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Distribution of *Escherichia coli* in a coastal lagoon (Venice, Italy): Temporal patterns, genetic diversity and the role of tidal forcing

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ABSTRACT

Despite its worldwide importance as fecal indicator in aquatic systems, little is known about the diversity of *Escherichia coli* in the environment and the factors driving its spatial distribution. The city of Venice (Italy), lying at the forefront of a large European lagoon, is an ideal site to study the mechanisms driving the fate of fecal bacteria, due to the huge fluxes of tourists, the city's unique architecture (causing poor efficiency of sewages treatment), and the long branching network of canals crossing the city. We summarize the results of a multi-year investigation to study the temporal dynamics of *E. coli* around the city, describe the population structure (by assigning isolates to their phylogenetic group) and the genotypic diversity, and explore the role of environmental factors in determining its variability. *E. coli* abundance in water was highly variable, ranging from being undetectable up to 10^4 Colony Forming Units (CFU) per 100 ml. Abundance did not display significant relationships with the water physico-chemical variables. The analysis of the population structure showed the presence of all known phylogroups, including extra-intestinal and potentially pathogenic ones. The genotypic diversity was very high, as likely consequence of the heterogeneous input of fecal bacteria from the city, and showed site-specific patterns. Intensive sampling during the tidal fluctuations highlighted the prominent role of tides, rather than environmental variables, as source of spatial variation, with a more evident influence in water than sediments. These results, the first providing information on the genetic properties, spatial heterogeneity and influence of tides on *E. coli* populations around Venice, have implications to manage the fecal pollution, and the associated waterborne disease risks, in coastal cities lying in front of lagoons and semi-enclosed basins.

1. Introduction

Escherichia coli is used worldwide as fecal indicator species to assess the water quality in aquatic environments (Alm et al., 2006). Phylogenetic analyses suggest that *E. coli* can be divided into seven major groups (A, B1, B2, C, D, E and F) and five criptic clades (Clermont et al., 2013). The cryptic clades include clade I, representing the most closely related lineage of *E. coli*, and the clades II to V in the *Escherichia* genus, genetically but not phenotypically distinct from *E. coli* (Luo et al., 2011; Vignaroli et al., 2015). Strains belonging to the different phylogroups and clades occupy separate ecological niches, and possess diverse physiological properties and ability to cause infections (Gordon et al., 2008; Clermont et al., 2013). Human ExPEC (Extraintestinal Pathogenic *E. coli*) associated with extraintestinal infections typically belong to phylogroups B2 and D (Walk et al., 2007), whereas many vertebrate commensal strains belong to A and B1 phylogroups. A and B1 strains are more frequently isolated in water and sediment (Walk et al., 2007; Luna et al., 2010; Vignaroli et al., 2012), suggesting potential adaptation to certain environmental stresses in non-host environments (Berthe et al., 2013). A recent study in a river basin showed that phylogenetic groups are seasonally affected by water temperature, with higher frequency of phylogroups A and B1 during low and high temperature seasons, respectively (Jang et al., 2014). Despite this growing body of information, there is currently scarce knowledge on the frequency and spatio-temporal patterns of *E. coli* phylogroups and cryptic clades in the aquatic environment.

A number of studies have investigated how *E. coli* behaves once released into the aquatic environment. Several studies addressed the survival ability of enteric bacteria in marine waters (Byrd and Colwell, 1993; Alkan et al., 1995). Some of them reported fast decay rates (Martinez et al., 1989), believed to be the consequence of the high salinity, which could influence the culturability and

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mortality rates (Menon et al., 2003). More recently, a number of evidences demonstrated that *E. coli* can persist for prolonged periods of time in secondary habitats, such as soil, sediment, water and the surface of macroalgae (Byappanahalli et al., 2003; Anderson et al., 2005; Ferguson and Signoretto, 2011). The ability to survive outside the intestine, and the discovery of environmentally-adapted populations, poses questions about the usefulness of this bacterium as reliable indicator (Luo et al., 2011). The survival and distribution of *E. coli* in aquatic systems depends upon a complex interplay of abiotic and biotic factors. Abiotic factors include temperature, salinity, nutrient concentration, pH and UV radiation (Barcina et al., 1997; Noble et al., 2004; Whitman et al., 2004), while biotic factors are grazing by protozoa, competition for resources with other microbes and viral infection (Menon et al., 2003; Feng et al., 2010; Wanjugi and Harwood, 2013). Recent studies pointed out the important role of rainfall in influencing presence and distribution, by conveying microbial pollutants due to terrestrial run-off and other mechanisms (Sidhu et al., 2012; Heaney et al., 2014).

Relatively less studies have been carried out on the influence of hydrodynamics forcing on fecal bacteria distribution and spatial variability. Enns et al. (2012) demonstrated that tidal action increased spatio-temporal variability at recreational beaches, by causing resuspension of enterococci from shoreline sediments, while Ge et al. (2012) documented transport of *E. coli* due to waves in nearshore waters. Desmarais et al. (2002) studied bacterial survival in tidally influenced river sediments, demonstrating that cycle of wetting and drying due to tides allowed *E. coli* re-growth. In streams, turbulent flow conditions remobilize FIB from sediments leading to decrease in downstream water quality (Yakirevich et al., 2013). Tides and waves can influence fecal bacteria distribution by horizontal advection, dilution and diffusion processes (Rippy et al., 2013). However, the role of physical processes, especially in urbanized lagoons and semi-closed systems characterized by tidal water exchanges, has been investigated in only few studies (Sanders et al., 2005; Schernewski et al., 2012; Dorsey et al., 2013).

The city of Venice (Italy) is among the best sites to study the fate and persistence of fecal bacteria in aquatic systems, and the influence of environmental drivers. The city is located at the forefront of the one of the largest Mediterranean lagoons, which is a semi-closed environment connected to the Adriatic Sea by three opening (inlets), through which the tidal semidiurnal regime ensures alternation of high and low tides every 6 h (Umgiesser et al., 2004) and guarantees large daily water exchanges with the open sea (Cucco and Umgiesser, 2006). The tide excursion can reach 1 m during spring tides (Ferrarin et al., 2010). The hydrodynamic regime around the city, and in its canals, is largely governed by the tide and wind, and the cleaning capacity of the basin is influenced by the characteristic of the tidal exchange with the adjacent open sea (Cucco and Umgiesser, 2006). Due to its history and unique building architecture, the historical center has never been provided with a main, efficient sewage treatment system (Coraci et al., 2007; Zaggia et al., 2007; Sfriso and Facca, 2013). Consequently, only partially treated effluents, deriving from a large number of domestic and commercial inputs, are discharged into the city canals (Coraci et al., 2007), determining fecal contamination and potential scenarios for the spread of waterborne diseases among residents and tourists (Lagi et al., 2013). However, only few studies have addressed the fecal pollution in the lagoon surrounding the city. Rose et al. (2006) enumerated the hepatitis A virus (HAV) and enterovirus, reporting a large fraction of positive water samples. Sherwin et al. (1993) investigated the accumulation of sewage in sediments and mussels by use of chemical markers, reporting highest values in the interior canals of the city. No one study has characterized the temporal patterns and genetic properties of *E. coli* populations around the city, nor have identified environmental factors which may drive the fate of fecal bacteria over different temporal and spatial scales. This lack of knowledge hampers to develop proper and scientifically-sound management policies to improve fecal pollution around the city.

We report the results of a multi-year investigation carried out to study the temporal dynamics of *E. coli* around Venice, describing the population structure and the presence of potentially pathogenic strains, characterizing the genotypic diversity, and exploring the role of environmental factors in influencing the spatial and temporal distribution around the city and particularly during the tidal cycle. This is the first study performed so far to describe the temporal variability and genotypic diversity of *E. coli* in this city, and to explore the role of environmental factors.

2. Materials and methods

2.1. Site description and sampling activities

The city of Venice (Italy) is built inside a coastal lagoon on more than a hundred islands, which are bordered by a complex network of almost 160 interlinked canals. The canals have mean depth ranging from 1 to 5 m, and reach the cumulative length of 40 km (Coraci et al., 2007). A semidiurnal tide regularly ensures large water exchanges from the nearby Adriatic Sea to the lagoon, and viceversa. The current speed in the city canals can vary from few cm s^{-1} to 50 cm s^{-1} and the salinity, having mean value of about 30 psu, is affected by tides and meteorological conditions (Zaggia et al., 2007). This study was conducted over a period of two and a half years at two sampling sites (Arsenale, 45°26′2.24″N, 12°21′0.32″E; Sette Martiri, 45°25′48.77″N, 12°21′16.56″E) located in two canals of the historical center of Venice, Italy (Fig. 1). The sampling activities were carried out, during 13 events, from 27th June 2012 to 18th November 2014. The sampling events were chosen in order to cover the widest variability in the environmental conditions in the area, by sampling in the four seasons and under the full range of temperature values. Sampling at the Arsenale site was not performed in two instances ($n = 2$) due to inability to operate. The sites were selected to represent different levels of putative microbiological contamination. Samples of surface water were collected in sterile containers (capacity 1 L) and transported immediately to the laboratory at *in situ* temperature in the dark. Samples were analyzed within 2 h from the sampling. For analyses of temporal patterns in abundance, sampling activities were carried out always during the peak of low tide, when water was not visibly moving, to minimize the variance induced by tides. Additional samples were collected in selected events during high tide conditions, to allow colony isolation and subsequent genetic analyses of the isolates. The total number of samples analyzed during the entire study was 66 (for water) and 36 (for sediment), an estimate which includes the sampling during the tidal cycle described below.

2.2. Sampling during the tidal cycle

We performed additional, intensive sampling activities to explore the effects of tides on the *E. coli* patterns in water and sediments. To do this, we collected samples at the same sites every 2 h over a 48-h period, according to the alternating semidiurnal tidal cycle which exhibits alternation of high and low tide every 6 h, and we included the ebb and flow phases. These activities were performed on 18th and 19th November 2014 (for water sampling), and 20th and 21st January 2015 (for sediment sampling) in separate events due to logistic needs. This sampling was repeated, at the Sette Martiri site, on 3rd and 4th August 2015. During this event,