Evaluation of the Microbiological Quality of Natural Waters

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Abstract: An evaluation of the microbiological water quality from the Algarve region was conducted. The parameters were determined using two semi-automated most probable number methods, Colilert and Enterolert (IDEXX Laboratories, Inc.). These methods were developed for the enumeration of coliforms and enterococci, respectively. Since they are less time consuming in what refers to sample manipulation, incubation and results reading, they represent an extremely important advantage when we are concerned about water quality monitoring. Natural water samples were analysed for microbiological parameters, pH and salinity. The objective of this work was to set up that the Colilert and Enterolert methods are suitable to monitor the microbiological quality of natural recreational waters.

Keywords: Colilert; Enterolert; coliforms; enterococci; natural waters; indicators

1 Introduction

The water is one of the most privileged vehicles in the disease transmission to men. Not just when consumed but also from direct contact, as many natural waters are used for recreational bathing purposes [1]. Under certain conditions, these waters may be adversely affected by fecal pollution from a variety of sources including municipal wastewater and raw sewage effluent from treatment plants, private septic disposal systems, and storm water runoff [2]. Once the contamination of natural waters with untreated fecal material may result in an increased risk of transmission of diseases to the humans who use those waters [3], it is necessary that the sanitary quality of recreational bathing areas is routinely monitored by public health authorities for the presence of microbiological contamination [2].

Historically, fecal coliforms and *Escherichia coli* have been used as indicators of choice when monitoring recreational water quality. Recent studies have shown that high densities of *E. coli* and

enterococci recovered from recreational waters have a stronger correlation with swimming associated gastrointestinal disease than do densities of fecal coliform bacteria [4].

There are two standard methods for the enumeration of those microorganisms from marine recreational waters. The multiple tube fermentation (MTF) technique, that provides a most probable number (MPN) analysis after growth in liquid medium, and the membrane filtration (MF) technique that, enumerates on the surface of agar by providing a CFU.100mL^{-1} count (APHA 1995) [5]. Both methods require confirmed and completed tests after the initial observation of a positive primary medium. A complete analysis can require an additional 24 to 72h for a final result [6]. Although these methods showed a good recovery for coliform and enterococci from marine and estuarine waters, the false-positive and false-negative rates were found to be 10.0 and 11.7%, respectively [2]. But the major limitation of the standard methods is the length of time required to complete the testing [5]. To overcome this question two semi-automated most probable number (MPN) methods, Colilert and Enterolert, had been developed for the enumeration of coliforms and enterococci, respectively. These methods require significantly less time than the MF procedure and less quality control testing [2].

Two nutrient-indicators, ortho-nitrophenyl galactopyranoside (ONPG) and 4-methyl-umbelliferyl-glucuronide (MUG) are the major sources of carbon in Colilert, and can be metabolized by the coliform enzyme *β*-galactosidase and the *E. coli* enzyme *β*-glucuronidase, respectively. As coliforms grow in Colilert, they use *β*-galactosidase to metabolize ONPG and change it from colourless to yellow. *E. coli* use *β*-glucuronidase to metabolize MUG and create fluorescence [7]. The Enterolert test utilizes a nutrient indicator substrate, 4-methylumbelliferone-*β*-D-glucoside, that fluoresces when metabolized by enterococci. Methylumbelliferyl derivatives have the advantage of being highly sensitive and specific, non-carcinogenic, and easily detected with UV light sources [2].

In this study, we used the IDEXX methodologies to evaluate the levels of bacterial contamination in natural waters. Since these methods are already used for routinely monitoring tidal marine and freshwater recreational bathing areas in such a rapid, feasible and economic way.

2 Materials and Methods

2.1 Sampling

Natural samples were collected in sterile plastic bottles from the subsurface layer (30cm) and transported to the laboratory in cold boxes at approximately 4ºC, where they were processed within the maximum of 3 hours after collection.

2.2 Physicochemical parameters

To determine the pH and salinity values a Crison GLP22 pH meter and a Crison GLP32 conductimeter were used, respectively.

2.3 Microbiological parameters

Total coliforms, *E. coli* and enterococci were enumerated by means of Colilert and Enterolert methods in more than 30 natural water samples. These methods provide an MPN result based on the colour change and presence or absence of fluorescence in 51 individual wells each containing a sample nutrient indicator mixture. A 1:10 dilution of the test water sample was prepared (90 ml of sterile deionised water plus 10 ml of sample) in a sterile polystyrene vessel. One package of powdered Colilert or Enterolert reagent was then added to the vessel, and the sample-reagent combination was mixed and then poured into a Quanti-Tray, a sterile plastic disposable panel containing 51 wells. The trays were then mechanically sealed, distributing the mixture into the wells, and the results read after 18h incubation at 35 ± 0.5ºC for Colilert and after 24h incubation at 41 ± 0.5ºC for Enterolert. For Colilert, yellow wells were interpreted as positive for coliforms, then, the Quanti-Trays were checked for *E. coli*, in a dark environment by placing it under a 365nm wavelength UV light. Any fluorescence in a well was considered a positive reaction for that well and thus indicating the presence of *E. coli* on the Colilert test. The Enterolert trays were also observed under the UV light and the fluorescent wells were considered positive for enterococci. Based on the number of positive wells and the dilution factor, MNP tables are used to calculate coliforms, *E. coli* and enterococci density per 100mL of sample.

3 Results and Discussion

Table 1 shows the values obtained for the pH and salinity (gNaCl.L^{-1}). On Table 2 it is shown the enumeration for total coliforms, *E. coli* and enterococci. Table 3 gives the percentage of samples analysed that were under and above the Portuguese legislation.

For pH values, we can see that it varied from 6.94 to 8.0 with a mean of 7.61 for coliforms and *E.*

coli samples. For enterococci samples the pH variation was between 7.23 and 8.38 and the mean value was 8.02. In samples employed for coliforms and *E. coli* enumeration, the salinity values varied from 0.43 to 1.00gNaCl.L^{-1}, with a mean value of 0.66gNaCl.L^{-1}. The maximum value of 33.8gNaCl.L^{-1} and minimum of 0.28gNaCl.L^{-1}, with a mean of 26.87gNaCl.L^{-1} was determined in samples used for enterococci enumeration. It is important to measure these parameters as the IDEXX principle is based on enzymatic activities, which are extremely dependent on physiological status of the bacteria. Changes in irradiation, salinity, temperature and nutrient concentration of the environment may cause stress on the bacteria and problems in recovery [8].

The enumeration of microbiological parameters (Table 2), showed a variation between zero and $48.4x10^4$MPN.100mL^{-1} for total coliforms and *E. coli*. This variation was between zero and $0.58x10^4$MPN.100mL^{-1} for enterococci. The Portuguese law states that microbiological water quality for recreational purposes should present a maximum value of 250UFC.100mL^{-1} for *E. coli*, and 100UFC.100mL^{-1} for the enterococci [9]. Table 3 showed that, 59% of the samples analysed for *E. coli* was under the maximum acceptable limit, and 41% above it. For enterococci samples 45% were under the limit and 54% above it. By the mean values obtained in this study we could say that 50% of the natural waters analysed were not adequate for recreational activities. Even though, we had almost 50% samples which results were under the acceptable limits. The precision of the test is an important issue, because we need to be self-confidence when saying that the water is adequate for recreational activities. To overcome this question we analysed natural water samples by the standard methods in parallel with IDEXX methods. In this way we can evaluate the performance of both methodologies for the detection of coliforms, *E. coli* and enterococci in waters. Considering that the IDEXX tests are not yet standardised in the European Union, the objective is to establish the effectiveness of Colilert and Enterolert methods against the normalised ones.

Some studies were already done in drinking and bathing waters for the enumeration of coliforms, *E. coli* and enterococci [10]. The authors concluded that in drinking water samples, the Colilert method was more sensitive to detect coliforms than the standard methods, multiple tube fermentation and membrane filtration, but equally sensitive to detect *E. coli*. Buckalew *et al*. 2006, also found a positive correlation between standard methods and Colilert in environmental waters samples for *E. coli* enumeration [11]. Wastewaters were as well analysed with Colilert method in parallel with the standard methods. Chihara *et al*. 2005, concluded that Colilert can be used for the quantification of fecal coliforms and *E. coli* bacteria in both agricultural and municipal wastewater samples, once they found small differences in bacteria concentrations detected by Colilert and the other two standard methods [12].

The Colilert and Enterolert methods represent a good solution for problems related to save time and money spent in monitoring the microbiological water quality by the standard methods. And besides that they also give the results in less then half of the time that the normalised methods. Anyway a more complete study needs to be undertaken to assume these methods as a reference.

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Table 1 – Values obtained for the pH and salinity of the natural samples analysed.

	pH			Salinity ($gNaCl.L^{-1}$)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Coliforms and ***E. coli***	6.94	8.00	7.61	0.43	1.00	0.66
Enterococci	7.23	8.38	8.02	0.28	33.80	26.87

Table 2 – Enumeration of total coliforms, *E. coli* and enterococci in the natural samples analysed.

	Total number of samples	Minimum ($MPN.100mL^{-1}$)	Maximum ($MPN.100mL^{-1}$) $\times 10^4$	Mean ± SD ($MPN.100mL^{-1}$) $\times 10^4$
Coliforms	34	0	48.40	6.38 ± 12.5
E. coli		0	48.40	3.67 ± 11.10
Enterococci	44	0	0.58	0.07 ± 0.13

Table 3 – Percentage of samples under and above the maximum limit acceptable in the Portuguese legislation for *E. coli* ($250UFC.100mL^{-1}$) and enterococci ($100UFC.100mL^{-1}$) for natural waters.

	% Samples < Limit	% Samples > Limit
E. coli	59	41
Enterococci	45	54