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Comparing the Total Coliform and Fecal Coliform for Recreational Waters in Public Swimming Areas in the Kingdom of Bahrain

Ali S. Bin Thani*, Sahar Baksh and Mariam Tanvir

Department of Biology, College of Science, University of Bahrain, P.O. Box 32038, Kingdom of Bahrain.

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Abstract

The Kingdom of Bahrain has an annual rainfall of about 78 mm with limited surface water resources and poor underground recharge. The rapid increase in urbanization, during the last 20 years, has increased the demands for additional water supplies. Recycling of such limited resources would also necessitate monitoring effluents coming off wastewater treatment plants. The monitoring process requires continuous and laborious work by dedicated water laboratories to verify water supply safety. Herein, the total number of coliforms in two public swimming areas/beaches (Zallaq and Hidd) were evaluated, and the results were compared to those from effluents of the main sewage treatment plant in Bahrain (Tubli Water Pollution Control Centre, WPCC). The results indicated a high Most Probable Number (MPN) for Tubli (MPN annual average value of 504 CFU/100 mL) compared to the other two sites Hidd (102 CFU/100 mL) and Zallaq (47 CFU/100 mL). The number of fecal coliforms was estimated using both biochemical and molecular approaches. According to estimates by EMB cultures and PCR among the total coliforms, *E. coli* (fecal indicator) constituted 37.3% for Tubli WPCC effluents, and less than 30% for the two public swimming areas in Hidd and Zallaq.

Keywords: Coliform; Fecal coliform; Recreational waters; Bathing waters; Kingdom Bahrain.

1. Introduction

Insufficient water resources in arid areas with scarce and erratic rainfalls are adding to the costs spent by developing countries to maintain the infrastructure of water facilities. In the Kingdom of Bahrain, the annual rainfall is about 78 mm with limited surface water resources and poor underground recharge. The rapid increase in urbanization during the last 20 years has increased the demands for additional water supplies. Recycling of such limited resources would also necessitate monitoring effluents coming off wastewater treatment plants. The monitoring process requires continuous and laborious work by dedicated water laboratories to test water supply safety. Many worldwide water safety programs test water samples and check for indicator microorganisms. Upon obtaining data, regulations and access to recreational waters are coordinated with local organizations and governmental agencies (Efstratiou *et al.*, 2009).

In 1914, the US Public Health Service Drinking Water Standards set the criteria for testing the quality of water for drinking and bathing (Efstratiou *et al.*, 2009). The test uses conventional techniques, e.g., the multiple tube fermentation/Most Probable Number (MPN) to test for presence of coliforms. Coliforms are Gram-negative, rod shape *Enterobacteriaceae*. A subset of this group is the fecal coliforms (e.g., *Escherichia coli*), which indicates contamination of test samples with human waste/sewage outlets (Rompre *et al.*, 2002). Fecal coliforms are used as indicator for the presence of pathogenic microorganisms (Efstratiou *et al.*, 2009).

The objectives of the present study are to monitor the total number of coliforms (TC) in two public swimming areas/beaches (Zallaq and Hidd) and to compare the results obtained with effluents of the main sewage treatment plant in Bahrain (Tubli Water Pollution Control Centre-WPCC). The present study also estimates the percentage of fecal coliforms at the three aforementioned seashores. The study used both conventional and molecular approaches to estimate and enumerate the microorganisms.

To the best of our knowledge, the two selected beaches have not been previously investigated for the presence of coliforms even though that these were public swimming areas. The numbers obtained would be useful as references for future studies and further analysis.

2. Materials and Methods

2.1. Isolates

Collection of samples was performed between October and June for three successive years (2014 - 2016). The samples were collected at 1.0-meter depth offshore. Collection of samples was performed during the daytime at

* Corresponding author. e-mail: abinthani@uob.edu.bh.

high tides (two liters for each location/site in sterile containers). The samples were processed within two hours after sampling by transfer of seawater into lauryl sulfate broth (for overnight incubation) and subsequent culture on Eosin Methylene Blue (EMB) for isolation (Figure 1). Fifty-one isolates were stored in 30 % glycerol at -20°C for further analysis and polymerase chain reaction.

2.2. Conventional Microbiology

Standard procedures for isolation and enumeration of coliforms using the presumptive (MPN) and confirmative tests (Grabow *et al*., 1981; SABS, 1984; Grabow, 1990; ISO, 1990; ISO, 1994; Standard Methods, 1995; Grabow, 1996) were followed by biochemical assessments to complete the identification of isolates.

2.3. Molecular Typing and Restriction Digests

Amplification of the 16S rDNA was carried in 100 µL total volumes. The reaction components were 2.5 units of HotstarTaq DNA polymerase (QIAGEN), 200 µM dNTPs and 0.5 µM of both primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACTT-3') (Jiang *et al*., 2006). The following run conditions were used (BIO-RAD iCycler): initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for one minute. The final extension was performed at 72°C for ten minutes (Noble *et al*., 2003; Kampfer *et al*., 2011; Aghababaee *et al*., 2012).

The produced amplicons (1.5 kb) were digested with *Eco*RI at 37°C for 2 hours (Figure 1). The PCR fragments and restriction digests were run on 0.8-1.3 % agarose gels for visualization.

2.4. Statistical Analysis

Logistic regression analysis, with the presence of coliforms as dependent variable and the investigated years as categorical independent variable, was used to assess whether there is a significant positive relationship between locations during consecutive years and the log values for the MPN numbers obtained for each site.

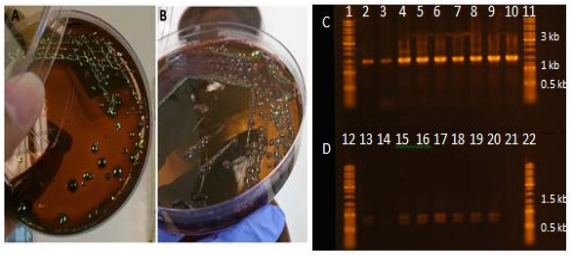

Figure 1. Obtained isolates from confirmatory tests on EMB plates and PCR. **A.** Metallic green sheen and fish-eye purple colony. **B.** Green sheen, purple un-nucleated and flat nucleated purple colony. **C.** Agarose gel electrophoresis of PCR products. The PCR amplicons indicates the detection of the 16S rDNA gene in the isolates. In numeric order from left to right, Lanes 1 and 11 is GeneRuler DNA ladder (ThermoFisher Scientific). Lanes 2 and 3 references strain *E. coli* MG1655. Negative control was run in (D) lane 21. Lanes 4-10 runs for selected isolates. Band size was 1.5 kb. **D.** Restriction digests of the PCR products in (C) by *Eco*RI. In numeric order from left to right, Lanes 12 and 22 is GeneRuler DNA ladder (ThermoFisher Scientific). Lane 13 reference strain *E. coli* MG1655. Lanes 14-20 runs for selected isolates. Band sizes 0.7 and 0.8 kb.

3. Results and Discussion

The present study was carried out during the months of winter and early summer as we expected high tides during these seasons. High tides might change the number of counted coliforms (St Laurent *et al*., 2014). The locations studied had the following coordinates: Dry-dock beach at Hidd (26.19595986, 50.662142), Tubli effluent (26.196866, 50.565727), Zallaq/Al Jazair beach (25.989600, 50.461081) (Figure 2).

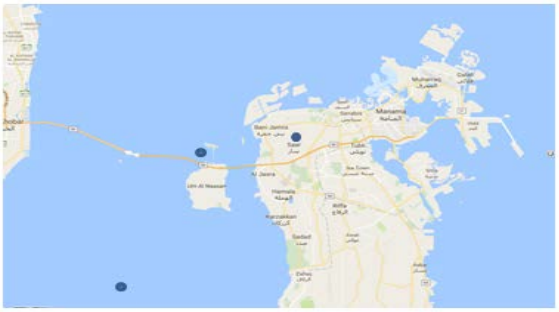

Figure 2. Map of the Kingdom of Bahrain showing study sampling sites. The blue marks, from left to right: Zallaq, Tubli, and Hidd.

Two of the studied areas are known public swimming beaches Zallaq and Hidd. The third location was selected as a control (Tubli-WPCC). Physical parameters for studied areas showed alkaline pH for both Hidd and Tubli (pH 8), while close to neutral for Zallaq (pH 7.2). Salinity was higher for Zallaq (54 PSU), than for Hidd (44 PSU) and Tubli (44 PSU). Figure 3 shows the total coliform values obtained for each site for three successive years (2014-2016). The numbers indicate high MPN for Tubli (MPN nine months' average value of 504 CFU/100 mL) compared to the other two sites; Hidd (102 CFU/100 mL) and Zallaq (47 CFU/100 mL). The number of fecal coliforms was estimated using both biochemical and molecular tests. According to estimates by EMB cultures and PCR among the total coliforms, *E. coli* (a fecal indicator) constituted 37.3 % for Tubli WPCC effluents, and less than 30 % for the two public swimming areas in Hidd and Zallaq.

Compared to previous data, Tubli WPCC continues to contribute to the total coliforms and fecal coliforms found in Tubli bay (Qureshi and Qureshi, 1990; Qureshi *et al*., 1993; Mahasneh *et al*., 1997). The effluents of Tubli bay potentially pose health issues due to the highly pathogenic species detected previously (Amin, 1988; Qureshi and Qureshi, 1992). They found that most of the species were resistant to a panel of routinely used antibiotics in the public health sector (Amin, 1988; Qureshi and Qureshi, 1992). Thus, there is a necessity for a quick intervention by local environmental agencies to control the spread of antibiotic resistant strains. However, in the current study, the obtained numbers for Tubli WPCC effluents (504 CFU/100 ml) were not significant in terms of introduced pathogenic species as set by Efstratiou, *et al.* (2009) (Figure 3). Efstratiou *et al.* (2009) indicated that a value of 1000 CFU/100 mL of total coliforms is needed to indicate the presence of pathogenic species, such as *Salmonella* spp. in seawater. Moreover, our values indicate a reduction in total coliforms compared to previous values obtained during a study in 1993-1994 (Al-Sayed *et al*., 2005). The

log mean values of CFU/mL were around 5-6 (Al-Sayed *et al*., 2005), while the mean log value in our study was around 0.7 CFU/mL. This indicates that in the last 20 years, Tubli WPCC has increased their standards and quality of released effluents, hence achieving a reduction in the TC by almost 86%. In regards to both Hidd and Zallaq, most numbers of total coliforms are still within the limits set for public use and are considered safe < 100 CFU/100 mL (Figure 2) (Efstratiou *et al*., 2009).

The 16S rDNA PCR and the restriction digest showed similar sizes and patterns as depicted by http://insilico.ehu.eus/PCR/ using the sequences of both primers 27F and U1492R as inputs and selecting - apply to all *Escherichia* as the target microorganism with allow a mismatch of 2 (San Millán *et al*., 2013). No new species were identified by PCR, as both typical PCR amplicon sizes (Figure 1) and the restriction fragments produced were identical to that obtained *in silico* (two DNA fragments of 0.7 and 0.8 kb) (Suardana, 2014).

The statistical analysis presented in (Table 1) and (Figure 3) shows a positive trend among different swimming areas and their corresponding log mean values for the MPN numbers upon successive years of study, $r(18)= 0.599$, $p < 0.005$. Indicating a significant relationship between the numbers of coliforms detected and the sites studied. The standard deviations obtained for readings of Zallaq and Tubli are smaller than that for Hidd (Table 1 and Figure 3). This variability in the reported readings of Hidd area could be attributed to the fact that Hidd is more open to the high tides and open sea as illustrated in (Figure 2).

Table 1. The log mean value CFU/100 mL at different sites

Year	Location	Nine months log mean values for the MPN TC coliforms (Standard deviation)
	Zallaq	1.66 (±0.024)
2014	Hidd	1.86 (±0.179)
	Tubli	2.69 (±0.017)
	Zallaq	1.70 (±0.024)
2015	Hidd	1.90 (±0.179)
	Tubli	2.70 (±0.017)
	Zallaq	1.65 (±0.024)
2016	Hidd	2.19 (±0.179)
	Tubli	2.72 (±0.017)

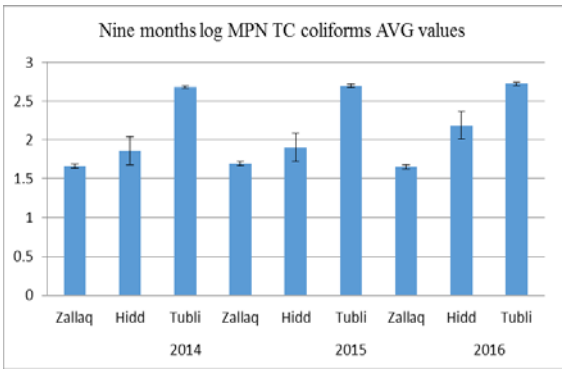

Figure 3. Total coliform estimates using the MPN method. Nine months' log average values (CFU/100 mL) of seawater for the three studied areas (years 2014-2016). Error bars refer to the standard deviation obtained for each site.

4. Conclusions

According to the estimates done by both EMB and PCR, *E. coli* (as fecal indicator) constituted 37.3% of the total coliforms isolated in Tubli WPCC effluents, and less than 30% for the two public swimming areas in Hidd and Zallaq. Compared to previous data, Tubli WPCC continues to contribute to the total coliforms and fecal coliforms found in Tubli bay. However, the obtained numbers for Tubli WPCC effluents are not significant in terms of introduced pathogenic species. Moreover, the data indicate a reduction in total coliforms compared to previous values obtained during a study in 1993-1994.

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