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Surveillance of human enteric viruses in coastal waters using concentration with methacrylate monolithic supports prior to detection by RT-qPCR

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ABSTRACT

This is the first surveillance study using methacrylate monolithic supports to concentrate environmental coastal water samples, prior to molecular target detection by RT-qPCR. Rotaviruses (RoV) and Noroviruses (NoV) were monitored in a polluted area at the Bay of Koper (Gulf of Trieste, Northern Adriatic Sea) and at a nearby bathing area and mussel farm areas.

RoV and NoV are released into the Bay of Koper, with higher rates close to the discharge of the wastewater treatment plant, however, they can be detected at recreational and mussel farming areas. Our results showed that water bodies considered safe based on FC concentrations, can still have low, yet potentially infective, concentrations of human viruses.

1. Introduction

Coastal areas are important climate change hotspots that are subjected to an increase of population and consequently an increase in economic activities that generate several environmental concerns (Giorgi, 2006; de Sherbinin, 2014). Nowadays, about 50% of world population resides in cities located near the coast, and the trend is expected to continue in the next decades. Estimations predict an increase in surface water temperatures, sea level rise and an intensification of inundation and flooding events (Brown et al., 2013; Small and Nicholls, 2003; WHO, 2015).

Pollution of coastal waters is a major global problem, since these areas are often recipients of waste waters and are used for recreation and aquaculture (Fiksdal et al., 1994; Goh et al., 2017). Contaminants that lead to pollution of coastal environments include feces and urine from humans, domesticated and wild animals (Bradshaw et al., 2016; Field and Samadpour, 2007). Pathogens discharged from wastewaters pose a health risk to everyone exposed to the polluted waters, mainly to recreational users (by swimming or participating in other water-related activities, such as surfing, snorkeling, sailboarding, among others) and consumers of harvested food from the contaminated area. The number of publications related to water quality and water-related diseases increased in the last decade and gastroenteritis is still a major health risk from exposure to contaminated waters (Sweileh et al., 2016).

Microbial quality of waters has been traditionally assessed by monitoring faecal indicator bacteria, such as *Escherichia coli*, intestinal *enterococci* and faecal coliform bacteria (FC) (Liang et al., 2015). They were selected as environmental indicators of human pathogens because they are abundant in surface waters, easy to culture under controlled conditions and widely available in the intestinal flora of humans and other warm-blooded animals (Bartram, 2001; Bradshaw et al., 2016; George et al., 2002). However, several studies have shown that the reliance on monitoring faecal coliform bacteria alone as indicators of faecal pollution is insufficient to protect human health (Bartram, 2001; Bradshaw et al., 2016; Gerba et al., 1979; Liang et al., 2015). One of the main limitations of coliform bacteria, as indicators of faecal pollution, is their poor correlation with the presence of non-bacterial pathogens, in particular with the presence of human viruses (Cook, 2013; Eslamian, 2016; Payment and Locas, 2011; Petterson et al., 2001). Viruses persist in marine environments for long periods (Suttle, 2005), which can lead to higher risk of human exposure (Updyke et al., 2015). Not surprisingly, human viruses are believed to cause the majority of waterborne diseases worldwide (Griffin et al., 2001, 2003).

A group of viruses that have significant impact on public health are enteric viruses (Griffin et al., 2003; Updyke et al., 2015). These pathogens are associated with a variety of human diseases, from ocular

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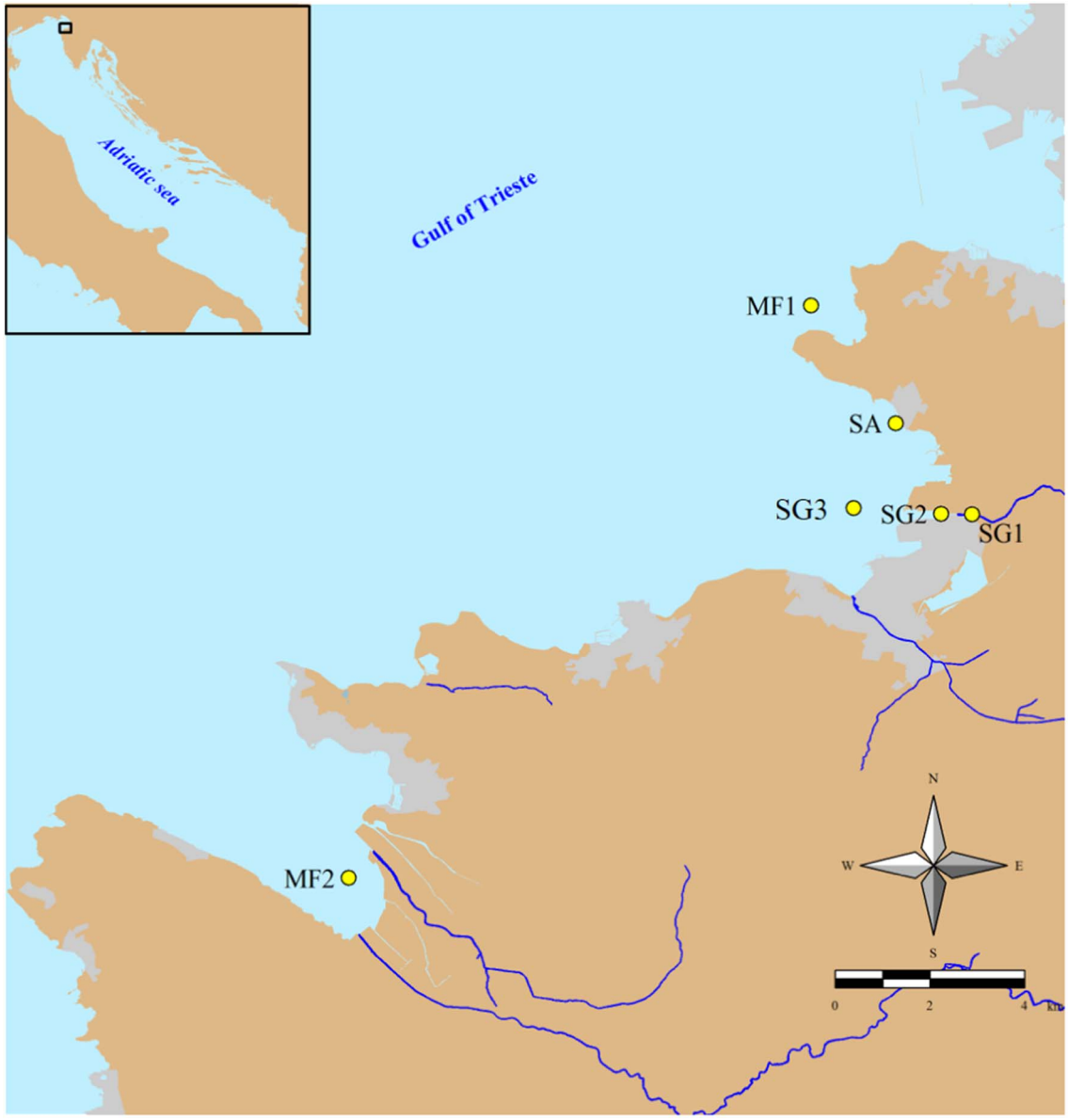

Fig. 1. Sampling locations at the inner part of the Bay of Koper and Bay of Piran (Gulf of Trieste, northern Adriatic Sea). Salinity/pollution gradient: Outlet of Koper WWTP located in river Rižana mouth (SG1), Rižana estuary (SG2), located at 700 m from the WWTP discharge and middle of the Bay of Koper (SG3). MF1 and MF2 are two locations used for mussel farming and SA1 is in a beach used for swimming and other recreational activities.

and respiratory infections to gastroenteritis, hepatitis, myocarditis and aseptic meningitis (Gerba et al., 1996). Infections by enteric viruses cause >2 million deaths each year (WHO, 2015), mainly in developing countries (Lin and Ganesh, 2013; Rezaeinejad et al., 2014; WHO, 2015). Rotavirus (RoV) and norovirus (NoV) are among the most commonly present enteric viruses in polluted coastal waters and the main agents of viral gastroenteritis worldwide (Bishop, 2009; Girones et al., 2010; Lin and Ganesh, 2013; Nwachcuku and Gerba, 2004; Robilotti et al., 2015).

Due to the low numbers of human viruses in natural waters and their low infection dose (as few as 10 particles), an efficient concentration step is critical for an effective detection (Balasubramanian et al., 2016; Bosch et al., 2005; Bosch, 2007; Gentry-Shields et al., 2013; Haas et al., 1993). Methods that use adsorption elution principle with electropositive or electronegative filters, sedimentation by flocculation, ultrafiltration and ultracentrifugation are some of the most widely used concentration methods for enteric viruses in environmental waters (Calgua et al., 2008; Fong and Lipp, 2005). However, these methods have poor recovery rates and slow processing times (Ikner et al., 2012). To overcome these limitations, in a recent study, CIM C4 hydrophobic interaction methacrylate monolithic columns, were successfully applied to concentrate RoV and NoV from water samples with different salinities, prior to molecular target detection with RT-qPCR. The procedure exhibited good recovery rates when compared with traditional methods, and allows fast concentration of enteric viruses in one step. The recovery rate of rotaviruses was 92% with a concentration factor of 307-fold. For noroviruses the recovery rate was 13% with a concentration factor of 43-fold (Balasubramanian et al., 2016).

In the current study, the presence of RoV and NoV genogroup II (from now on written as RoV and NoV, respectively) were monitored using a recently described CIM C4 concentration method, in combination with one-step RT-qPCR detection, to survey coastal water quality in the Gulf of Trieste (northern Adriatic Sea). During a period of one year, RoV and NoV were seasonally monitored, along a salinity/pollution gradient at the inner part of the Bay of Koper and at areas used for mussel farming and recreation. Possible correlations between the presence of RoV and NoV with the concentrations of FC and physico-chemical parameters in coastal water samples were concurrently