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Full Length Research Paper

Estimation of coliform contamination rate and impact of environmental factor on bacterial quality of tube well water supplies in Khorramdarreh County, Iran

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In this study, the presence of fecal coliforms in tube wells of Khorramdarreh County, Iran, and impact of environmental factors on quantity and quality of these organisms were evaluated. In a cross-sectional survey, 76 tube well water samples from March to September 2010 were tested to determine presence of fecal coliforms, by measuring the most probable number (MPN), and identification of three enteric bacteria namely, *Escherichia coli*, *Klebsiella* sp. and *Salmonella* sp. Out of 76 wells, 24 (31.57%) and 25 (32.89%) wells showed presence of fecal coliforms, during rainy and dry seasons, respectively. In contrast to seasonal changes, depth of the wells showed significant correlation with type of isolated bacterial indices (P = 0.0005). On the other hand, depth of the well and seasonal variations did not show any significant correlation with fecal coliform contamination rate. The level of bacterial contamination of tube wells, in terms of presence of fecal coliforms is alarming. This was only an indicative study, and therefore, appropriate studies are required to determine the extent, risk factors and nature of the problem.

Key words: Fecal coliforms, tube well, *E.coli*, *Klebsiella* sp., *Salmonella* sp.

INTRODUCTION

Water is an increasingly scarce resource worldwide. One of the most significant changes has been the growth of cities to unprecedented sizes and rising demand for water (Mazari-Hiriart et al., 2008). Moreover, due to rapid increase of population, rapid industrialization, flow of pollution from upland to lowland, and too much use of fertilizers and pesticides in agriculture, there is a growing concern over the safety and fate of this valuable source of water (Jothivenkatachalam et al., 2010). Globally, Iran is situated in a relatively dry zone and only 10% of the country receives enough rainfall and remaining part rely heavily on groundwater to meet its need (Ravilious, 2008). Ground water is usually defined as water found underground in the saturated zone of rocks and exploited by means of tube well and depending on geological constitution of the soil in different region, the depth usually varies from 50 to 500 m (Reshma and Prakasma, 2007). Water used for drinking should be of potable nature which means it could be consumed in desired amount without adverse effect on health (Jiban et al., 2009). Vast varieties of microorganisms are water borne and due to this fact, conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population (Omezuruike et al., 2008). For more efficient hygienic supply of water the World Health Organization (WHO) proposed a scheduled program of water assessment, including microbial assay (WHO, 2008). When water is submitted to an accredited laboratory, for testing, it will also be analyzed for the bacteriological property, particularly fecal coliforms status. While coliforms are themselves not normally causes of serious illness, their presence can be used as an indication for other pathogenic organisms of fecal origin. Microbial character

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of well water can be influenced by certain factors like seasonal variations and depth of the well, which should be considered while assessing these water sources (Egwari and Aboaba, 2002). Ground water is one of the main sources of water supply in Iran and since it is used for human consumption, it should meet required microbial standards. Regular microbial analysis of water at source must be carried out to monitor the effectiveness of treatment process. In present study, our aim was to determine the presence of fecal coliforms contamination in tube well water of Khorramdarreh County, Iran, and investigate the impact of environmental factors, namely seasonal variations and depth of the well, on quantity and quality of these organisms.

MATERIALS AND METHODS

Site description

This descriptive study was performed between March and September 2010 in Khoramdarreh. Khorramdarreh County, with an area of 407 km² and population of 60499 (2006), is situated in Zanjan province, which is in North West part of Iran. This county has a mostly rural population which mostly rely on ground water supply for domestic and agriculture purposes. According to water resource management organization of Iran, on the bases of depth, the tube wells are classified in to three category of deep (> 50 m), semi deep (30 to 50 m) and shallow (less than 30 m). All wells in study area were equipped with electric motor for water draining.

Sampling strategy

Sample size was calculated (precision 5%, confidence interval 95%) and out of 190 available well, 76 locations were randomly selected for this study, out of which it was comprised of 32 deep and semi deep wells each, and remaining 12 were shallow wells. Samples were collected twice, one in rainy and the other in dry season from selected wells at different locations. The rainy season in Khoramdare region starts from March to middle of June and from then onward up to September, it is relatively dry. In order to reduce microbial contamination during sample collection, the water was pumped continuously for 3 min first of all and then exiting mouth of the well was flame-sterilized with a portable gas flame and finally midstream flow was collected in a 300 ml sterile plastic bottle. About 3 to 4 ml of the bottle was left empty to prevent any probable contamination of sample with external periphery, while tightening the screw cap. In order to avoid any error between collection and analysis, well number, location, time and date of sampling were labeled on each bottle. Water samples were placed in ice containing cool box and dispatched within 30 to 45 min to micro-biology diagnostic laboratory for analysis.

Laboratory evaluation

Determination of fecal coliforms contamination rate was based on most probable number (MPN) method, (Sutton 2010) which estimates the concentration of total coliforms (TC) and thermophilic coliform (*Escherichia coli)* per 100 ml of water sample. It is based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculums placed into a set number of culture media tubes. A serial dilution was prepared with sample inoculums for detection of total fecal coliforms. The sterility of each batch of test medium was confirmed by concomitant incubation of non inoculated tubes, as a control. The presumptive test was followed by the confirmative test. In confirmative test, positive tubes (gas production) were sub cultured on eosin methylene blue (EMB) agar, for selective isolation of *E. coli*. At the same time, selective isolation of two other enteric bacteria namely*, Klebsiella* sp. and *Salmonella* sp., as model organisms of enteric pathogen, was attempted. In present study the Hicrome Coliform Agar w/SLS-M1300 from Himedia Company, India was used as selective and differential media for identification of *Klebsiella* sp. and *Salmonella* sp. On this media, *Klebsiella* sp. appears pink and *Salmonella* sp. as colorless colonies. The pure cultures of the bacterial isolates on EMB and Hicrome Coliform Agar were subjected to conventional morphological and biochemical test methods for identification of the above mentioned organisms, with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). With the help of Statistical Package for Social Sciences (SPSS, Version 11) software package, the data was analyzed, using X2 tests, to test the significance of associations between categorical variables.

RESULTS

Following bacterial analysis of collected water samples out of 76 wells of different depth, 24 (31.57%) and 25 (32.89%) locations showed fecal coliforms contamination during rainy and dry seasons, respectively (Table 1). The MPN for the presumptive TC of water samples ranges from 3.6 to 460 and 3.6 to 1100 MPN/100 ml during rainy and dry seasons, respectively (Table 2). As it is evident in Table 2, *E. coli* comprises the dominant genes of isolated bacteria in water samples, and incidence of *Klebsiella* sp. and *Salmonella* sp. are only evident in shallow wells. Among the contaminated wells, well no. 73 showed concomitant isolation of *Salmonella* sp. and *Klebsiella* sp. In contrast to seasonal changes, depth of the wells showed significant correlation with type of isolated bacterial indices (P = 0.0005). On the other hand, depth of the well and seasonal variations did not show any significant correlation with TC contamination rate.

DISCUSSION

Coliforms are normal inhabitants of digestive tracts of animals, including human, and are found in their wastes, besides soil material (Jiban et al., 2009). They are also considered as indicator organisms of water pollution caused by fecal contamination which is a serious problem due to the potential for contracting diseases from pathogens (disease causing organisms). Although the concentrations of pathogens from fecal contamination are small, the possible occurrence of a number of different pathogens is large. On the other hand, coliforms are not specific indicators of fecal pollution and the presence of *E. coli* (thermophilic coliforms) is proved to be the most appropriate group of coliforms to indicate fecal pollution from warm-blooded animals (Ashbolt et al., 2001). According to WHO standard, potable water should be free of coliform organisms (WHO, 2008), hence, some of the water samples, in our study, did not comply with the

Table 1. Frequency distribution of contaminated wells of different depth in rainy and dry season.

Depth of well	Contaminated well	
	Rainy season	Dry season
Deep	8	7
Semi deep	10	11
Shallow	6	7
Total	24	25

Table 2. Frequency distribution of MPN/100 ml and isolated bacterial sp. in rainy and dry seasons.

Contaminated well number	Depth of the well	Total coliform MPN/100 ml		Isolated bacterial sp.	
		In rainy season	In dry season	In rainy season	In dry season
5	D	15	43	*E. coli*	*E. coli*
8	D	43	43	*E. coli*	*E. coli*
13	D	15	43	*E. coli*	*E. coli*
14	D	3.6	3.6	*E. coli*	*E. coli*
18	D	23	23	*E. coli*	*E. coli*
19	D	15	43	*E. coli*	*E. coli*
20	D	15	23	*E. coli*	*E. coli*
25	SD	15	3.6	*E. coli*	*E. coli*
34	SD	15	9.1	*E. coli*	*E. coli*
37	SD	240	460	*E. coli*	*E. coli*
44	SD	3.6	3.6	*E. coli*	*E. coli*
46	SD	9.1	6.1	*E. coli*	*E. coli*
47	SD	9.1	3.6	*E. coli*	*E. coli*
49	SD	23	9.1	*E. coli*	*E. coli*
50	SD	9.1	15	*E. coli*	*E. coli*
53	SD	43	43	*E. coli*	*E. coli*
54	SD	15	23	*E. coli*	*E. coli*
56	SD	0	9.1	-	*E. coli*
65	S	210	210	*Klebsiella*	*Klebsiella*
67	S	21	7.3	*Klebsiella*	*Klebsiella*
68	S	120	120	*Klebsiella*	*Klebsiella*
69	S	15	43	*E. coli*	*E. coli*
70	S	120	120	*Klebsiella*	*Klebsiella*
73	S	460	1100	*Klebsiella Salmonella*	*Klebsiella Salmonella*
76	S	9	20	*E. coli*	*E. coli*

D, Deep; SD, semi deep; S, shallow; MPN, most probable number.

recommended value. In comparison with other similar findings, the results from this study are considered to show slightly lower but also, in some cases, higher contamination rate. Bacteriological analysis of the ground water of District Nainital (Uttarakhand, India) did not show any sign of bacterial contamination in tube-well water samples (Jain et al., 2010) whereas assessment of groundwater in Bangalore, India, showed 50% bacterial contamination in the groundwater (Shankar et al., 2008). In our study, no significant correlation could be established between TC contamination rate and seasonal variation, which contradict the similar findings in a Niger (Egwari and Aboaba, 2002) India (Jiban et al., 2009) and Mexico City (Mazari-Hiriart et al., 2008). The water quality index (WQI) value, as a function of various physic-chemical and bacteriological parameters, was determined for groundwater obtained from a total of 21 locations in Bhandara District of central India. The WQI during pre-monsoon season varied from 68 to 83, while for post-monsoon, it was between 56 and 76. Significantly ($P < 0.01$) lower WQI for the post-monsoon season was observed, indicating deterioration of the groundwater overall in corresponding season (Rajankar et al, 2011). In a study on rural drinking water at supply and household levels, 61% of tube-well water samples met the Bangladesh and WHO standards of fecal coliforms and the rate of water contamination was highest during the February to May period (Hoque et al., 2006). Study in Karnataka state of India revealed that the level of contamination is beyond the permissible limit for indicator organisms in groundwater during rainy season which can render the consumer more vulnerable to health risks (Jiban et al., 2009). Recently Iran is experiencing a relatively low rain fall level as per the official reports released by Iranian Meteorological organization, which can be a probable explanation for non significant correlation between contamination rate and seasonal variations.

In contrast to seasonal changes, depth of the wells showed significant correlation with type of isolated bacterial indices (P = 0.0005). At the same time no significant correlation could be found between contamination rate and depth of the well. Following deep article review, we could not find any related article; hence we do not pass any comment on this issue. The isolated bacterial species in this study were matching with some of those commonly encountered in water and aquatic environments reported by other researchers (Omezuruike et al., 2008). The identified isolates include *E. coli, Salmonella* sp. and *Klebsiella* sp. These data confirm what other studies have noted that tube wells are commonly contaminated with fecal organisms (Luby et al., 2008; Omezuruike et al., 2008). There are certain index and model organism which their presence in water sample is of great concern, for example evidence of E*. coli* as an index for *Salmonella* sp. and F-RNA coliphages as models of human enteric viruses (Ashbolt et al., 2001). Hence presence of coliforms group in these water samples suggests that there are chances of mixed contamination with more dangerous microorganisms. Upon bacterial analysis we could isolate *Salmonella* sp. from shallow well no. 73, which might be due to the presence of a dairy farm in vicinity of this specific well region.

Conclusion

In this study, the coliform contamination of tube well in Khoramdarreh was studied. The bacterial analysis showed that out of 76 wells, 24 (31.57%) and 25 (32.89%) locations are contaminated with coliform during rainy and dry seasons, respectively. In adition to *E. coli*, *Klebsiella* sp. and *Salmonella* sp*.* were also isolated. There was a significant correlation between depth of the wells and type of isolated bacterial indices (P = 0.0005). It is recommended that the government and other relevant actors in Khoramdarreh establish a comprehensive drinking water system that integrates water supply, quality, handling and related educational programs in order to ensure the safety of drinking water supplies.

Conflict of interest

No conflict of interest to declare.

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