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Study the bacteriological quality of bottled water in Baghdad province and Inhibitory effect of bacteriocin extracted from *Pseudomonas aeruginosa*

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Abstract:

Three hundred and nineteen samples of bottled water belonging to ten different local and imported brands (37 imported and 282 local samples) collected from the Iraqi (in Baghdad) market with different size ranged (500 ml to 20 litter) during January tile June 2014 was analyzed bacteriologically.

Heterotrophic plate count (HPC) was determined using R2A agar culture medium also all the collected samples were analyzed for the presence of coliform bacteria, fecal coliform bacteria, *E. coli* ,and, *P. aeruginosa.* Around 5 % of the water samples exhibited HPC counts ranged between 5- 500 cfu/ml.

The bacteriological tests of water showed that the bacterial failure percentage was 5% of bottled water samples, the most probable number of total coliform ranged between 1.1 to 23 CFU/ 100ml. These results didn't agree with national and international standard characters that detected no coliform bacteria / 100 ml of treated water.

In this study three isolates of *Pseudomonas aeruginosa* (most dominant bacteria) were tested for bacteriocin production and antimicrobial activities were measured by using the agar well diffusion method on some Gram positive and Gram negative pathogenic bacteria which involved (*Aeromonas hydrophilia, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis*) were used as indicator isolates. Three isolates of *Pseudomonas aeruginosa* were positive for bacteriocin production with a wide range effect on gram positive and negative bacterial growth, with diameter (2-20) mm.

Introduction

Sales and consumption of bottled water have skyrocketed in recent years. From 1988 to 2002, the sales of bottled water globally have more than quadrupled to over 131 million cubic meters annually [1].

The public's concern over increased water pollution and the so common belief among people that bottled water is superior to municipal water in that it contains no microorganisms [2 ,3]. In addition, bottled water is often recommended for patients with immune-system deficiencies as well as marketed as ideal for infant nutrition and reconstitution of foods [4].

From health risk point of view, the great distribution of *Pseudomonas* spp. and *Aeromonas* spp. as the main components of heterotrophic bacteria in bottled water and their increased resistance to clinically available antimicrobial agents has been considered as health concern particularly to immunocompromised patient and because there is the risk of transferring the resistance to other bacteria present in the human body and some pathogenic ones [5,6,7].

By taking into account that there are many local and imported brands of bottled water in Baghdad market, and the increased consumption of such waters raises the question as to whether they are hygienically safe. The refore, the aim of the study is to provide an adequate information on the microbiological quality using in 319 local and imported drinking bottled water samples collected from different parts of Baghdad.

The most common group of indicator organisms used in water quality monitoring are coliforms. These organisms are representative of bacteria normally present in the intestinal tract of mammals including human, [8,9]. In bottled water, total coliform bacteria may be indigenous from the natural source of the water or may be introduced during processing. It has also been established that a number of these bacteria could multiply during storage to reach infective doses for consumers [10].

Material and Methods

1. Sample collection:

During the period from January tile December 2014, samples of different sizes ranged (500 ml to 20 litter) were collected randomly from local markets national and imported brands of drinking water particularly those within consuming validity. 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 :

-Total viable plate count: Total viable plate count (TVPC) was performed by pour plate technique. Bottled water samples were mixed in R2A media and incubated at 28 ^{o}C for 5–7 days. After incubation colonies were counted with the help of colony counter then cultures were examined for distinct colonies, the colonies were transferred on to surface of nutrient agar in plates and incubated at 37 0 C for 18- 24 h. All the colonies were tested for colony morphology and colony color. The identification tests for genera and species of each isolate were performed using conventional bacteriological methods [11], and confirmed by using Bio Mic kit (Biomerieux) France**.**

-Detection of coliform bacteria

The MTF method was performed as a 10 tube MPN test. According to the *standard methods*, each tube containing 10 ml of double-strength lactose broth was inoculated with 10 ml of drinking water sample and incubated at 35ºC for 24-48 h. The tubes that were positive for total coliforms, as indicated by the production of acid or gas were then confirmed in brilliant green lactose bile (BGLB) broth and EC broth for the presence of total and fecal coliforms, respectively [12].

Detection of *P. aeruginosa*

Presumptive test

-One hundred of water samples were filtered through a membrane filter with a pore size of 0.45 µm by vacuum pumping .

- The membrane was placed in flask with Asparagine broth (100 ml)**,** then incubated at 37 ^{o}C for 24h or 48h. Formation of green color is a positive presumptive test.

Confirmed test : Confirmed positive results by streaking loop full of positive growth on to surface of MPA agar in plates and incubated at 41 ^{o}C for 48 h [13].

Screening for bacterioc in production and antibacterial activity.

The agar well diffusion assay was used to determine antibacterial activities *P. aeruginosa*. Single colony from nutrient agar plate was inoculated in5ml in Brain heart Infusion (BHI) broth and incubated at 37°C for 18 -24 hr .

After incubation cell free filtrate was obtained by centrifuging the bacterial culture at 10.000 rpm for 10 minute and sterilized by filtration through 0.2 mm pore size filter. Inhibitory activity was detected by the agar-well diffusion method [14], Portions (100 µl) of cell free supernatant were added to wells (5mm) cut into the plate, which was inoculated with (*Aeromonas hydrophilia, Escherichia coli, Staphylococcus aureus, Streptococcus faecali*) (as the indicator isolates) and the plate was incubated for 18 hour at 37°C, then the diameter of the inhibition zone around each well was measured in mm.
Contaminated bottled water poses great health risk especially when it is used by elderly persons, infants, hospitalized patients and immuno-compromised ones[15].

In the present study the bacteriological examination of 319 different bottled water samples was carried out .Table 1 summarized total viable plate count (TVPC) of imported and local bottled water samples.

Around 5 % of the water samples exhibited HPC counts ranged between 5- 500 cfu/ml.

Table 1: Total viable plate count (TVPC) (cfu/ 100 mL) in contaminated bottled water samples

Brand	TVPC range CFU/100ml	No. and% of samples
Imported	5-300	10(3%)
Local	5-500	6(2%)
Total	16	5%

Imported brand showed the maximum pollution with range 5-300 CFU/ 100ml, Whereas local recorded range5-500 CFU/ 100ml. The TVPC count shows the presence of heterotrophic bacteria in the water samples, which indicates the bacterial pollution of drinking water [12].

Results of recent study were lower than [16] who found that bacterial failure percentage was 26.67% of bottled water samples, also [17] found (36%) of samples did not comply with WHO standard guidelines for drinking water.

All these colonies isolated on R2A were confirmed by using the BioMic kit for the identification to indicate the presence of (eight) different species of bacteria, table (2).

Table 2. Total number and (%) of the identified species of isolated from bottled waters

Bacterial species	No. and% of isolats isolated from bottled waters
P. aeruginosa	42 (13.2)%
P. fluorescens	24(7.5%)
Klebsiella spp	2(0.6%)
Aeromonas hydrophila	22(6.9%)
Serratia spp.	4(1.3%)
Enterobacter cloacae	8(2.5%)
Pantoea spp.	31(9.7%)
Acinetobacter	32(10%)

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 current study [17].

These organisms cause infection mainly among people with impaired natural defense mechanisms. These people include the very old, the very young, immuno compromise people and the patients in hospitals [18].

In the other hand 8 (2.5%) bottled water samples were contaminated with T. coliforml and 8(2.5%) fecal coliforml bacteria ,on other word all samples gave positive results to TVPC gave positive results to. coliforml and 8 (2.5%) fecal coliforml.

The presence of coliform bacteria in drinking water suggests the possible presence of pathogenic enteric microorganisms thus unsafe for drinking. Several previous studies have shown that bottled drinking water is contaminated with pathogenic bacteria [16].

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 [19]. In a study conducted in Brazil, 20 liters water bottles were found contaminated with coliform bacteria [20]. In Egypt, Abd El-Salam *et al* . [21] studied quality of bottled water brands and Total Coliforms were detected in 28.6 % of the examined bottled water Samples.

The rapid growth of bacteria after the water is bottled may be due to the increased surface area from the bottle, the increase in temperature during storage, and the trace amounts of nutrients arising from the bottle [22].

The presence of coliforms in bottled water samples not only indicates the potential presence of enteric microorganisms but also refer to the efficiency and integrity of production system. It is therefore recommended to monitor the complete water processing system to avoid major public health problems [17].

In recent study, *P aeruginosa* had the higher recovery rate among the identified species of isolated from bottled waters that due to it can survive longer in low nutrient environment by slowing down its metabolic activity and its resistance against disinfectants which used for the treatment of water [23].

Recent results came in agreement with Daood [24] who found *P. aeruginosa* and *A. hydrophila* in bottled Water, presence of these bacteria in bottled water is considered as an indicator of the quality of water. Different studies have shown that bottled drinking water is not always safe [22, 25, 26].

Other researchers found that contaminated bottled water are reason of Many outbreaks [21, 27, 28].
There are many reasons for the bacterial pollution of bottled water such as improper or impaired disinfection, infiltration of contaminated water, leakage points. In addition, the improper storage of bottled water enhanced bacteria to grow up to harmful levels by providing favorable conditions [17].

The other side of study ,agar well diffusion bioassay was used for the evaluation of antimicrobial activity of bacteriocin (cell free supernatant) of *Pseudomonas aerugenosa* isolates against some Gram positive and Gram negative pathogenic bacteria and the diameter of the inhibition zone was measured. All the three isolates of *P. aeruginosa* isolates produce bacteriocin effected on indicator isolates growth as shown in table 3, figure 1 .

Table 1: Zones of inhibition (mm.) Producing by three isolates of *Pseudomonas aeruginosa*

Indicator isolates	Zones of inhibition (mm) Producing by three isolates of *P. aeruginosa*		
	P1	P2	P3
Escherichia coli	20	18	16
Aeromonas hydrophila	11	16	19
Acinetobacter baumannii	12	13	10
Streptococcus faecalis	9	10	14
Staphylococcus aureus	2	5	3

The results showed that *P. aeruginosa* P1 inhibit the bacterial growth of the tested isolates with higher inhibition zone (average 2-20 mm), while P2, P3 inhibit the bacterial growth of the tested isolates with average of inhibition zone (5-18),(3-19) mm respectively. The results of recent study found that bacteria *Escherichia coli* was the most affected bacteria by bacteriocin of *P. aeruginosa* with a range of inhibition zone between (16-20) mm, followed by *Aeromonas hydrophila* (11-19) mm. *Streptococcus faecalis* (9-14) mm , *Acinetobacter baumannii* (12-13) and *Staphylococcus aureus* was most resistant to bacteriocin (2-5) mm, after 24 hours incubation period.

Figure 1: Inhibition bacterial growth zones by bacteriocin of *P. aeruginosa* isolates

References

[1] **Gleick, P. H.** The myth and reality of bottled water. In The World's Water: The Biennial Report on Freshwater Resources 2004 2005; Island Press: Washington, DC, USA. 2004.

[2] **Papapetropoulou, M., Tsintzou, A. and Vantarakis, A.** Environmental mycobacteria in bottled table waters in Greece. Can. J. Microbiol. Vol. 43, pp. 499-502. 1997.

[3] **Hunter, P. R.** The microbiology of bottled natural mineral waters. *J. Appl. Bacteriol*.Vol. 74, pp. 345–352. 1993.

[4] **Warburton, D. W.** A review of the microbiological quality of bottled water sold in Canada. Part 2. The need for more stringent standards and regulations. Can. J. Microbiol. Vol. 39, pp. 158–168. 1993.

[5] **Rosenberg, F.A. and Hernandez- Duquino, H.** Antibiotic resistance of *Pseudomonas* from German mineral waters. Toxicity Assessment Vol. 4, pp. 28l-294. 1988.

[6**] Massa, S., Petrucciolli, M., Fanelli, M. and Gori, L.** Drug resistant bacteria in non carbonated mineral waters. Microbiol. Res. Vol. 150, pp. 403–408. 1995.

[7] **Mary, P., Defives, C. and Hornez, J. P.** Occurrence and multiple antibiotic resistance profiles of non-fermentative Gram-negative microflora in five brands of non-carbonated French bottled spring water. Microbiol. Ecol. Vol. 39, No. 4, pp. 322–329. 2000.

[8] **Fewtrell, L. and Bartram, J.** Water Quality: Guidelines, Standards and Health London, UK: IWA Publishing; pp. 289-315. 2001.

[9] **Stevens, M.; Ashbolt, N. and Cunliffe, D.** Review of Coliforms as Microbial Indicators of Drinking Water Quality-Recommendations to change the use of Coliforms as Microbial indicators of drinking water quality. NHMRC, Biotext Pty Ltd, Canberra, Australia. 2003.

[10] **Bharath, J., Mosodeen, M., Motilal, S., Sandy, S., Sharma, S., and Tessaro, T., et al.** Microbial quality of domestic and imported brands of bottled water in Trinidad. International Journal of Food Microbiology, Vol. 81, pp. 53-62. 2003.

[11] The Microbiology of water, Part 1- Drinking water Report on Public Health and Medical subjects. Methods for the examination of water and Associated Methods. London, HMSO. No.71. 1995.

[12] **APHA, AWWA, WEF**, Standard Methods for the Examination of Water and Wastewater, 21st edition. Washington, DC. 2005.

[13**] Eaton A. ; Wef E. and Arnold E.** Standered method for the examination of water and waste water. 21 st. edition. American public health association. 2005.

[14] **Tagg, J. R. and Mc Civen, A. R.** Assay system for bacteriocins. Appl. Microbiol. Vol. 21, pp. 943- 948.1971.

[15] **H.,Schwartzbrod, L.; E.Dei-cas.,** Microbial agents associated with waterborne diseases. Crit. Rev. Microbiol., Vol. 28, pp. 371-409. 2002.

[16] **Taema , B. R. , Hnoush , A. H. and saad, T. M**. An assessment of the bacterial quality of various bottled water marketed in Al-Muthanna province. JMAS . Vol. 1, No. 1. 2013.

[17] **Khatoon, A. and Pirzada, Z. A.** Bacteriological Quality Of Bottled Water Brands In Karachi, Pakistan. Biologia (Pakistan), Vol. 56 , No. 1,2, , pp. 137-143. 2010.

[18] **Sharma, D. Kanchantm Arsenic Filter**; removal of bacteria (total coliform) of Gem 505 model. A Thesis in partial fulfillment for the requirements for Bachelor Degree in Environmental Science, School of Science, Kathmandu University. 2005.

[19] **Jeena, M. I., Deepa, P., Mujeeb, R. K. M., Shanthi R.T. & Hatha, A.A**. Risk assessment of heterotrophic bacteria from bottled drinking water sold in Indian markets. Int. J. Hyg. Environ. Health, Vol. 209, pp. 191-196. 2006

[20] **Zamberlan da Silva, M.E., Santana, R.G., Guilhermetti, M., Filho, I.C., Endo, E.H., Ueda-Nakamura, T., Nakamura, C.V. & Dias Filho, B. P.** Comparison of the bacteriological quality of tap water and bottled mineral water. Int. J. Hyg. Environ. Health. Vol. 211, pp. 504-509. 2008.

[21] **Abd El-Salam, M.M.M.; El-Ghitany, E. M. A. and Kassem, M. M. M.** quality of bottled water brands in egypt, part II: biological water examination. J Egypt Public Health Assoc, Vol. 83, No. 5- 6, pp. 466-486. 2008.

[22] **Venieri, D., Vantarakis, A., Komninou, G., and Papapetropoulou, M.** Microbiological evaluation of bottled non-carbonated water from domestic brands in Greece. International Journal of Food Microbiology. Vol. 107,pp. 68–72. 2006.
23-Mena, K. D. & Gerba, C.P.. Risk assessment of Pseudomonas aeruginosa in water. Rev. Environ. Contam. Toxicol. Vol. 201: pp.71-115. 2009.

[24] **Daood, N.** Risk Assessment of Heterotrophic Bacteria from Bottled Mineral Water Consumed in Syria. Damascus university journal for basic sciences, Vol. 25, pp. 21-42. 2009.

[25] **Zeenat, A., Hatha, A.A., Viola, L. & Vipra, K.** Bacteriological quality and risk assessment of the imported and domestic bottled mineral water sold in Fiji. J. Water Health,Vol.7, pp. 642-649. 2009.

[26] **Taj, A. & Baqai, R.** Antimicrobial effects of Alum and Sulphur on bacteria isolated from Mineral and Hospital water. Infectious Diseases Journal. Vol. 16, pp.10-13. 2007.

[27] **Reynolds, K. A., Mena, K. D. & Gerba, C. P.** Risk of waterborne illness via drinking water in the United States. Rev. Environ. Contam. Toxicol. Vol. 192, pp.117-158. 2008.

[28] **Addo, K. K., Mensah, G. I., Donkor, B., Bonsu, C. & Akyeh, M. L.** Bacteriological quality of bottled water sold on the Ghanaian market. African journal of food agriculture nutrition and development. Vol. 9, pp.1378- 1387. 2009.