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Original article

The threats of microbial contamination and total dissolved solids in drinking water of Riyadh's rural areas, Saudi Arabia

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Background: Water is a valuable resource in Saudi Arabia. There are untreated wells scattered throughout villages that are sources of drinking water for some rural residents.
Objectives: The present study was conducted to assess the quality of bottled and tap water as well as water from wells in the rural areas of Riyadh, Saudi Arabia.
Material and Methods: Water samples (n = 300) were randomly collected from bottles, taps, and wells. Bacteriological examination of samples included total and fecal coliforms. Screening was performed using wet mounts, trichrome stain, and a modified Ziehl–Neelsen technique. Total dissolved salts (TDS) were determined using a conductivity meter.
Results: There were no coliforms in samples taken from bottled water, whereas, they were detected in samples taken from tap and well water with percentages of 11%, 30% respectively ($p = 0.0001$). No fecal coliforms were detected in any of the bottled water samples. However, they were isolated from tap (7%) and well water samples (22%), $p = 0.001$. *Escherichia coli* content was found to have the highest percentage distribution compared with other coliforms subtypes in both tap and well water. Parasitological analysis detected only few cysts of *Entameba coli* in both tap (3%) and well (4%) samples while *Giardia lamblia* cysts (2%) were only detected in well water. There was a wide variation in concentrations of TDS in the 300 water samples. Chemical analysis of well water showed levels above the maximum limits of Saudi and international recommended standards and guidelines for salinity of drinking water.
Conclusion: Water derived from wells in rural areas of Riyadh showed microbial contamination and high total dissolved solids.

Keywords: Coliforms, contamination, parasites, Riyadh, TDS, water

Water is a scarce and valuable resource in Saudi Arabia. Well water is still and will continue to be one of the main sources of drinking water in the kingdom, especially in rural central areas including the Riyadh region [1]. Many villages use well water for drinking without prior treatment [2]. Bottled water is widely sold and consumed in Riyadh and is preferred over tap water. This requires regular checks of the quality including bacterial, parasitic, or chemical contamination [2-5]. Bacteriological pollution of drinking water is a continuing source of diseases worldwide [6].

Cholera accounted for several outbreaks after the Hajj in Mecca 1984–86. Safer water supply and sewage systems in this area have eliminated cholera outbreaks since then [7]. Unsafe water supply will not only fail to protect consumers against waterborne diseases, but will also serve to disseminate these diseases more widely [8].

Previous studies in Saudi Arabia revealed high prevalence rates of infection with intestinal microbes. Moreover, microbial elements have been detected from water collected from different regions of the kingdom. Furthermore, many studies have linked the frequency of intestinal parasites with some environmental factors such as environmental temperature, solar radiation, rain, wind and others [3, 9, 10].

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Identification of pathogens in drinking water is expensive and time-consuming. Indicator organisms are therefore used to monitor water quality. The indicator bacteria are known as coliforms, and are a collection of microorganisms that live in large numbers in the intestines of man and animals and aid in the digestion of food. A specific subgroup of this collection are so called fecal coliforms of which the most common member is *Escherichia coli (E. coli)* and other subgroups of coliforms including *Klebsiella* species, *Enterobacter* species, and *Citrobacter* species [11, 12].

The presence of coliforms in water indicates that water has been contaminated with fecal material and suggests contamination by pathogens which can survive in water [13-15]. Previous studies of parasitic organisms associated with drinking water in the kingdom, including the Riyadh region, create uncertainties of the overall quality of drinking water [16, 17]. To clarify this, there is a need for more extensive studies of drinking water in our communities. Common examples of parasitic protozoa, often encountered in untreated well water, include *Cryptosporidium parvum*, *Giardia lamblia*, *Entameba* spp., *Microsporidia* spp., and *Cyclospora* spp. [18, 19]. Total dissolved solids (TDS) affect taste of drinking water. Water with content above 1500 mg/L can taste poor. Generally, TDS levels of less than 500 mg/L are acceptable to households [United States Environmental Protection Agency (US-EPA 1986)].

According to the limits set by Saudi Arabian Standards Organization (SASO), Gulf Cooperation Council Standards (G.C.C.S), World Health Organization (WHO), and US-PEA, water with salinity level beyond 1500 mg/L is considered unsuitable for dinking, but could be used for irrigating crops with good salt tolerance such as date palm trees [20]. High salinity can be the result of, excessive pumping, runoff water, soil weathering, and agricultural drainage water [1, 21]. This study concerned the quality and safety of bottled, tap, and well water in the rural areas of the Riyadh region in Saudi Arabia.

Materials and methods

Commercially available bottled and mineral water was purchased at random from different local supermarkets within Riyadh's rural region. Well water samples were collected from open wells scattered throughout villages in Riyadh's rural areas, using systematic random sampling. In this method, sampling points were predetermined based on the number of samples needed. One randomly selected point was the first sample (e.g. creating a grid of the sampling area and taking a sample from every third grid). This method was practical for sampling water and was applied for a total of 13 samples from each type of water (bottled, tap, and well), collected every week for 2 months.

Water samples were subjected to bacteriological, parasitological, and chemical analysis to assess suitability for potable purposes.

Sampling

The current study was carried out on 300 water samples collected from different locations and sources namely bottled, tap, and well water.

Bottled water sampling

In this study, commercially available bottled water of different brands was randomly purchased from different local supermarkets within Riyadh region.

Tap water sampling

Tap water samples were collected either directly from the reservoir or from taps. Prior to sampling, water was allowed to run for several minutes to avoid external contamination.

Well water sampling

Well water samples were collected from water wells scattered throughout villages in Riyadh region. Water was allowed to run for several minutes before taking the samples.

Taking into consideration the standard methods of both gathering and handling of water samples, 140 ml of water samples were collected in sterile screw capped, 150-ml plastic bottles These representative samples were then examined to assess their bacteriological and parasitological characteristics and suitability for potable purposes [18, 22].

Bacteriological, parasitological, and chemical studies

In the laboratory, each bottle was adequately shaken then divided into three parts for detection of bacteria, parasites, and TDS respectively. All samples were properly labeled and recorded.

Bacteriological study

The number of total and faecal coliforms was determined using the most probable number (MPN) method. Statistical tables were used to interpret the results to give the MPN of the bacteria. From each dilution 1 ml was added to each of triplicate tubes containing 5 ml of MacConkey broth. The tubes were then incubated at 37°C for 48 hours for total coliforms and at 44°C (in water bath) for 24 hours for faecal coliforms. The positive tubes were streaked on eosin methylene blue (EMB) agar plates using sterile loop and incubated at 37°C for 24 hours. Microscopic examination was carried out to ensure Gram-negative, non-spore forming rods [18, 22, 23].

Parasitological study

After centrifugation of water at 2000 rpm for 5 minutes, the supernatant fluid was discarded, and the remaining sediments were subjected to the following [23, 24].

Wet mounts

This method was used for the detection of the most common parasites. A drop of sediment was placed on a slide and mixed with a drop of Lugol's iodine solution using an applicator stick. The mixture was then covered and examined microscopically.

Trichrome stain

Trichrome stain is a special stain used for detection of *Giardia lamblia* and *Cryptosporidia*. A drop of sediment was spread in a thin smear then fixed in Schaudinn's fixative. The slides were then transferred into iodine-alcohol solution, followed by staining with trichrome stain for 8 minutes. Destaining was done with acetic acid-alcohol followed by dehydration in 95% absolute alcohol.

Modified Ziehl–Neelsen method

A modified Ziehl–Neelsen method was used for detection of *Giardia lamblia* and *Cryptosporidia*. Fixation was done in methanol followed by staining in cold carbol-fuchsin for 5 minutes then differentiated in 1% hydrochloric acid-ethanol for 5 minutes. Rinsing and counter-staining was finally done with 0.25% malachite green.

Analysis of total dissolved solids (TDS)

Total dissolved solids (TDS) were determined by measuring the water electric conductivity using a conductivity meter (Myron L Delux DS meter, 532 T1, Calif., USA) [22].

Comparison of the TDS in water samples was conducted in accordance with the Saudi and international standards; Saudi Arabian Standards Organization (SASO), World Health Organization (WHO), and United States Environmental Protection Agency (USEPA) [25-27].

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences SPSS, version 15 (SPSS, Chicago, Ill.) [28]. The statistical test used was as follows: arithmetic mean, standard deviation, for categorized parameters, a Chi-square test was used. While for parametric data to compare between more than two groups, an ANOVA test (*F* test) was used. The level of significance was set at $p < 0.05$.

Results

No coliforms could be detected in any of the bottled water samples of the current study (**Table 1**). However, total coliforms were detected in 11% of tap water samples and 30% of well water samples. The bacterial count detected in the well water samples were (between 10–100 CFU/100 ml in 5 samples, 100–1000 CFU/100 ml in 8 samples, 1000–10000 CFU/100 ml in 5 samples, and 10000–100000 CFU/100 ml in 12 of the samples) while the bacterial count detected in the tap water were (between 10–100 CFU/100 ml in 9 of the samples and 100–1000/100 ml in 2 of the samples). The total coliform counts in tap water was significantly greater that that in bottled water ($p = 0.0001$).

Table 1. Total coliforms counts/100 ml water samples collected from different water sources of Riyadh rural areas, Saudi Arabia

Types of water	+ve samples for total coliforms	χ^2 (*p*)	Most probable number\100ml			
			$>10–10^2$	$>10^2–10^3$	$>10^3–10^4$	$>10^4–10^5$
Bottled water (n = 100)	0	29.5	0	0	0	0
Tap water (n = 100)	11%	0.0001*	9	2	0	0
Well water (n = 100)	30%		5	8	5	12

*There were significantly more positive samples for total coliforms in well water compared with bottled and tap water ($p = 0.0001$). In addition there were significantly more positive samples for total coliforms in tap water compared with bottled water

No fecal coliforms were detected in bottled water (**Table 2**), whereas they were detected in 22% of well water samples (the fecal coliforms counts were between 10–100 CFU/100 ml in 4 samples, 100–1000 CFU/100 ml in 6 samples, 1000–10000 CFU/100 ml in 8 samples, and 10000–100000 CFU/ 100 ml in 4 of the samples). On the other hand, fecal coliforms were detected in 7% of tap water samples (the fecal coliform counts were between 10–100 CFU/100 ml in 5 of the samples and 100–1000 CFU/100 ml in 2 of the samples). There were significantly more positive samples for fecal coliforms in well water compared with bottled and tap water ($p = 0.001$).

The distribution of the different coliform subtypes isolated from the examined samples showed that, when the 11% and 30% positive samples isolated from tap and well water were subtyped, *E. coli* was found to have the highest percentage distribution over those of other coliforms subtypes (*Klebsiella* species, *Enterobacter* species, *Citrobacter* species) in both tap and bottled water (**Table 3**).

E. coli was found to have the highest percentage distribution than those of other coliforms subtypes (*Klebsiella species Enterobacter specie*, *Citrobacter species*) in both tap and well water.

Parasitological studies revealed that cysts of *Entameba coli* were isolated only from three tap water samples and four well water samples with percentage of 3% and 4% respectively, while Cysts of *Giardia lamblia* were identified in two samples collected from the well water (2%) as can be seen in **Table 4**.

Analysis of the 300 water samples for TDS (**Table 5**), showed a wide variation in concentrations. TDS ranged from 123–2053 mg/L with a mean of 715.56 mg/L and (SD = 467.32) in well water samples. The value of standard deviation showed that there was a wide variation among the samples with respect to their TDS ($F = 59.45$, $p = 0.0003$).

Data and recommended standards and guidelines for salinity of drinking water are shown in **Table 6**. Screening the well water samples revealed that 8%, 16%, and 62 % of the samples studied were above the maximum limits of recommended standards and guidelines.

In Saudi Arabia, the most used water for drinking is bottled water. It complies with Saudi and international recommended standards and guidelines for salinity of drinking water. Meanwhile 4% of the tap water samples exceeded these standards.

Table 2. Fecal coliforms (*Escherichia coli*) counts/100 ml water samples collected from different water sources of Riyadh rural areas, Saudi Arabia

Types of water	+ve samples for fecal coliforms	χ^2 (*p*)	Most probable number/100ml			
			$>10–10^2$	$>10^2–10^3$	$>10^3–10^4$	$>10^4–10^5$
Bottled water (n = 100)	0	22.36	0	0	0	0
Tap water (n = 100)	7%	(0.001)*	5	2	0	0
Well water (n = 100)	22%		4	6	8	4

*There were significantly more positive samples for fecal coliforms in well water compared with bottled and tap water ($p = 0.001$).

Table 3. Distribution of the isolated coliforms groups (%) in different water samples from Riyadh rural areas, Saudi Arabia

Types of water		Isolated coliforms		
	Escherichia coli No (%)	*Klebsiella species* No (%)	*Enterobacter species* No (%)	*Citrobacter species* No (%)
Bottled water (n = 100)	0 (0)	0 (0)	0 (0)	0 (0)
Tap water (n = 100)	6 (55)	0 (0)	2 (18)	3 (27)
Well water (n = 100)	18 (60)	3 (10)	2 (7)	7 (23)

Table 4. Percentages of parasites detected in water samples collected from different water sources of Riyadh rural areas, Saudi Arabia

Types of water	Cysts of *Entameba coli*	Cysts of *Giardia lamblia*
Bottled water (n = 100)	0	0
Tap water (n = 100)	3%	0
Well water (n = 100)	4%	2%

Table 5. Total dissolved solids (TDS) (mg/L) in different water sources of Riyadh rural areas, Saudi Arabia

Types of water	TDS variables (mg/L)			*F*	*p*
	range	mean	SD		
Bottled water (n = 100)	89–348	181.72	73.04	59.45	0.0003*
Tap water (n = 100)	18–543	185.00	120.34		
Well water (n = 100)	123–2053	715.56**	467.32		

*There is a highly significant increase in TDS parameters in well water than tap and bottled water ($p = 0.0003$).

Table 6. Comparison of total dissolved solids (TDS) (%) in different water sources of Riyadh rural areas, Saudi Arabia with the Saudi and international standards

Types of water	Percentage of samples above SASO standards (1500)	Percentage of samples above WHO Guidelines (1000)	Percentage of samples above USEPA (500)
Bottled water (n = 100)	0	0	0
Tap water (n = 100)	0	0	4%
Well water (n = 100)	8%	16%	62%

SASO = Saudi Arabian Standards Organization, WHO = World Health Organization, USEPA = United States Environmental Protection Agency

Discussion

The quality of Saudi Arabian drinking water is currently receiving attention from environmental and water scientists [20, 29]. Data recorded in the present study indicated that no total coliforms, fecal coliforms or parasites were present in any samples taken from bottled water from the Riyadh Region. The absence of *E. coli*, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species indicates no coliform contaminations in bottled water. These data confirmed that bottled water is satisfactory for human drinking purposes. Previous studies in Yanbu analyzed total and fecal coliform bacteria of bottled water and indicated that all their samples were negative with no single colony of any type. This study in Yanbu also indicated that all of the collected brands were safe for drinking without additional treatment [3].

A previous study reported two different species of bacteria, *Bacillus* spp. *and Pseudomonas* spp. in supposedly bacteria-free commercially available bottled mineral water. This report created great concern [8]. Whether the species of bacteria present in the two samples were contaminants, pathogenic or not, the fact that they were present at all, raises concerns that such a health risks to consumers cannot be taken for granted and that ongoing studies are needed.

The increased prevalence of diarrhea in the kingdom could be the result of the possible presence of pathogenic coliforms and parasites in tap water [10]. Bacteriological analysis of water samples revealed that coliforms were detected in tap and well water samples. These results indicated that the examined samples from wells and tap water exceeded the guideline values recommended in accordance with the local and international standards and are considered dangerous and may reflect the possibility of potential health hazards [6]. *Giardia lamblia* encountered in our study, are potential pathogens associated with waterborne related diarrheal outbreaks in healthy people and more severely in immunocompromised. In view of the increased prevalence of immuno-suppressed patients in our communities, it is essential to promote the general awareness of the public over the need for safe drinking water. The presence of *Giardia* can also be attributed to contamination by cattle, cats, and dogs that are well-known carriers [30-33]. Although *Entameba coli* cysts are considered as nonpathogenic, they also are indicators of fecal pollution of drinking water [31, 32].

The elevated level of coliforms and parasites in tap water indicates a failure of the treatment process of rural areas in Riyadh's household water supply system. This could be in the distribution pipes of Riyadh in which, sea water is extracted from the Arabian Gulf and piped over an area of about 235,000 km^2. The water is then treated in several desalination plants, and transported via several independent pipeline systems over hundreds of kilometers through the desert to Riyadh, where it is stored in reservoirs and fed into the distribution system. The contamination could be at any point of this complex system. Further investigation of this issue needs to be conducted on a continuing basis. Previous study by Abu-Zeid et al. supports our results that drinking only from tap water sources was associated with diarrhea as compared with drinking only from bottled water. The prevalence of diarrhea could be explained by the presence of pathogenic bacteria and parasites emphasized in our study and confirmed by others [9, 34-36]. However, other studies, such as the study conducted in Yanbu did not detect any coliform bacteria in any drinking tap water samples [37]. This indicated that the supplied tap water in Yanbu city is safe and free from bacteriological contamination [3]. These results can be explained by the presence of the residual chlorine within the prescribed limits. On the other hand, residual chlorine has been investigated in tap water of districts of Riyadh and estimated between 173.5 mg/L–177 mg/L which is below the SASO standards of 250 mg/L [38].

In the present study, it was noticed that total coliforms isolated from the drinking well water was higher than those detected in tap water and the difference was statistically significant ($p = 0.0001$). This was expected because the well water does not receive any chlorination before consumption in some rural areas scattered in Riyadh region. Contamination may also be the result of intensive application of organic fertilizers and animal waste or leakage from septic tanks and waste water discharged to nearby drinking wells [39]. Contamination of well water might also be the result of contamination of hoses used to deliver water from wells, or exposure of these hoses to dust. Moreover, the livestock and animal activity near to the wells may lead to further microbial contamination and more bacterial input. Likewise, coliform counts were previously recorded in well water samples collected from different areas [35, 36]. Subcultures of total coliforms included in the present study revealed the presence of fecal coliforms (*E. coli*, *Klebsiella* species, *Enterobacter* species, *and Citrobacter* species). Their presence is an indicator of serious public health risks [40]. Ongoing investigation of pathogenic bacteria detected and numerical analysis of any other type of bacteria isolated from Riyadh water samples is clearly indicated. Although they are few in number, the presence of protozoan cysts in tap and drinking well water in the current study is alarming and should not be neglected. These results are consistent with those of Nabil et al. who found that the highest TDS values of well water were found in Riyadh and Qassim regions in comparison to other Southern, Northern, and Western parts of the kingdom [41].

Conclusion

The current study concluded that water derived from drinking wells showed increases in most of the investigated microbial parameters; followed by tap water. Fortunately, bottled water was free from contamination indicating that it is satisfactory for human drinking. Contamination of tap or well water may occur during storage in the house reservoirs or from the wells' delivery hoses.

Bottled water samples were comparable to Saudi and international recommended standards and guidelines for salinity of drinking water, while the well water samples were above the maximum limits of these standards

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