An assessment of the bacterial quality of various bottled
water marketed in Al-Muthanna province

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Abstract:

This study was carried out at Al-Muthanna province, it aims to determine bacterial quality of three (Oasis, Mina and Al-Rawdhateen) brands of bottled water from different sources that are consumed in Al-Muthana province and compare it with national and international standards. This study was conducted from August 2011 to February 2012. A survey of 60 duplicate water sample. The multiple-tube fermentation technique was used for the determination of total coliform in all samples. All bacterial tests was carried in researches laboratory of science collage. The statistical analysis of results using analysis of variance showed that there are significant differences among bottled water sample. The bacteriological tests of water showed that the bacterial failure percentage was 26.67% of bottled water samples. the most probable number of total coliform ranged between 12.30 to 81.0 CFU/ 100ml. These results didn't agree with national and international standard characters that detected no coliform bacteria / 100 ml of treated water.

Introduction:

Clean water is so basic to human life that water droplets, bubbling brooks, and waterfalls are enduring symbols of the life force [1, 2]. A sufficient, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe
 as possible [3]. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases [4].However, bottled

water is not necessarily safer than tap water, and over the years, concerns have been raised about the quality of bottled water marketed worldwide. Also, the commercialization of processed tap water, authorized by many countries, may attract unscrupulous manufacturers toward illegal activities such as bottling tap water and selling it as mineral water. Literature revealed that the levels of some water constituents in bottled water may be in violation of action levels for various parameters[5]. 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 [6]. However, given the widespread increase in nonpoint anthropogenic pollutant sources arising from rapid urbanization and development in production areas, the actual quality and origin of bottled water should be carefully evaluated. Infectious diseases caused by pathogenic bacteria, viruses, fungi and protozoa or parasites are the most common and wide spread health risk associated with drinking water . These pathogenic organisms responsible for many thousands of diseases and deaths each year, especially in tropical and subtrobical regions with poor sanitation.All water borne human pathogens are not of equal public significance. Some of them present a serious risk of disease whenever they are consumed in drinking water and are given high priority for health significance. Examples include strain of *Escherichia coli, Salmonella, Shigella, and Vibrio Cholera.* On the other hand, some organisms may be "opportunistic". 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. Examples of these organisms include *Pseudomonas, Klebsiella* and *Legionella* [7].

Materials and Methods:

1. Sample collection:

Three brands of the most popular bottled water samples : Oasis, Mina and Al-Rawdhaten that are consumed in Al-Muthanna province were purchased from local supper markets. Four bottles of 500 mL size for 5 production times were purchased for each brand in the determination of bacterial quality.

2. Procedure:

A series of MacConky broth (Himedia, India)   primary fermentation tubes(3 double strength and 6 single strength) are inoculated with graduated quantities of the sample to be tested

(3 tubes with 10 ml ,3 tubes with 1 ml and 3 tubes with 0.1ml) . The inoculated tubes are incubated at 35 + 0.5 °C for 24 + 2 hr, at which time the tubes are examined for gas formation. For the tubes in which no gas is formed, continue incubation and examine for gas formation at the end of 48 + 3 hr. Formation of gas in any amount within 48 + 3 hr is a positive presumptive test. The number of possative tubes recorded in each set and determine the MPN according to Standard methods for the examination of water and wastewater[8].

The confirmed stage is used on all primary fermentation tubes showing gas formation during the 24-hr and 48-hr periods. Fermentation tubes containing brilliant green lactose bile broth are inoculated with medium from the tubes showing a positive result in the presumptive test. Inoculation should be performed as soon as possible after gas formation occurs. The inoculated tubes are incubated for 48 + 3 hr at 35 + 0.5°C. Formation of gas at any time in the tube indicates a positive confirmed test.The completed test is performed on all samples showing a positive result in the confirmed test. It can also be used as a quality control measure on 20% of all samples analyzed. One or more plates of Eosin Methylene Blue (Himedia, India) are streaked with sample to be analyzed. The streaked plates are incubated for 24 + 2 hr at 35 + 0.5 °C. After incubation, transfer one or more typical colonies (nucleated, with or without metallic sheen) to a MacConky broth fermentation tube and a nutrient agar slant. The fermentation tubes and agar slants are incubated at 35 + 0.5°C for 24 + 2 hr, or for 48 + 3 hr if gas is not produced. From the agar slants corresponding to the fermentation tubes

in which gas formation occurs, gram-stained samples are examined. microscopically. The formation of gas in the fermentation tube and the presence of gram-negative, non-spore-forming, rod-shaped bacteria in the agar culture may be considered a satisfactorily completed test, demonstrating the positive presence of coliform bacteria in the analyzed sample [8].Gram strain method was used to describe the bacterial cells. Description includes gram reaction, shape, cells arrangement, spores and chains formation[9]. bacterial media used to determine different growth characteristics of bacteria.

3. Statistical Data Analysis

Data were recorded, organized and summarized in sample descriptive statistics methods using SPSS-PC statistical package (SPSS 17 for windows version) , Genstat program . Results were designed as factorial experiment, and these results were presented in Complete Randomized Design (C.D.R.) such as tables and graphs[10].

Results:

The failure rate of bacterial was 26.7% of bottled water samples The results in the table (1) showed that Mina and Rawdhaten brands samples showed equal bacterial pollution percentage 20% and represented 6.7% from total bottled water samples. In Mina brand the pollution acted by *E. coli, Kl. oxytoca,* where as they acted by *Escherichia coli* and *Klebsiella oxytoca* in Rawdhaten brand. At Oasis brand samples some types of bacteria detected such as *E. coli, Kl. oxytoca, Kl. Pneumonia* and *Shigella spp.* and 40% of brand samples was contaminated which acted 13.3% of total bottled water samples.

Table (1) Bacterial pollution percentage of bottled water samples.

Brand	N= 20	N= 60
Mina	20	6.7
Oasis	40	13.3
Rawdhaten	20	6.7
Total	_	26.7

There was a significant differences in total coliform count between the samples of bottled water samples as shown in table (2), AL-Rawdhateen brand showed the maximum pollution with mean 81.00 CFU/ 100ml, then Oasis with 25.4 CFU/ 100ml. Whereas Mina recorded a minimum pollution 12.3 CFU/ 100ml.

Table (2) Bacterial Count MPN of bottled and tap water samples.

Brand	MPN*(CFU/100ml)
Mina	12.3
Oasis	25.4
Rawdhaten	81.0
L.S.D.0.05	10.3
* Values are presented as means (n=20)	

Fig. (1) Bacterial Count (MPN) of bottled water samples.
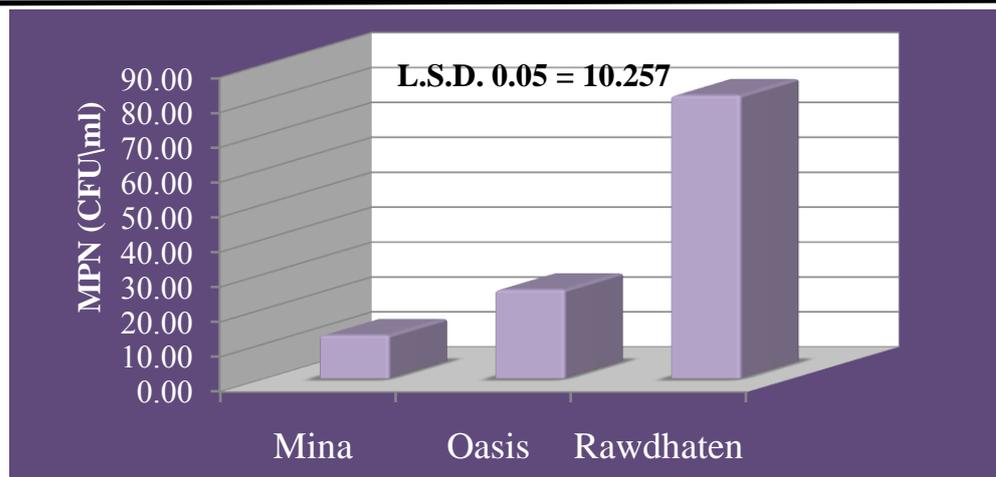
Discussion:

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, so they provide a general, albeit adequate, index of faecal

pollution of drinking water [11,12]. The percentage of contaminated bottled water samples was 26.67%. 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 [13]. In Egypt, Abd El-Salam *et al.*[14] studied quality of bottled water brands and Total Coliforms were detected in 28.6 % of the examined bottled water samples, these results compatible with the results of this study. Semerjian [15], Klont *et al.*[16] and

Abayasekara *et al.* [17] Their findings are in agreement with results of this study. This results not agreed with the results of Ehlers *et al.*[18] who found no total coliform bacteria in any of the ten bottled water samples in his study. 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 [19].Bottled water samples showed that there was a significant differences in total coliform count. The total coliform mean ranged between 81.00 - 12.30 CFU/ 100ml.According to Leclerc and Moreau [20] after bottling, the number of viable counts increases rapidly, attaining 10^4-10^5 cfu ml^{-1} within 3-7 days. During the following weeks, the bacterial counts decrease slowly or remain fairly constant. At the end of 2 years storage, colony counts are still about 103 CFU ml-

1.The present study results approach to the results of Lalumandier et al.[21] where he found the bacterial colony counts ranged from less than 0.01 to 4900 CFU/ml among the 57 samples of bottled water tested. This result did not agree with that founded by Mardani et al. [22] from Iran where gram negative bacilli and coliforms were not identified in study of Surveying common bacterial pollution in bottled mineral water. According to table (3) most bottled water samples exceeded the international standards of bacterial quality.

Table (3) Comparison of current results with some national and international standards.

standards	Total Coliforms CFU/100 ml
WHO	0
EPA	0
FDA	<4
Iraq	-
Syria	0
Jordan	< 1.1
Mina	12.3
Oasis	25.4
Rawdhaten	81.0

Recommendations:
There is a need for more stringent standardization and supervision of the bottled water market, particularly with regard to quality control, labeling, and continuous monitoring.

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تقييم النوعية البكتيرية لعدد من المياه المعبأة المستهلكة في محافظة المثنى

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المستخلص:

أجريت هذه الدراسة في محافظة المثنى حيث كانت تهدف الى تقييم المحتوى البكتيري لثلاث شركات من المياه المعبأة (منى, الواحة, الروضتين) من مصادر مختلفة والمستهلكة في محافظة المثنى ومقارنتها مع المحددات المحلية و الدولية   وللمدة من آب   2011 إلى شباط 2012 . درست 60 عينة مياه بشكل مكررات ثنائية, استخدمت طريقة العد الأكثر أحتمالاً لتحديد تواجد العدد الكلي لبكتريا القولون ولجميع العينات المشمولة بالدراسة. اظهر التحليل الإحصائي للنتائج باستخدام التصميم كامل العشوائية وجود فروق معنوية بين عينات المياه المعبأة. كما بينت الفحوصات البكتيرية للمياه أن نسبة الفشل البكتيري للعينات كانت 26,67% وأن العد الكلي لبكتريا القولون تراوح بين 12,3 إلى 81,0 خلية لكل 100 مل وهذا غير مطابق لما ذكر في المحددات المحلية والدولية حيث اشترطت خلو المياه المعالجة من بكتريا القولون لكل 100 مل.