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Use of Bacterial Indicators for Contamination in Drinking Water of Qom, Iran

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Abstract: This study is performed to investigate the bacterial contamination of the water resources and the pipeline networks of the city. Faecal coliforms (such as *Escherichia coli* (*E. coli*) as the most important indicator of water contamination), non-faecal coliforms and *Pseudomonas aeruginosa* (which have recently been introduced as a new indicator of water contamination) as well as the number of phototrophic bacteria are examined. Samples collected from all wells, a big reservoir supplying big part of the city's water (called Khordad 15 Dam), main pipeline networks, settling and resting reservoirs and finally treated water consumed by people under a sterile condition. The results showed 25% of samples from main water resources of Qom city were contaminated with confirm but no contamination detected in treated water. 16.7% of were samples were contaminated with *Pseudomonas aeruginosa* after subculturing to Acetamid broth, but 11.8% of treated water samples confirmed to be contaminated with this bacterium in the second subculture. Although the results of this study demonstrated the potential threats of bacterial contamination (such as coliforms) in water resources, Qom's fresh water is safe and water treatment facilities there work quietly efficient and remove all coliform contaminations. The appearance of *Pseudomonas aeruginosa* contamination in drinking water is a sign of pipeline network cracks and erosions which require new efforts to repair and renew them.

Key words: Water contamination, *E. coli*, *Pseudomonas aeruginosa*, Qom

INTRODUCTION

Water is basic to life and health; over 1 billion people worldwide have no access to safe drinking water and insurance of its quality and health have been a very important issue from the beginning (Peeler *et al*., 2006). History shows that from the beginning, human found that there was a relation between the incidence of diseases and water quality and he also determined the primary factors of water quality such as colour, taste and smelling Yassin *et al*., 2006). Such experiences as the outbreak of diseases with many deaths occurred by drinking unsafe water convinced him that those indicators are not sufficient to trust water safety (Hirata *et al*., 2007).

Water may be contaminated in many ways. The different forms of contamination come from different sources and are dealt with in different ways (Fall *et al*., 2007; Rozemeijer and Broers, 2007). The three main forms of water contaminations are physical, bacterial and chemical (Mor *et al*., 2006). Water has played a significant role in the transmission of human diseases and the indicator microorganisms have been used to suggest the presence of pathogens (Peeler *et al*., 2006; Serrano and Delorenzo, 2007). Today we understand the possible reasons for the presence or absence of indicators and pathogenic bacteria in water (Papini *et al*., 2005).

Microorganisms are widely distributed in nature and their abundance and diversity may be used as indicators for suitability of water (Popko *et al*., 2006). Bacteriological assessments, particularly for coliforms, the indicators of contamination by faecal matter are therefore routinely earned out by many public health authorities to ascertain the quality of water to ensure prevention of further dissemination of pathogens through the agency of water (Swistock and Sharpe, 2005). The use of bacteria as water quality indicators can be viewed in two ways: first the presence of such bacteria can be taken as an indication of faecal contamination of water and thus as a signal to determine why such contamination is present, how serious it is and what steps can be taken to eliminate it; second, their presence can be taken as an indication of the potential danger of health risks that faecal contamination posses (Papini *et al*., 2005; Ryu *et al*., 2005; McQuaig *et al*., 2006; Jaji *et al*., 2007).

The use of bacteria to indicate the presence of faecal contamination in water originated 125 years ago when a German named Von Fritsch reported that certain types of microorganisms are characteristics of human faecal contamination and indications of water contamination (Yassin *et al*., 2006). Therefore, monitoring the quality of water is one of the essential issues of drinking water management (Trevett *et al*., 2004; Dieter and Muckter,

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2007). *E. coli* (*Escherichia coli*) is a type of faecal coliform bacteria commonly found in the intestines of animals and humans (Edrington *et al*., 2006; Alam *et al*., 2007; Mallin *et al*., 2007). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Although most strains are harmless, some strains produce a powerful toxin and can cause severe illnesses. Infection often causes severe bloody diarrhea and abdominal cramps; sometimes the infection causes non-bloody diarrhea (Derlet and Carlson, 2006; Blatchley *et al*., 2007).

The ratio between total coliforms has been proposed as a means of distinguishing between human and animal-derived faecal matter (Anderson *et al*., 2005; Guber *et al*., 2007). However, this method is no longer recommended and none of the currently-used bacterial indicators distinguishes confidently different sources of faecal matter when used alone (Skuratov *et al*., 1998; Bonadonna *et al*., 2005; Shah *et al*., 2007). The most widely used indicators of bacterial contaminations are the coliform bacteria or total coliform bacteria (Sankararamakrishnan and Guo, 2005). The contamination of lakes and rivers by faecal material increases the risk to the populations due to waterborne diseases (Albinana-Gimenez *et al*., 2006; Kassenga, 2007).

Qom is situated in the Central North of Iran, (34° 39N, 50° 54E) and due to the undesirable quality of its underground water, there is a separate network of fresh water pipelines there. To maintain high water demands by the society and also the industries around the city, 56 deep wells and a big dam have been built in nearby city (Khordad 15 dam) which are serving as the main water suppliers. Since the major watersheds of Qom are salty and brackish because they are in contact with salty dimes, so their monitoring is very significant. With regard to the importance of the safety of water and its quality control, this study looked at the bacterial contamination of Qom's drinking water by estimating faecal coliforms (such as *E. coli* as the most important indicator of water contamination with human and animal wastes), non-faecal coliforms, *Pseudomonas aeruginosa* (which has recently been introduced as a new indicator of water contamination) and the number of phototrophic bacteria (Okagbue *et al*., 2002; Wingender and Flemming, 2004; Lal and Kaur, 2006; Kassenga, 2007). *Pseudomonas aeruginosa* is an opportunistic pathogen bacterium which is prevalent in water and can cause various diseases such as rash and otitis (especially for those who swim in the polluted water) (Itah and Ekpombok, 2004; Baumgartner and Grand, 2006).

MATERIALS AND METHODS

Samples were collected from all 20 wells of Ali-Abad field, 36 deep wells of Qomrood river, Khordad 15 dam, resting pools and treated drinking water (10 sites). The samples were in pre-sterilized stopper glass bottles, transported to the laboratory in ice boxes and processed within 2 to 4 h of collection. To eliminate chloride activity, sodium thiosulfate was added to treated samples. Water samples were collected twice from winter to spring of 2005-2006. The water quality was determined by Most Probable Number (MPN) method (15 tube-tests). Coliforms were detected by inoculation of samples into tubes of Lactose Broth (LB) and incubation at 37±1°C for 48 h. The positive tubes were subcultured into Brilliant Green Lactose Broth (BGLB) and *E. coli* Broth (EC) and were incubated at 35±1 and 44.5±1°C, respectively. Gas production in BGLB at 35°C was used for the detection of total coliform after 48 h incubation. Faecal coliforms were detected by inoculation of water samples in EC Broth and incubated at 35±1°C for 24-48 h. All the culture media were obtained from Merck (Darmstadt, Germany).

Pseudomonas aeruginosa were detected by inoculation of samples into tubes of Aspargin Broth and incubation at 37±1°C for 48 h. The positive tubes having fluorescence were subcultured into Acetamid Broth which was incubated at 35±1°C. The colour of positive tubes changed to pink.

In order to determine heterotrophic bacteria, Heterotrophic Plate Count (HPC) method was used. Water samples cultured in Plate Count Agar (PCA) by the spread method and were incubated at 37±1°C for 48 h and colony forming units were determined by colony counter.

RESULTS

The average temperature of untreated water was 16°C ranging from 10 to 20°C. The average Electro-Conductivity (EC) of untreated Qom's water sources was 2220 S m^{-1} with a maximum and minimum of 1670 and 3320 S m^{-1}, but the average EC for treated water was 2424 S m^{-1} with maximum 4440 S m^{-1} and minimum 1525 S m^{-1}. Qomrood river which originates from Khordad 15 dam showed increase in EC during its passage through agricultural fields (1700 to 5700 S m^{-1}) but the maximum EC recorded (10800 S m^{-1}) from the point where it leaves the city of Qom (the river goes through the city and finally ends in Hoze-Soltan salt lake) (Table 1). Maximum and minimum turbidity recorded were 5.87 NTU (well No. 3) and 0.4 NTU (Zad Street), respectively, with average of

2 NTU. pH showed the least value at the entrance of Khordad 15 dam (pH = 6.65), the most in the lake behind the dam (pH = 8.21) with average of pH 8.

The results for untreated water (samples from all wells and Khordad 15 dam) showed 58.33% of samples cultured on LB were coliform probable positive. Those probable positive tubes were subcultured into Brilliant Green Lactose Broth (BGLB) and *Escherichia coli* Broth (EC) and the results showed 25% of original samples were positive in this stage which clearly confirm *E. coli* existence and contamination in 25% of water sources in Qom (Table 2). From those samples, 50% of them were probable positive and 16.7% were confirmed to be contaminated with *Pseudomonas aeruginosa* after subculturing to Acetamid broth (Table 2). In total heterotrophic assay, the average Heterotrophic Plate Count (HPC) method showed colony forming unit (cfu) was about 85 and the maximum numbers in untreated and treated water belonged to Jafarea well No. 2 (about 500 mL^{-1}). For treated water (water supplying through city pipeline networks), the average of chloride content assayed was 69.2 mL; the maximum and minimum belonged to Sadughi Blvd. (1.2 mL), Zad Street and Modarres Street (0.5 mL), respectively. No coliform contamination was seen in BGLB and EC subcultures of treated water, although in the first LB tubes 17.6% were positive (Table 3). But from 23.5% of probable positive *Pseudomonas aeruginosa*, 11.8% confirmed to be positive in the second subculture (Table 3).

Table 1: Increases in Qomrood river EC from when it passes through agricultural fields and then through Qom (the highest recorded EC measured when it leaves the city)

Location	EC (S m^{-1})
Behind Khordad 15 dam	1700.0
Neizar bridge	3780.0
Abdollah holy shrine	4410.0
Ghalecham	4540.0
Khalajabad bridge	4660.0
Laks Hamvar	5700.0
Enghelab bridge	10800.0
Average	5084.3

Table 2: Probable positive and confirmed positive of untreated water samples to *coliforms* and *Pseudomonas aeruginosa* of Qom water resources

Test	Probable No. (%)	Confirmative No. (%)
Coliform	58.33	25.0
Pseudomonas aeruginosa	50.00	16.7

Table 3: Probable positive and confirmed positive of treated water samples to *coliforms* and *Pseudomonas aeruginosa* of Qom fresh water

Test	Probable No. (%)	Confirmative No. (%)
Coliform	18.0	0
Pseudomonas aeruginosa	23.5	18

The mean of cfu showed 56.2 and the maximum and minimum belonged to Day Street (more than 60 mL^{-1}) which is in the end of one of the water pipeline network and Amir Kabir Street (30 mL^{-1}), respectively.

DISCUSSION

E. coli is a type of faecal coliform bacteria commonly found in the intestines of animals and humans (Peeler *et al*., 2006). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination (Hoque *et al*., 2006). Sewage may contain many types of disease-causing organisms (Yassin *et al*., 2006). During rainfalls, snow melts, or other types of precipitation, *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater (Guber *et al*., 2006). When these waters are used as sources of drinking water and the water is not treated well or inadequately treated, *E. coli* may end up in drinking water (Djuikom *et al*., 2006; Guber *et al*., 2006).

Pseudomonas bacteria contamination is an emerging health concern that most pools and spa companies are unaware of and most water testing performed by these companies do not check for these bacterial contamination (Wingender and Flemming, 2004). *Pseudomonas* species are opportunistic pathogens that are part of the normal bacterial flora of the pharynx, mucous membranes and skin of humans (Nedachin *et al*., 2005). It flourishes in warm, moist areas which makes pools and hot tubes an ideal environment for these bacteria (Zunino *et al*., 2002; Wingender and Flemming, 2004). In recent years, the number of reported *Pseudomonas aeruginosa* outbreaks in recreational waters has risen dramatically (Skuratov *et al*., 1998). *Pseudomonas* is capable of causing significant illnesses including skin rashes (folliculitis) (Bhatia and Brodell, 1999), ear infections (swimmer's ear) (Golledge, 1996), urinary tract infections, pneumonia and corneal ulcers (keratitis) (Ladhani and Bhutta, 1998). *Pseudomonas aeruginosa* is one of the leading (and most damaging) causative agents of microbial keratitis in contact lens wearers (Insler and Gore, 1986). This bacterium is of major concern in hospitals, since it can cause severe secondary infections, such as pneumonia, in burn victims or immunocompromised persons (Insler and Gore, 1986).

The prior studies have shown that although the existence of coliforms in water cannot be regarded as contaminated water with human and animal wastes but it may be related to water contamination with other pathogenic and non-pathogenic bacteria (Zhang and Lulla, 2006). About a quarter of Qom's water resources were contaminated with coliforms which is really high and it could be due to pumping water from the watershed located exactly beneath the city. It is important to remember that recently some efforts have been made to build city sewage system and to prevent all houses' wastewater to enter directly underground wells and afterward leak to underground watershed. Due to high level of coliform contamination, some wells inside the city have been closed recently. But no coliform contamination was seen from samples taken after treating water, which could be due to high concentration of chloride in water (as shown to be high in Qom's drinking water) which kills all bacteria before water is consumed.

It has also been shown that the *Pseudomonas* contamination might not be due to original water sources' contamination but it may occur in defects of pipeline network system; pipeline erosions may be the first probable cause. The results of this study also confirm that *Pseudomonas* contamination occurs during water transmission and it is not from main sources (wells or dam). The pipeline networks in Qom has been made over fifty years ago and the poor maintenance plus high erosive characters of soil and water in this area have made it vulnerable to puncture and erosion. *Pseudomonas* can enter pipelines through those holes and punctures and so it would be reasonable to find *Pseudomonas* bacteria contamination in Qom's drinking water. Recently new efforts have been made to change the pipelines to more resistant ones.

Although coliforms are known as the best index for monitoring water microbial quality, in some cases this index is not very efficient. For example, when the number of heterotrophic bacteria is not high, it cannot show all kinds of contaminations. Some believe that the excess growth of heterotrophic bacteria result in suppress of the coliforms. Therefore, it is recommended to use other bacteria as an alternative index in water microbial quality control and *pseudomonas* can be one of these indicators.

Finally present results showed that Qom's drinking water is safe for consumers although potential threats (such as coliforms) have been traced in water resources, but water treatments are working quiet efficiently and no contamination reaches the consumers. *Pseudomonas* contamination in drinking water urges new efforts to repair and renew the pipeline network.

Regarding present findings in this study, more work should be done to find the sources of erosions in Qom water pipelines and to prevent drinking water from contamination with opportunistic and pathogenic bacteria.

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