Quality of Bottled Water Brands in Egypt

Part II: Biological Water Examination

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ABSTRACT

People can survive several days without food, but just a few days without water. People buy bottled water for a variety of reasons, including convenience, fashion, and taste or because they think it is safer than tap water. The taste of the water has to do with the way it is treated and the quality of its source, including its natural mineral content. However, taste does not always indicate safeness. Refrigeration has a significant effect on the bacteriological quality of the purchased bottle. To assess the quality of bottled water in Egypt, samples of 14 Egyptian brands of uncarbonated natural bottled water were evaluated within 6 months. Biological examinations of a total of 84 samples were carried out using standard methods comparing them with the Egyptian standards No. 1589/2005. Also bacteriological examinations of 56 samples were carried out after "1-3" months and "3-6" months storage time at room temperature to detect the effect of storage on their quality. More than half (54.8%) of biological parameters were violated the Egyptian standards. A percentage of 28.6% of all bottled water samples were contaminated with coliform, but surprisingly fecal coliforms and E.coli were not detected.

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Moreover, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from 5.95% and 3.6%, respectively of all samples. *Giardia lamblia* cysts has been found in 2.4% of samples, while absence of *Cryptosporidium* oocysts in all samples was reported. More than half (52%) of the unrefrigerated samples were unacceptable compared to only 19.4% of the refrigerated bottles. These results suggest the need for continuous monitoring for evidence of contamination at source or during the bottling process.

**KEY WORDS:** Bottled water, water quality, water standards, water storage, water packaging, water processing

**INTRODUCTION**

Bottled water consumption has been steadily growing in the world for the past 30 years. It is the most dynamic sector of all the food and beverage industry. Consumption in the world increases by an average of 12% each year, in spite of its high price compared to tap water. Consumers may have various reasons for purchasing bottled drinking-water, such as taste, convenience, or fashion, but for many consumers, safety and potential health benefits are important considerations because they believe bottled water is safer than tap water. There are concerns about chlorine by-products, contaminants such as lead, nitrates, and microorganisms contamination in municipal water supplies. However, some microorganisms, which are normally of little or no public health significance, may grow to higher levels in bottled waters. Although certain mineral waters may be useful in providing essential micro-nutrients, such as calcium and magnesium, World Health Organization (WHO) is unaware of any convincing evidence to support the beneficial effects of consuming such mineral waters and has no scientific information on the benefits or hazards of consuming bottled...
waters with very low mineral content.\textsuperscript{(3)} Also, a recent study by the International Bottled Water Association (IBWA) revealed that 25\% of all bottled water are simply tap water placed in a bottle which is a valid method of bottling water by the FDA under certain good manufacturing practices (GMPs) regulations.\textsuperscript{(4)} In addition, the quality of bottled water can also substantially vary among brands as well as with time and with different production runs depending on its source, treatment technology, manufacturing operation, packaging material, and shelf-life before use.\textsuperscript{(5)} Although, bottled water should have a shelf life of 30 days unopened, most bottled water companies' label showed that their water is valid for 1 to 2 years. On the other hand, bottled water is most commonly disinfected with ozone, which provides a residual disinfection for a limited time and subsequently does not leave a residual taste like tap water, which uses chlorine as a final disinfectant. The length of time chlorine and ozone remains active in water depends on many factors, including temperature. However, bottled water may be in distribution and storage conditions for several weeks which may adversely affect its quality.\textsuperscript{(6)}

The Centers for Disease Control and Prevention (CDC) reported a 1994 outbreak of cholera in the United States associated with bottled water. The brand of water involved was not named. The bottled water plants producing the water involved in this outbreak reportedly obtain their water mainly from municipal water and some of the wells used tested positive for fecal coliform bacteria. In Portugal, although cholera outbreak occurred in the mid-1970s due to the use of bottled water from a contaminated limestone aquifer by broken sewers from a nearby village but it demonstrates the continuing potential for contaminated bottled water to spread waterborne
disease.\textsuperscript{5} Therefore, this study was conducted to assess the quality of bottled water in Egypt by examining biological parameters and comparing with the Egyptian standards in order to gauge the safety of bottled water for human consumption.

**MATERIAL AND METHODS**

The present study was carried out during a 6 month period on a total of 84 uncarbonated natural bottled water samples of 14 commercial brands produced in Egypt and were designated from A to N (6 specimens each). These specimens were purchased from several retail outlets in Alexandria and Cairo, 42 specimens were purchased unrefrigerated from the shelves and other 42 specimens were purchased refrigerated and kept this way (at 4°C) till the time of examination. The volumes of the bottles ranged from 600 to 1500 mL. These bottles have a validity date of one year. Bottled water samples were collected monthly, preserved, and examined biologically according to the Standard Methods for the Examination of Water and Wastewater.\textsuperscript{7} Then, 56 samples were stored refrigerated at 4°C (4 specimens for each brand) for 2 days. The bacteriological examinations were carried out for them and also after "1-3" months and "3-6" months storage time at room temperature to detect the effect of storage on their safety. Data were tabulated and analysed using Statistical Package for Social Sciences (SPSS) version 11.0 computer software package.\textsuperscript{8}
RESULTS AND DISCUSSION

Biological water quality

According to the Egyptian standards No. 1589/2005(9), 54.8% of the examined bottles were bacteriologically unacceptable (Table 1). Lower figures were obtained by EL-Batouti(10) who noticed that 38.3% of the examined bottled water samples in Alexandria/Egypt were bacteriologically unsatisfactory and failed to meet the Egyptian standards.

Table (1): Results of Biologically Compliance and Noncompliance of the Examined Bottled Water Samples with the Egyptian Standards According to Their Brand

<table>
<thead>
<tr>
<th>Bottled water brands</th>
<th>Examined samples</th>
<th>Acceptable samples</th>
<th>Unacceptable samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>3</td>
<td>50.0</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>4</td>
<td>66.7</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>G</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>H</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>J</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>K</td>
<td>6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>L</td>
<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>3</td>
<td>50.0</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>38</td>
<td>45.2</td>
</tr>
</tbody>
</table>

It is evident from table (2-a,b) that although twelve of the fourteen brands were contaminated with Heterotrophic Plate Counts (HPC), eight brands were above the enforcement standards and 30.9% of the examined bottled water samples showed HPC that exceeded $10^2$ Colony Forming Units (CFU)/ml and only 7.14 % exceeded $10^3$ CFU/ml at 37°C. Although HPC is
a potential indicator of overall sanitation in bottling and water source; it may be harmless of themselves, but in some cases may indicate presence of infectious bacteria. This study agrees with Kassenga\textsuperscript{(11)} who detected HPC in 92\% of the bottled water samples analyzed. Lower results were obtained by EL- Batouti\textsuperscript{(10)}, Richards \textit{et al.},\textsuperscript{(12)} and the Natural Resources Defense Council (NRDC)\textsuperscript{(5)} who reported that the percentage of bottled water samples that had unacceptable HPC at 37\textdegree C constituted 29.3\%, 8\%, and 17\%, respectively. Warburton \textit{et al.},\textsuperscript{(13)} added that carbonated bottled water had a lower HPC which may be attributed to the use of carbonation as a final step of disinfection; a process which can lower the pH of the product and significantly reduce the bacterial load. Abdel Aziz \textit{et al.},\textsuperscript{(14)} found that 85\% of the examined bottled water samples collected from Egypt had uncountable HPC. In accordance with other studies, the present study has shown that the HPC had a wide variation that ranged from <10 up to 10\textsuperscript{3} CFU/ml within the different brands and even within the same brand.\textsuperscript{(15,16)} Obiri-Danso \textit{et al.},\textsuperscript{(17)} found that HPC ranged from 1.0 to 4.60 × 10\textsuperscript{2} CFU/ml of bottled water. Higher results were obtained by Manaia \textit{et al.},\textsuperscript{(15)} in Portugal and Ogan\textsuperscript{(18)} in Nigeria who found that 34.5\% and 53.5\% of the examined samples, respectively had counts that exceeded 10\textsuperscript{4} CFU/ml. Much higher counts (10\textsuperscript{4} to 10\textsuperscript{6} CFU/ml) were reported by Abdel Karem and Hassan\textsuperscript{(19)} who analyzed four Egyptian brands of bottled water. High numbers of heterotrophic bacteria in bottled water might come from the non-sterile plastic bottles arrived at the plant in cardboard cartons and are shipped without caps, thus the interiors are exposed to airborne contamination and the presence of foreign matter or contaminated equipment during bottling.\textsuperscript{(20)} However, high HPC values may indicate also poor GMP during the processing of
bottled water and it may be used to determine the suitability of water for use in the manufacture of food and drink. In order to minimize spoilage, the numbers must be low.\textsuperscript{(21)}

The presence of indicator organisms indicates that water is contaminated by potentially dangerous fecal matter and hence their absence denotes in general the water safety. Although coliform organisms may not always be directly related to the presence of fecal contamination or pathogens in drinking water, the coliform test is still useful for monitoring the microbial quality of drinking water.\textsuperscript{(22)} Neimi \textit{et al.},\textsuperscript{(23)} confirmed that only \textit{E. coli} is considered as a specific and reliable indicator of fecal pollution of water; since the more general test for Fecal Coliforms (FC) also detects thermotolerant non-fecal coliform bacteria. In the present study, although Total Coliforms (TC) were detected in 28.6\% of the examined bottled water samples, neither FC nor \textit{E. coli} were found. NRDC also revealed that, although TC bacteria were present in a few samples, no FC bacteria or \textit{E. coli} bacteria were found.\textsuperscript{(5)} Lower percentages were reported by Selka (2-3\%)\textsuperscript{(24)}, Warburton \textit{et al.}, (3.7\%)\textsuperscript{(25)}, NRDC (4\%)\textsuperscript{(5)}, and EPA (8\%)\textsuperscript{(20)}. However, no coliforms were isolated by Richards \textit{et al.},\textsuperscript{(12)} Abdel Aziz \textit{et al.},\textsuperscript{(14)} or Tsai and Yu\textsuperscript{(16)}.

Obiri-Danso \textit{et al.},\textsuperscript{(17)} showed that none of the microbial indicators of fecal contamination (TC, FC, and enterococci) were detected in bottled water. EL-Batouti\textsuperscript{(10)} showed that the percentage of bottled water samples that contained TC constituted 13.7\% where FC represented 8.0\% and \textit{E. coli} 2.3\%. Additionally, Kassenga\textsuperscript{(11)} found that total and fecal coliform bacteria were present in 4.6\% and 3.6\%, respectively.
In the present study, Fecal Streptococci (FS) were not detected in any of the examined samples. Abdel Aziz et al.\textsuperscript{(14)} reported presence of \textit{Enterococcus faecalis} in bottled water samples collected from Egypt.

Investigations on \textit{Staphylococcus aureus} (\textit{S. aureus}) are recommended to indicate the poor hygienic practices during the bottling process as staphylococci are part of the commensal skin flora.\textsuperscript{(26)} In the present study, \textit{S. aureus} was detected from only 5.95% of the samples. Higher detection rate of \textit{S. aureus} was reported by EL-Batouti\textsuperscript{(10)} (10.7%). This suggests that the bottled water may be subjected to contamination, not only from the containers, but also from the physical surroundings and the people who come in contact with any part of the bottling operation.\textsuperscript{(20)} However, Selka\textsuperscript{(24)} found that only 3.3% of the bottled water from retail stores and processing plants contained coagulase-positive \textit{S. aureus}. Mavridou\textsuperscript{(27)} in Scotland and Abdel Karem & Hassan\textsuperscript{(19)} in Egypt revealed similar findings.

\textit{Pseudomonas aeruginosa} (\textit{P. aeruginosa}) has been advocated as a mean of monitoring the hygienic quality of drinking water. It is used to assess the quality of bottled water as its presence suggests non-compliance with GMP.\textsuperscript{(25)} Contamination may be the result of colonization of the bottling plant equipment; as rubber seals, lining or coating, washers, and even disinfecting soap, that may all provide nutrients for this organism. It can grow in low-nutrient water such as deionized and distilled water and reach $10^4$ CFU/ml in mineral water thus increasing the public health risks. The presence of \textit{P. aeruginosa} may suppress standard coliform enumeration procedures and can degrade water colour, turbidity, and taste.\textsuperscript{(28)} The present study revealed that 3.6% of the examined bottled water samples were
contaminated by P. aeruginosa representing 50% of brand N. Approximately similar results were obtained by Richards et al.,\textsuperscript{(12)} who isolated P. aeruginosa from 4% of bottled water samples. Lower percentage was reported by Warburton et al.,\textsuperscript{(25)} where 1.2% of the examined samples were contaminated by P. aeruginosa. On the other hand, EL-Batouti\textsuperscript{(10)}, Hernandez-Duquino and Rosenbeg\textsuperscript{29}, Manaia et al.,\textsuperscript{(15)}, Papapetropoulou et al.,\textsuperscript{(30)} and Hunter\textsuperscript{(26)} isolated P. aeruginosa from 5.7%, 10.2%, 11%, 18.8%, and 29% of the examined bottled water samples, respectively. It was also found to be the predominant isolated strain by Tamagnini and Gonzalez.\textsuperscript{(31)} It was found that two out of four brands had a mean bacterial count for P. aeruginosa that ranged from $1.7 \times 10^4$ to $3.7 \times 10^5$ CFU/ml.\textsuperscript{(19)} It has been shown that the adherence, survival and colonization of P. aeruginosa on plastic bottle surfaces especially Polyvinyl Chloride (PVC) enhances the capacity of bacteria to resist disinfection and starvation.\textsuperscript{(31)}

The present study revealed that none of examined bottled water samples was contaminated with mold or yeast. Tsai and Yu\textsuperscript{(16)} showed that mold and yeast were detected in 38.6% and 18.8% of domestic and imported samples, respectively. Additionally, another study found that yeasts or molds were recovered from 14.6% of samples.\textsuperscript{(32)}

The potential harm posed by carry-over of orally toxic metabolites of organisms, producing algal (cyanophytic) blooms, was considered.\textsuperscript{(33)} In this study nine of the fourteen brands were not contaminated with algae, five brands were contaminated with counts ranging from $2.40 \times 10^2 \pm 3.16$ to $2.40 \times 10^3 \pm 3.44$ unit/L.

One of the main concerns about overall drinking water safety is microbial contaminants such as Cryptosporidium or Giardia.
Since Cryptosporidium and Giardia are primarily surface water contaminants, water originating from a protected natural or underground water source is unlikely to contain Cryptosporidium or Giardia. However, some bottled water may also come from treated municipal supplies. So, at least one of three processing methods (reverse osmosis, one-micron absolute filtration, and distillation) for effective removal of microbial (surface-water) contaminants, including Cryptosporidium or Giardia is recommended. Ozonation may also prove to be an effective treatment for Cryptosporidium and Giardia removal. In the current study, although none of the examined bottled water samples had Cryptosporidium oocysts, Giardia lamblia cysts were observed in 2.4% of total bottled water samples.

Bottled water is usually disinfected to remove harmful organisms, but is not intended to sterilize the water so, bacteria are found in most bottled water. To maintain the purity of bottled water, Health Canada recommends the refrigeration of the bottled water once it is opened, and preferably once bought. Check the bottling date and best-before-date on the bottle to determine how fresh the product is. In 1988, Health Canada study of bottled water kept at room temperature for 30 days showed a substantial increase in the bacterial count especially HPC. This finding supports the results of this study where more than half (52%) of the samples of the purchased unrefrigerated bottles were unacceptable and failed to comply with the Egyptian standards, compared to only 19.4% of the refrigerated bottles. This pattern was consistent in all the brands. It was also noticed that 57.2% of the bottled water samples that were originally purchased refrigerated and kept at 4°C for 2 days maintained the same HPC compared to only 39.3% in unrefrigerated bottles. Moreover, only 7.1% of the
samples of the refrigerated bottles showed increased HPC compared to 14.3% of the unrefrigerated bottles (Table 3).

Table (3): Heterotrophic Plate Counts of the Examined Bottled Water Samples According to the Condition of the Purchased Bottles (Refrigerated and Unrefrigerated) and Storage at 4°C for 2 Days

<table>
<thead>
<tr>
<th>Heterotrophic Plate Counts</th>
<th>Storage at 4°C for 2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Condition of the purchased bottles</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Same</td>
<td>16</td>
</tr>
<tr>
<td>Increase</td>
<td>2</td>
</tr>
<tr>
<td>Decrease</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
</tr>
</tbody>
</table>

Nearly, similar results were obtained by EL-Batouti\textsuperscript{(10)} who found that the percentage of unacceptable samples of the unrefrigerated bottles constituted 54.0%, compared to 22.7% for the refrigerated bottles. Refrigerated samples also showed a higher tendency to maintain the same HPC after refrigeration for 2 days at 4°C than in unrefrigerated samples (52% and 40%, respectively). Nsanze et al.,\textsuperscript{(36)} found that out of 10 bottled water samples that were stored at 4°C for 2 days, only 30% had initial counts <1CFU/ml, while 20% of the samples increased in HPC and 50% decreased in their counts. They also demonstrated that organisms multiplied more easily between 25°C and 37°C; the common indoor temperature, whereas refrigeration temperature (4°C) reduced or maintained the bacterial status for at least 3 days. On the other hand, Gonzalez et al.,\textsuperscript{(37)} demonstrated the
survival and multiplication of *P. aeruginosa* in bottled water stored at 20, 30, and 37°C. It was found to compete well with the natural bacterial population, reaching values greater than $10^4$ CFU/ml and surviving longer than one year. This organism can slow down its metabolic activity and is therefore considered as a persistent contaminant\(^{(38)}\). In agreement with other studies in different countries\(^{(16,27)}\), the present study showed that the longer the time elapsed between the production date and date of purchasing, the higher the percentage of unacceptable samples, where 57.1\% of the samples stored at room temperature for "4-6" months were nonacceptable compared to only 32.1\% of those stored for "1-3" months (Figure 1).

**Figure 1:** Percentage of Acceptable and Unacceptable Bottled Water Samples According to the Storage Time

*Storage Time*

*Figure 1*: Percentage of Acceptable and Unacceptable Bottled Water Samples According to the Storage Time
EL-Batouti\textsuperscript{(10)} revealed that the higher percentage of unacceptable bottled water samples was found during the "4-6" months storage time than during the "1-3" months storage time (52.5% and 33.2%, respectively). In absence of treatment with ultraviolet irradiation, carbonation, ozonization, or others, bacterial multiplication may commence 1-2 days after bottling and can continue for 1-3 weeks, resulting in a population of $10^5$-$10^6$ CFU/ml.\textsuperscript{(39)} This study reinforces the view that uncarbonated bottled waters have a large bacterial population after storage. In Spain, according to Gonzalez \textit{et al.},\textsuperscript{(37)} the initial population of bottled water increased from $10^2$ CFU/ml to $10^5$-$10^6$ CFU/ml after 3 days storage at 20 °C. Morais and da Costa\textsuperscript{(39)} in Portugal and Mavridou\textsuperscript{(27)} in Scotland demonstrated that the HPC rapidly increased at room temperature from an initial count of 1-5 CFU/ml at the day of bottling to a maximum of $10^4$-$10^5$ CFU/ml only two weeks later. Tsai and Yu\textsuperscript{(16)} found similar results after storage for one month at 25°C. Morais and da Costa\textsuperscript{(39)} have stated that the reason for altered growth behavior may be due to the oxygenation of the water during the bottling process, the increased surface area provided by the bottle, trace amounts of nutrients arising from the bottle such as carbon, or the multiplication and feeding of bacteria on the products of lysis or on the metabolites of autotrophs. In the present study, it was found that brand L represented the best brand as regards the bacteriological quality and its compliance with the Egyptian standards for natural drinking bottled water No.1589/2005.\textsuperscript{(9)} This may be due to the fact that its plastic polyethylene terephthalate (PET) bottle is sterilized and automatically filled, as indicated by the label on its bottle. Although PVC is still used, PET is increasingly used for many reasons: it is brighter than PVC, very transparent, and it almost looks like glass. PET is
shatter-resistant and easy to work on. Its light weight (20% lighter than PVC) enables to reduce plastic quantities needed to make a bottle. It is compressible, so volumes of waste are smaller. PET is in addition easy to recycle or remanufacture: it can be turned into polyester carpets, fabrics, and fibers for the textile and clothing industry, plastic films, eggs boxes, industrial strapping, and new PET bottles. When burnt, it doesn’t release chlorine into the atmosphere, contrary to PVC, whatever type of incinerator is used. Still, the manufacture of plastic bottles also can cause release of phthalates, and other by-products of plastic-making, into water, air, or other parts of the environment.\(^{(3)}\) Brand K was the worst brand where its percentage of unacceptable samples was about 6 times that revealed in brand L. As for brands C and N approximately one-third of their samples were non-compliant with the Egyptian standards.\(^{(9)}\) While for brands A, E, F, G, H, I, and J more than half of their samples were violating the Egyptian standards.\(^{(9)}\)

**RECOMMENDTIONS**

Protection of well from contamination and improvement of processing practice to comply with GMP. The choice of packaging materials should be considered. Health education of community on the proper purchasing and storing of bottled water practices should be launched. Further studies on the effects of packaging materials and storage conditions on final product quality are required.

**REFERENCES**


