

# Microbiological quality analysis of fresh vended fruit juices and water sold in roadside stalls in Dhaka Metropolis by MPN method

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Quality of drinking water and juice is very important because if the quality deteriorates by the contamination of faecally originated microorganisms, it may cause serious diarrhea associated problems leading to death. In overpopulated countries like Bangladesh, this is a common scenario to experience diarrheal diseases due to drinking non-potable water as well as contaminated fresh juices. Present study was conducted to determine the quality of drinking water and juice by detection of indicator bacteria *Escherichia coli* by MPN (Most Probable Number) method which was performed by three consecutive steps including presumptive test, confirmed test and completed test. Other gram negative bacteria were also identified by biochemical methods. The indicator bacterium *Escherichia coli* was detected in two water samples out of 15 samples and one juice sample out of fifteen samples respectively during the MPN test method. Other Gram negative bacteria found in both water and juice samples included *Klebsiella* spp., *Alcaligenes* spp., *Pseudomonas* spp. and *Proteus* spp. The quality of drinking water and juice was found to be good in Bangladesh but proper hygiene should be maintained more strictly to avoid the contamination by other gram negative bacteria which are also capable of causing disease.

**Key words:** Fruit juice; Drinking water; Most Probable Number (MPN); Quality; Portability

Water is one of the five major dietary components required for good health of human. Though water is one of the most abundant components found in the environment but the availability of pure drinkable or potable water is a matter of question nowadays due to overpopulation, unhygienic living conditions, pollution, damage of ecosystem, destruction of the environment by human activities, changing weather etc. (1-3). Water plays an important role in regulating metabolic activity as well as maintain physiological activities but at the same time can cause disease if consumed focally contaminated water (4-5). According to World Health organization, every year approximately four billions cases of diarrhea with few millions of enteric diseases occur due to drinking non potable water (6). Underground water supply is usually free from microbial contamination, but it can occur by nearby overflowed sewage line, underground leakage from sewage line and cause serious health associated problems (1-4, 7-9, 10, 11). Majority of people in Bangladesh drink water directly after collecting from pump or some processing like filtering, boiling, chemical treatments etc. (1-3). Contamination by human and animal waste can serve a source of pathogenic bacteria causing different diseases like

dysentery, diarrhea, typhoid, salmonellosis, shigellosis, listeriosis, viral infections like Hepatitis A, parasitic infections and so on (12-16). Some pathogenic microorganisms contaminating the water include *Salmonella* spp., *Escherichia coli*, *Listeria* spp., *Shigella* spp., *Vibrio* spp., *Yersinia* spp., *Campylobacter* spp., Hepatitis A and Hepatitis E virus, some parasites etc. (17-19). On the other hand, fruit juices are non-alcoholic beverages which are very popular among the people of all ages, especially in summer season. Health conscious people these days prefer fresh juices more than soft drinks. They supply vitamins and minerals (calcium, phosphorous, sodium), bioactive compounds (glycosides, flavanone antioxidants hydroxycinnamic acid) which help to improve human health status, helps in maintaining blood lipid profile in patients suffering from hypercholesterolemia, inhibit breast cancer, defeat urinary tract infections, give protection from congestive heart failure (20-27). Juices are prepared by squeezing the fruit pulp and mixing it with significant amount of water, ice, syrup and other ingredients depending on the recipe (28). Contaminated fruit juice can be responsible for gastrointestinal disorders and even death if consumed (29-37).

The easiest way to determine the quality drinking water is to detect the indicator bacteria *Escherichia coli* in the water as well as fruit juices which determines the equivalent presence of focally contaminated other pathogenic bacteria which are difficult to culture and also

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present in very low numbers (38-40). One easy method to detect the indicator bacteria *Escherichia coli* from the water sample is MPN method which is also known as multiple tube method comprising three consecutive tests as presumptive test, confirmed test and completed test (41-43). Present study was designed to determine the quality of drinking water and fruit juices available in roadside tea stalls in Dhaka Metropolis. Fifteen water samples and fifteen fresh fruit juice samples were collected randomly from different areas of Dhaka city for microbiological quality analysis by most probable number (MPN) technique.

## MATERIALS AND METHODS

**Study area and sample processing.** Fifteen fruit juice and fifteen water samples were collected from different areas of Dhaka city. After aseptically collection of samples, the samples were transferred immediately to the laboratory for microbial quality analysis by following the procedures proposed by American Public Health Association. (43).

### MPN test procedure

**Presumptive test.** Presumptive test is the primary presumption for the presence of Gram negative coliform bacteria in the samples which can be detected by the appearance of gas in the Durham tubes placed inside the lactose

broth of 1x concentration) is required for each sample under analysis. Each test tube contained 10 ml of fermentation broth and inoculated with the juice and water samples individually in a sequential order of 10 ml in five of each 2X lactose fermentation broth, 1ml in five of each 1x lactose fermentation broth and finally 0.1 ml in five of each 10 ml 1X lactose fermentation broth. All the test tubes were incubated with half circled screw caps at 37°C for 48 hours. By this procedure all of the 15 samples were subjected to the presumptive test individually (1-2).

**Confirmed test.** Positive samples indicated by the production of gas in the Durham tubes placed inside the lactose fermentation broth were selected for the confirmed test procedures to determine whether the indicator bacteria of fecal origin *Escherichia coli* was present or not. One loop full sample from the positive test tubes was inoculated on EMB by streaking and incubated at 37 °C for 24 hours and then observed for the production of green metallic sheen. EMB media was used as differentiate media to detect and separate *Escherichia coli* from other Gram negative coliform bacteria by the production of green metallic sheen in the media. The presence of green metallic sheen in EMB confirms the presence the indicator bacteria *E. coli*. (1-2).

**Completed test** From the green metallic sheen showing streaking line produced by *E. coli* on EMB media was inoculated into LFB 1X media containing Durham tube to re-confirm the positive lactose fermentation by the detection of gas. From the same place of EMB media, *E. coli* inoculum was taken to observe microscopically for their Gram reactions. This was the final stage of the MPN method where we can decide that whether the juice under analysis was potable or not. Finally, the standard biochemical tests were performed to confirm the identification of isolates other than *Escherichia coli* found on EMB agar plates for all of the 15 of fruit juice samples by the standard biochemical tests (43-44).

**Identification of other bacteria found on EMB.** To determine the presence of other gram negative bacteria, the inoculum from the growth on EMB media were subjected to biochemical identification.

TABLE 1. Presumptive test results for juice

Sample	5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each	MPN/100ml	Lower limit of 95% confidence	Upper limit of 95% confidence
01	2	0	0	5	<0.5	13
02	4	4	1	21	7	42
03	5	5	3	920	220	2600
04	5	5	5	≥2400	700	0
05	5	5	5	≥2400	700	0
06	5	5	3	920	220	2600
07	5	5	5	≥2400	700	0
08	5	5	3	920	220	2600
09	5	5	5	≥2400	700	0
10	5	5	3	920	220	2600
11	2	0	1	7	1	17
12	4	0	0	13	4	35
13	1	0	0	2	0.3	10
14	2	0	0	5	<0.5	13
15	5	3	2	140	52	400

TABLE 2. Presumptive test results of water

Sample	5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each	MPN/100ml	Lower limit of 95% confidence	Upper limit of 95% confidence
01	5	5	5	≥2400	700	0
02	5	5	5	≥2400	700	0
03	5	5	5	≥2400	700	0
04	5	5	4	1600	400	4600
05	5	5	1	350	100	710
06	1	0	0	2	0.3	10
07	5	1	1	46	14	120
08	5	4	0	130	36	400
09	5	5	4	1600	400	4600
10	5	0	0	23	7	70
11	2	0	1	7	7	17
12	3	1	0	11	5	35
13	5	1	1	46	14	120
14	5	5	4	1600	400	4600
15	3	0	0	8	2	22

## RESULTS AND DISCUSSIONS

Food borne infections and intoxications are very common in developing countries like Bangladesh (45-50). Quality of drinking water and juice is very important because it directly influences the health status of a particular locality. Juices can transmit pathogenic microorganisms which can be incorporated by different ways like raw materials used in juice preparation (water, ice which are main source), fruit quality, equipment and hygiene of preparation area as well as the juice makers (51-55). On the other hand, water is not only used directly for drinking but also for other purposes like cleaning, washing, food preparation, juice and other drinkable item preparation, cooking, maintaining proper hygiene and so on. The contaminated water with pathogenic bacteria can come from fecal contamination and transfer to human by fecal oral route and cause serious enteric diseases. has been found out that each year 2 million death

occurred due to enteric diseases resulting from contaminated drinking water (7, 15). The problem is more common in densely populated country including Bangladesh where the maintenance of proper hygiene, processing a huge quantity of water for drinking and maintain proper disposal of sewage waste which might increase the contamination rate as well as diarrheal incidents (1-3, 10, 15).

Current study was conducted to determine the quality of fresh fruit juices and water by most probable number (MPN) method which only target the presence of indicator coliform bacteria *Escherichia coli*. If this bacteria is present in the juice then it could be assumed that the quality of juice is not good and it is non-potable. The contamination might come from the water and ice used in the juice preparation.

In presumptive test, four juice samples (sample no. 4, 5, 7 and 9) showed maximum growth of lactose fermenting coliform bacteria with the  $\geq 2400$  MPN/100ml juice (Table 1). Second highest growth was present in sample

TABLE 3. Confirmed test results of juice

Sample no.	Growth of coliform on EMB media	Production of green metallic sheen	Presence of <i>Escherichia coli</i>	Result
01	Present	Negative	Absent	Potable
<b>02</b>	<b>Present</b>	<b>Positive</b>	<b>Present</b>	<b>Non-potable</b>
03	Present	Negative	Absent	Potable
04	Present	Negative	Absent	Potable
05	Present	Negative	Absent	Potable
06	Present	Negative	Absent	Potable
07	Present	Negative	Absent	Potable
08	Present	Negative	Absent	Potable
09	Present	Negative	Absent	Potable
10	Present	Negative	Absent	Potable
11	Present	Negative	Absent	Potable
12	Present	Negative	Absent	Potable
12	Present	Negative	Absent	Potable
13	Present	Negative	Absent	Potable
14	Present	Negative	Absent	Potable
15	Present	Negative	Absent	Potable

TABLE 4. Confirmed test results of water

Sample no.	Growth of coliform on EMB media	Production of green metallic sheen	Presence of <i>Escherichia coli</i>	Result
01	Present	Negative	Absent	Potable
02	Present	Negative	Absent	Potable
<b>03</b>	<b>Present</b>	<b>Positive</b>	<b>Present</b>	<b>Non potable</b>
04	Present	Negative	Absent	Potable
05	Present	Negative	Absent	Potable
06	Present	Negative	Absent	Potable
07	Present	Negative	Absent	Potable
08	Present	Negative	Absent	Potable
09	Present	Negative	Absent	Potable
10	Present	Negative	Absent	Potable
11	Present	Negative	Absent	Potable
12	Present	Negative	Absent	Potable
13	Present	Negative	Absent	Potable
<b>14</b>	<b>Present</b>	<b>Positive</b>	<b>Present</b>	<b>Non potable</b>
15	Present	Negative	Absent	Potable

no. 3,6, 8 and 10 with 920 MPN/100ml juice sample. Lowest number of coliform bacteria with lactose fermenting capability was present in sample no. 13 (2 MPN/100ml juice).

In the same step for water test, three water samples (sample no. 1, 2, 3) showed maximum growth of lactose fermenting coliform bacteria with the  $\geq 2400$  MPN/100ml water (Table 2). Second highest growth was present in sample no. 4, 9 and 14 with 1600 MPN/100ml drinking water. Lowest number of coliform bacteria with lactose fermenting capability was present in sample no. 6 (2 MPN/100ml water).

All the test tubes with positive result (gas formation in the Durham tube) were then selected for second stage of MPN method, the confirmed test method. One loop of sample was taken to streak onto EMB agar plates and after 24 hours incubation, among fruit juice samples we found green metallic sheen suggesting the presence of *Escherichia coli* only in sample 02 (Table 3). Rest of the samples showed growth of coliform bacteria other than *Escherichia coli*. The presence of *Escherichia coli* indicates the faecal contamination of the juice probably from contaminated water source or for poor personal hygiene. And from the water samples, we found green metallic sheen suggesting the presence of *Escherichia coli* only in sample 3 and 14 (Table 4). Rest of the samples showed growth of coliform bacteria other than *Escherichia coli*.

The presence of *Escherichia coli* was confirmed by the completed test (Tables 5 & 6). In lactose fermentation broth it showed gas production. Gram staining was also performed to visualize the cell morphology and gram reaction as a part of completed test procedure. After MPN test method applied on fifteen fruit juice and fifteen water samples, it was found out that only one juice sample and two water samples were non-potable. Other samples were potable in the sense that they were free from faecal contaminations. Such result indicates that the overall quality of fresh fruit juices is good. The processing method, maintenance of hygiene, quality of water and ice used here were followed properly which reflected on the results.

As other coliform bacteria was found on EMB media for all of the juice samples as well as water samples (Tables 7 & 8), we performed biochemical identification of them. After biochemical identification we confirmed the presence of *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *Alcaligenes* spp. These microorganisms can also cause intestinal disorders. The presence of such bacteria indicates that though the juice preparation procedure was free from faecal contamination, still there are some lacking which should be minimized to protect public health safety.

## CONCLUSION

The overall quality of fresh fruit juices and water is good because only one juice and two water sample was contaminated with *Escherichia coli* suggesting of faecal contamination. Others were potable according to the absence of the indicator bacteria. The people are becoming more aware about sanitation and hygiene and the quality of processing non-alcoholic beverage items has been increased. But it is needed to be more careful on using water which must be properly purified, and sanitation process should be prioritized more to reduce the number of other contaminating bacteria.

## CONFLICT OF INTEREST

Authors have no conflict of interest.

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TABLE 5. Completed test results for juice

Sample no.	Gas in lactose fermentation broth	Gram reaction	Result
02	Positive	Gram negative, short rod	<i>Escherichia coli</i> confirmed

TABLE 6. Completed test results for water

Sample no.	Gas in lactose fermentation broth	Gram reaction	Result
03	Positive	Gram negative, short rod	<i>Escherichia coli</i> confirmed
14	Positive	Gram negative, short rod	<i>Escherichia coli</i> confirmed

TABLE 7. Presence of other gram negative bacteria in the juice samples other than *Escherichia coli* on EMB media

Sample no.	<i>Proteus</i> spp.	<i>Pseudomonas</i> spp.	<i>Klebsiella</i> spp.	<i>Alcaligenes</i> spp.
01	+	-	+	-
02	+	-	-	+
03	-	+	-	+
04	-	-	+	+
05	-	+	-	-
06	+	-	-	-
07	-	+	-	+
08	-	-	+	-
09	+	-	-	+
10	-	+	-	+
11	+	-	-	-
12	+	-	-	-
13	+	+	-	+
14	-	-	-	-
15	-	-	+	+

TABLE 8. Presence of other gram negative bacteria in the water samples other than *Escherichia coli* on EMB media

Sample no.	<i>Proteus</i> spp.	<i>Pseudomonas</i> spp.	<i>Klebsiella</i> spp.	<i>Alcaligenes</i> spp.
01	-	+	-	-
02	-	+	+	-
03	-	-	-	-
04	+	-	+	-
05	+	-	-	+
06	+	+	-	+
07	-	+	-	-
08	-	-	+	-
09	-	-	+	-
10	+	+	-	-
11	-	-	-	+
12	+	-	+	-
13	-	+	-	-
14	-	-	+	+
15	-	-	+	-

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