MICROBIAL ANALYSIS OF PACKAGED DRINKING WATER SOLD IN ALLAHABAD CITY

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Abstract: Due to the drift towards modern life style and poor quality of available supply water there has been a tremendous increase in the use of packaged drinking water in the recent decades. Packaged drinking water comes in various shapes and form which include plastic bottles, glass water, large gallons, jars and sachets. Before these are packed, the water pass through a series of purification processes as per the standards set by the national and international bodies. But recent studies have shown that the microbiological quality of even the packaged water cannot be trusted blindly. Hazardous microbial contamination has been found during earlier studies. This study was conducted to assess the microbiological quality of the different packaged water available throughout the markets of Allahabad city which included a total of 20 samples belonging to 5 national and international brands. The study showed that heavy number of coliform (*E. coli)*, *Staphylococcus aureus* and faecal contamination (*Streptococci)* was found in the packaged drinking water and hence this water is not completely fit for human consumption. Consumption of such water may lead to several health hazards and serious diseases. This calls for an urgent need of quality assessment and monitoring of these packaged water by the local and national government bodies during their manufacturing and distribution.

IndexTerms **- Packaged drinking water, coliforms, faecal contamination, *E. coli, Staphylococcus aureus, Streptococci.***

I. INTRODUCTION

Water is the most essential requirement for the all living beings including man and for the same reason; the provision for safe drinking water for all was one of the eight components of health care as identified by the International Conference on Primary Health Care in 1978. Being a developing country, in India access to completely safe and clean drinking water is still far-sighted dream and water borne diseases are common and remains a serious issue as about 70% of surface water resources are being contaminated by toxic biological organic and inorganic pollutants. (Gangil *et al.*, 2013). Bottled drinking water can be defined as any potable water, which is sealed in food-grade bottles for human consumption (Sharma *et al.*, 2015), and people rely on them expecting it to be free of microbial contaminations and health hazards.

As per Manual for Packaged Drinking Water published by Bureau of India Standards (BIS), January 2005 (Doc No.: SM/IS14543/01), "Packaged drinking water means water derived from any source of potable water which may be subjected to treatments such as, decantation, filtration, combination of filtrations, aeration, filtration with membrane filter, depth filter, cartridge filter, activated carbon filtration, demineralisation, re-mineralization, reverse osmosis or other such methods to comply with the prescribed standard and then packed. The water is disinfected to a level that will not lead to harmful contamination in the drinking water." (BIS, 2005)

The packaged drinking water can be produced must be in accordance with the standards of food grades of the PFA Act, 1954. Disinfection can be done via chemical and/or physical methods to control micro-organisms to a level that does not compromise food safety or suitability for consumption. The processed water is packed in sealed containers of various types/sizes/shapes, such as Jars, Pouches, Plastic Bottles, Glass bottles and Cups, made from the plastic materials permitted under ISS. Two manual are published by BIS as Indian Standards for Packaged Drinking Water namely IS13428 for Packaged Natural Mineral Water and IS 14543 for Packaged Drinking Water. Both the products are under mandatory certification. The Indian Standard specification for packaged drinking water IS 14543:2004 prescribes Physical, Chemical, Radioactive and Microbial (parameter as per Cl. 5.19) requirements for processed water as well as the standard requirements for packaging i.e. containers and material used for manufacturing the containers.

Indian population, recently, has shown an astronomical increase in the consumption of packaged water (bottled and sachet drinking water) which can be attributed to (a) unavailability of safe municipal water, (b) changes in fashion towards the consumption of designer water, (c) increased concerns about the safety of the piped water supply (Obiri-Danso *et al.*, 2003). The growing sale and consumption of such water products raises the question as to whether they are hygienically produced (Dada, A.C, 2008). Not

just the bottled but also packed sachet drinking water is also used by masses in larger quantity because they are available in smaller volumes, locally available and are inexpensive.

However, studies conducted earlier revealed that most of the samples of bottled water were unfit for human consumption. The microorganisms most frequently found were *Pseudomonas*, *A. hydrophila*, *Escherichia coli*, *Flavobacterium*, and *Mycobacterium*. (Sharma *et al.*, 2015) and Coliform bacteria. The common sources of bacteria are waste, septic systems, and surface water that gets into the well. Microbial contamination of bottled water can be influenced by variety of factors such as source of bottled water or it may be contaminated during processing and packaging.

In view of the above mentioned important aspects of packaged drinking water and its microbial safety, the present study entitled **"Microbial Analysis of Packaged Drinking Water sold in Allahabad city"** was conducted to evaluate the microbial quality of bottled water available in Allahabad city, U.P., India with the following objectives: -

- To check the quality of packaged drinking water whether it is safe for drinking purpose or not by the microbial analysis of water sample (bottles and sachet) using standard method as describes in BIS manual.
- To detect the presence of pathogenic bacteria in packaged drinking water sold in Allahabad city.

II. MATERIALS AND METHOD

The study was designed to analyze the microbial quantity of the packaged drinking water sold in Allahabad city to check the level of contamination. Reliable laboratory methods and analysis are a pre-requisite to ensure the drinking water characteristic.

2.1 Place of work

The present study entitled "**Microbial Analysis of Packaged Drinking Water sold in Allahabad City**" was carried out in Post Graduate Department of Botany, Ewing Christian College, Allahabad, Uttar Pradesh, India.

2.2 Study samples

During the study a total of 20 samples (16 bottles and 4 sachet water) were collected. The samples include 4 major brands of bottled drinking water and 1 local brand of sachet packed drinking water. The study was performed within 24 hours of buying the water samples.

2.3 Collection and transportation

All the samples were collected randomly from the local markets around the Allahabad city. The collected samples of water bottle include 1000ml, 500ml and 250 ml water bottle. The sachet water of 100ml was bought. During collection, it was ensured that both the water bottles and sachet did not exceed the expiry date, were sealed packed and none of their seal was open until the start of experiment. The bottles were transported to the Microbiology laboratory of the Post Graduate Department of Botany under the controlled conditions.

2.4 Isolation of target organisms

In this study three target organism were chosen *viz. Escherichia coli, Staphylococcus aureus,* Faecal *Streptococci.* The microbial analysis was done within 24 hours of the sample collection. The water samples were analyzed for **Colony Forming Units (CFU),** using the Serial Dilution Pour Plate technique, (Dilution-10^{-4}); of the target bacteria present in 1ml of water sample on selective media Eosin Methylene Blue agar, Mannitol Salt Agar and MacConkey Agar respectively and isolates were identified on the morphological basis using Gram Staining Technique.

2.4.1 isolation of *E. coli*

One ml water sample was subjected to a series of dilution (up to 10^{-4}) using sterile water blanks; 1ml of it was placed in the center of sterile Petri dish using a sterile micropipette. Molten cooled selective culture medium Eosin Methylene Blue (approx. 15mL) was then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the media, the petri-plates was inverted and incubated at 37°C for 24-48 hours using the incubator (**Sanders, 2012)**. Then after, the results were analyzed and photographs were taken.

2.4.2 isolation of *S. aureus*

Mannitol Salt Agar was chosen as the selective media for the isolation of the bacteria; rest of the process remains the same as mentioned in section 2.4.1.

2.4.3 Isolation of Faecal *Streptococcus*

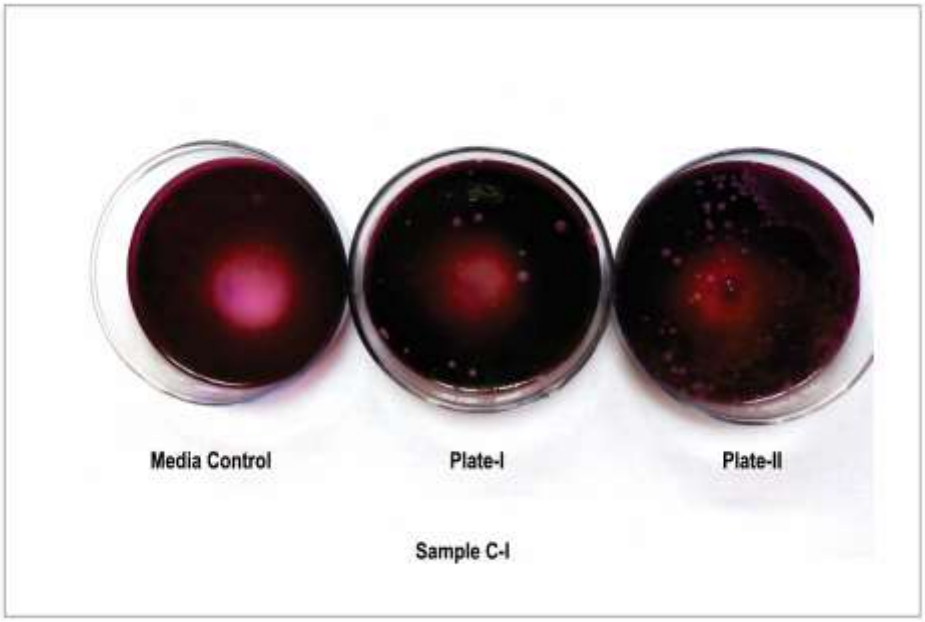
MacConkey Agar was chosen as the selective media for the isolation of the bacteria; rest of the process remains the same as mentioned in section 2.4.1.

The growth of micro-organisms occurs on both the surface and within the medium. Colonies that grow within the medium generally are small and may be confluent; the few that grow on the medium surface are of the same size and appearance. Each (both large and small) colony was carefully counted (using magnifying colony counter if needed). Each colony represents a "colony forming unit" (CFU).

2.5 CULTURAL AND MORPHOLOGICAL EXAMINATION

The characteristics of colonies such as colony shape, colour, elevation etc. were studied after incubating different sample plates for 24-48 hours.

The morphological examination of isolated bacteria was done by Gram Staining method as per the procedure given by Dr. R. P. Singh in his book **Microbiology Second Edition, 2008**.



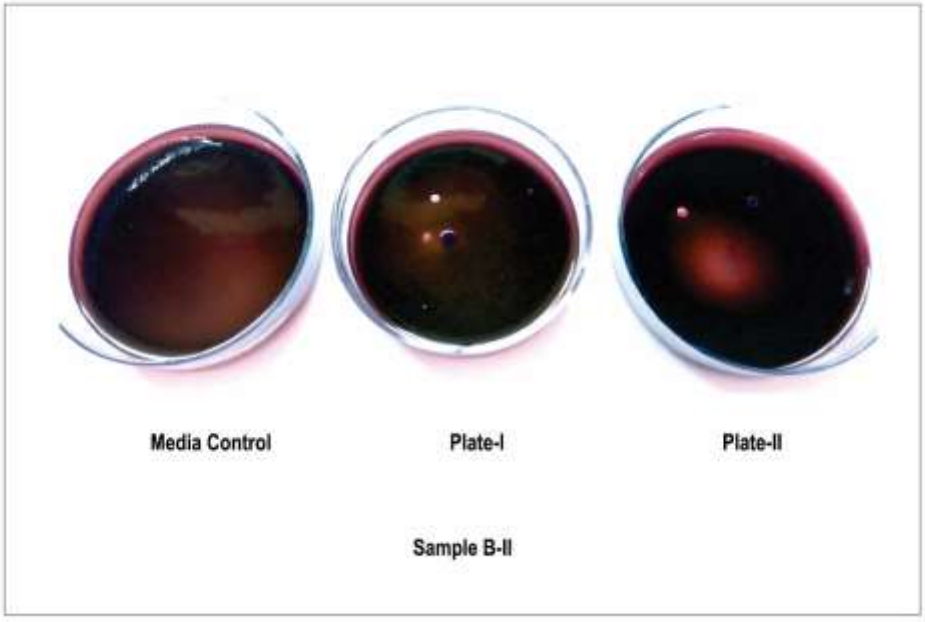


Plate 1: Plates of EMB showing colonies of *E. coli*

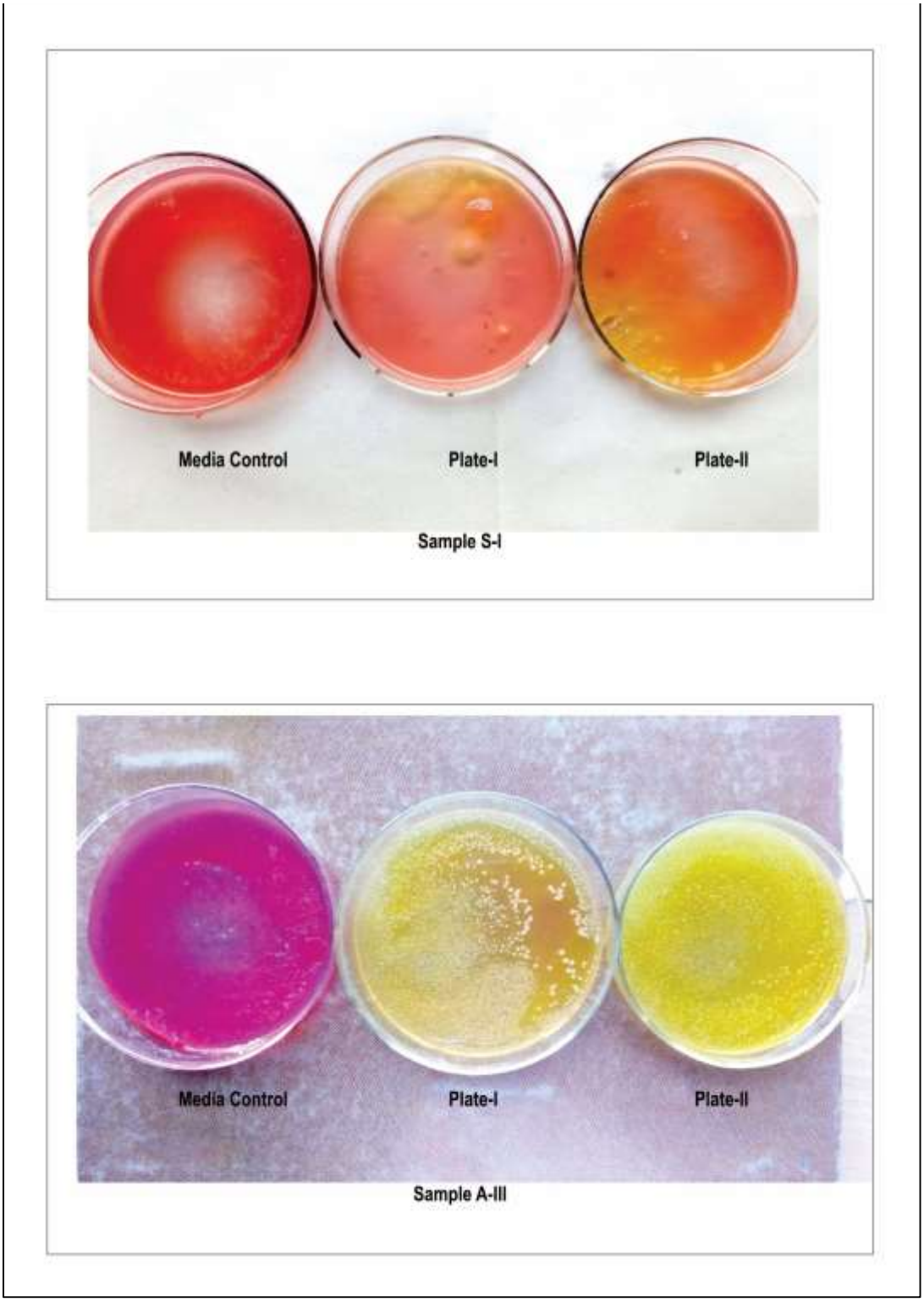


Plate 2: Plates of MSA showing colonies of *Staphylococcus aureus*

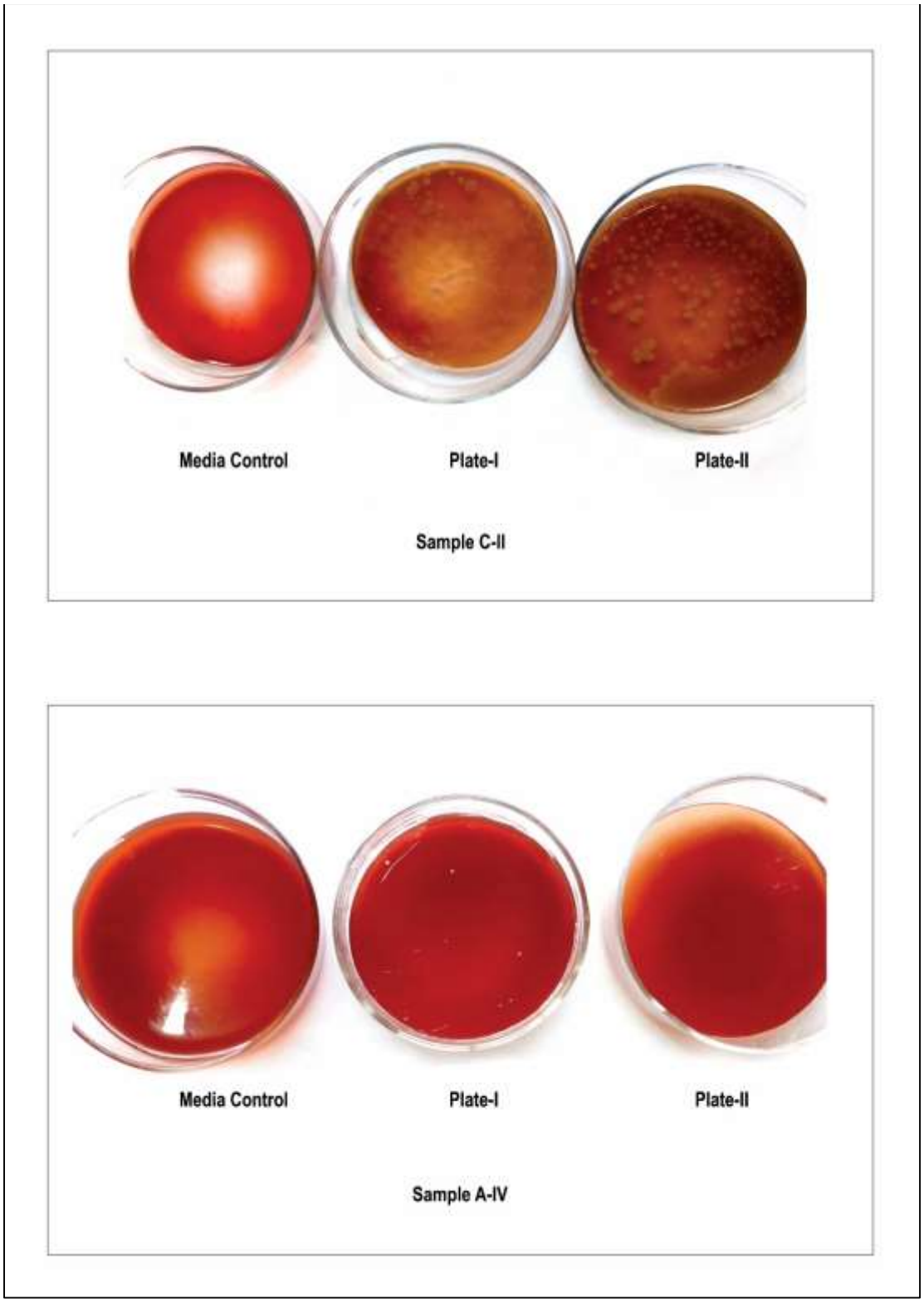


Plate 3: Plates of MacConkey showing colonies of Faecal *Streptococci*

III. RESULT AND DISCUSSION

The study revealed that none of the 5 brands complied with the national and international standards as given by BIS, 2004 and WHO, 2015 which says that the average count of *E. coli*, coliform and faecal contaminations in the packaged drinking water must be nil. After incubation of the petri plates for 24-48 hr, the morphological characteristics of the colonies isolated on the selective media was studied and the characters were tabulated.

Table I: colony characteristics

MEDIA	ELEVATION	SHAPE	COLOUR	CELLULAR ARRANGEMENT	GRAM REACTION	Identified bacterium
EMB	Flat	Circular, entire	Purple with dark centre, on incubation more than 48 hr show green metallic sheen	Isolated rods	-ve	*E. coli*
MSA	Convex	circular, entire	Golden yellow with yellow zones	Cocci in grape-like clusters	+ve	*S. aureus*
MAC	Convex	circular, entire	Light pink	cocci arranged in chain like fashion	+ve	Faecal *Streptococci*

Table II: Sample wise distribution of target organisms (in CFU/ml)

BRAND	SAMPLE	*E.coli* (No.x10^4)	*S. aureus* (No.x10^4)	Faecal *Streptococci* (No.x10^4)
A	I	**3**	**0.5**	**0.5**
	II	**2.5**	**0**	**2**
	III	**1.5**	**241.75**	**1**
	IV	**3**	**1**	**4**
B	I	**33.5**	**92**	**0.5**
	II	**23**	**47.5**	**3.5**
	III	**3**	**3**	**1**
	IV	**2.5**	**1.5**	**2.5**
C	I	**468**	**6**	**178**
	II	**3.5**	**40**	**211**
	III	**0.5**	**1.5**	**0.5**
	IV	**3**	**2**	**0**
	I	**2.5**	**5.5**	**1**
	II	**4**	**3.5**	**1**

BRAND	SAMPLE	*E.coli* (No.x10^4)	*S. aureus* (No.x10^4)	Faecal *Streptococci* (No.x10^4)
D	III	0	0	0.5
	IV	149.5	3	0
SACHET	I	29.5	36.5	0.5
	II	0.5	6.5	0
	III	2	1.5	0
	IV	0	1	1

Table III: Brand wise distribution of target organisms (in CFU/ml)

BRAND	*E.coli* (No.x10^4)	*S. aureus* (No.x10^4)	Faecal *Streptococci* (No.x10^4)
A	2.5	60.81	1.87
B	15.5	36	1.87
C	118.75	12.37	97.38
D	39	3	0.625
SACHET	8	11.37	0.38

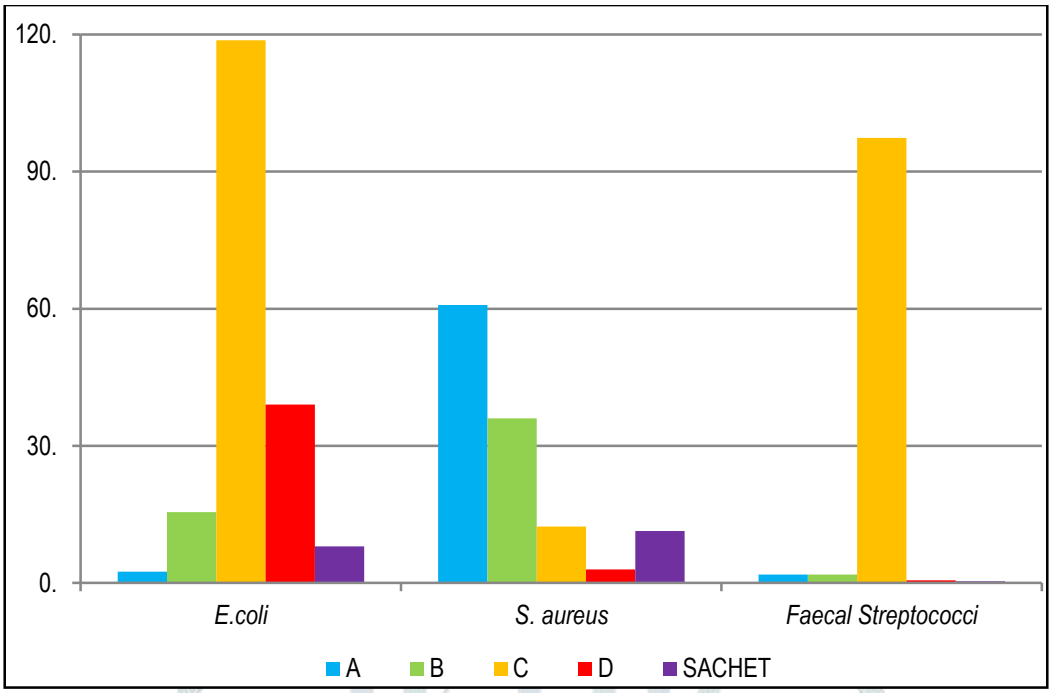


Fig I: Brand wise average distribution of target organisms (in CFU/ml)

3.1 *E. coli* COUNT OF THE SAMPLES-

The study reveal that the highest *E. coli* count was obtained in brand C, 118.75 x10^4 CFU/ml which was followed by brand D, 39x10^4 CFU/ml, brand B 15.5x10^4 CFU/ml and brand A 2.5x10^4 CFU/ml. Whereas the sachet water was found to have 8x10^4 CFU/ml of *E. coli* count.Although none of the samples comply with the IS14543 standard of BIS 2004, the lowest count was obtained in brand A.

3.2 *S. aureus* COUNT OF THE SAMPLES-

S. aureus is a harmful coliform bacterium which must be absent in packaged drinking water. However, this study reveals that large amount of these bacteria was found in all the 20 samples analyzed highest count being in brand A, 60.81x10^4 CFU/ml followed by brand B 36x10^4 CFU/ml. Brand C and the sachet water show almost similar *S. aureus* count of 12.37x10^4 CFU/ml and 11.37x10^4 CFU/ml respectively. Brand D had the lowest count of 3x10^4 CFU/ml amongst all the samples analyzed.

3.3 FAECAL CONTAMINATION (*Streptococci*) COUNTS OF THE SAMPLES-

Any kind of faecal contamination in drinking water whether packaged or not is impermissible by the national as well as international standards. However, large amount of faecal *Streptococci* contamination was found in brand C having a count of 97.38x10^4 CFU/ml. The other brands show much lesser contamination, 1.87x10^4 CFU/ml in brand A and B both followed by brand D, 0.625x10^4 CFU/ml. The count obtained from sachet, 0.38x10^4 CFU/ml was found to be the least.

3.4 COMPARATIVE AVERAGE DISTRIBUTION OF TARGET ORGANISMS IN BOTTLE AND SACHET-

Table IV: Average distribution of target organisms (in CFU/ml)

SAMPLE	*E.coli* (No.x10^4)	*S. aureus* (No.x10^4)	*Faecal Streptococci* (No.x10^4)
BOTTLES	**43.93**	**28.045**	**25.435**
SACHET	**8**	**11.37**	**0.375**

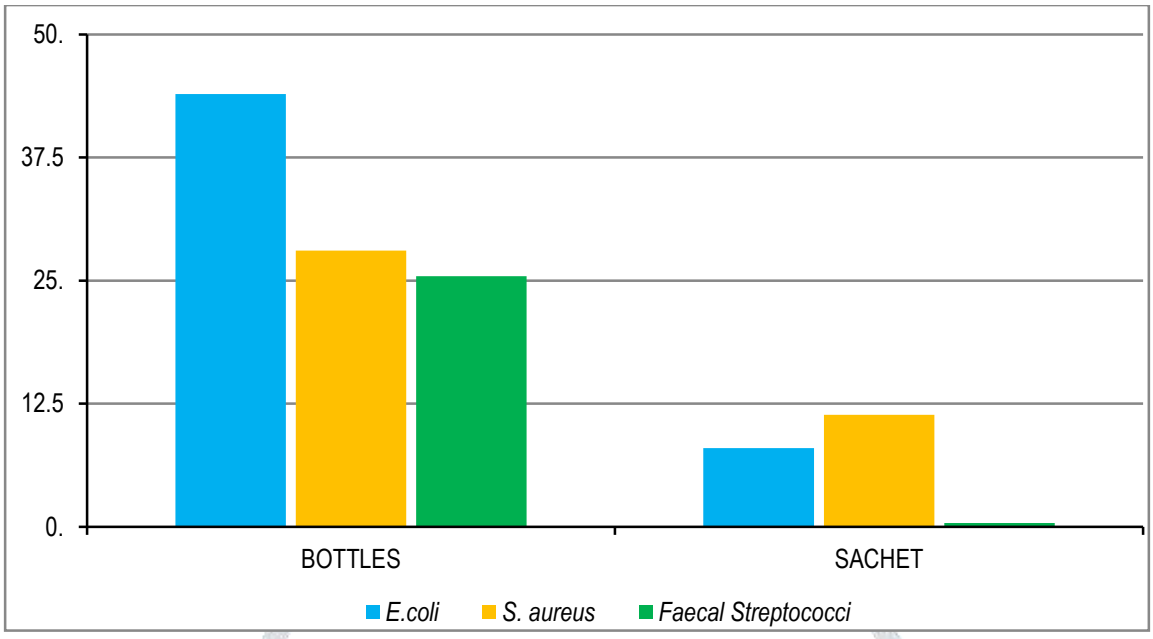


Fig. II: Average distribution of target organisms (in CFU/ml)

The comparative study of 4 brands A, B, C and D comprising 16 out of 20 samples reveal that the counts obtained in bottled drinking water was much higher than that of the sachet waters. The *E. coli* count of bottled water $43.93x10^4$ CFU/ml was almost 5 times than that of sachet water which was $8x10^4$ CFU/ml. The *Staphylococcus aureus* count in bottle samples was $28.05x10^4$ CFU/ml and that of sachet was found to be $11.37x10^4$ CFU/ml. The faecal contamination in bottled water was $25.43x10^4$ CFU/ml as compared to $0.38x10^4$ CFU/ml of sachet.

3.5 TOTAL AVERAGE COUNT OF TARGET ORGANISMS IN ALL THE SAMPLES-

Table V: total average count (In CFU/ml)

SAMPLE	***E.coli*** **(No.x10^4)**	***S.aureus*** **(No.x10^4)**	**FAECAL *STREPTOCOCCI*** **(No.x10^4)**
TOTAL AVERAGE COUNT	**25.965**	**19.7**	**12.91**

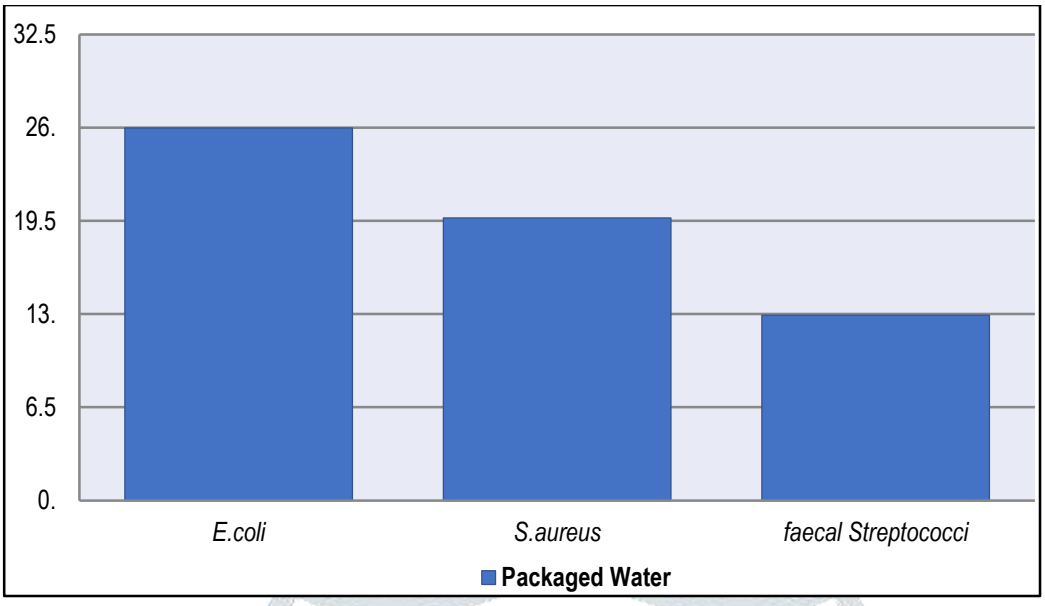


FIG. III: Overall average occurrence of microbes in all the packaged drinking water samples (In CFU/ml)

The total average count of all the 20 samples analyzed during the study was 25.965×10^4 CFU/ml for *E. coli*, 19.7×10^4 CFU/ml for *Staphylococcus aureus* and 12.91×10^4 CFU/ml for faecal *Streptococcus*. Out of the total 20 samples analyzed 90% (n=18) had *E. coli* and *S. aureus* while faecal *Streptococci* contamination was found in 80% of the samples (n=16).

In 1995 a study in Yale, USA was performed by **Edberg *et al.* (1995)**in which coliform bacteria were only isolated from those bottled water that used filling lines which alternated between milk and water. These mixed lines use a single set of pipes for both milk filling and water filling which was the major cause of contamination.

A similar Study in Kumasi, Ghana by **Obiri-Danso *et al.* (2003)** revealed that faecal coliforms and *Enterococci* were not isolated in any of the 8 brands of bottled drinking water. 4.5% of the 88 factory plastic-bagged sachet drinking water contained coliforms whereas 43% of the hand-filled hand-tied and 5% of the factory-bagged sachet waters were contaminated with bacteria of faecal origin. **Dada, A.C (2008)** conducted a study in Nigeria, in which he reported an average of 22 %(*n*=100) of all the identified packaged water did not meet the existing standards prescribed by NAFDAC. Though E. *coli* was not detected in all packaged water samples tested in the study but contamination might have occurred due to some reasons after processing of water. 6.67% (P1, *n*=30) showed contamination after production, 40% (P2, *n*=30) of the samples were contaminated due to improper storage, while the highest level of contamination (45%) occurred due to distribution chain (P3, *n* = 40). **Ajayi *et al.* (2008)** had reported an earlier study of packaged drinking waters in Ibadan, Nigeria in which larger proportions of sachet water were found to show positive coliform counts compared to bottled waters. Study of bottled and sachet water in Nigeria was performed by **Oyedeji, 2009** in which total coliforms and *E. coli* were detected in only one of the 16 brands of bottled drinking waters. Whereas all the 20 brands of sachets were found to contain total coliforms that ranged between 2 and 140 100 per ml**.** *E. coli* was also detected in four of the brands (20%) while two of the brands (10%) had *E. faecalis*. A survey entitled "Microbiological safety of bottled water" was conducted by food safety authority of Ireland in 2011where Nineteen (2.5%) of the 748 bottled water samples were categorized as unsatisfactory and unsafe for human consumption because the harmful microorganisms such as *E. coli* and *Enterococci* were detected in them. A similar study in Lahore, Pakistan (**Tahir *et al.*, 2011**) reveals that coliform bacteria were not detected in any of the bottled water sample but total viable count of all bottled water samples was much higher than that prescribed by the IBWA and PCRWR standards. The microbial quality of bottled water samples was not up to the mark because samples were showing higher bacterial count so it will effect on the health of the consumer. **Gangil *et al.*, 2012** conducted a study at Jaipur microbial evaluation of all water samples revealed that, out of twenty, 50% samples were found satisfactory in standard plate count. On the other hand, coliforms, *E. coli* and Staphylococcal counts revealed that 45%, 20%, and 5% samples respectively were found unfit for human consumption as per Bureau of Indian Standards (BIS) for drinking water. Overall 45% of samples proved to be fit for consumption, while 55% samples had higher bacteriological values than BIS standard. It seems that the organism has survived in water for pretty long time because the bottled water was kept under chilled conditions. Such contaminated water also leads to gastrointestinal diseases and also responsible for food poisoning outbreaks **(Gangil *et al.*, 2012**). In Chennai, **Devi Venkatesan, *et al.*, 2014** screened 36 sachet drinking water, out of which 28 (77.7%) met the WHO standard of zero coliform, 1 (2.7%) of sachet water had 1-3 coliform, 3 (8.3%) had 4-10 coliform and 4 (11.11%). Total coliform and faecal coliforms were not isolated in any of the five different brands (15 samples) of bottled drinking water. A study conducted in Chandigarh (**Sharma *et al.*, 2015**) suggests that the samples were free of coliforms. Out of the 46 samples analyzed, 19 samples showed bacterial growth and out of these in 8 (17%) samples, the cfu/mL value exceeded the limit of 100 cfu/mL set by BIS. Around 2% of the samples showed bacterial counts higher than 1,000 cfu/mL. A survey performed in Riyadh, Saudi Arabia by **Al-Sulaiman 2016** revealed that only 5% of the samples were contaminated with *Pseudomonas* species and 0.8% contaminated with *E. coli*.

The contaminations and poor quality of packaged drinking water can be attributed to a number of factors. Firstly, the material used for packaging (plastics of bottles and sachets) is produces in large quantities and stored for a long period of time before they are used for packaging. Secondly, Warburton *et al.* (1992) showed that unused, stored bottles may contain bacteria which can lead to potential contamination after packaging. The packaged water is stored for a long length of time before they are distributed and used by the customer. During this period the growth and multiplication of bacteria is favoured. However, it is possible that bacterial growth is suppressed when bottles and sachets stored in chilled condition, but the already present microbes under such storage are not killed. Thirdly, Hunter and Burge (1987) showed that the ceramic and charcoal filters used during the purification process to remove objectionable taste and odour could lead to contamination of drinking water because the concentrate both bacterial and organic matter. Fourthly, polythene bags used in bagging sachet water are imported as hoses that are cut into appropriate sizes and one side heat sealed before being sent to printing houses for labeling, all of which can lead further contamination (Obiri-Danso, 2003). Compared to *E. coli* and *S. aureus* lesser concentration of *Streptococci* was observed in the samples but this concentration was indicative of faecal contamination. The contamination in the sachet water could possible occur due to back seepage in the pouches that are not properly sealed (WWIS, 2007). Lesser faecal contamination may be attributed to use of protective sealed caps on bottles, improved and hygienic filling system and use of non-returnable plastic containers (Oyedeji, 2009). The contamination of packaged water may also be influenced by the source of raw water used for its production and the treatment process that is being used. Surface water such as river or lake water is highly contaminated because they are open to any kind of environmental or human contaminations. The high demands of packaged water with the changed in life style and status has imposed a heavy pressure for the production of packaged drinking water due to which many times their quality is sacrificed.

IV. CONCLUSION

The study entitled "**Microbial Analysis of Packaged Drinking Water Sold in Allahabad City"** was conducted at the Post Graduate Department of Botany, Ewing Christian College, Allahabad to assess the quality of branded bottled and sachet water sold around the city. 20 samples (16 bottles and 4 sachets) of packaged drinking water were collected from the local market of the city from different places which represented 5 different brands. The isolation of the target organisms (*Escherichia coli, Staphylococcus aureus* and Faecal *Streptococci)* was done on the different selective media *viz.* Eosin Methylene Blue Agar, Mannitol Salt Agar and MacConkey Agar respectively. The sample water was diluted to a dilution of 10^{-4} and was plated using serial dilution agar plate method. After incubation, the colonies were counter to determine the CFU/ml. The organisms were identified using Gram Staining method. Based on this study it can be concluded that: -

- None of the 20 samples complied with the standards of BIS, Government of India.
- *E. coli* was detected from 90% (n=18) of the sample whose count ranged between 0.5 to 149.5x 10^4 CFU/ml.
- *S. aureus* was detected from 90% (n=18) of the samples that ranged between 0.5 x 10^4 CFU/ml to as high as 241 x 10^4 CFU/ml.
- 80% (n=16) samples show Faecal *Streptococci* contamination was ranging from 0.5 x 10^4 CFU/ml to 211 x 10^4 CFU/ml.
- On an average, the highest contamination was reported from the bottled water of brand A while the sachet water showed the least contamination.

This contamination may occur due to unhygienic packaging material (plastic bottles and polythenes of sachet), improper purification process, and storage conditions or may be due to distribution. Such contaminated water is extremely unsafe for human consumption and may lead to severe gastro-intestinal diseases and health hazards. Based on my study I would like to make the following recommendations: -

Recommendations: -

- Strictly adhering to the prescribed standard methods of water purification, storage, distribution and the kind of material to be used for the manufacturing of the packaged drinking water.
- Regular surveys and vigilance by the local and national authorities to ensure the quality of the water being sold in the market.
- The companies must themselves ensure the quality of their products.
- Customers must be aware of the product they are using and themselves be aware of the quality limits of the packaged drinking water.

V. ACKNOWLEDGEMENT

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