). The normal habitat of faecal coliforms is the intestinal tracts of man and animals and they are not known to be found in nature in the absence of faecal contamination from the above sources. They are excluded out of animal body through excretion process, in the form of faeces. Some of them are pathogenic and cause diseases like typhoid, dysentery and enteric fever etc. Thus, the presence of these organisms in water and fruit juices is dangerous for human consumption (9).

In view of the great demand for fresh fruit juices during summer in Bellary city, present study was undertaken for detection and identification of possible sources of faecal coliforms in street vended fresh fruit juices sold along the road sides and assessed its safety for human consumption.

Materials and Methods

In the present study, the different parameters were undertaken to evaluate the safety level in street vended fruit juices sold along the road sides at four different public places of Bellary city. MPN tests, microscopic examination, colony characteristics and rapid biochemical tests were used here for detection and identification of the presence of different types of coliforms in freshly squeezed fruit juices.

Collection of the samples: The fresh squeezed orange juices as well as water samples (used in preparation of juices) were collected aseptically from four different public hot spot areas of Bellary city, India. are vegetable market, Royal circle, Gandhi Nagar market and Cantonment areas and placed them in sterile containers, stored at 4°C, then transferred to the laboratory for sample analysis.

Serial Dilution Technique: For sample analysis, 1ml of homogenized juice was diluted ten folds using sterile distilled water and then this was further diluted serially to 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). These dilutions of fruit samples are necessary to perform MPN counts and plating to obtain isolated colonies (10).

MPN of Food: The MPN test is a combination of presumptive, confirmed and complete tests. It is used to detect and estimate coliform population in a food / or juice sample. This method employs the use of Lauryl sulphate Tryptose Broth (LTB) for presumptive test and Brilliant Green Lactose Bile Broth (BGLBB) for confirmed and complete tests. 0.1 ml of (10^{-3}, 10^{-4} and 10^{-5}) each dilution was inoculated into three test tubes of LTB and a Durham's tube. After incubation period of 24 hours the number of tubes in each set of three that show positive for acid and gas production was counted. A count of number of tubes showing positive results for complete test were noted, this count gives the MPN ratio which when checked against standard probability table gives us the MPN for the sample. Calculation of MPN was done from the complete test results table ratio of the number of tubes positive in each of the three dilutions gives the MPN ratio. The same MPN ratio was checked against standardized MPN probability tables to obtain the Most Probable Number of coliforms for the sample (10).

MPN of water: A modified procedure was used to calculate the MPN of water. In this test, we used double strength (ds) and single strength (ss) LTB for the presumptive and single strength BGLBB for the confirmed and complete tests. Serial dilution of the water sample was not made. Instead, we directly used 10ml, 1ml and 0.1ml to the media tubes (10).

Isolation: All the positive tubes (obtained in MPN food and water tests) were sub-cultured onto to Blood agar, Eosin Methylene Blue [EMB] agar, Mac Conkey's agar, Nutrient agar, Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 18-24 hours. Four typical characteristic colonies were picked up and identified them as coliform bacteria based on their unique cultural characteristics. In addition to these studies, organisms of suspected colonies were further examined microscopically for Gram staining and bacterial motility (10).

Rapid Biochemical tests: To confirm the identity of different members of Enterobacteriaceae, a standard combination of four tests were used, namely Indole test, Methyl Red test, Voges-Proskauer test and Citrate test. In addition to these, few carbohydrates fermentation tests were also undertaken. They are Glucose fermentation, Adonitol fermentation, Arabinose fermentation, Lactose fermentation, Sorbitol fermentation, Mannitol fermentation, Rhamnose fermentation and Sucrose fermentation.

The interpretation of the results of these biochemical tests were made as per the standard table-1.

Table-1 Interpretation table for positive results.

Test	Biochemical Reaction	Colour change of medium
IMViC tests		
Indole	Tryptophanase production/ Indole production from tryptophan	Cream to pink
Methyl Red	Mixed acid production from glucose	Cream to Red
Voges-Proskauer.	Voges-Proskauer reagent to detect acetoin	Cream to Red
Citrate	Citrate used as sole carbon source	Green to Blue
Carbohydrate Fermentation		
Glucose	Glucose fermentation	Red to yellow
Adonitol	Adonitol fermentation	Red to yellow
Arabinose	Arabinose fermentation	Red to yellow
Lactose	Lactose fermentation	Red to yellow
Sorbitol	Sorbitol fermentation	Red to yellow
Mannitol	Mannitol fermentation	Red to yellow
Rhamnose	Rhamnose fermentation	Red to yellow
Sucrose	Sucrose fermentation	Red to yellow

Results

MPN Food (Juice): The presumptive, confirmed and complete test results of juice sample-1 showed that, all the tubes were positive for all the three dilutions (10^{-3}, 10^{-4} and 10^{-5}). This has given the MPN ratio as 3:3:3. and its concurrent MPN probability table value was found to be 140+ coliforms/ inoculum of the sample. This indicates that sample-1 was highly contaminated. In case of sample-2, the presumptive, confirmed and complete tests showed positive in two tubes but absent in one tube to 10^{-3} dilution. For 10^{-4} dilution, only one tube was positive to all the three tests, remaining two tubes were negative to all tests. In case of 10^{-5} dilution all three tubes were negative to all the three tests, hence the MPN ratio of sample-2 was 2:1:0 and its concurrent probability table value was 1.5 coliforms /inoculum of the sample. Similarly the MPN ratio of sample-3 and sample- 4 were 3:3:2 and 1:0:0 respectively and their concurrent probability table value was 110.0 coliforms/ inoculum of the sample-3 and 0.4 coliforms / inoculum of the sample-4 (Table-2; Fig-1).

MPN water: The results obtained from Presumptive, confirmed and complete test reports showed that all the tubes of water sample-1, 2 and 3 were positive for all the three concentrations (ie., 10ml, 1ml and 0.1ml). and displayed the MPN ratio as 3:3:3. Thus, the MPN probability table value was found to be 1,100+ coliforms/ 100ml of the sample. Where as, the MPN ratio obtained for sample-4 was 2:2:1 and its concurrent probability table value was 28+ coliforms / 100ml of the sample (Table-3).

Isolation: The morphological colony characteristics of bacteria were studied on different types of selective solid media namely, Blood agar, Eosin Methylene Blue agar, Mac Conkey agar, Nutrient agar and Xylose Lysine Deoxycholate agar. The characteristics of the colonies were appeared to be small sized, with smooth surface. The majority of the colonies in different selective media were round in shape. The elevation may differ from raised, convex and flat types for different enterobacteria. The surface of the colonies was smooth due to the presence of capsule around the organisms. This confirms that, these organisms are highly pathogenic in nature. In case of blood agar media, the characteristic colouration of the colonies are grey, for EMB agar medium – the colonies are green metallic sheen, Mac Conkey agar – pale pink colour, nutrient agar- transparent colonies and XLD agar – yellow colouration leads to the indication of positive for fecal coliforms. The characteristic specificity of these coliforms is summarized in detail into the tables (Table-4 and Table-5).

Biochemical Tests: Based on the rapid biochemical tests, the identities of the coliform bacteria on different selective media of both food and water samples were confirmed (Table -6 and Table-7). The isolated colonies were subjected to Indole test, Methyl Red test, Voges-Proskauer test, citrate test, carbohydrate fermentation tests such as glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose fermentation tests. *Klebsiella pneumoniae* showed positive to Voges-Proskauer test, citrate test, glucose fermentation, adonitol fermentation, arabinose fermentation, lactose fermentation, sorbitol fermentation, mannitol fermentation and sucrose fermentation. Where as, *Citrobacter freundii* displayed positive to Methyl Red test, citrate test, glucose, arabinose, lactose, sorbitol and sucrose fermentation tests. In case of *Enterobacter aerogens* responded positively to some of the biochemical tests like, Voges-Proskauer test, citrate as well as all the carbohydrate fermentation tests. *Escherichia coli* showed positive response to Indole test, Methyl Red test and the entire all the carbohydrate fermentation tests, except, adonitol fermentation test. Based on the microscopic studies it was confirmed that, all the above identified coliform bacterias were found to be Gram negative as well as motile in nature, except *Klebsiella pneumoniae was* found to be non motile (Table-8).

Discussion

MPN results showed that sample-1 was most contaminated with a count of 1, 40,000 coliforms per 100 ml of the juice sample. The second highest contamination was seen in juice sample-3 with a count of 1, 10,000 coliforms per 100 ml. The third highest contamination was observed in juice sample-2 with count of 1,500 coliforms per 100 ml. The least contamination was observed in juice sample- 4 with a count of 400 coliforms per 100 ml. The first three water samples were also found to be totally contaminated with a count of 1,100+ microorganisms per 100 ml. Based on the above studies, it has been confirmed that, water used in the preparation of fruit juices was highly contaminated with faecal coliforms. In addition to this, the contamination of juices was also due to the use of unhygienic conditions of water storage and use of unclean utensils and unhygienic physical and biological contaminants. The results of the present studies clearly indicated the presence of four different types of fecal coliforms namely, *Klebsiella pneumonia, Citrobacter freundii, Enterobacter aerogens* and *Escherichia coli* in all fruit juice

samples. These organisms are highly pathogenic and may cause serious diseases in human beings. *Klebsiella pneumonia* species cause urinary tract infections, chronic broncho-pulmonary diseases, pneumonia, septicemia, meningitis etc. *Citrobacter freundii* causes urinary tract infections, infections in gall bladder, middle ear etc. *Enterobacter aerogens* are responsible for urinary tract infections and hospital sepsis etc. *Escherichia coli* cause diarrhea, urinary infections, pyogenic infections and septicemia etc (11-13). Several researchers contributed similar type of investigations in different places with different street vended fruit and vegetable juices. Tambekar *et al* (2009) (14) reported the food borne illness associated with the consumption of road side freshly squeezed fruit juices at public places in Amaravati city, India and samples were also analyzed for the presence of dominant enteric bacterial pathogens were *Escherichia coli* (40%), followed by *Pseudomonas aeruginosa* (25%), *Salmonella* spp (16%), *Proteus* spp (9%), *Staphylococcus aureus* (6%), *Klebsiella* spp (3%) and *Enterobacter* spp (1%). Sandeep *et al.,* (2004) (2) have detected total *Staphylococcus* counts or coliform counts in three samples of carrot juices and Kinnow-mandarin juices obtained from two different areas of the Patiala city. Moushumi *et al.,* (2004) (15) explained the presence of faecal coliforms in fresh squeezed carrot juices and explained the possible entry points of bacterial pathogens in carrot at several points during course in the distribution chain and hence into carrot juice. Overall the results of the present study indicate that, majority of the street vended fresh fruit juices in many parts of the city showed contamination with faecal coliforms.

Conclusion

The MPN analysis showed high levels of contamination in juice samples sold along the road sides of Bellary city. This would be possible because of the poor quality of water was used in juice preparation; moreover, water is one of major sources of sewage contamination. The results of the present findings clearly demonstrated that, the road side ready fresh juices did not meet public health standards and many kinds of enteropathogenic bacteria were found namely, *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii* and *Enterobacter aerogens.* Such foods lead to hazardous effects to the consumers. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for juice preparations, especially street vended fruit juices.

Table-2 MPN result sheet of four different orange fruit juice samples

Samples	Dilution	Incubation period (hrs)	10^{-3}			10^{-4}			10^{-5}		
			1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube
Sample No-1 (Food) (vegetable market)	Presumptive Test	24	+	+	+	+	+	+	+	+	+
	Confirmed Test	24	+	+	+	+	+	+	+	+	+
	Completed Test	24	+	+	+	+	+	+	+	+	+
Sample No-2 (Food) (Royal circle)	Presumptive Test	24	+	+	−	+	−	−	−	−	−
	Confirmed Test	24	+	+	−	+	−	−	−	−	−
	Completed Test	24	+	+	−	+	−	−	−	−	−
Sample No-3 (Food) (Gandhi Nagar Market)	Presumptive Test	24	+	+	+	+	+	+	+	−	−
	Confirmed Test	24	+	+	+	+	+	+	+	+	−
	Completed Test	24	+	+	+	+	+	+	+	+	−
Sample No-4 (Food) (Cantonment)	Presumptive Test	24	+	−	−	−	−	−	−	−	−
	Confirmed Test	24	+	−	−	−	−	−	−	−	−
	Completed Test	24	+	−	−	−	−	−	−	−	−

Table-3 MPN result sheet of four different water samples (used in juice preparation)

Samples	Dilution	Incubation period (hrs)	10ml			1ml			0.1ml		
			1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube	1st tube	2nd tube	3rd tube
Sample No-1 (Water) (vegetable market)	Presumptive Test	24	+	+	+	+	+	+	+	+	+
	Confirmed Test	24	+	+	+	+	+	+	+	+	+
	Complete Test	24	+	+	+	+	+	+	+	+	+
Sample No-2 (Water) (Royal circle)	Presumptive Test	24	+	+	+	+	+	+	+	+	+
	Confirmed Test	24	+	+	+	+	+	+	+	+	+
	Complete Test	24	+	+	+	+	+	+	+	+	+
Sample No-3 (Water) (Gandhi Nagar Market)	Presumptive Test	24	+	+	+	+	+	+	+	+	+
	Confirmed Test	24	+	+	+	+	+	+	+	+	+
	Complete Test	24	+	+	+	+	+	+	+	+	+
Sample No-4 (Water) (Cantonment)	Presumptive Test	24	+	+	−	+	+	−	+	−	−
	Confirmed Test	24	+	+	−	+	+	−	+	−	−
	Complete Test	24	+	+	−	+	+	−	+	−	−

Table-4 Colony characteristics of fecal coliforms of food samples (orange juice) on selective agar media

Colonies on selective media	Size of the colony	Whole colony	Edge	Elevation	Surface	Colour	Conclusion
EMB agar	Small	Round/ Round with raised margin	Smooth	Raised	Smooth	Opaque green metallic sheen	Lactose fermenting coliforms, especially E.coli.
Blood agar	Small	Round with raised margin	Smooth	Convex	Smooth	Opaque grey colour colonies	Non-haemolytic on blood agar
Nutrient agar	Small	Round	Smooth	Convex / Raised	Smooth	Transparent colourless colonies	Bacteria present in sample.
XLD agar	Small	Round / L-form	Smooth	Flat	Smooth	Opaque yellow colonies	Xylose fermenting, lysine non-decarboxylating bacteria
Mac Conkey agar	Small	Round / Round with raised margin	Smooth	Raised / Crateriform	Smooth	Opaque pink colour colonies/ Transparent colourless colonies	Lactose fermenting organisms

Table-5 Colony characteristics of fecal coliforms of water samples on different selective agar media

Colonies on selective agar media	Size of the colony	Whole colony	Edge	Elevation	Surface	Colour	Conclusion
EMB agar	Small	Round / L-form	Smooth	Raised / Flat	Smooth	Opaque green metallic sheen / Dark centered metallic sheen	Lactose fermenting coliforms,especially E.coli.
Blood agar	Small	Round	Smooth	Convex	Smooth	Opaque grey colour colonies	Non-haemolytic on blood agar
Nutrient agar	Small	Round	Smooth	Convex	Smooth	Transparent colourless colonies	Bacteria present in sample.
XLD agar	Small	Round	Smooth	Flat	Smooth	Opaque yellow colonies	Xylose fermenting, lysine non-decarboxylating bacteria
Mac Conkey agar	Small	Round with raised margin	Smooth	Drop like /Raised	Smooth	Opaque pink Colonies / Transparent colourless colonies	Lactose fermenting organisms

Table-6 Biochemical profile of Food / Juice samples on selective Mac Conkey, Eosin Methylene Blue agar media Nutrient agar

Tests	Mac Conkey Agar			Eosin Methylene Blue Agar			Nutrient Agar		
	Colour change of medium	**Biological Reaction**	**Results**	**Colour change of medium**	**Biological reaction**	**Results**	**Colour change of medium**	**Biological reaction**	**Results**
IMViC test				**IMViC test**			**IMViC test**		
Indole	No change of colour	Indole was not produced	Negative	No colour change	Indole was not produced	Negative	Cream to Red	Indole was produced	Positive
Methyl red	Cream to yellow	Mixed acid was not produced	Negative	Cream to Yellow	Mixed acid was not produced	Negative	Cream to Red	Mixed acid was produced	Positive
Voges-Proskauer	Cream to Pink	Acetoin was produced	Positive	Cream to Pink	Acetoin was produced	Positive	No colour change	Acetoin was produced	Negative
Citrate	Green to Blue	Carbon utilized	Positive	Green to Blue	Carbon utilized	Positive	No colour change	Carbon was not utilized	Negative
Carbohydrate Fermentation Test				**Carbohydrate Fermentation Test**			**Carbohydrate Fermentation Test**		
Glucose	Red to Yellow	Utilization of glucose	Positive	Red to Yellow	Utilization of glucose	Positive	Red to Yellow	Utilization of glucose	Positive
Adonitol	Red to Orange	Adonitol utilized	Positive	Red to Yellow	Adonitol utilized	Positive	Red to Pink	Adonitol was not utilized	Negative
Arabinose	Red to Yellow	Arabinose utilized	Positive	Red to Yellow	Arabinose utilized	Positive	Red to Yellow	Arabinose utilized	Positive
Lactose	Red to Yellow	Lactose utilized	Positive	Red to Yellow	Lactose utilized	Positive	Red to Yellow	Lactose utilized	Positive
Sorbitol	Red to Orange	Sorbitol utilized	Positive	Red to Orange	Sorbitol utilized	Positive	Red to Orange	Sorbitol utilized	Positive
Mannitol	Red to Pink	Mannitol is not utilized	Negative	Red to Yellow	Mannitol utilized	Positive	Red to Orange	Mannitol utilized	Positive
Rhamnnose	Red to Yellow	Rhamnose utilized	Positive	Red to Yellow	Rhamnose utilized	Positive	Red to Yellow	Rhamnose utilized	Positive
Sucrose	Red to Yellow	Sucrose utilized	Positive	Red to Yellow	Sucrose utilized	Positive	Red to Orange	Sucrose utilized	Positive

Table-7 Biochemical profile of water samples on selective Xylose Lysine Deoxycholate and Eosin Methylene Blue agar media

Test	Xylose Lysine Deoxycholate agar			Eosin Methylene Blue Agar media		
	Colour change of medium	**Biological Reaction**	**Results**	**Colour change of medium**	**Biological Reaction**	**Results**
	IMViC test			**IMViC test**		
Indole	No colour change	Indole is not produced	Negative	No colour change	Indole is not produced	Negative
Methyl Red	Cream to Yellow	Mixed acid was not produced	Negative	Cream to Red	Mixed acid was produced	Positive
Voges-Proskauer	Cream to pink	Acetoin is produced	Positive	No colour change	Aacetoin was not produces	Negative
Citrate	Green to Blue	Carbon utilized	Positive	Green to Blue	Carbon utilized	Positive
Carbohydrate Fermentation Test				**Carbohydrate Fermentation Test**		
Glucose	Red to Orange	Slow utilization of glucose	Positive	Red to Yellow	Utilization of glucose	Positive
Adonitol	Red to Yellow	Adonitol utilized	Positive	Red to Pink	Adonitol was not utilized	Negative
Arabinose	Red to Yellow	Arabinose utilized	Positive	Red to Orange	Arabinose utilized	Positive
Lactose	Red to Yellow	Lactose utilized	Positive	Red to Yellow	Lactose utilized	Positive
Sorbitol	Red to Yellow	Sorbitol utilized	Positive	Red to Orange	Sorbitol utilized	Positive
Mannitol	Red to Yellow	Mannitol utilized	Positive	Red to Pink	Mannitol was not utilized	Negative
Rhamnose	Red to Pink	Rhamnose utilized	Negative	Red to Pink	Rhamnose was not utilized	Negative
Sucrose	Red to Yellow	Sucrose utilized	Positive	Red to Yellow	Sucrose utilized	Positive

Table- 8 Summary of the microscopic and biochemical profile of clinical isolates of both food and water samples.

Gram's reaction	Motility	Indole test	MR test	VP test	Citrate test	Carbohydrate fermentation test								
						Gluc	Adon	Arab	Lact	Sorb	Mann	Rham	Sucr	Remarks
−	-	−	−	+	+	+	+	+	+	+	+	−	+	*Klebsiella pneumoniae*
−	+	−	+	−	+	+	−	+	+	+	−	−	+	*Citrobacter freundii*
−	+	−	−	+	+	+	+	+	+	+	−	+	+	*Enterobacter aerogenes*
−	+	−	−	+	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*
−	+	+	+	−	−	+	−	+	+	+	+	+	+	*Escherichia coli*

Note: MR-Methyl Red, VP-Voges-Proskauer, Gluc-Glucose, Adon-Adonitol, Arab-Arabinose, Lact-Lactose, Sorb -sorbitol, Mann-Mannitol, Rham-Rhamnose, Sucr-Sucrose,

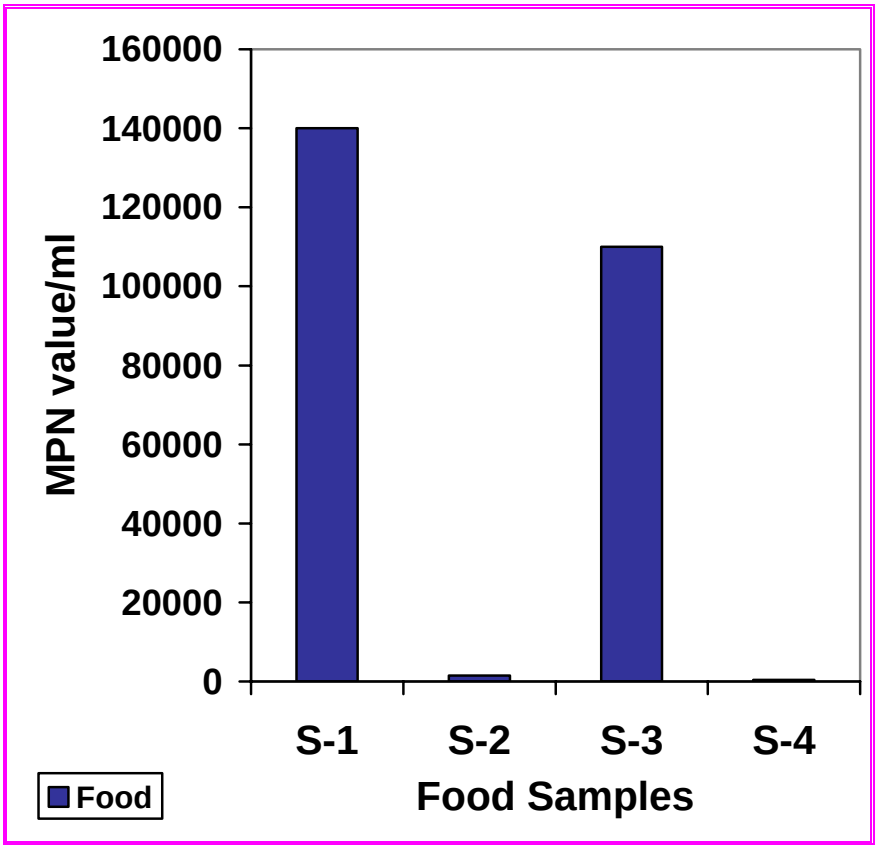

Fig -1 MPN results for food (juice) samples sold along road sides at Bellary City, India

Note: S-1 Sample No. 1[Orange juice from Vegetable Market], S-2 Sample No. 2[Orange juice from shop-2], S-3 Sample No. 3 [Pineapple juice from shop-3], S-4 Sample No. 4[Grape juice from shop-4]

Scale : X-axis-1unit =1 sample ; Y-axis-1unit =20,000 coliforms/ ml

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