Marine Pollution Bulletin 109 (2016) 163–170

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Detection and risk assessment of diarrheagenic *E. coli* in recreational beaches of Brazil

Vanessa F.V. Rodrigues [a,b], Irma N.G. Rivera [a], Keah-Ying Lim [b], Sunny C. Jiang [b,*]

[a] Biomedical Science Institute, Microbiology Department, University of São Paulo, Estado de São Paulo, SP 05508-000, Brazil
[b] Department of Civil and Environmental Engineering, University of California, Irvine, CA 92697-2175, USA

ARTICLE INFO

Article history:
Received 3 January 2016
Received in revised form 25 May 2016
Accepted 2 June 2016
Available online 11 June 2016

Keywords:
Diarrheagenic *E. coli*
Recreational health
Quantitative microbial risk assessment
Brazilian beaches

ABSTRACT

Marine beaches are important recreational and economic resources in Brazil, but the beaches' water quality is negatively impacted by the discharge of domestic sewage effluent. The occurrence of diarrheagenic *Escherichia coli* among the *E. coli* isolated from three Brazilian marine beaches was investigated. Multiplex and single step PCR were used to screen 99 *E. coli* isolates for ten target toxin genes. Six toxin genes, *stx*1, *eae*, *estp*, *esth*, *ast*A, and *bfp*A, were identified in 1% to 35% of the isolates. A quantitative microbial risk assessment (QMRA) of human exposure to diarrheagenic *E. coli* during marine recreation was carried out. The results indicated that the diarrheagenic *E. coli* risk is well below the U.S. EPA's recommended daily recreational risk benchmark. However, the overall recreational health risk due to all pathogens in the water could be much higher and exceeded the U.S. EPA's benchmark.

1. Introduction

Marine recreational waters are often affected by both point source pollution from sewage outfalls and industrial discharges, and non-point source pollution from diffused sewage discharges, stormwater runoff, re-suspension of contaminated sediments, human bather's shedding, and animal fecal inputs (Hamilton et al., 2010; King, 2013; Rivera et al., 2008; Rivera and Martins, 1996; U.S. EPA). In Brazil, marine beaches are important recreational and economic resources, but the beaches' water quality is negatively impacted by agriculture discharges and effluent of partially treated or untreated domestic sewage. According to Trata Brazil Institute, only 62.3% of human sewage generated in Brazil in 2013 was treated (Instituto Trata Brasil, 2014). The scenario is the worst in coastal regions — in Baixada Santista region (São Paulo state), only 60% of human sewage was collected by sewer systems with the remaining 40% released directly to the environment as diffused source of discharge. Of the 60% sewage collected through sewer systems, only 10% was fully treated using conventional wastewater treatment process, whereas the remaining was partially treated through gross removal of solids and chlorination (Companhia de Tecnologia de Saneamento Ambiental (CETESB), 2005). Discharge of untreated or partially treated human sewage to beaches is usually elevated during peak recreational season due to the influx of tourist population that generates excess sewage, which overwhelms the sewer collection system resulting in direct discharge of raw sewage to the beaches as diffused sources. The excess sewage collected through the sewer system is only partially treated because of the limited treatment capacity of the local wastewater treatment plants (Companhia de Tecnologia de Saneamento Ambiental (CETESB), 2005).

The untreated or improperly treated sewage contains toxic chemicals, pathogens, and high concentrations of organic and inorganic nutrients, which can have adverse health effects on recreational bathers through inhalation of aerosols, accidental ingestion of water, and wound infection (Abdelzaher et al., 2010; Abessa et al., 2005; Braga et al., 2000; Westrell et al., 2004; World Health Organization, 1999). Gastrointestinal illnesses (GI) are the most commonly reported and documented recreational water diseases, which are caused by enteric viruses, bacteria, and protozoa that are commonly associated with human sewage (Centers for Disease Control and Prevention; Fleisher et al., 2010; Turbow et al., 2008). The high nutrient concentration associated with sewage discharge can also trigger the proliferation of native marine opportunistic pathogens such as *Vibrio spp.*, which causes both GI and wound infections (Dickinson et al., 2013). Eyes, ear, and skin infections among recreational bathers are also well documented, but their infective agents have not been well identified and quantified in recreational waters impacted by both point source (Haile et al., 1999; Wade et al., 2010) and non-point source pollution (Colford et al., 2012; Colford et al., 2007; Fleisher et al., 2010).

In order to protect the health of beachgoers in Brazil, the environmental sanitation agency of São Paulo State performs routine monitoring of the microbial water quality of beaches for fecal indicator bacteria (thereafter abbreviated as "FIB" that include enterococci, thermotolerant coliform, and *Escherichia coli*). The current water quality criteria for recreational waters are based in Conama Resolution no. 274/2000, which

* Corresponding author at: 844E Engineering Tower, University of California, Irvine, CA 92617, USA.
E-mail address: sjiang@uci.edu (S.C. Jiang).

http://dx.doi.org/10.1016/j.marpolbul.2016.06.007
0025-326X/

classifies beaches into two categories of use: "Proper" or "Improper". For a beach to be considered "Proper" for safe recreational activities, the water samples must contain less than 100 enterococci, 1000 thermotolerant coliform, and 800 *E. coli* per 100 mL in 80% of the five samples collected from the same sampling point for five consecutive weeks (Conselho nacional do meio ambiente (CONAMA), 2008). However, it is unclear whether these water quality criteria are protective of the health of bathers.

The level of FIB in recreational water is traditionally used to indicate human sewage contamination of the water; a higher FIB level generally corresponds to a higher recreational health risk. This is, however, a gross generalization because a specific type of FIB (e.g. enterococci, thermotolerant coliform, or *E. coli*) may be more suitable to indicate the recreational risk in a specific type of water (e.g. fresh or marine). Putting this into context, the U.S. EPA set different thresholds of FIB level based on the type of recreational waters; *E. coli* are only used for indicating the health risk of recreational freshwater whereas enterococci are used for both marine and freshwater. Furthermore, these thresholds are established based on a large-scale epidemiological study in the U.S.—the National Epidemiological and Environmental Assessment of Recreational (NEEAR) water studies, wherein the threshold level of each FIB corresponds to a range of NEEAR-gastrointestinal illness (NGI) rates of 32 or 36 illness cases per 1000 bathers(U.S. EPA, 2012). These illness rates also serve as a measure for the level of protection provided to assure the safety of people recreating in the waters. In comparison, there is no clear rationale for the selection of the FIB standard set by the Conama Resolution no. 274/2000. For example, the threshold level of 800 CFU/100 mL *E. coli* concentration set in Conama Resolution no. 274/2000 (regardless of the type of recreational water) is significantly higher than the 126 CFU/ 100 mL *E. coli* threshold (geometric mean of samples collected over thirty days and no sample exceeds 410 CFU/100 mL) set by U.S. EPA for sewage-impacted freshwater. Again, the 100 CFU/100 mL enterococci threshold of Conama Resolution is also more than twice as high as the U.S. EPA threshold of 35 CFU/100 mL. Ultimately, this leads to the important question regarding the adequacy of Conama Resolution no. 274/2000's water quality criteria for recreational health protection due to the unknown health risk that is associated with these criteria.

In the absence of a costly epidemiological study, recreational health risks of sewage-impacted beaches can be estimated through understanding the occurrence of pathogen, the level of human exposure, and the pathogen dose-response relationship, following the Quantitative Microbial Risk Assessment (QMRA) framework. Towards assessing the recreational health risks of Brazilian beaches, we investigated the fraction of diarrheagenic *E. coli* among the total *E. coli* population isolated from water samples of three Brazilian coastal regions impacted by different levels of human sewage contamination in order to understand the recreational risk specific to this group of pathogen.

Diarrheagenic *E. coli* is a well-known etiological agent of human diarrheal diseases (Kaper and Hacker, 1999; Nataro and Kaper, 1998; Russo and Johnson, 2000), which represents a small portion of the diverse serotypes and strains of *E. coli* found in human and other warm-blooded animal guts. Diarrheagenic *E. coli* are grouped into six categories: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohaemorrhagic (EHEC), enteroagregative (EAEC), and *E. coli* with diffuse adherence (DAEC) (Nataro and Kaper, 1998). The pathogenicity of *E. coli* in the diarrheagenic categories is usually assessed through searching for virulence-associated genes, such as *estp*, *esth* and *elt* in ETEC; *bfpA* and *eae* in EPEC; *stx1*, *stx2* and *eae* in EHEC; *invE* in EIEC, and *aggR* and *astA* in EAEC (Fujioka et al., 2013). In spite of the significant burden of diarrheagenic *E. coli* to human health, its presence/abundance has not been investigated extensively in recreational beach waters due to the technical challenges for detecting toxin genes. Here we report the detection of EPEC, ETEC, EIEC, EHEC and EAEC in three coastal regions in the São Paulo state using PCR methods. A QMRA model was then used to estimate the health risks associated with exposure to diarrheagenic *E. coli* during recreational activities at those beaches. Finally, the total recreational illness risk is then inferred through analyzing the contribution of diarrheagenic *E. coli* to the total risk of recreating in sewage-impacted water.

The role of *E. coli* as an indicator of marine recreational water health risk has been investigated in several previous studies. For example, Bonamano et al. (2015) modeled the dispersion of viable and total *E. coli* cells in an artificial semi-enclosed bathing area of an Italian beach. The study showed that a high concentration of *E. coli* was found during the summer tourist season, which was attributed to the overflow of sewage treatment plants. However, the limitations of *E. coli* as an indicator for marine recreational beach safety is also well known, which is further highlighted by the U.S. EPA recommendation of using enterococcus as the indicator due the sensitivity of *E. coli* to osmotic stress (U.S. EPA, 2012). A primary goal of this study is to improve the current understanding of *E. coli* in marine waters through evaluation of the contribution of diarrheagenic *E. coli* to recreational health risks. The results of this study provide scientific ground that can be used for supporting the further development of the recreational water quality criteria set by the Conama Resolution no. 274/2000.

2. Materials and methods

2.1. Study sites

The water samples were collected between 2005 and 2007 from three coastal regions in the São Paulo State, namely, Baixada Santista (BS), São Sebastião Channel (SS), and Ubatuba (U) (Fig. 1). Each region was impacted by different levels of anthropogenic pollution from both sewage, industrial outfalls, and diffused discharge of untreated raw sewage and agriculture runoff (Burbano-Rosero et al., 2011). Sewage generated in these coastal regions is either discharged into the coastal environment directly untreated, or is collected by sewer systems and sent to treatment plants. Of the sewage collected, a portion is treated using conventional biological wastewater treatment process and discharged directly to surface waters; the remaining portion is partially treated through the removal of gross solids followed by chlorination. This partially treated sewage is released into the ocean through submarine outfalls that are situated 8–24 m below the water surface (Companhia de Tecnologia de Saneamento Ambiental, (CETESB)). Little is known of the impact of these minimally treated sewage outfalls to recreational beaches. Neither the transport pattern of sewage plumes nor the microbiological quality of the surrounding waters was monitored. These are in dramatic contrast to the highly regulated submarine outfalls in the U.S.

Santos/São Vicente Bay, in Baixada Santista, is the entry point of two main urban waterways to the Atlantic Ocean. Santos and São Vicente Channels collect diffused urban runoff and sewage effluents from two of the most populated coastal cities in São Paulo State — São Vicente and Santos with a combined population of ~743,000 inhabitants in 2005 (Companhia de Tecnologia de Saneamento Ambiental (CETESB), 2014). Baixada Santista coast is also the home of four submarine sewage outfalls as shown in Fig. 1. Samples for this study were collected offshore from the mouth of São Vicente (BS1) channel, a preserved area (BS2), and near the mouth of Santos channel (BS3).

São Sebastião channel is 25 km in length and flows between the city of São Sebastião and the Ilhabela island. The two cities were reported to have ~73,000 and ~25,000 inhabitants in 2005, respectively. The region is famous for its beaches, which makes it a popular tourist destination, especially for people from the state of São Paulo. Four outfalls were identified in the region — three submarine outfalls releasing partially treated sewage, and one releasing industrial sewage (Fig. 1). Samples were collected offshore near the Marine Biology Center – CEBIMar – USP in front Praia do Segredo (SS1) and near the Barequeçaba beach (SS2).

The last region included in this study, Ubatuba, is a small tourist city with ~79,000 inhabitants. Approximately 33% of the sewage generated in the region is collected and treated by sewage treatment plants. The region is also not known to have any submarine outfalls. Samples