

## Bacteriological Evaluation of Commercially Bottled Water in Al-Laith, Saudi Arabia

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### Abstract

Although bottled water meets quality standards, a variety of bacteria can be detected in most commercially bottled water. The present study was carried out to evaluate the bacteriological quality of bottled Drinking water in Alaith city, Makkah province, KSA. Forty five bottles of different 9 brands of bottled water were randomly selected from different supermarket stores to be microbiologically evaluated. Heterotrophic plate counts (HPC), *E. coli*, coliforms count (TC) and most probable number (MPN) were determined. The results showed that HPC were 9 out of 45 (20%) while total coliforms bacteria were detected in 3 out of 45 (7%) of the bottled water and there was no *E. coli* detected in the samples. Identified bacteria were *Klebsiella* spp., *Pseudomonas* spp., *Agrobacterium* spp., *Streptococcus* spp 1 (2.2%) and *Bacillus* spp 1 (2.2%). It was concluded that bottled drinking water in Al-Laith is safe, free of fecal indicators and within local and international guidelines recommendations.

**Keywords:** Colorectal Cancer; Review; Screening; Treatment

### Introduction

Water is essential to life. An adequate, safe and healthy water must be available to everybody. When safe drinking water is accessible, then there will be an improving of the public health. Bottled water is drinking water packaged in plastic water bottles. Sizes are variables, they are ranging from small bottles to large ones for water coolers. Efforts should be implemented to improve the quality and the safety of drinking water [1]. The bottled waters demand led to the increasing of manufacturing's and marketing in the Kingdom Saudi Arabia which becomes one of the biggest consumer of bottled water in the world. The manufacturers represent 70 percent of the total number of bottled water plants in the Gulf. Bottled water must meet certain local and international standards before it may be distributed. However, even within safety guidelines, a variety of bacteria can still be detected in most commercial bottled water available in the world. There are nonpathogenic and easily isolated bacteria called indicator organisms can be used indirectly for detection of pathogens. Examples of these indicators are coliforms bacteria. Total coliforms bacteria are Gram-negative rod-shaped, non-spore forming, lactose fermenting with acid and gas, motile or non-motile bacteria. They are always used indicator of quality of foods and water. The presence of total coliforms in any bottled water sample is not necessarily indicative of faecal contamination of the water [2]. The coliform genera could include *Klebsiella*, *Citrobacter*, *Enterobacter*, *Hafnia* and *Escherichia*. Coliform bacteria can also be found in the environment and may lead to biofilm formation [3]. Total coliform bacteria may include fecal coliform bacteria in addition to other bacteria. They live in the colon of man and animals and are almost always present, even in healthy persons. *Escherichia coli* (*E. coli*) has ability to grow and ferment lactose at 44°C which

is distinguished featured test. They are also identified by the growth and color reaction on special culture media. *E. coli* can be cultured on differential medium such as eosin methylene blue (EMB), to give a metallic green colonies. The presence of *E. coli* suggesting the fecal origin and thus indicating confirming fecal contamination.

When fecal coliforms is present in water, that means there is a contamination and poor quality of the water source [3,4]. Gram-positive bacteria contaminating waters have been sometimes reported such as "coryneform-like" and bacillus bacteria, Staphylococci and Micrococci [5]. Many other rare bacterial species have been reported to contaminate drinking-water systems. Heterotrophs microorganisms that need to grow in organic carbon for growth. The microorganisms recovered through heterotrophic bacterial count (HPC) tests generally include bacteria, yeasts and moulds. Heterotrophs in this spectrum are *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Aeromonas* spp., *Bacillus* spp., *Nocardia*, *Acinetobacter* spp., *Alcaligenes* spp., *Comamonas* spp., *Flavobacterium* spp., *Sphingomonas* spp., *Moraxella* spp. and atypical *Mycobacterium* spp [6]. Heterotrophs when grow at 22°C, and 37 °C, should be less than 100/ml, and 20/ml Colony forming unit(CFU), respectively" [7]. Due to the contamination of drinking water, acute microbial diarrheal diseases are a major public health problem in developing countries. According to the World Health Organization (WHO), The death due to water associated diseases exceeds 5 million/year. From these cholera being the [1]. It has been estimated that 560,000 people/year were suffering from waterborne diseases, while 7.1 million/year with moderate infections, resulting in the death of 12,000/year [1]. The present study was carried out to evaluate the bacteriological quality of bottled water in Alaith city, Makkah province, KSA.

## Materials and Methods

Forty five bottles of different 9 brands of bottled water were selected randomly from different supermarket stores to be evaluated during 2016, and were tested for bacteriological quality. All samples were collected in plastic containers and were well capped. bottled waters were in terms of brands, and volumes. The samples were checked to be capped and in good condition before purchase. Collected samples were transferred in an ice box to the Microbiology Laboratory in Department of microbiology, Health College, Umm Al Qura University at Alaith city, Saudi Arabia. The HPC, total coliforms, fecal coliforms, *E. coli* and enterococci count were determined, in addition to the most probable number (MPN) of coliform bacteria. The counting of HPC was done out using both the serial dilution and the pour plate technique. The preparation of serial fold 10 dilutions in sterile water were done. One ml of each dilution was placed in sterile petri-dishes. Molten plate count agar (20 ml) (Oxoid) was cooled to 45 - 50°C, then was placed to plate and mixed with the prepared sample (one ml) thoroughly. The mixture was allowed to cool (solidify) and then incubated at 22°C and 37°C for 1 - 3 days. The bacterial count was done per milliliter and the isolates were identified according to their biochemical characteristics using API identification strips (bioMerieux-France). MPN method done according to American Public Health Association standard methods (APHA) [8]. Briefly, 10 tubes of MacConkey's broth (Oxoid) were arranged in two rows with a 100 ml bottle. The first row was inoculated with 10 ml of test sample and 50 ml double strength MacConkey broth was inoculated with 50 ml of test sample. Second row containing MacConkey's broth medium (1 ml single strength) was inoculated with 1 ml water sample and incubated at 44°C for 24h. Then the number of bottles that showing lactose fermentation with acid and gas was counted. Enterococcus spp. was detected by membrane filter technique by using bile esculin agar. The bacterial isolates were identified through colony morphology, Gram's staining, and biochemical properties. The Gram-positive, catalase-negative cocci that isolated from azide dextrose broth and grown on bile aesculin agar at 45°C, is considered faecal streptococci belonging to the genera *Enterococcus* which confirmed by the growth at pH 9.6, 10° and 45°C and in 6.5% NaCl resistance to 60°C for 30 minutes and capable to reduce methylene blue [9].

## Results and Discussion

The results of the bacteriological analysis of drinking water from Al-Laith area showed that most of bottled drinking water are free of contamination when compared with guidelines of drinking water recommended by the International Bottled Water Saudi Food and Drug Authority (SFDA) [7]. Out of 45 samples collected, the number of samples with HPC were 10 (22.2%) i.e. contaminated with single (13.3%) or with more than one type (mixed) (8.9%) of organisms (Tables 1-3). While Coliforms bacteria (MPN) were detected in 4 out of 45 (8.9%) of the samples (Table 1). Identified Gram-negative bacteria in bottled water samples were *Pseudomonas* spp 6 (13.3%), *Enterobacter* spp 4 (8.9%), *Klebsiella* spp 2 (4.4%) and *Agrobacterium* spp (2.2%) (Tables 1 and 3). No *E. coli* contamination detected in any water sample. Gram-positive bacteria identified in bottled drinking water was *Bacillus* spp 1 (2.2%) (Table 2). No water sample was contaminated with *Streptococcus* spp (Table 2). However, The HPC of samples collected from 22.2% different brands were in the range of 71 to 218 CFU/ml levels (Table 1) which is less than recommended for drinking water (500 CFU/ml) [10]. The range is lower than similar studies [10-13]. In Taif, KSA, Shahaby, *et al.* [11] reported 1.9% bottled mineral water contamination [11]. HPC serves as an indicator of general microbial population and thereby as indirect indicators of water safety. Kassenga reported that heterotrophic bacteria were detected in 92% of the bottled mineral waters samples in addition to the presence of total and fecal coliform bacteria were present in 4.6% and 3.6%, respectively, in Tanzania [14]. Drinking water should contain no any coliform in 100 mls of water [15]. However, in the

Brand	No	HPC CFU/ mL	MPN/100ml	Fecal coliforms	E. coli CFU/mL	Enterococci CFU/mL	Species
B1	1	0	0	0	0	0	neg
	2	115	115	0	0	0	<i>Enterobacter spp</i>
	3	0	0	0	0	0	neg
	4	0	0	0	0	0	neg
	5	0	0	0	0	0	neg
B2	6	0	0	0	0	0	neg
	7	218	121	0	0	0	<i>Klebsiella sp</i>
	8	0	0	0	0	0	neg
	9	0	0	0	0	0	neg
	10	0	0	0	0	0	neg
B3	11	0	0	0	0	0	neg
	12	0	0	0	0	0	neg
	13	0	0	0	0	0	neg
	14	147	0	0	0	0	<i>Bacil and pseud</i>
	15	0	0	0	0	0	0
B4	16	85	0	0	0	0	<i>Agro and pseudo</i>
	17	0	0	0	0	0	neg
	18	0	0	0	0	0	neg
	19	0	0	0	0	0	neg
	20	0	0	0	0	0	neg
B5	21	0	0	0	0	0	neg
	22	111	0	0	0	0	<i>Pseudomonas spp</i>
	23	0	0	0	0	0	neg
	24	0	0	0	0	0	neg
	25	0	0	0	0	0	neg
B6	26	123	38	0	0	0	<i>Kleb and entero</i>
	27	151	98	0	0	0	<i>Pseud and entero</i>
	28	0	0	0	0	0	neg
	29	0	0	0	0	0	neg
	30	0	0	0	0	0	neg
B7	31	0	0	0	0	0	neg
	32	0	0	0	0	0	neg
	33	28	0	0	0	0	<i>Pseudomonas spp</i>
	34	0	0	0	0	0	neg
	35	0	0	0	0	0	neg
B8	36	0	0	0	0	0	neg
	37	0	0	0	0	0	neg
	38	125	91	0	0	0	<i>Enterobacter spp</i>
	39	0	0	0	0	0	neg
	40	0	0	0	0	0	neg
B9	41	0	0	0	0	0	neg
	42	0	0	0	0	0	neg
	43	71	0	0	0	0	<i>Pseudomonas spp</i>
	44	0	0	0	0	0	neg
	45	0	0	0	0	0	neg

**Table 1:** Bacteriological results of bottled drinking water samples.

present study, coliforms bacteria were detected in (8.9%) of water samples. Coliforms have been reported to in similar studies in Egypt and Nigeria [16,17]. Coliforms are indicators of poor water quality and opportunistic contamination during the process of bottling of the water [18]. The reason for the bacterial bottled water contamination with coliforms is the poor hygienic practices of the producers, such hand hygiene, knowledge, attitudes and practices of sellers [18]. In present study the *Pseudomonas* spp was (13.3%), *Enterobacter* spp was (8.9%), *Klebsiella* spp was (4.4%) and *Agrobacterium* spp was (2.2%). There is no fecal indicators contamination in this study which is lower than past research studies [19,20], thus pointing to the free microbial contamination of bottled water in the studied area. The presence of *Pseudomonas* spp has been previously reported in bottled water [10,19]. Several species of *Pseudomonas* can cause disease in humans. *P. aeruginosa* is sometimes used as an indicator of fecal bacterial contamination besides being a pathogen [20]. *Klebsiella* spp. has been previously reported in bottled water contamination [4,11,22]. Several *Klebsiella* spp. may be pathogenic to humans. *Klebsiella* have been reported to be isolated from aquatic environments mixed with wastewaters, food with a high content of sugars, plant and vegetables products, and others [1]. Gram-positive bacteria identified in bottled drinking water was only *Bacillus* spp (2.2%). There is no fecal Streptococci indicators contamination in the present study. Faecal Streptococci are those Gram-positive bacteria in chains living in humans and animals faeces. Results are closed to the data of reported by international researchers [6,11]. *Streptococcus* are rarely multiply in contaminated water, in addition they are more persistent than other coliform bacteria. *Bacillus* spp. are widely spread Gram-positive bacilli bacteria which may be recovered from water samples. They are spore-producing bacteria i.e. resistant to environmental stresses and disinfection. *Bacillus* may be found in two forms; non-harmful (free living) and harmful (pathogenic species) [23]. *Bacillus thermoglucosidasius* is typically found in soil samples but It has not been reported as a human pathogen. Bottles favored growth of bacteria may be due to the fact that, plastic materials such as ethylene-propylene and latex surface support bacterial growth more than glass and steel [10]. In addition, bacteria adhere more readily to rough surfaces [24,25] and the characteristic of the plastic bottles, beside that there is possibility of microorganisms' growth in favorite conditions in bottled water [26]. It could be concluded that bottled drinking water in Al-Laith is safe, within local and international guidelines recommendations and free of fecal indicators contamination.

Test	No positive	%
HPC	10	22.2
Coliforms (MPN)	4	8.9
Fecal coliforms	0	0
<i>E. coli</i>	0	0
<i>Enterococci</i>	0	0

Table 2: Result of Microbiological analysis of bottled drinking water.

Species	No positive	%
<i>Enterobacter</i> spp	4	8.9
<i>Bacillus</i> spp	1	2.2
<i>Klebsiella</i> spp	2	4.4
<i>Agrobacterium</i> spp	1	2.2
<i>Pseudomonas</i> spp	6	13.3
Single growth	6	13.3
Mixed growth	4	8.9

Table 3: Predominant bacteria identified in the study.

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