Bacteriological quality of bottled water brands in Karachi, Pakistan

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ABSTRACT

In the present study, bacteriological quality of 187 different mineral water bottle brands marketed in Karachi was analyzed from September 2009 to June 2010. All the collected samples were analyzed for the presence of coliform bacteria, fecal coliform bacteria, *E. coli*, fecal entero cocci, *P. aeruginosa* and total viable plate count (TVPC). Alarmingly, 67 (36%) of these samples did not comply with Pakistan and WHO standard guidelines for drinking water. The majority of the specimens (39 samples) were contaminated with higher TVPC and *P. aeruginosa*, while many were contaminated with both coliform (20 samples) and fecal coliform bacteria (11 samples). Total heterotrophic bacterial number in 49 samples was higher than even 200 cfu/mL. The presence of coliform bacteria in drinking water suggests the possible presence of pathogenic enteric microorganisms thus unsafe for drinking. The data presented here clearly raise the concerns regarding the quality of drinking mineral water and highlights the danger posed to the public health.

Key words: Bottled, water, coliform, bacteria, contamination

INTRODUCTION

Water is an essential requirement of all life forms. Satisfactory supply of clean, safe and hygienic water is imperative for health. Drinking unsafe and unhygienic water can cause high prevalence of waterborne diseases like diarrhoea, typhoid and cholera (Oyedeji et al., 2010, Fawell & Nieuwenhuijsen, 2003). As a preventive measure, consumption of bottled water has increased in recent years in developing countries and elsewhere. Sale of bottled water has gone to more than 35 billion US dollars (Raj, 2005) and by an average of 12% increase per year the world over (El-Salam et al., 2008). People from all over the world drink about 13x10^10 liters of bottled water annually (Samadi et al., 2009). In Pakistan the consumption of bottled water increased from 5.9% to 9.5% during 2003-2007 (Samadi et al., 2009).

In general people are concerned about the poor quality of tap water that is why they have switched over to bottled water perceiving it to be clean and safe. Although, people consider bottled water safe but it can be also be contaminated with chemical and biological agents (Khaniki et al., 2010). Presence of coliform bacteria, *E coli* or *Pseudomonas* in bottled water can pose a great threat to the public health. Infants, young children, debilitated and immuno-compromised people are at high risk of waterborne diseases, even at lower infective doses (WHO, 2005).

According to world health organization (WHO) recommendations, potable water should have <20 cfu/mL heterotrophic bacterial count with
complete absence of coliform bacteria, fecal coliforms, *E. coli*, enterococci and *Pseudomonas aeruginosa*. Although, coliform organisms may not always be considered as indicator of fecal contamination but their presence in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp. and *Vibrio cholera*. *Pseudomonas aeruginosa* is an opportunistic pathogen causing a variety of infections like urinary and respiratory tract infections, particularly in patients who are severely immuno-compromised (Zamberlan da Silva et al., 2008). Presence of *P. aeruginosa* is a great risk to human health because Pseudomonas species are multi drug resistant organisms having the ability to transfer its resistance genes to other bacteria in the human body.

The main objectives of the current study are to analyze the bacteriological quality and bacterial contamination of bottled mineral water from Karachi markets and to check their compliance with the standards.

**MATERIALS AND METHODS**

**Sample collection:** A total of 187 different local brands of commercially available bottled water were collected randomly from supermarkets and various shops of Karachi from September 2009 to June 2010. Bacteriological analysis of all the samples was conducted within 2 hours of collection.

**Sample analysis:** All the collected water samples were analyzed for the following parameters according to WHO and Pakistan standard guidelines and as described previously (Liguori et al., 2010):

- **Total viable plate count:** Total viable plate count (TVPC) was performed by pour plate technique. Bottled water samples were mixed in R2A media (Merck) and incubated at 37°C for 48 hours and at 22°C for 72 hours for thermophilic and psychrophilic bacteria respectively. After incubation colonies were counted with the help of colony counter.

- **Coliform bacteria:** Membrane filter technique was performed to isolate the coliform bacteria. Briefly, 250mL of each sample was filtered through 0.45 μm pore size cellulose nitrate membrane filter (Sartorius) and placed on Tergitol 7 TTC agar (Merck) and plates were incubated at 37°C for 18-24 hours. Plates were checked for the presence of coliform bacteria by routine diagnostic tests like TSI, Citrate, SIM.

- **Escherichia coli:** *E. coli* was also detected from the same plates that were used for the isolation of coliform bacteria. Identification of *E coli* was done by routine diagnostic tests like TSI, Citrate, SIM.

- **Fecal coliforms:** 250 mL of each sample was filtered through 0.45 μm cellulose nitrate membrane filter. Filter membrane was placed on MFC agar (Merck) & plates were incubated at 44.5°C for 18-24 hours. After incubation plates were observed for dark blue colored colonies. Fecal coliform bacteria were confirmed by the gas formation in EC broth (Merck) incubated at 44.5°C for 18-24 hours.

- **Fecal enterococci:** 250 ml of each sample was filtered through 0.45 μm cellulose nitrate membrane filter which was then placed on Slanetz-Bartley agar (Merck) and plates were incubated at 37°C for 48 hours. After incubation plates were observed for typical colonies. Isolated colonies were confirmed as fecal enterococci by bile esculin azide test.
**Pseudomonas aeruginosa**: Similarly, 250 ml of each sample was filtered through 0.45 µm cellulose nitrate membrane filter and then membrane was placed on *Pseudomonas* CN agar (Citrimide-Nalidixic agar; Merck) and plates were incubated at 37°C for 24-48 hours. Identification of *P. aeruginosa* was done by routine diagnostic tests like TSI, Citrate, SIM and oxidase test.

**RESULTS AND DISCUSSION**

Karachi city is a highly urbanized area of Pakistan, where approximately 200 brands of bottled water are marketed. To the best of our knowledge, very few studies have been conducted to analyze the quality of bottled mineral water from Pakistan. Therefore this study was undertaken to analyze the bacteriological quality of bottled water from Karachi markets and to check their compliance with the standards. According to the guidelines of national and international recommendations, all the specimens were checked for the following parameters: TVPC at 22°C or 37°C, presence of coliform bacteria, fecal coliforms, *E. coli*, fecal enterococci and *P. aeruginosa*.

In the present study the bacteriological examination of 187 different bottled mineral water samples was carried out. Alarmingly, 67 (36%; figure 1) of these samples did not comply with the standard guidelines of Pakistan and WHO for drinking water. In these specimens TVPC at 37°C was found higher than the recommended range which is <20 cfu/mL (Figure 2). TVPC was found even higher than 200 cfu/mL in 49 (26%) mineral water samples (Table 1). TVPC count shows the presence of heterotrophic bacteria in the water samples, which indicates the bacterial pollution of drinking water. Although heterotrophic bacteria are themselves non-pathogenic but there are chances that the higher heterotrophic bacterial count is associated with the presence of coliform bacteria or other pathogens, as indicated in our results. Out of 67 non-complied samples only 8 failed due to higher TVPC count parameter alone, while 39 samples failed because TVPC was associated with *P. aeruginosa*. Whereas, rests of the samples failed because higher TVPC was associated with *P. aeruginosa*, coliform and *E. coli* in different combinations as indicated in figure 2.

Shockingly, 20 (11%; figure 2) mineral water bottles were contaminated with coliform bacteria. Although all the analyzed bottled samples were negative for fecal coliform and fecal enterococci but 11 (6%; figure 2) of these were found positive for *E. coli*, an indicator of fecal contamination. The presence of coliforms and *E. coli* in bottled water samples not only indicates the potential presence of pathogenic enteric microorganisms but also questions the efficiency and integrity of production system. It is therefore recommended to monitor the complete water processing system to avoid major public health problems.

Yet another point of concern was the presence of *P. aeruginosa* in 45 (24%; figure 2) mineral water samples. In this study, the main reason for the non-compliance of the majority of the mineral water samples to standards was that these were contaminated with *P. aeruginosa* along with higher TVPC (Figure 3). One of the main reasons for *P. aeruginosa* isolation in high percentage was that it can survive longer in low nutrient environment by slowing down its metabolic activity and its resistance against disinfectants commonly used for the treatment of water (Mena & Gerba, 2009). Presence of *P. aeruginosa* in mineral water is
considered as an indicator of the quality of water. In a recent study, majority of *Pseudomonas* spp. isolated from bottled mineral water were found to be multi drug resistant (Daood, 2009), which can cause significant health hazards to the consumers.

Different studies have shown that bottled drinking water is not always safe (Venieri *et al*., 2006, Zeenat *et al*., 2009). In India 40% of the bottled drinking water samples were failed in TVPC parameter and most of the isolated were highly resistant to many antibiotics (Jeena *et al*., 2006). In a study conducted in Brazil, 20 liters mineral water bottles were found contaminated with coliform bacteria, fecal coliform and *P. aeruginosa* (Zamberlan da Silva *et al*., 2008). Many outbreaks have also been resulted due to contaminated bottled water (Reynolds *et al*., 2008). CDC reported in 1994 that Cholera out break in US was associated with bottled water (El-Salam *et al*., 2008). Similarly, bottled water was reported to be one of the vehicles for *Vibrio cholera* transmission in Portugal (Addo *et al*., 2009). According to a previous study in Pakistan the majority of the mineral water samples were contaminated with *P. aeruginosa* and *E. coli* (Taj & Baqai, 2007).

Contaminated mineral water poses great health risk especially when it is used by elderly persons, infants, hospitalized patients and immuno-compromised ones (Leclerc *et al*., 2002). There can be many reasons for the bacterial contamination like improper disinfection, infiltration of contaminated water, leakage points. Furthermore, the improper storage of the products provides favorable conditions for the bacteria to grow up to harmful levels. Therefore, a regular checking of mineral water bottles is necessary to prevent the spread of diseases and to keep vigilance on quality of water available in the market. In addition, studies about the chemical analysis of these brands are equally important. The data presented here clearly raise the concerns regarding the quality of drinking mineral water; danger posed to public health and highlights the importance of a continued surveillance system to minimize the chances of contamination in the mineral water.

Table 1: Total viable plate count (TVPC) denoted in cfu/mL in mineral water samples.

<table>
<thead>
<tr>
<th>TVPC range (cfu/mL)</th>
<th>Number of samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>75</td>
<td>40%</td>
</tr>
<tr>
<td>15-20</td>
<td>45</td>
<td>24%</td>
</tr>
<tr>
<td>20-200</td>
<td>18</td>
<td>10%</td>
</tr>
<tr>
<td>200-300</td>
<td>25</td>
<td>13%</td>
</tr>
<tr>
<td>&gt;300</td>
<td>24</td>
<td>13%</td>
</tr>
</tbody>
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**Fig. 1:** Total number of samples having compliance and non-compliance with Pakistan and WHO standards.

<table>
<thead>
<tr>
<th>Total no of Samples</th>
<th>187 100 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complied with the standards</td>
<td>120 64 %</td>
</tr>
<tr>
<td>Not complied with the standards</td>
<td>67 36 %</td>
</tr>
</tbody>
</table>

**Fig. 2:** Pass and fail ratio of all the parameters used to analyze the quality of mineral water samples.
Profile of samples fail in different parameters

Coliform, E.coli, P. aeruginosa and TVPC (02)
Coliform, P. aeruginosa and TVPC (04)
Coliform, E.coli and TVPC (09)
P. aeruginosa and TVPC (39)
Coliform and TVPC (05)
TVPC (08)

Fig. 3: Profile of samples failing in different parameters.

REFERENCES


