***Short Communication***

# MICROBIAL EXAMINATION OF BOTTLED WATER AVAILABLE IN LOCAL MARKET OF LAHORE

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## ABSTRACT

The present work describes the microbial examination of six samples of bottled water collected from Defence market of Lahore. Six samples (Wah, Classic, Nestle, Askari, Aqua Safe, and Sparkletts) were analyzed for total viable count and coliform count. The serial dilution such as $10^{-2}$ was prepared for the study of microbial load. The pour plate method was employed for the estimation of microorganisms. The total viable count ranged from 1.0 x $10^2$ to 16.80 x $10^2$ TVC/ml. The results reported are the average of three replicates. The MPN method was employed for the detection of coliform. The coliform bacteria were not detected in any of the bottled water sample. It was however; found that total viable count of all bottled water samples was much higher than the IBWA and PCRWR standards. The microbial quality of bottled water samples were not up to the mark because samples were showing higher bacterial count so it will effect on the health of the consumer.

***Key Words****:-Bottled water, MPN method, Coliform*

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## INTRODUCTION

The object of microbial examination of any food or food product is to look their freedom from fecal pathogens, toxic bacteria and high viable count. The most common water contaminants are Coliform bacteria. Common sources of bacteria are waste, septic systems, and surface water that gets into the well. The presence of indicator organisms within recommended limits is of prime importance in the quality of bottled water (Tamine, 1981; Batzing, 2001). The limit for total viable count is less than 200 TVC/ml (IBWA, 1989). Total viable count reflects the conditions in which the food was produced, packed, stored and handled. Total viable count can be used to predict the shelf life or keeping quality of the product. Epidemiological studies suggest a positive relationship between high concentrations of *E. coli* and enterococci in ambient waters and incidents of

gastrointestinal illnesses associated with recreational activities (Nagra et al., 2011). Research supports use of *E. coli* and enterococci rather than the broader group of fecal coliforms as indicators of microbiological pollution. Consistent quality and taste are two of the principle differences between bottled water and tap water (Adler *et al*., 1993).

Pathogens enter water via fecal contamination that can lead to severe and widespread human illness from drinking, swimming and bathing waters (Hendricks, 1978). Sources of fecal contamination in surface waters include wastewater treatment plants, septic systems, domestic and wild animal manure, and storm runoff. Direct testing of individual pathogens isn't cost-effective or practical, scientists have identified indicator organisms to indicate the presence of more harmful pathogens (WHO, 1996).In view of this the present research work was carried out to determine the status of the bottled water being sold in local market of Lahore whether they are free from microbial examination.

## MATERIALS AND METHODS

### Sampling:

Six samples of bottled water were collected (Table 01) from a local market. These all samples are easily available in any market of Lahore. Samples were carefully selected while examining their sealed packing. Samples were taken to L.C.W.U and stored in the refrigerator for microbial examination.

### Preparation of nutrient media:

Nutrient agar medium (g/L) peptone 5.0, Meat extracts 3.0, Agar Agar 12.0, was used for determining the total viable count of the bottled water.

### Sampling techniques

### Total viable count:

The total microbial colonies of the samples were counted by pour plate method and incubated for fixed period at $30^0$C for 24 – 48 hours. Colony count method was used to estimate the bacteriological load of the organism.

### Detection of coliform

The coliform organisms were determined by standard multiple tube fermentation technique (DHSS, 1985).The results were compared with International (Table 1)) standards drafted for total viable count and coliform count (IBWA).

## RESULTS & DISCUSSION

The data of table 5 showed that Nestle bottled water had lowest total viable count which was 1.0 x $10^2$ TVC/ml; it was with in permissible range and is according to national and international standards (IBWA, 1989, PCRWR, 2004). It must be recommended that a low total viable count does not always represent a safe product as other factors and parameters should also be considered, and the product should be recommended on an

aggregate basis (Christian, 1963). Some organisms produced toxin that remain stable under condition that may not favor survival of microbial cell (Kundsin, 1997).

Highest contamination was observed in Sparkletts bottled water and Askari bottled water that was 16.40 x $10^2$ to 16.80 x $10^2$ TVC/ml respectively (Table 6&8). In both samples gram positive and gram negative bacteria were found. The high total viable count not only indicated improper hygienic condition in processing but it was also definitely contradictory to the claim of manufacturers. Total counts of microorganisms can be used as an indicator of the sanitary quality of bottled water. Total viable count may reflect the handling history of freshness of the product. Total viable count may be taken to indicate type of sanitary control exercised in the production, transport and storage of bottled water.

**Table 1: List of manufacturers of different bottled water samples**

| SAMPLE | MANUFACTURERS |
|---|---|
| **Wah** | Wah Valley Cooperation, Farooq Center, Mcleod Road,LHR. Pakistan. |
| **Classic** | Classic Needs Pakistan (Pvt) Ltd. |
| **Nestle** | Nestle Milk Pack Ltd, 308 Upper Mall, LHR. Pakistan |
| **Sparkletts** | Sparkletts ( Pvt.) Ltd . P.O. Box 1670 , Islamabad, Pakistan |
| **Askari** | Askari Pharmaceuticals (A project of army welfare trust) 37 commercial area, Cavalary Ground LHR. Pakistan |
| **Aqua Safe** | Aqua Safe Cooperation, 96 Mcleod road LHR. Pakistan |

**Table 2: Standards drafted by International Bottled Water Association (IBWA)**

| Total Viable Count/ml | No more than 100 |
|---|---|
| Most Probable Number (MPN) | < 101 subject to the frequency of opportunity for water analysis. |

**Table 3: Microbial Examination of Wah Bottled Water**

| Sr. No. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Colour | Morphology | Gram Staining | |
| 1. | $12.48 \times 10^2$ | White | Cocci | + | - |
| 2. | $11.20 \times 10^2$ | Off white | Rod | _ | - |
| 3. | $12.80 \times 10^2$ | White | Cocci | _ | - |
| Mean | $12.16 \times 10^2$ | - | - | - | Nil |

**Table 4: Microbial Examination of Classic Bottled Water**

| Sr. No. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Colour | Morphology | Gram Staining | |
| **1.** | $15.68 \times 10^2$ | Yellow | Cocci | + | - |
| **2.** | $14.80 \times 10^2$ | Off white | Rod | _ | - |
| **3.** | $14.32 \times 10^2$ | White | Rod | _ | - |
| **Mean** | $14.93 \times 10^2$ | - | - | - | Nil |

**Table 5: Microbial Examination of Nestle Bottled Water**

| Sr. No. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Colour | Morphology | Gram Staining | |
| **1.** | $1.0 \times 10^2$ | Off white | Cocci | _ | - |
| **2.** | $1.3 \times 10^2$ | White | Rod | _ | - |
| **3.** | $1.25 \times 10^2$ | Yellow | Cocci | + | - |
| **Mean** | $1.18 \times 10^2$ | - | - | - | Nil |

**Table 6: Microbial Examination of Askari Bottled Water**

| Sr. No. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Colour | Morphology | Gram Staining | |
| **1.** | $16.80 \times 10^2$ | Off white | Rod | _ | - |
| **2.** | $15.68 \times 10^2$ | White | Cocci | + | - |
| **3.** | $14.64 \times 10^2$ | White | Cocci | _ | - |
| **Mean** | $15.7 \times 10^2$ | - | - | - | Nil |

**Table 7: Microbial Examination of Aqua Safe Bottled Water**

| Sr. no. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Colour | Morphology | Gram Staining | |
| **1.** | $12.64 \times 10^2$ | White | Cocci | _ | - |
| **2.** | $15.84 \times 10^2$ | Off white | Rod | | - |
| **3.** | $14.24 \times 10^2$ | White | Cocci | _ | - |
| **Mean** | $14.24 \times 10^2$ | - | - | - | Nil |

**Table 8: Microbial Examination of Sparkletts Bottled Water**

| Sr. No. | TVC/ml | Characteristics of Predominant Colonies | | | Coliform |
|---|---|---|---|---|---|
| | | Cooler | Morphology | Gram Staining | |
| **1.** | $15.84 \times 10^2$ | Yellow | Cocci | + | - |
| **2.** | $14.88 \times 10^2$ | White | Cocci | + | - |
| **3.** | $16.40 \times 10^2$ | Off white | Rod | _ | - |
| **Mean** | $15.7 \times 10^2$ | - | - | - | Nil |

Microbial contamination of bottled water can be influenced by variety of factors such as source of bottled water; water may be contaminated during processing and packaging. The mean value of Wah ,classic and aqua fresh bottled water sample were $12.16 \times 10^2$ TVC/ml(Table 3) which is not in accordance with the standards of Pakistan (PCRWR, 2004) and with the international standards (IBWA, 1989). It is much higher than the limits. The results revealed after testing the bottled water available in market show poor water quality, even for some renewed brands. The bacteria examined during tests were mostly cocci. Total viable count of Aqua Safe bottled water and Classic bottled water were also higher that was $15.84 \times 10^2$ TVC/ml and $14.93 \times 10^2$ TVC/ml respectively. In Aqua Safe bottled water there were only the gram negative bacteria were found but in Classic bottled water both gram positive bacteria and gram negative bacteria were found. It was found that bacterial population varied quite markedly in each of the samples.

High bacterial counts among different samples may be due to unclean utensils and containers, particularly place and atmosphere where bottled water were processed. The coliform bacteria were absent in all the samples tested. Coliform counts are generally used as an indicator of possible fecal contamination, potential for the presence of pathogenic species and reflect the hygiene standards adopted in the food's preparation (Tamine, 1981; stevens *et al*, 2001; Alder *et al*, 1993). Improper handling and storage can allow the level to increase.

The results of present study depicts that the total viable count of all bottled water samples were higher than the permissible range (IBWA,1989, PCRWR, 2004). The coliform bacteria were absolutely absent in all bottled water samples. This is a matter of great satisfaction that pathogenic bacteria in particular have not been found in all water samples. The total viable count shows that mostly non pathogenic bacteria were present. The overall quality of bottled water sample is satisfactory but still need further precautionary measures are suggested in order to improve the quality of bottled water.

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