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Original article

Quality Control Evaluation of the Microbial Contamination of Bottled Drinking Water and House Hold Reverse Osmosis Water in Tripoli, Libya

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ABSTRACT

Background and aims. *Access to clean and safe water is essential for human life. Among the quality control requirements of drinkable water are the microbiological monitoring tests to prevent any microbial contamination. Bottled water is generally considered to be safe for use by people. However, several reports have reported that bottled water does not always meet the accepted standards. House hold Reverse Osmosis (RO) stations are commonly used in houses which purified the supply water using membrane filters, it has high efficiency in removing dissolved salts, chemicals, impurities and microorganisms from the water. This study aimed to assess the microbiological quality of RO water samples and some domestic bottled waters marketed in Tripoli-Libya.* ***Methods***. *About 9 samples of different bottled drinking water and 18 samples of RO drinking water were collected from different parts in Tripoli.* ***Results***. *The microbiological analysis tests of this study showed that the total bacterial counts of bottled waters and one sample of RO water were below 10 CFU/100 ml. A total of 16 RO samples had a total of the bacterial count in the range between 10 and 300,000 CFU/100 ml and one sample was found with a bacterial count of more than 300,000 CFU/100 ml. However, total coliform bacteria, yeast and mold were not detected in all bottled and RO water samples.* ***Conclusion***. *The tested domestic bottled waters sold in markets and shops in Tripoli have bacteriological contents within the accepted ranges based on WHO standards, whereas almost all the RO samples have high bacterial count that can be risky to human health. Accordingly, the public should be aware of the proper use of RO stations and monitoring the validity of filters in order to be efficient to produce safe water that is free of microbial and chemical contamination.*

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INTRODUCTION

Water is an essential requirement of life of almost all living organisms. It can be dangerous to the health if it is contaminated by microorganisms [1,2]. Access to safe and healthy drinking water is of important issue that creates a challenge for public health supplies around the world [3,4]. It is estimated that five million people die every year as a result of waterborne illness [5]. Contaminated drinking water can be source of waterborne diseases such as diarrhea, cholera, dysentery, typhoid and polio [6]. Waterborne diseases are still considered as a major public health concern because of the presence of coliforms and other pathogens in drinking water that may be related to the ineffective or poor treatment processes such as sanitation, disinfection and purification methods [7-9].

Recently, as people become more awareness regarding waterborne diseases, bottled water is preferred to be used as an alternative to tap water [10]. It is perceived by consumers that bottled water has better quality and taste compared to tap water. However, several researches reported that bottled water is not of high quality and thus not suspected to be safe because of the presence of viruses, bacteria and fungi with the percentages above the standard limit [10,11] and accordingly it leads to various severe gastrointestinal diseases [12]. Sometimes, the presence of bacteria is harmless, in which the disease that is associated with bottled water consumption is uncommon [13]. Contamination of bottled water could be resulted from using contaminated source of water or during process of bottling either from the environment or equipment [14].

Currently, the microbiological assessments of bottled waters are projected to include the determination of the microbial count, identification of the pathogenic flora and the investigation of the presence of specific indicators of human or environmental contamination [15]. Reverse Osmosis (RO) is a water purification technique that it basically depends on the use of semipermeable membranes for water filtration [16-18]. It can physically separate and remove the unwanted components such as dissolved solids, bacteria, viruses, and heavy metals from the water [19-22]. The RO system is also known as a filter media with the characteristics of having the smallest pore size of 0.0001 microns compared to other filters [23-25].

Reverse Osmosis membrane processes are commonly used with the advantages of having high elimination of constituents such as dissolved solids, organic compounds and inorganic ions. It can work continuously with reasonable low energy consumption and it can be combined with other separation processes with a good performance under adjustable operating conditions [26-28]. However, membrane fouling is a major problem for this process in the drinking water especially with the presence of high concentrations of natural organic and inorganic matters [29].

Fouling is that method that leads to loss of the membrane performance because of the accumulation of suspended or dissolved materials on its surface or within the pores [30-31]. Thus, RO membrane will have low efficacy in the elimination of bacteria from the drinking water which a regular maintenance cleaning of the filters every 6 months is recommended to enhance its efficacy in removing the contaminants. This study was conducted to evaluate the microbiological quality of bottled waters in Tripoli, and to determine the efficacy of RO system to eliminate bacteria from the drinking water.

METHODS

Sample collection

A total of 9 samples of different bottled drinking water and 18 samples of RO drinking water were collected from different parts in Tripoli. All steps of collection were performed under septic conditions in 250 ml pre autoclaved containers and tightly closed with parafilm.

Enumeration and identification of bacterial species

Culture media and incubation conditions were used according to the standard procedure. Plate count agar for heterotrophic bacteria count, Sabouraud chloramphenicol agar for yeast and mould count, Violet Red Bile Agar for *Escherichia coli*. Cetrimide agar for *Pseudomonas spp*, bismuth sulphite agar medium for *Salmonella spp*, MacConkey agar for *Klebsiella spp*, MSA dried agar for *Staphylococcus spp* and MacConkey broth medium for coliforms.

Statistical analysis

The obtained data are presented as average ± SD for the triplicate measurements of each of the tested samples.

RESULTS

The microbiological analysis in the terms of the occurrence of pathogenic bacteria and total coliform bacteria are shown in Table 1 and 2. The viable aerobic microbial counts of all bottled water samples and one sample of RO water were below 10 CFU/100 ml. A total of 16 RO water samples were found to be in the range of 10-300,000 CFU/100 ml with the highest number was between 100,000-300,000 CFU/100 ml. However, only one sample was found with a count of more than 300,000 CFU/100 ml. In addition, the results showed that total coliform bacteria, yeast and mould were not detected in all tested bottled and RO water samples.

Table 1. Total microbial count of bottled drinking water samples

Sample	Total viable aerobic microbial count CFC/100 ml	Total yeast and mould count	Total coliforms
B 01	< 10	ND	ND
B 02	< 10	ND	ND
B 03	< 10	ND	ND
B 04	< 10	ND	ND
B 05	< 10	ND	ND
B 06	< 10	ND	ND
B 07	< 10	ND	ND
B 08	< 10	ND	ND
B 09	< 10	ND	ND

Results were the average value of three independent measurements (n = 3); ND is not detected.

Table 2. Total microbial count of RO water samples

Sample	Total viable aerobic microbial count CFC/100 ml (mean ± SD)	Total yeast and mould count	Total coliforms
R01	$(128 \pm 12.32) \times 10^3$	ND	ND
R02	$(122 \pm 10.82) \times 10^3$	ND	ND
R03	$(132 \pm 5.69) \times 10^3$	ND	ND
R04	$> 3 \times 10^5$	ND	ND
R05	< 10	ND	ND
R06	$(28 \pm 6.51) \times 10^4$	ND	ND
R07	$(3 \pm 8.75) \times 10^5$	ND	ND
R08	$(3 \pm 5.01) \times 10^5$	ND	ND
R09	$(29 \pm 8.65) \times 10^3$	ND	ND
R10	$(3 \pm 6.78) \times 10^4$	ND	ND
R11	$(3 \pm 4.55) \times 10^4$	ND	ND
R12	$(4 \pm 3.61) \times 10^4$	ND	ND
R13	$(35 \pm 3.22) \times 10^3$	ND	ND
R14	$(28 \pm 6.67) \times 10^3$	ND	ND
R15	$(38 \pm 3.58) \times 10^3$	ND	ND
R16	(8000 ± 7.58)	ND	ND
R17	(6400 ± 8.79)	ND	ND
R18	$(24 \pm 4.88) \times 10^4$	ND	ND

Results were the average value of three independent measurements, n= 3 (mean ± SD); ND is not detected.

The prevalence of various groups of bacteria was found to be negative for all tested bottled water samples. None of the *Salmonella spp*, *Escherichia coli* or *Staphylococcus spp* was detected in all RO water samples. However, three samples (17%) of RO water were found to be contaminated with *Klebsiella spp*. *Pseudomonas spp* was detected in two samples (11%). Seven samples (39%) were found to be positive for non-fermenting gram negative bacteria (Table 3).

Table 3. Microbial isolates from RO water samples

Types of pathogens	Number of positive water samples	Count range (as per WHO guidelines)
Pseudomonas spp	2 (11%)	Should be absent
Salmonella spp	ND	Should be absent
Escherichia coli	ND	Should be absent
Staphylococcus spp	ND	Should be absent
Klebsiella spp	3 (17%)	Should be absent
Non fermenting gram-negative bacteria	7 (39%)	Should be absent

ND is not detected

DISCUSSION

The bacteriological examination confirmed that no aerobic bacteria were detected in all bottled drinking water. The reason for not detected bacteria in the bottled water could be possibly related to the good quality of the purification techniques. The obtained results revealed that 17 (94%) of RO water samples shown positive results for bacterial growth with a total bacterial count varied from 10 to 300,000 CFU/100 ml. One sample (6%) was shown to be not contaminated with the total microbial count was within the accepted limits specified by WHO which recommended to be less than 20 CFU/100 ml [32]. Also, the results showed that the samples were contaminated by *Klebsiella spp*, *Pseudomonas spp* and non-fermenting gram negative bacteria with percentages of 17, 11 and 39% respectively as shown in Table 3. However, some of gram-negative bacteria such as *Salmonella spp* and *Escherichia coli* were not detected in the samples. All samples were shown to be free from gram-positive bacteria represented by *Staphylococcus spp* as shown in Table 3. This result was consistent to other studies where most bacteria found in the drinking water were gram-negative bacteria. These bacteria were known to be naturally present in the water [2, 33]. In fact, the presence of some bacterial types such as *E. coli* in drinking water could be related to its presence in high numbers among intestinal flora of humans and other animals, as it is expected to be found in fecal wastes. Therefore, if *E. coli* is detected in a high percentage more than other pathogenic bacteria, this can be used as an index of the potential presence of entero-pathogens in water environments [34]. Accordingly, the presence of *E. coli* in water is considered as a sign of faecal pollution and the water would be harmful to the human health [35].

The present study showed negative results for the presence of *E. coli*. This indicates that the water was free from faecal contamination as *E. coli* is one of the main indicators for the faecal contamination in drinking water. Furthermore, some types of bacteria such as *Pseudomonas aeruginosa* tend to survive longer in the environment as they have an ability to resist the chlorination. Thus, the presence of this kind of bacteria in chlorinated water could indicate an ineffective chlorination process or contamination of water after chlorination [36].

The ability of the water filter to remove bacteria depends on some factors such as the pore sizes where the filtration process is effective in removing bacteria when pore sizes of filters are less than the sizes of bacteria. Other factors such as the age and the cleaning of the water filter play an important role in removing bacteria as the filters will allow bacteria to colonize on their surface as soon as they get wet [37]. Also, the bacterial growth is not inhibited as there are no toxic chemicals or substances in the cartridges of the filters, so the bacteria will reach the detectable levels after 7-8 months and dangerous levels after 12-24 months. In order to inhibit the bacterial growth or aggregation, the filter cartridges should be changed every 6 months, irrespective of use [36]. Nearly all water samples were of poor quality and non-potable for human consumption. The presence of high pathogenic bacteria such as *Klebsiella spp* can be a source of severe waterborne diseases in the population.

CONCLUSION

This study indicated that domestic bottled waters have bacteriological contents that are within the accepted ranges for WHO standards. Despite the fact that companies producing bottled drinking water by using good purification processes, they were sometimes not effective for eliminating bacteria and thus a number of bacteria were found to be present in the water. The RO water samples were found to have bacterial count above the permitted range. This indicated that the RO membrane filter showed low and limited effect in elimination of bacteria from the drinking water. The health authority should have the responsibility for supervising manufacturers and monitoring the quality of bottled drinking water to ensure that relevant health procedures are followed. Furthermore, the repeat cleaning to the filters of RO system every 6 months is recommended to be used and maintained in order to decrease the microbial count aiming to preserve consumers' health. Further work is needed to evaluate the health hazardous due to deficiency of some ions such as potassium, sodium and other important minerals that should be present in drinking water.

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تقييم مراقبة الجودة للتلوث الميكروبي لمياه الشرب المعبأة والمياه المنزلية المفلترة في طرابلس، ليبيا

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المستخلص

الخلفية والأهداف. إن الحصول على المياه النظيفة والآمنة أمر ضروري لحياة الإنسان. ومن متطلبات مراقبة جودة المياه الصالحة للشرب اختبارات المراقبة الميكروبيولوجية لمنع أي تلوث ميكروبي. تعتبر المياه المعبأة بشكل عام آمنة للاستخدام من قبل الناس. ومع ذلك، فقد أفادت عدة تقارير أن المياه المعبأة لا تلبي دائمًا المعايير المقبولة. تُستخدم محطات المفلترة (RO)بشكل شائع في المنازل التي تقوم بتنقية مياه الإمداد باستخدام المرشحات الغشائية، وتتميز بكفاءة عالية في إزالة الأملاح الذائبة والمواد الكيميائية والشوائب والكائنات الحية الدقيقة من الماء. هدفت هذه الدراسة إلى تقييم الجودة الميكروبيولوجية لعينات مياه المفلترة وبعض المياه المعبأة المحلية المسوقة في طرابلس, ليبيا. **طُرق الدراسة**. تم جمع حوالي 9 عينات من مياه الشرب المعبأة المختلفة و18 عينة من مياه الشرب RO من مناطق مختلفة في طرابلس. **النتائج**. أظهرت اختبارات التحليل الميكروبيولوجي لهذه الدراسة أن إجمالي التعداد البكتيري للمياه المعبأة وعينة واحدة من ماء التناضح العكسي كان أقل من 10 CFU / 100 مل. كان إجمالي 16 عينة RO يحتوي على إجمالي عدد البكتيريا في النطاق بين 10 و 300000 CFU / 100 مل وتم العثور على عينة واحدة تحتوي على عدد بكتيري يزيد عن 300000 CFU / 100 مل. ومع ذلك، لم يتم اكتشاف البكتيريا القولونية الكلية والخميرة والعفن في جميع عينات المياه المعبأة ومياه المفلترة. **الخاتمة**. تحتوي المياه المعبأة المحلية التي تم اختبارها والتي تباع في الأسواق والمحلات التجارية في طرابلس على محتويات بكتريولوجية ضمن النطاقات المقبولة بناءً على معايير منظمة الصحة العالمية، في حين أن جميع عينات التناضح العكسي تقريبًا تحتوي على عدد بكتيري مرتفع يمكن أن يشكل خطراً على صحة الإنسان. وعليه، يجب أن يكون الجمهور على دراية بالاستخدام السليم لمحطات التناضح العكسي ومراقبة صلاحية المرشحات لتكون فعالة لإنتاج مياه آمنة خالية من التلوث الميكروبي والكيميائي.

الكلمات الدالة. التلوث البكتيري، مراقبة الجودة، المياه المعبأة، المياه المفلترة