Enterococci as Fecal Indicator in a Tropical Beach: A Case Study

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ABSTRACT

Fecal enterococci are generally not virulent; however, multidrug-resistant strains have emerged as leading causes of hospital-acquired infections. Thus, periodic enterococci monitoring should be included in highly populated cities to control the dissemination of multidrug-resistant strains to the marine environment. This study aimed to quantify enterococci bacteria from water and intertidal sediment samples in a beach located near Rio de Janeiro touristic spots. We also intended to accomplish if enterococci should be included in touristic beaches sanitary monitoring. Toward this approach, we monitored from August to December 2014 fecal indicator bacteria (FIB) at a beach close to some touristic spots through multiple tube method. Although FIB quantification was within sanitary standards of Brazilian legislation, high enterococci densities (=30 MNP.100 mL^{-1}) were detected in the water collected in August. Thus, enterococci monitoring should be included in touristic beaches to avoid the risk of multidrug-resistant bacteria dissemination among swimmers and beachgoers.

Keywords: Bacteria; Fecal Indicator; Pollution Monitoring; Public Health.

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About 50% of the global population lives within a few hundred kilometers from the sea (Shuval 2003). Cities with more than 10 million inhabitants grew along coastal areas without proper environmental policies. Thus these ecosystems suffer from industrial and socioeconomic development pressures (PBMC 2014). Urban beaches located in highly populated cities can receive urban pollution from untreated wastewaters, marine outfalls, polluted rivers, industrial discharges, surface runoff or from visitors like humans and animals (Moura et al. 2009; Shuval 2003). These sources can promote pathogens proliferation that may cause outbreaks and economic damage to public health (Dwight et al. 2005; Shuval 2003).

Contaminated water is the primary transmission source of infectious diseases in developing countries, thus water and sediment quality survey should be continuously checked to reduce risk of primary human contact with high levels of fecal pollution (Boehm and Sassoubre 2014; Boehm and Soller 2012; Cabelli 1983; Sabino et al. 2014; Wade et al. 2006; Wiedenmann et al. 2006). In the United States, the fecal pollution standard for recreation bathing waters was initially set using concentrations of total coliforms. These guidelines were based on a US Public Health Service study conducted on Lake Michigan in Chicago in 1948 (Boehm and Sassoubre 2014). After those studies, researchers determined that enterococci concentrations measured in recreational marine waters polluted by treated wastewater were strongly correlated to the number of swimmers becoming sick with gastrointestinal illness (Boehm and Soller 2012; Cabelli 1983). The epidemiological studies in the 2000s supported the association of enterococcal concentrations and swimmer health in recreational freshwaters and marine waters (Boehm and Sassoubre 2014; Wade et al. 2006; Wiedenmann et al. 2006). Given this evidence, the United States (US), Europe Union (EU), and the World Health Organization (WHO) recommend the adoption of enterococci as an indicator of recreational water quality and risk of swimmer illness (Boehm and Sassoubre 2014; WHO 2003).

The enterococci (ENT) is adopted as indicators of fecal contamination in recreational waters worldwide(Boehm and Sassoubre 2014). However, recreational water quality standards vary by country. The policy for each country requires the use of a specific enterococcal enumeration method generally relating bacterial counts to a geometric mean and a statistical threshold value (WHO 1999). In all cases, the technique is culture-based and involves the use of selective and differential media in solid or liquid form (Edge and Boehm 2011). A new standard method for measuring enterococci in water has been developed based on quantitative polymerase chain reaction (qPCR) in conjunction with a hydrolysis probe (EPA 2012). However, inter- and intra-laboratory variation of this method is still being debated (Boehm and Sassoubre 2014; Shanks et al. 2012; Whitman et al. 2010). Besides its weak reproducibility when compared to culture-based methods, it is important to note that qPCR methodology is still distant from developing countries financial and technical reality. Thus, the International Organization for Standardization (IOS) methods based on multiple-tube technique are currently relevant worldwide (APHA 2005; IOS 2000, 1998).

Bringing this discussion to South America, in Chile, the concentration of total coliform bacteria (TC), thermotolerant coliforms (TEC) and fecal streptococci (FS) are quantified through the fermentation technique in multiple tubes as described in APHA (2005). With this method, indicator bacteria may be easily detected and quantified by economical and straightforward methods (Rivera et al. 2010). However, the Chilean recommendation for monitoring indicator bacteria corresponds only to the coliform group formed by bacillary, facultative anaerobes, Gram-negative, non-spore-forming, and lactose fermenters bacteria (Chile 2002, 2004).

Rio de Janeiro and São Paulo are the two largest metropolises by population in Brazil (IBGE 2010). Thus, regulation recommends constant monitoring of coastal recreative water. Although São Paulo state follows universal instruction and monitors enterococci densities all through the coast (CETESB 2018), the state of Rio de Janeiro restricts enterococci quantification to a few beaches: Ilha do Governador, Ramos, Paquetá, and Sepetiba (INEA 2018). Those beaches receive clandestine sewage discharges and are distant from touristic spots (Fistarol et al. 2015). However, considering all touristic beaches located in Rio de Janeiro city, the local environment agency (INEA) does not monitor enterococci density. Only total coliforms (TC) and thermotolerant coliforms (TEC) are included on its weekly beach report (INEA 2018). The same scenario can be observed in other highly populated Brazilian cities including Salvador and Fortaleza, where *E. coli* and thermotolerant coliforms are, respectively, the fecal bacteria indicator quantified in marine waters instead of enterococci (INEA 2018; SEMACE 2018). Although fecal enterococci are generally not virulent, multidrug-resistant strains have emerged as leading causes of hospital-acquired infections (Boehm and Sassoubre 2014). Thus, periodic monitoring should also be included in these cities to control multidrug-resistant strains dissemination to the marine environment.

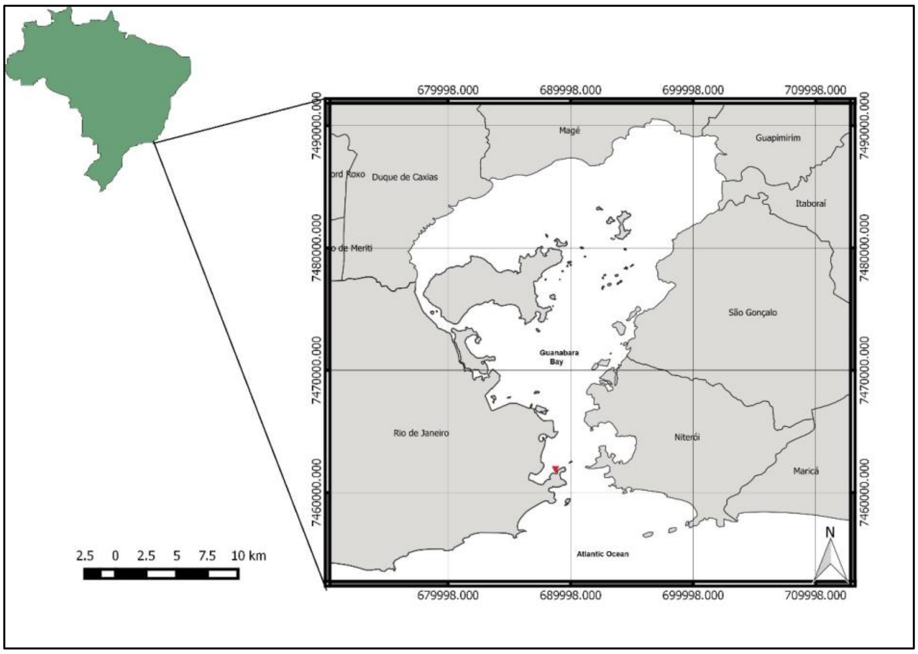
Therefore, this case study aimed to quantify enterococci bacteria from water and intertidal sediment samples in a beach located near touristic spots and bathed by Guanabara Bay waters, Rio de Janeiro, Brazil. We also aimed to accomplish if enterococci should be included in Rio de Janeiro touristic beaches sanitary monitoring program. Toward this approach, we chose the small São João beach for fecal indicator bacteria (FIB) monitoring from August to December 2014 through multiple tube method.

MATERIALS AND METHODS

CHARACTERIZATION OF THE STUDY AREA

Rio de Janeiro is a tropical coastal city that attracted 6.6 million tourists in 2016 due to the Olympic Games. Nevertheless, Rio de Janeiro city is densely populated and discharges much of its wastewaters by marine outfalls without prior treatment. Along the northeast coast of Rio de Janeiro city (Fistarol et al. 2015), there is the second largest bay on the coast of Brazil, the Guanabara Bay (Figure 01). Although Guanabara bay houses a sewage treatment plant, much of its contamination come from improper sewage disposal from its surroundings (Fistarol et al. 2015). The western and northwestern parts of this Bay present poor water quality because it receives most of metropolitan Rio de Janeiro drainage (Ribeiro and Kjerfve 2002). When microbial pollutants discharge into shallow coastal waters, they are diluted or diffuse in the water column; however, they still represent a potential risk for public health (Kacar and Omuzbuken 2017).

Figure 01. Localization of São João beach (red triangle) in Guanabara Bay, Rio de Janeiro, Brazil.



Source: The Authors.

For this case study, we selected the São João beach, a sandy tropical beach of small length (140-meters), shallow waters (≤ 4 meters), low hydrodynamics and thin sand. Bathed by Guanabara Bay waters during outflow, it stands close to the touristic Sugarloaf Mountain. This beach is inside the São João Fortress in Urca, a neighborhood from the city of Rio de Janeiro, Brazil at coordinates 22°56'S; 43°9'W (Fig. 01). Although with limited access, the proximity to Guanabara Bay waters may contribute to its contamination, which makes it an attractive, spot to investigate enterococci quantification.

Considering the small length of São João beach, a total of three stations with approximately 50-meter far from the center of the beach were investigated in this study. Each station is representing a sampling replica.

SAMPLING AND LABORATORY ANALYSIS

Water (150 mL) and sandy sediment (50 g) samples from three intertidal zone stations were collected aseptically with sterilized material at ebb tide monthly from August to December 2014. Each station was interpreted as a beach replica. Collected samples were transported to the laboratory for analysis within four hours after sampling following APHA (2005).

Tide was consulted at Brazilian Navigation and Water Resources Directory website Diretoria de Hidrografia e Navegação – DHN (DHN 2014). Measurement of water abiotic variables (pH, salinity, and temperature in °C) was with HANNA Hi 98282.2 multiparameter meter. Precipitation volume (mm) in Urca station (Rio de Janeiro, Brazil) 96 hours before sampling was consulted at municipality database (Prefeitura do Rio de Janeiro 2014).

The fecal indicator bacteria (FIB) monitored in this study comprises enterococci (ENT) and total (TC) and thermotolerant (TEC) coliforms. Multiple-tube fermentation Technique (APHA 1992) quantified TC, TEC and ENT. Details of this method are available at APHA (1992). The same sample mass (sediment or water) was used in all analysis to ensure an appropriate comparison. Thus, sediment samples were weighted (50 g) and transferred to 100 mL of sterile dilution water (APHA 2005). The samples were agitated for 10 minutes for bacterial carriage to the liquid phase (Pinto et al. 2012; Wang et al. 2010). Hence, the inoculation of three dilutions (10^0, 10^{-1}, and 10^{-2}) and five replications for each station followed water guidelines of APHA (1992). For water samples, 100 mL of each sample were inoculated and incubated on test tubes following sediment procedures (APHA 1992). Positive results showed bacterial growth (turbidity) with gas formation.

ENT quantification was adapted from Monteiro (*unpublished data*) as follows. Dilutions of water and sediment samples were inoculated into 10 mL of azide dextrose broth (Acumedia, USA), incubated in a bacteriological stove for 48h at 35°C. For ENT confirmation, growth was spread through m-*enterococcus* agar medium (Acumedia, USA) and incubated at 35°C for 48h. Light or dark red colonies from each plate were spread on inclined agar heart brain infusion (BHI agar, Acumedia, USA) and incubated for 24h at 35°C. Colonies were Gram-stained, and catalase tested for ENT attestation. Positive colonies were spiked into 05 mL of BHI broth and 03 mL of BHI broth with 6.5% NaCl and incubated at 45°C for 48h and 35°C for 72h respectively. Turbidity on both tubes confirmed the presence of enterococci. All FIB values were expressed as the most probable number in 100 mL of a sample (MPN.100 mL^{-1}), including sediments. The sediment samples were suspended in sterile dilution water before analysis.

DATA ANALYSIS

Descriptive statistical analysis of data was conducted contrasting the values of FIB to the sampling dates, precipitation and environmental matrices (water or sediment) at R software 2.2.0 for Microsoft Windows operating system. The Bootstrap statistical model is a resampling method (Odeck 2009) applied in this study to determine the relative difference in the quantity of FIB in environmental matrices (water or sediment). The test was performed using a resampling of 1,000 bootstrap samples at R software.

RESULTS

The water and sediment collections were performed in three seasons: winter (August), spring (September and October) and summer (November and December). Table 01 shows water abiotic variables (salinity, pH, and temperature in °C). The average water temperature and pH were 22.32ºC and 8.13 respectively. Salinity average from September to November was representative of saline waters (34.70); however, in August and December salinity was characteristic of brackish water (≤ 29).

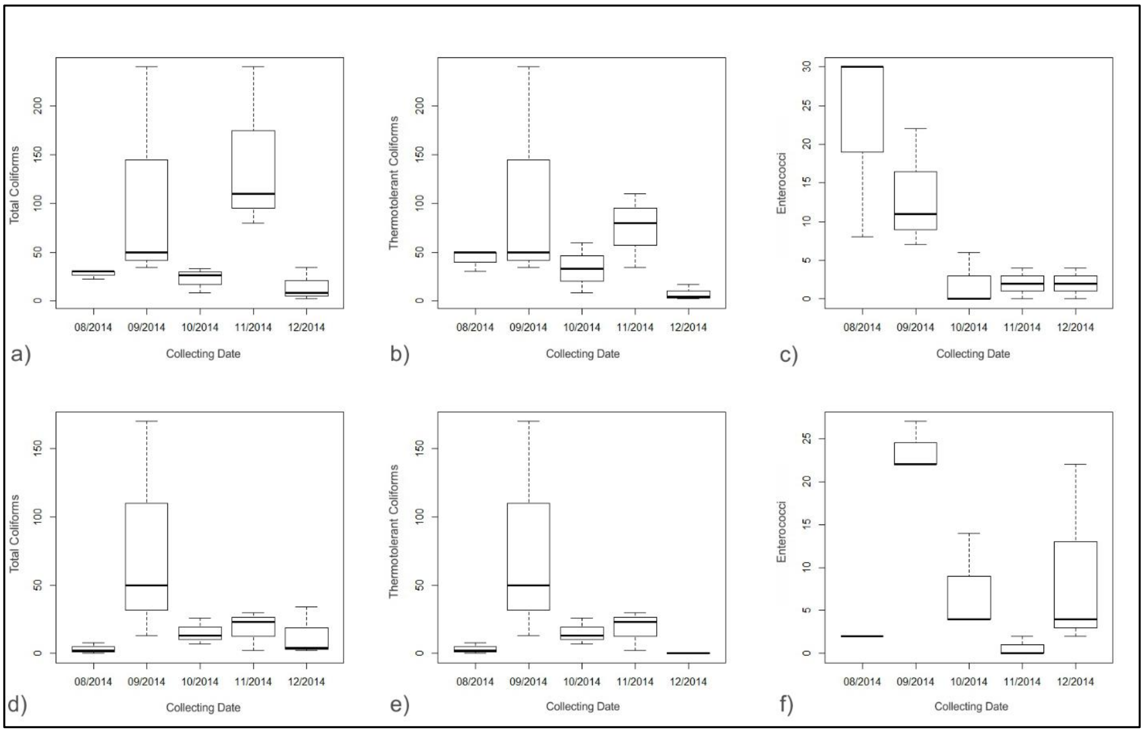
Table 01. Localization of São João beach (red triangle) in Guanabara Bay, Rio de Janeiro, Brazil.

VARIABLES		AUG/2014 WINTER		SEP/2014 SPRING		OCT/2014 SPRING		NOV/2014 SUMMER		DEC/2014 SUMMER	
		Median	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Abiotic variables (water)											
TEMP. (°C)		21.56	-	20.83	-	24.09	-	22.35	-	22.78	-
pH		8.43	-	7.81	-	8.21	-	8.34	-	7.87	-
SAL		27.76	-	34.20	-	34.78	-	35.21	-	29.07	-
Fecal indicator bactéria											
WATER (n=3)	TC	30	22-30	50	34-240	26	8-33	110	80-240	8	2-34
	TEC	50	30-50	50	34-240	33	8-60	80	80-110	4	2-17
	ENT	30	8-30	11	7-22	0	0-6	2	0-4	2	0-4
SEDIMENT (n=3)	TC	2	0-8	50	13-170	13	7-26	23	2-30	4	2-34
	TEC	2	0-8	50	13-170	13	7-26	23	2-30	0	0-0
	ENT	2	2-2	22	22-27	4	4-14	0	0-2	4	2-22

Source: The Authors.
Legend: TEMP - temperature; SAL – salinity; TC – Total coliforms; TEC – Thermotolerant coliforms; ENT – Enterococci.

Table 01 and Figure 02 present fecal indicator bacteria (FIB) densities in each sampling. ENT densities varied with time and source. In water, the highest density of ENT (30 MNP.100 mL^{-1}) was found in August and September (22 MNP.100 mL^{-1}). In sediments, the highest ENT values (27 MNP.100 mL^{-1}) were found in September and December (22 MNP.100 mL^{-1}). ENT densities found in sediment in September, October and December were higher (14 ≤ ENT ≥27 MNP.100 mL^{-1}) than those found in water samples (4 ≤ ENT ≥22).

Figure 02. Fecal indicator bacteria (FIB) quantified in water and sediment (n=3) according to the collecting dates in MPN.100mL^{-1}.



Source: The Authors.

a) Total coliforms (TC), b) Thermotolerant coliforms (TEC) and c) Enterococci (ENT) in water; d) Total coliforms (TC), e) Thermotolerant coliforms (TEC) and f) Enterococci (ENT) in sediment.

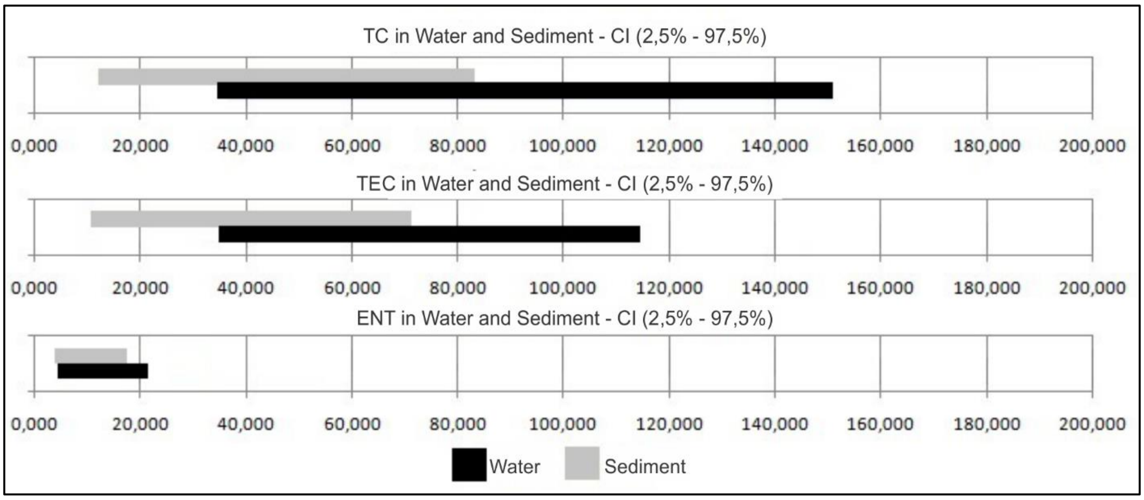
TC and TEC densities were higher than ENT in most of the time for both matrices samples (Table 01; Figure 02). In water, the highest TC and TEC numbers were found in September (240 MNP.100 mL^{-1}) and repeated in November for TC (240 MNP.100 mL^{-1}). In sediment, the highest TC and TEC numbers were also found in September (170 MNP.100 mL^{-1}). Despite September and November peaks, the number of all FIBs was sparse (≤ 60 MNP.100 mL^{-1}) in the other collections. However, according to Brazilian legislation, the ENT density found in the water was higher than the recommended for “excellent” water classification in August.

Nonetheless, the TC and TEC densities in water were higher than in the sediment (Table 01). The bootstrap confidence intervals for FIB densities in water and sediment overlap in the three groups (Figure 03), suggesting no difference in FIB quantity when comparing environmental matrices.

When water temperature was contrasted with FIB abundance, the highest temperature (24.09°C) was followed by the absence of enterococci in water (Table 01). In opposition, in August and

September, water temperature was between 20.83ºC and 21.56ºC; and ENT density was higher (Table 01). When FIB numbers (TC, TEC, and ENT) were confronted with accumulated precipitation, there was no significant correlation between these indicators. However, the highest FIB numbers detected in water and sediment (Figure 02) were obtained in September collection. TC densities presented another peak in November. Following municipality database (Prefeitura do Rio de Janeiro 2014) for Urca station, precipitation accumulated 96h before collections were reported in September (11.6 mm) and November (5.6 mm).

Figure 03. Representation of confidence intervals of the fecal indicator bacteria (FIB) number in water and sediment generated by Bootstrap resampling method based on 1,000 bootstrap samples.



Source: The Authors.

TC - Total coliforms; TEC - Thermotolerant coliforms; ENT - Enterococci. Unit: MPN.100mL^{-1}.

DISCUSSION

Contaminated water is the primary transmission source of infectious diseases, particularly in developing countries (Madigan et al. 2010). Several studies have linked exposure to contaminated recreational waters with the occurrence of various diseases, mainly gastrointestinal, respiratory and cutaneous (Boehm and Sassoubre 2014; Boehm and Soller 2012; Cabelli 1983; Pruss 1998; Shuval 2003; Wade et al. 2006; Wiedenmann et al. 2006; Yau et al. 2009). Besides public health risk, clean beaches attract visitants and move the local economy. Thus, water and sediment quality survey should be continuously checked to reduce the risk of primary human contact with high levels of fecal pollution (Boehm and Sassoubre 2014; Boehm and Soller 2012; Cabelli 1983; Wade et al. 2006; Wiedenmann et al. 2006). Therefore, this was the first study to quantify enterococci bacteria from water and intertidal sediment of a beach located at Rio de Janeiro, Brazil, bathed by Guanabara Bay waters during outflow. Enterococci densities were compared with TC, TEC, and Brazilian recreation waters limits, to search for evidence if ENT should also be included at Rio de Janeiro touristic beaches sanitary monitoring program following international recommendations (Boehm and Sassoubre 2014; EPA 2012; WHO 2003).

Concerning abiotic variables, the beach water presented typical characteristics of shallow coastal waters. In September (spring) and December (summer) São João water pH was below 08 (Table 01). Although there was no register of precipitation in December, in September, it rained 11.2 mm 96 h before collection. The pH shifts in saline waters are low due to high solute concentrations. However, local biota physiological activities, such as respiration rates and photosynthesis, or factors such as temperature and geological formations can cause pH oscillations (PBMC 2014). Thus, the pH of the aquatic system is a relevant, indicator of the water quality and the extent of the pollution in coastal areas (Kacar and Omuzbuken 2017). Slight fluctuations at pH values may be explained by urban runoff that carries debris and waste from streets to the sea, decreasing beach sanitary quality (Shuval 2003). Sewage also contributes as it brings a massive amount of biodegradable organic matter that will be decayed releasing CO_2 and decreasing pH. Thus, pH value below eight alerts for domestic sewage dumping (Brasil 2000; Kacar and Omuzbuken 2017; WHO 1999).

Salinity and temperature fluctuated with samplings in this study, changing with time from saline to brackish waters. These salinity shifts were not related to the season of collection. Kacar and Omuzbuken (2017) also found significant differences in water quality parameters especially salinity values (25.7 to 41.2), across places and time at eastern Aegean Sea monitoring. Salinity and temperature variations occur markedly in coastal waters. In tropical environments, heat exchange at the interface air and water are more intense (PBMC 2014). Salinity can shift because of evaporation, precipitation, and runoff. During rainy season an intense inlet of fresh water, streams, and non-point pollution areas can decrease salinity values.

On the contrary, salinity values were quite high in shallow waters because of evaporation during summer (Kacar and Omuzbuken 2017). Likewise, water temperature decreases with increasing depth of the water column. At low depths, surface water interacts with air and sunlight (Pereira and Soares-Gomes 2002). These oscillation factors are most intense in shallow and surface water.

The lowest values of ENT in water (≤ 6 MNP.100 mL^{-1}) were found in October, November, and December when the temperature was between 22.35°C and 24.09ºC (Table 01). Oppositely, the highest ENT densities were detected in August and September when water temperatures were between 20.83ºC and 21.56ºC. Lessard and Sieburth (1983) observed that decay rates of *E. coli* and enterococci populations in sewage were closely related to temperature variation. In marine environments, there is a greater decreasing of ENT populations at high temperatures (Byappanahalli et al. 2012). High temperatures and sunlight can cause a decrease of the microorganism populations by desiccation and the incidence of UV light (Byappanahalli et al. 2012). TEC, however, are more resistant to shallow marine waters high temperatures (Griffin et al. 2001).

In sediments highest ENT values were found in September, October and December (14 ≤ ENT ≥27 MNP.100 mL^{-1}) while in water, densities were between 04 and 22 MNP.100 mL^{-1} on these collections (Table 01). Enterococci numbers can be quite variable in time and space (Boehm and Sassoubre 2014). Enterococci can reach recreational waters through different sources including sewage, agricultural and urban runoff, stormwater, direct input by animals via defecation, bather shedding, boats, plant debris, polluted groundwater, soils, sediments, and sands (Boehm and Sassoubre 2014). Additionally, beach sands have been shown to harbor enterococci which can grow (Zubrzycki and Spaulding 1962) and be transported into the adjacent waters (Yamahara et al. 2007). Planktonic enterococci can settle to sediments at the bottom of the water column or can attach to larger particles that settle to the bottom of the water column. Settling velocity is a function of particle size, shape and density, and fluid density and viscosity (Boehm and Sassoubre 2014). Thus, ENT will settle faster if attached to a wide particle. Resuspension of enterococci that have been previously deposited in the sediments can occur when the sediment is disturbed, and experiences shear stresses greater than the critical shear stress (Boehm and Sassoubre 2014; Nevers and Boehm 2011).

A comparatively small number of studies have considered the interaction between enterococci and particles in natural waters. These studies can be consulted at Boehm and Sassoubre (2014). More research is needed to understand if kinetic models of bacterial attachment to particles in surface waters, and to understand the mechanisms by which the attachment of enterococci to particles occurs. A single study has characterized the surface properties of *E. faecalis* and found the bacterium is negatively charged at all pH, even in the presence of ions (Schinner et al. 2010). A mechanistic understanding of how electrostatic, hydrophobic, and other surface-surface interactions control enterococci adhesion to particles would be useful (Boehm and Sassoubre 2014).

Studies investigating long-term spatially and temporally concentrations of enterococci in marine waters indicates that they vary at predictable time scales, due to various fate and transport processes including rainfall, tides, sunlight insulation, and turbulence. Rainfall can be more substantial in some areas during El Niño events due to inputs of stormwater, thus leading to higher concentrations of enterococci in waters (Boehm et al. 2002). Enterococci concentrations also vary due to the tides

(Boehm et al. 2002; Yamahara et al. 2007). Tides control transport of enterococci in marine waters through tidal currents, and tides also can modulate enterococci inputs (Boehm and Weisberg 2005). Sunlight suppresses enterococci concentrations near high noon, due to photoinactivation (Boehm et al. 2009). Finally, enterococci concentrations vary at high frequencies in marine waters, due to mixing processes that generate patches and ligaments of enterococci in free-enterococci waters (Boehm 2007).

The statistical bootstrap model generated confidence intervals of bootstrap averages for each FIB analyzed in water or sediment (Figure 03). These intervals overlap in three groups of FIB suggesting no difference in FIB amount when water and sediment matrices were compared. However, according to literature, it is not correct to affirm the similarity of these variables. Once bacteria can adhere to sand particles as discussed previously, the sand can present microbiological contamination greater than adjacent water (Boehm and Sassoubre 2014; Nevers and Boehm 2011; Sabino et al. 2014; Yamahara et al. 2007; Zubrzycki and Spaulding 1962). Solo-Gabriele et al. (2000) observed patterns of *E. coli* concentrations in the sediment. *E. coli* concentrations increased in storm events and after these events returned to baseline levels and varied in a cyclic pattern which was related to tidal cycles. The flow tide presented the highest *E. coli* concentrations. In contrast, lower densities were found at ebb tide. It was concluded that this cyclical pattern was caused by the growth of *E. coli* within riverbank soils which were later washed by high tide.

In a broader approach, FIB numbers in both environmental matrices were between zero and 50 MNP.100 mL^{-1} in August and December when salinity was low – equivalent to brackish water (29.07) – and the temperature was close to average (22.32ºC) (Table 01). The TC and TEC as a fecal indicator (<50 MNP.100 mL^{-1}) indicate "excellent" waters, according to Brazilian legislation for recreational waters (Brasil 2000). However, when we take enterococci into account, water quality cannot be considered as "excellent." The enterococci water density was higher than Brazilian limits (30 MNP.100 mL^{-1}). Thus, in August, water was considered as "good" according to regulation (<50 MNP.100 mL^{-1}) (Brasil 2000). In the United States, EPA marine recreational water quality criterion for enterococci in water is not more than 35 colony forming units/100 mL (geometric mean standard) and 10^4 colony forming units (CFU)/100 mL (single-sample standard) (Boehm and Sassoubre 2014; EPA 2012). In the EU, bathing water standards for enterococci range from limits of 100-400 CFU/100 mL, depending on whether the beach is marine or fresh, and whether the beach is rated as "excellent" or "sufficient" (The Council of the European Union 2006).

The FIB group are composed of bacteria derived from the gastrointestinal tract of warm-blooded animals. Once released in the environment, the FIB is subjected to stresses, such as high salinity, that lead population declination over time (Griffin et al. 2001). Schulz and Childers (2011) evaluated the effect of temperature variation and salinity in diversity and decay of fecal *Bacteroidales*. They noticed at higher temperatures that decay rate was elevated, although diversity was smaller at higher salinities, with lower decay rates. Okabe and Shimazu (2007) observed that the decrease in FIB decay at elevated salinities was indirectly related to the decrease in predation. According to the authors, predators would be impacted due to salinity. Thus, at low salinities grazing may be enhanced. Also, the temperature may also influence FIB grazing. Sherr et al. (1988) evaluated the effect of temperature on digestion rates of phagotrophic flagellates and ciliates while feeding fluorescently labeled bacteria. It was observed that the digestion rates increased exponentially at temperatures between 12°C to 22°C in both types of protozoa and the digestion time appeared to be significantly influenced only by protozoan cell size (or type) and by temperature. Concerning enterococcal predation, it can happen by bacterivorous protozoa such as amoebas, forams, nanoflagellates, and ciliates; and various other zooplankton (Boehm and Sassoubre 2014).

Although there was an observable increase in TC and TEC densities in water in September and November ($110 \leq \text{FIB} \geq 240$ MNP.100 mL^{-1}), no correlation was found when FIB values were confronted with the accumulated precipitation (5 mm < PRECIP > 11.2 mm) 96h before collection (Table 01). Ackerman and Weisberg (2003) evaluated rain volume and the FIB densities in coastal areas over five years in Californian beaches. They noticed an increase in FIB after precipitation exceeding 06 mm. The same was not observable when precipitation was <2.5 mm. Although for Zhang et al. (2013) there was no significant difference in water temperature, salinity, and pH before and after the rain in China. They found a clear relationship between rainfall and concentrations of dissolved oxygen, enterococci and fecal coliforms. Their results revealed that the maximum level of indicator bacteria was attained within about six hours following the incidence of rain. However, the time required for water quality recovery depends on the intensity and duration of rainfall (Zhang et al. 2013). Generally, FIB numbers remain elevated for five days after rainfall, although decreases to quality standard levels within three days. Noble et al. (2003) quantified FIBs 36h after precipitation at southern California coast. After the rainfall, shoreline water exceeded by 60% quality limits compared to dry weather. Concerning the urban areas adjacent to runoff outlet, water limits exceeded by more than 90% after rain. Jiang and Chu (2004) related the occurrence of FIB and human viral on beaches close to rivers after rain events. They concluded that the negative impacts of urban runoff in the quality of coastal waters are more significant during the wet season. The opposite effect also occurs in dry weather caused by high temperatures and sunlight bacteria desiccation, especially in the tropics (Tallon et al. 2005).

In developed countries, sewage is typically well-treated before discharge through an outfall that is usually located far from recreational waters. Direct inputs of untreated sewage, however, can impact recreational waters during storm events in regions that have combined sewer overflows and in regions with leaking sewer lines (Sercu et al. 2009). However, surface waters throughout the world are plagued by high concentrations of fecal bacteria. In the US, 24% of surface water bodies are listed as impaired, due to elevated levels of fecal indicator bacteria, and a subset of those are impaired because of high concentrations of enterococci (Boehm and Sassoubre 2014; EPA 2012).

Brazilian legislation follows WHO (2003) recommendation and considers thermotolerant coliforms, enterococci, and *E. coli* as fecal bacteria indicators of seawater quality. CONAMA resolution (Brasil 2000) classifies water as excellent when TEC $\leq$250 MNP.100 mL^{-1} and ENT $\leq$25 MNP.100 mL^{-1}. Above this limit (250 $\leq$ TEC $\geq$ 500 MNP.100 mL^{-1} and 25 $\leq$ TEC $\geq$ 50 MNP.100 mL^{-1}) water is classified as good. In consonance with Brazilian resolution (Brasil 2000), the water of São João beach was classified as excellent when TEC was considered (TEC$\leq$240 MNP.100 mL^{-1}). However, when ENT was analyzed (ENT = 30 MNP.100 mL^{-1}), water was considered as good (Brasil 2000). Despite Brazilian (Brasil 2000) and WHO (2003) recommendation, cities including Rio de Janeiro, Salvador, and Fortaleza do not consider enterococci densities at water monitoring reports of touristic beaches (INEA 2018; INEMA 2018; SEMACE 2018). Although fecal enterococci are generally not virulent, multidrug-resistant strains have emerged as leading causes of hospital-acquired infections (Boehm and Sassoubre 2014). Thus, periodic monitoring should also be included in these cities to control the dissemination of multidrug-resistant strains to the marine environment. Enterococci sources, fate, and transport knowledge can help the creation of models that predict enterococcal concentrations in surface waters. When surface water is known to contain concentrations of enterococci that exceed regulatory standards, actions must be taken to reduce their concentrations (Boehm and Sassoubre 2014). Thus, even though water is still considered as good at São João beach, ENT quantification must be included at water monitoring program of touristic beaches once its density that varies with time and space represents a potential risk of illness to swimmers.

CONCLUSIONS

The case study aimed to quantify enterococci bacteria from water and intertidal sediment samples in a beach located near touristic spots and bathed by Guanabara Bay waters. The density of enterococci found in water in August collection was higher (30 MNP.100 mL^{-1}) than Brazilian limits, being considered as good (<50 MNP.100 mL^{-1}). According to literature, although fecal enterococci are generally not virulent, multidrug-resistant strains have emerged as leading causes of hospital-acquired infections. Thus, even though water still considered as good at São João beach, ENT quantification must be included at water monitoring program of touristic beaches once its variable densities represent a potential risk of illness to swimmers. Despite the small-scale approach, these results show that enterococci can indicate more faithfully classification of water quality than total and thermotolerant coliforms. Thus, this study support that the density of enterococci in recreational waters should be monitored periodically.

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Enterococos como Indicador Fecal em uma Praia Tropical: Um Estudo De Caso

RESUMO

Enterococos fecais geralmente não são virulentos, entretanto, cepas multirresistentes emergiram como principais causas de infecções hospitalares. Assim, o monitoramento periódico de enterococos deveria ser incluído em cidades com alta densidade populacional para controlar a disseminação de cepas multirresistentes no ambiente marinho. O objetivo deste estudo foi quantificar enterococos em amostras de água e sedimento intermaré em uma praia localizada perto de pontos turísticos do Rio de Janeiro. Também pretendemos avaliar se os enterococos devem ser incluídos no programa de monitoramento sanitário das praias turísticas. Para essa abordagem, monitoramos de agosto a dezembro de 2014 as bactérias indicadoras fecais (FIB) em uma praia próxima a pontos turísticos através do método de tubos múltiplos. Embora o FIB estivesse dentro dos padrões sanitários da legislação brasileira, altas densidades de enterococos (= 30 MNP.100 mL^{-1}) foram detectadas na água coletada em agosto. Assim, o monitoramento de enterococos deve ser incluído em praias turísticas para evitar o risco de disseminação de bactérias multirresistentes entre nadadores e frequentadores da praia.

Palavras-Chave: Bactérias; Indicador Fecal; Monitoramento da Poluição; Saúde Pública.

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