

Bacteriological Assessment of some domestic bottled waters marketed in Basrah governorate, Iraq.

Nassir Abdullah Hillo

Ecology Department, Science College, University of Basrah

E-mail: nassirmicro@gmail.com

Abstract:

Contaminated water can cause a spectrum of disease ranging from self-limiting gastrointestinal disturbances to severe life threatening infections. This study was conducted about bacteriological assessment of domestic bottled waters commonly sold in Basrah governorate. Eighty samples of bottled waters were collected randomly from ten brands. The samples were analyzed about total bacterial count, total coliform bacteria, faecal coliform bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, According to standard methods for examination of water. Results showed that 19(23.75%) of samples contained total bacterial count above the acceptable standard. Total coliform bacteria were detected in 11(13.75%) water bottles including *E. coli*, *Klebsiella pneumonia* and *Enterobacter* sp, while a faecal coliform bacteria were detected in 1(1.25%) samples were *E. coli* and *Pseudomonas aeruginosa* were detected in 2(2.5%) samples, while none of the bottled water samples were positive for *Staphylococcus aureus*.

Key words: bottled water, bacteriological quality, coliform count, total bacteria count, Basrah governorate.

Introduction

The water consumed by human being comes in various forms and from various sources (Deutsch, 1997 and Baba *et al.*, 2008). The water quality is often related to the degree of bacterial contamination and other factors (Khaniki *et al.*, 2010). Contaminated water can cause a spectrum of diseases

ranging from self-limiting gastrointestinal disturbances to severe life threatening infections (Pant *et al.*, 2016). As a preventive measure, consumption of bottled waters has increased in recent years in all over the world. It is generally perceived as pure, clean of good quality and

protected (Khatoon and Pirzada, 2010). Sales of bottled water have increased dramatically in recent years, with worldwide sales of more than 35 billion US dollars, largely because of the public perception of purity, safety and public concern about the quality of tap water (Raj, 2005). The manufacturers have promoted the bottled water as pure and clean water, ideal for infants, elderly and immune-suppressed individuals. In addition, the presence of odors and unpleasant taste in the water supplying by municipal as well as to fluoride, chlorine and other additives led to increase the consumption of bottled water in the world (Semerjian, 2011). However, the concern about the microbial quality of bottled water have been raised over the recent years. Several studies have been detected the coliform and heterotrophic bacteria in bottled water with counts which far exceeded national and international standards set for potable water for human consumption (Bharath *et al.*, 2003 and Kkaniki *et al.*, 2010). Different studies in different countries (India, Brazil, US, Pakistan and Portugal) have shown that the bottled water is contaminated with potential pathogens such as *Pseudomonas aeruginosa*, *Vibrio cholerae* (Khatoon and Pirzada, 2010) *Staphylococcus aureus*, *Aeromonas hydrophila*,

Salmonella spp and *Shigella spp* (Ahmed *et al.*, 2013). The bacteria in bottled waters may be indigenous from the natural source of the water or may be introduced during processing. The number of these bacteria could multiply during storage to reach infective doses for consumers (Bharath *et al.*, 2003).

The manufacturing and consumption of bottled water in Iraq become very popular during ten years ago. The growing demand for bottled water in Iraq attributed to different reasons such as water scarcity, change in the characteristic of drinking water quality and weak confidence of consumer in the drinking water supplied from government projects (Ismael *et al.*, 2013). The aim of this study was to evaluate the bacteriological quality of some domestic bottled water commonly sold in Basrah governorate and check compliance with Iraqi and international standard of drinking water.

Materials and methods:

Sample collection: Total of 80 sample of domestic bottled water commonly sold in Basrah governorate were collected randomly from various shops and markets in different location of Basrah governorate during the

period of September 2016 – December 2016. Overall 80 sample belonging to 10 different domestic brands, the details of each brand are given in (table 1). 20 bottled of water, two from each

brand with different production dates were selected monthly and transferred directly to the analytical laboratory for bacteriological analysis at suitable conditions.

Table 1: Information of tested domestic bottled waters

Brands name	No. of tested samples	Location of production	Methods of disinfection
Alem	8	Basrah	Ozone
Al waha	8	Babel	Ozone
Al-khaleej	8	Basrah	Ozone
Al wafi	8	Baghdad	Ozone &U.V.
Al Janaaen Al mualaka	8	Basrah	Ozone
Al Malika	8	Basrah	Ozone
Basrah pearl	8	Basrah	Ozone &U.V.
Miah al janoob	8	Basrah	Ozone
Salsal	8	Basrah	Ozone &U.V.
Veneza	8	Baghdad	Ozone

Sample analysis: All the bottled water samples were analyzed for the following bacteriological parameters according to WHO standard guidelines (WHO, 2011).

Total bacterial count: Total bacterial count was performed by using both pour plate and serial dilution technique. Serial dilutions of the bottled water were prepared in sterile normal saline, 1ml of sample was transferred to sterile, empty petri dish. Melting nutrient agar was poured into the petri dish containing the sample, and mixed thoroughly. The mixture was allowed to solidify

and the plate incubated at 37 C° for 48 hours. The bacterial colonies that developed were counted and recorded as colony-forming unit per milliliter (cfu/ml).

Total coliform bacteria count: Total coliform bacteria count were determined by using membrane filter technique. 100 ml of each sample was filtered through 0.45 µm pore size cellulose nitrate membrane filter (Sartorius, Germany) and placed on eosine methylene blue agar (Himedia) and incubated at 37 C° for 24 hours.

Faecal coliform count: Faecal coliform count were determined by using membrane filter technique. 100 ml of each sample was filtered through 0.45 µm pore size cellulose nitrate membrane filter and placed on Mf-c agar (Himedia) and incubated at 44.5 C° for 24 hours in waterbath.

Detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus*: The presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were determined by filtering 100 ml of each sample (as described above). The filter membranes were placed on nutrient agar plates and incubated at 37 C° for 24 hours. Colonies arising after incubation were checked by Grams staining for the presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and streaking onto fresh *Pseudomonas* agar (Himedia) and Mannitol salt agar (Titan Biotech) respectively.

Results:

This study was conducted to investigate the bacteriological quality of bottled waters from Basrah markets because there aren't studies have been carried out in Basrah governorate about domestic bottled water. The results of the total bacteriological

count analyses of 80 different domestic bottled water samples representing the products of 10 companies (table 2) were ranged between zero and 510 cfu/ml. 19 (23.75%) of the samples had total bacterial count above the acceptable limits which is < 20 cfu/ml (figure 2). The brands of bottled water (Al Malika, Miah al janoob and Salsal) which had 510, 167 and 223 content of total bacterial count respectively while (Alem, Al waha, AL-khaleej, al wafi, Al janaaen Al mualaka, Basrah pearl and veneza) which had zero content of total bacterial count.

The total coliform bacteria in 100 ml of bottled water were detected in 11 (13.75 %) of samples (figure 1(B) and figure 2). Six samples of Al Malika brand out of 8 samples was contaminated with coliform bacteria with mean number is 167 (table 3), including *Klebsiella pneumonia* and *Enterobacter* sp. Four samples of Miah al janoob brand out of 8 samples was contaminated with coliform bacteria with mean number 13, including *E. coli* and *Enterobacter* sp. One sample of Salsal brand out of 8 samples contaminated with coliform bacteria with mean number 23, including *Enterobacter* sp in the sample.

Table 2: Total bacterial count of analysis of domestic bottled waters.

Brands name	No. of tested samples	No.(%) samples positive for aerobic bacteria	Mean (cfu/ml)
Alem	8	0 (0 %)	0
Al waha	8	0 (0 %)	0
Al-khaleej	8	0 (0 %)	0
Al wafi	8	0 (0 %)	0
Al Janaaen Al mualaka	8	0 (0 %)	0
Al Malika	8	8 (100 %)	510
Basrah pearl	8	0 (0 %)	0
Miah al janoob	8	6 (75 %)	167
Salsal	8	5 (62 %)	223
Veneza	8	0 (0 %)	0
Overall	80	19 (23.75 %)	

Table 3: Prevalence of coliform bacteria in tested domestic bottled waters.

Brands name	No. of tested samples	No.(%) samples positive for coliform bacteria	Mean (cfu/100ml)
Alem	8	0 (0 %)	0
Al waha	8	0 (0 %)	0
Al-khaleej	8	0 (0 %)	0
Alwafi	8	0 (0 %)	0
Al Janaaen Al mualaka	8	0 (0 %)	0
Al Malika	8	6 (75 %)	187
Basrah pearl	8	0 (0 %)	0
Miah al janoob	8	4 (50 %)	13
Salsal	8	1 (12.5 %)	23
Veneza	8	0 (0 %)	0
Overall	80	11 (13.75 %)	

The faecal coliform bacteria were detected in one sample 1(1.25%) in Miah al janoob brand (figure 1(A) and figure 2) although three samples of this brand was contaminated with *E. coli*.

Pseudomonas aeruginosa was detected in two samples (2.5%) in

Salsal brand and Al malika brand (figure 2).

Staphylococcus aureus which are the other indicator organisms was investigated in all water samples, the analyzed bottled samples were negative for *Staphylococcus aureus* (figure 2).

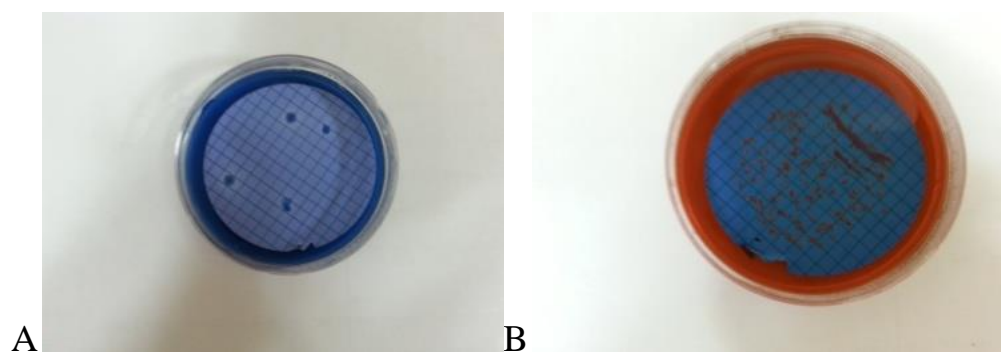


Figure 1: A: Faecal coliform bacteria, B: Total coliform bacteria.

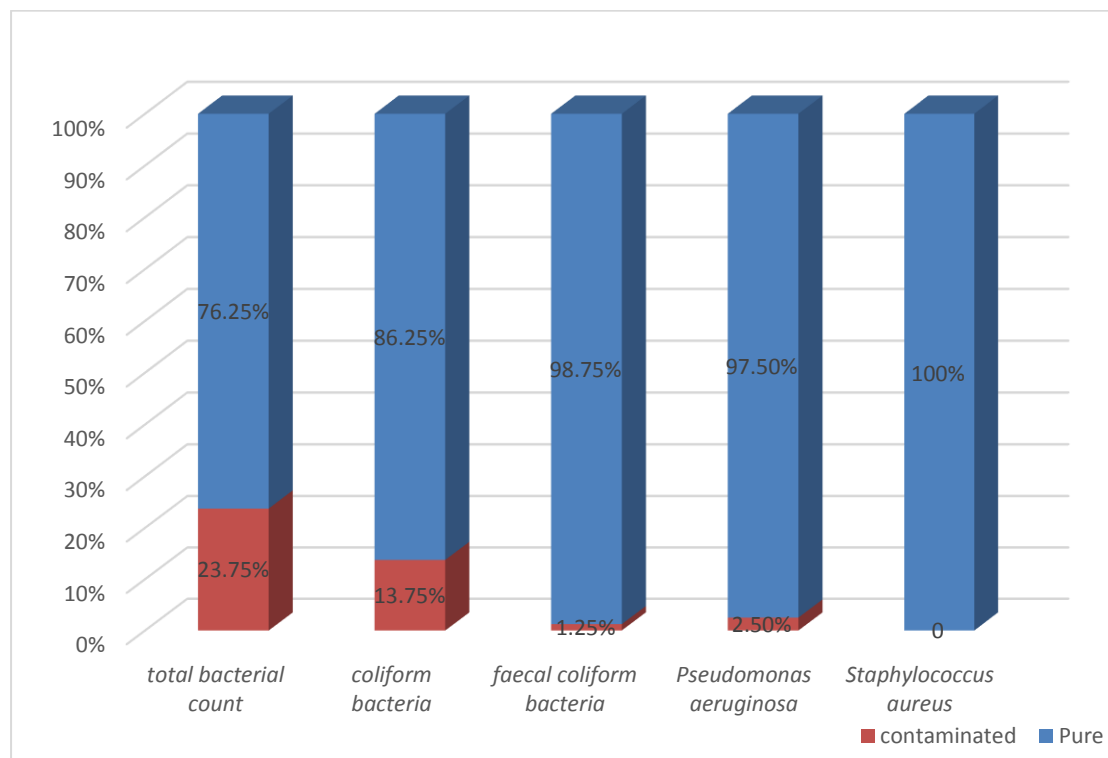


Figure 2: Profile of tested samples with different bacterial counts

Discussion:

The present study the bacteriological analysis of 80 samples of domestic bottled water were carried out. The total bacterial count was ranged from 0 to 510 cfu/ml among the bottled water examined. 19 (23.75 %) samples of examined bottled water didn't comply with standard guidelines of WHO was recommended range which is > 20 cfu/ml. The result showed lower than (Khatoon and Pirzada, 2010) who found the total bacterial count is 36% of samples more than of standard guidelines of WHO.

In a study conducted in Baghdad by (Olewi *et al.*, 2016) in 2014 were reported 5% of bottled water samples failed to meet Iraqi and WHO standard which are showed high number of heterotrophic bacteria in the water samples. The enumeration of total bacterial counts is used as indicator of overall bacteriological quality. The presence of high bacterial counts might play a main role in the presence of potential pathogens in drinking water (Khanbiki *et al.*, 2010).

The total coliform bacteria were detected in 11 (13.75 %) out of 80 tested samples. The coliform bacteria must not be detected in any 100 ml sample according to WHO standard. The result of present study was showed the presence of

coliform bacteria more than the result reported by Rabee *et al.* (2012) in Baghdad city which was 9.5% of tested samples, while Igbeneghu and Lamikanra (2014) in Nigeria was reported 16.3% of the bottled water samples contaminated with coliform bacteria. The presence of coliform in bottled water samples are indicated the potential presence of pathogenic enteric bacteria and ineffectiveness of the treatment process, therefore appropriate treatment processes should be utilized to avoid major public health problems (Khatoon and Pirzada, 2010).

The faecal coliform bacteria were detected in one sample out of 80 tested samples. Samuel *et al.* (2016) in Nigeria reported in their study that 20 % of tested samples was contaminated with faecal coliform bacteria, while Olewi *et al.* (2016) reported 2.5 % of tested samples give positive results to faecal coliform bacteria. Faecal coliform bacteria are considered as important indicator of recent contamination of faecal matter because coliform bacteria can exist in the environment. Faecal coliform bacteria are found in the intestine tract of warm blooded animals. Hence its detection is indicate of faecal contamination from animals or human and also the potential

presence of pathogenic enteric bacteria (Venkatesan *et al.*, 2014).

The prevalence of *Pseudomonas aeruginosa* was detected in 2 (2.5 %) samples along with samples that contained higher total bacterial count. *Pseudomonas aeruginosa* is an opportunistic pathogen and also known for its resistance to many antimicrobial agents making the treatment of infection by this bacteria a difficult task (Igbeneghu and Lamikanra, 2014). Different studies have shown that bottled drinking water are contained *Pseudomonas aeruginosa* in high percentage. Oleiwi *et al.* (2016) were recorded 13.2 % of investigated bottled water samples contaminated with *Pseudomonas aeruginosa*, while Khatoon and Pirzada (2010) reported that 24 % of water samples were contaminated with *Pseudomonas aeruginosa*.

The pollution of domestic bottled waters samples with heterotrophic bacteria, coliform bacteria and other species of bacteria examined in this study referred to don't follow stipulated guidelines in their production processes like improper disinfection, infiltration of contaminated water and the improper storage of the products provides favorable conditions for the bacteria to grow up to harmful levels (Khatoon and Pirzada, 2010), therefore there is need for stringent monitoring the

water factories and impose comply with proper health procedure.

Conclusions:

The result of this study revealed that some domestic bottled waters available in markets and shops of Basrah governorate have bacteriological contents above unacceptable limits to Iraqi and WHO standard and another contaminated with coliform bacteria. Since domestic bottled waters are in high demand in Basrah governorate, therefore there is need to improve treatment of the water before bottling as well as adequate post-bottling handling. The health ministry considered responsible for monitoring manufacturers to follow strictly the work of manufacturers and impose comply with proper health procedures.

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تقييم المحتوى البكتيري لبعض عينات المياه المعبأة المحلية المستهلكة في محافظة البصرة، العراق

ناصر عبدالله حلو

كلية علوم-قسم البيئة /جامعة البصرة

المستخلص:

تسبب المياه الملوثة يسبب الكثير من الامراض والتي تتراوح بين اضطرابات معوية الى اصابات خطيرة تهدد حياة الافراد. الدراسة الحالية تضمنت تقييم المحتوى البكتيري لعدد من المياه المعبأة المحلية الصنع والاكثر استهلاكاً في محافظة البصرة. جمعت 80 عينة ماء عشوائياً تعود لعشر علامات تجارية خلال المدة الممتدة من تشرين اول 2016 الى كانون ثاني 2017. درست العينات باستخدام عدة فحوصات بكتيرية وهي حساب العدد الكلي للبكتريا الهوائية, العدد الكلي لبكتريا القولون, بكتريا القولون البرازية والتحري عن بكتريا *Pseudomonas aeroginosa* و *Staphylococcus aureus* وفق المعايير القياسية لفحص الماء. اظهرت النتائج ان 19 عينة (23,75%) تحتوي على بكتريا هوائية اعلى من المقاييس الموضوعه من قبل منظمة الصحة العالمية, في حين اظهرت النتائج وجود بكتريا القولون في 11 عينة (13,75%) وتتضمن بكتريا *E.coli*, *klebsiella pneumonia* و *Enterobacter sp* وبكتريا القولون البرازية في عينة واحدة (1,25%) وتعود لبكتريا *E.coli*, و بكتريا *Pseudomonas aeroginosa* في عينتين اثنتين (2.5%), بينما لم يسجل وجود بكتريا *Staphylococcus aureus* في اي من العينات المفحوصة.