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Evaluation of equivalence between different methods for enumeration of fecal indicator bacteria before and after adoption of the new Bathing Water Directive and risk assessment of pollution

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ABSTRACT

The quality of bathing water is of considerable public importance due to the possibility of fecal contamination. In 2009, Croatia implemented the new European Bathing Water Directive (BWD, 2006/7/EC) establishing stricter microbiological standards for new parameters with new reference methods. This study aims to evaluate the equivalence of different methods according to the old and revised BWD and to provide the possibility of data comparison. Furthermore, the directive requires the establishment of the bathing water profile (BWP) for pollution risk assessment. The estimation of consistency of pollution risk assessment with obtained microbiological results was also performed.

Six marine beaches of the Municipality of Rijeka (Croatia) were examined during the 2009 season. Statistical analysis showed equivalence between determination methods for fecal contamination indicators. Based on the current water classification results, the need for correction of estimated pollution risks and recommendations for inclusion of historical microbiological data during BWP enactment was noticed.

1. Introduction

The Maritime Domain and Seaport Act (Official Gazette No. 158 of 2003, 100 of 2004 and 141 of 2006) stipulates the beach as "the integral part of the maritime domain and the common good of public interest for the Republic of Croatia." It is under Government protection, and its use is regulated in the manner prescribed by the Act. Clean beaches are extensively used for promoting healthy ways of spending leisure time as well as for contribution to tourism development.

Water quality standards related to the microbiological quality of bathing waters vary throughout the world. The Bathing Water Directive EEC (76/160/EEC, 1976) was implemented back in 1976 specifying 19 parameters to be monitored at two levels (mandatory and guide). Yet, the priority had been given to two microbiological parameters – total coliforms (TC) and fecal coliforms (FC) as well as to guideline standards for fecal streptococci (FS).

However, since that time, the progress of science and technology, and almost 30 years of experience in applying the parameters from the old Directive, led the Commission to its revision. A new Bathing Water Directive (2006/07/EC 2006) came into force in 2006, completely repealing the existing 1976 Directive in all EU states by 2015. The revised Directive attempts to improve the safety of bathers' health through more stringent standards as well as through monitoring methodologies and management measures (Mansilha et al., 2009). The EU Directive sets out the minimum standards allowing the introduction of stringent criteria into the national regulations of the EU member states. Croatian national regulation has stipulated lower values, meaning more stringent conditions of assessment than EU directive. The reason is the insistence on maintaining high sea water quality on the beaches of the Croatian Adriatic where use of the European Directive threshold would not allow early detection of adverse changes in the environment, or the rapid implementation of remedial measures. Strict national criteria contribute to swimmer safety, especially of children, pregnant women and elderly persons, who are more susceptible. It is particularly important to consider that tourists can be more sensitive to pathogens given that they may not have developed an adequate immune response (Wiedenmann et al., 2006). This greater sensitivity should be taken into account when defining the limitation criteria. As an EU accession country and with coast

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belonging entirely to the Mediterranean, the Republic of Croatia has started the transfer to the new BWD during the year 2009.

The essential amendment of the revised Directive has been to reduce monitored parameters from 19 to only 2 microbiological indicators of fecal contamination. These were *Escherichia coli* (substituted fecal coliforms) and intestinal enterococci (substituted fecal streptococci), thus simplifying testing methodology and significantly reducing the costs of analysis.

Furthermore, the revised BWD requires the establishment and maintenance of the bathing water profile (BWP) for pollution risk assessment. A system of bathing water profiles is an innovative approach which provides a better understanding of risks as a basis for prevention management measures (proactive approach).

The primary aim of this paper has been to determine equivalency using two different methods for fecal indicator bacteria enumeration, respectively the former and the revised BWD. In this way, it was possible to compare the past and current information as well the utilization of the data from previous monitoring to assess trends. The second goal of this study was to determine the coastal water quality and the consistency of pollution risk assessment with obtained microbiological quality of water.

2. Methods

2.1. Sample collection and study area

Natural water samples from six locations (beaches) were collected bimonthly during the bathing season from June to September 2009. Water samples were obtained following the directions of the BWD (2006), i.e. approximately 30 cm below the sea surface, filled in sterilized bottles and delivered to the laboratory of the Teaching Institute of Public Health in Primorsko-goranska Region where all the analyses were carried out within 4 h following the sampling. Sampling was performed at the six local beaches (Fig. 1) that had been previously selected based on various relevant characteristics: proximity to the pollution sources and historical data of water quality.

The study area was divided into two stations where previous examinations have shown very low concentrations of examined microbiological parameters, and four stations that recorded fecal contamination and the significant impact of wastewater. In total, 60 samples were collected.

2.2. Microbiological analyses

Water samples were analyzed for six microbiological parameters – total coliforms (TC), fecal coliforms (FC), *E. coli* (EC), fecal streptococci (FS), intestinal enterococci (EN), *Clostridium perfringens* (CP) – using International Organization for Standardization (ISO) methods and *Standard Methods,* 21st edition (2005).

100-ml aliquots of samples (without dilutions) taken from the same vessel of a carefully mixed test sample were filtered through a sterile 0.45-μm pore size hydrophilic mixed cellulose ester filter, diameter 47 mm (GN-6 Metricel, Pall Life Sciences, USA). Afterwards, membranes were incubated on an agar plate intended to yield the growth of the target organisms while suppressing the growth of all other organisms, at the appropriate temperature for 24–48 h.

TC, FC and FS were analyzed using Standard Methods (2005) while EC and EN were examined in accordance with ISO 9308-1 and ISO 7899-2, respectively. CP was analyzed using the Directive of the Council of the European Union, recommendations 98/83/EC (1998).

Growth on membranes was monitored by counting the colonies formed during the recommended period of incubation cultivated on the agar plates under selective conditions (cfu = colony forming unit).

Biolife's LES Endo agar (sodium sulfite, fuchsin, sodium desoxycholate and sodium lauryl sulfate, lactose) was used for total coliforms enumeration according to the *Standard Methods* (9222 B). After an incubation period of 20–24 h at 35 °C ± 0.5 °C, all red colonies on the filter having characteristic metallic sheen were counted. Biolife'sM-FC (Rosolic acid, bile salts, aniline blue, lactose) medium was applied to cultivate and enumerate the fecal coliform group of bacteria according to the *Standard Methods*, (9222 D). Petri dishes were incubated at 44.5 °C for 22–24 h. Lactose utilization (blue color) has been the basis for identification of the fecal coliforms bacteria which have various shades of blue on M-FC medium. Fecal streptococci were detected using Biolife's Kanamycin Aesculin Azide Agar (KEA – kanamycin sulphate, sodium azide,

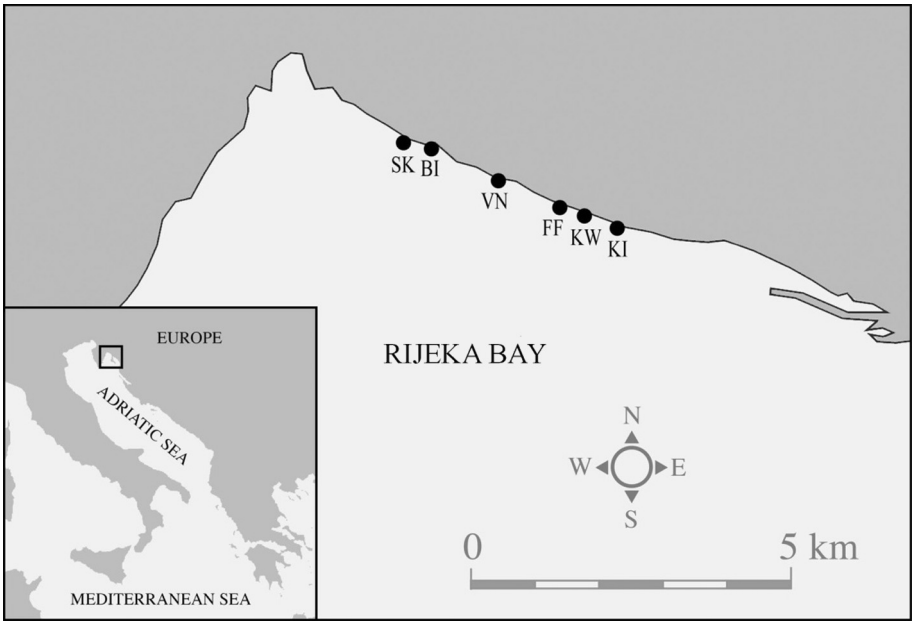

Fig. 1. Map showing sampling locations on the coast in coastal water of the western part of the Municipality of Rijeka.