



## ENTEROCOCCI PRESENCE IN INTERSTITIAL WATER IN INTERTIDAL AREAS OF SANDY BEACHES FROM VERACRUZ-BOCA DEL RÍO, GULF OF MÉXICO

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### Abstract

In this study the presence of enterococci was analyzed in interstitial water samples collected in the intertidal zone of Villa del Mar, Mocambo and Arroyo Jiote beaches located in Veracruz-Boca del Río, México. Sampling conducted in March 2013, included 18 points in each beach considering the level of the tide. The genus *Enterococcus* was present in 100 per cent of the analysed samples, where a significant difference among the studied beaches was observed. The highest concentrations of the organism occurred in Villa del Mar beach, where 44 percent of the samples exceeded the maximum permissible limit for recreational water. Arroyo Jiote was the beach where the lowest concentrations were recorded. It is necessary to make a follow up to this type of research since the observed levels are indicative of the possible presence of pathogens that could pose a risk to the health of beach users.

**Keywords:** Enterococcus; intertidal, sandy beaches, interstitial water.

### 1. Introduction

The concentration of bacteria in the role of pollution indicators in coastal waters has been used for decades as a security measure for recreational use. Although not all are necessarily pathogenic bacteria, they are found in abundance in human input downloads, where there is a risk that pathogens are present (Noble *et al.*, 2004). Bacteria commonly identified as indicators are: total coliforms, fecal coliforms, Enterococci and *Escherichia coli* (Halliday *et al.*, 2011). In 2012 the water quality criteria was defined by the United States Environmental Protection Agency (EPA), which reaffirmed the enterococci as fecal indicator bacteria in marine environments (Cui *et al.*, 2013).

The term enterococci includes all species of the genus *Enterococcus* and corresponds to Gram positive, facultative halophilic microorganisms, which develop at pH 9.6 and temperature of 10° to 45° C, also show resistance at 60° C for 30 minutes and to a concentration of 6.5 percent of sodium chloride (Vergaray *et al.*, 2007, Díaz-Pérez *et al.*, 2010). These characteristics give it the distinction of being a useful tool to evaluate the microbiological quality of marine environments that have been impacted by fecal contamination (Phillips *et al.*, 2011, Halliday *et al.*, 2011).

In Mexico, the microbiological criteria for water beaches for recreational use were based on studies by the World Health Organization (WHO), in which a number of features of microbiological indicator enterococci are defined, and are associated with an increment in the frequency of various kinds of diseases. Different studies with scientific bases relate gastrointestinal symptoms to acute febrile respiratory illness (AFRI) with the presence of enterococci in waters that do not have the quality that is set for recreational use (SEMARNAT 2012). In this context, the Ministry of Health (2013) considered a level of 200 NMP enterococci / 100 mL for an estimated 5-10 percent risk of gastrointestinal diseases, and 1.9 to 3.9 percent for AFRI. Therefore, the criteria to classify the beaches for recreational use within the Clean Beaches Program in Mexico, established as suitable beach water containing NMP 0-200 enterococci / 100 mL. While it is considered unsuitable, enterococci water values greater than 200 MPN / 100 mL. Health authorities use this approach in order to make the decision to close the beaches as a measure to protect the health of users when the quality of the recreational water is deteriorated.

The issue before us in this work, is that people who visit the beach are not only in contact with water, but enjoy playing in the sand, mostly children, without thinking that this activity could pose a greater risk to their health, as described in Whitman *et al.*, (2009). Recent studies have documented high levels of enterococci in beach sand with higher concentrations than the one corresponding to beach water. In addition, these international standards are generally not suited to local or regional situations, such as the structure of the beach, the dynamic marine or epidemiological situation (Whitman *et al.*, 2014) and ignore the measurement of microbial indicators in interstitial water (water that fills the spaces between grains of sediment) in the intertidal zone.

In this regard, Wright (2008) showed that the "washing" of sediment and the porous water or interstitial represent an important role when controlling the enterococci levels in the water column. Meanwhile, Wheeler *et al.* (2003) and Whitman *et al.* (2014) consider that the levels of enterococci in water could decrease when they are dragged out and therefore unable to develop due to lack of nutrients, competition with native microorganisms and / or inactivated by sunlight.

#### 1.1 The case of the Corridor of Veracruz-Boca del Río

The sandy beaches of Veracruz-Boca del Río represent a major tourist destination in Mexico (INEGI 2012) where various recreational activities are performed. These beaches are frequently visited by national and international tourism, they are semi-protected by over 20 reef structures that belong to the Veracruz Reef System National Park (PNSAV). It is common to observe in them shore protection structures (Valadéz-Rocha and Ortiz-Lozano 2013) that increase their

accretion and thus stop the natural dynamics, which could favor the presence of microorganisms, including the genus *Enterococcus* (Halliday and Gast, 2011) whose health risk is unknown in our country, therefore represents an important issue to investigate. For that reason, the purpose of this information is to determine the presence of enterococci in the pore water of the intertidal zone in the sandy beaches of Veracruz-Boca del Río, so it can be considered as a measure that contributes to a better prediction of the microbiological safety of recreational waters in general.

## 2. Material and Methods

### 2.1 Study Area

Sampling was done in the tourist corridor Veracruz-Boca del Río in March 2013 matching the north winds season in the Gulf of México. The collection was conducted in three beaches whose characteristics were considered representative of the corridor.

Villa del Mar beach. It has an area of approximately 360 meters, adjoins the urban area and is located between two rocky cliffs to the north and south, which according to Valadéz-Rocha and Ortiz-Lozano (2013) dissipate energy waves, change the pattern of sediment transport and favor accretion. Along the beach there are restaurants, tourist palapas, umbrellas and service tables located toward the intertidal zone. There are also boats used for fishing and taking tourists on short trips. It is considered one of the oldest tourist corridor beaches of Veracruz-Boca del Río (Gallegos-Jiménez 2008) and is the beach with the highest number of visitors.

Mocambo beach. It has an area of approximately 350 meters, not directly adjacent to urban areas. The presence of restaurants, tourist palapas and boats is moderate. It is located between two rocky cliffs to the north and south, which according to Olivares-Cruz (2010) make the coastal current almost parallel to the coastline.

Arroyo Jiote beach. It has an area of approximately 400 meters, adjoins small to moderate dunes (0.5-2 m) covered with vegetation. The presence of any type of construction, boats or tourists is low to moderate. It is the beach with more natural features, when compared with Villa del Mar and Mocambo beaches.

### 2.2 Sample Collection

In each beach two representative sites were settled and placed similarly in each way. At each site pore water was collected at three stations placed at different levels, high, medium and low tide on a central transect perpendicular to the waterfront. Two replica transects were considered, each located laterally and 5 meters away from the central transect, with a total of nine samples per site and 18 per beach.

Pore water samples were collected with sterile serological 10 mL pipettes, at a depth of 10 centimeters and poured into Whirlpak sterile bags, previously labeled and then placed in a container with ice at 4°C to be transported to the laboratory. The environment was determined by measuring the temperature, salinity, pH, and dissolved oxygen parameters, using a multi sensor YSI 556 MPS. It was supplemented by determining the percentage of organic matter by dry weight variation (Dean 1974) and sediment type conducting a sieve analysis for sand (Buchanan 1984).

### 2.3 Microbiological analysis

Enterococci analysis was performed by the method of the chromogenic substrate (Enterolert IDEXX) according to manufacturer's instructions.

### 2.4 Data Analysis

The data obtained were subjected to an analysis of one-way variance (ANOVA) to observe the variability in the concentration of enterococci among beaches. In order to establish the significant differences a posteriori Tukey test with a significance level of  $P = 0.05$  was applied, using the package STATISTICA 7.0 (StatSoft Inc. USA).

## 3. Results

The environmental parameters temperature, salinity, pH and dissolved oxygen showed a similar behavior with average values of 21.72° C, 37.04, 7.47, and 8.60 respectively. The composition of the sediment was predominantly fine sand (0.063 mm) in the studied beaches in percentages of 71.3 percent to 96.6 percent. The content of organic matter in the sediment was  $2.15 \pm 0.4$  percent (mean  $\pm$  1 SD n = 6).

The genus *Enterococcus* was found in the 54 analyzed samples of interstitial water, their values ranged from 10 MPN / 100 mL to 18171.7 MPN / 100 mL.

The results of the enterococci density for the three evaluated beaches are shown in Figure 1. The concentration of enterococci in the Villa del Mar beach ranged from 15 to 18,171.7 NMP / 100 mL. The Arroyo Jiote beach showed enterococci values between 10 and 20.5 MPN / 100 mL.

When applying a statistical analysis significant differences were observed in enterococci concentration between the beaches ( $P < 0.05$ ). When performing multiple comparisons of means and gathering information by the method of Tukey, it appears that there are significant differences ( $P < 0.05$ ) between the concentration of enterococci in Villa del Mar beach regarding Mocambo and Arroyo Jiote beaches (Figure 1).

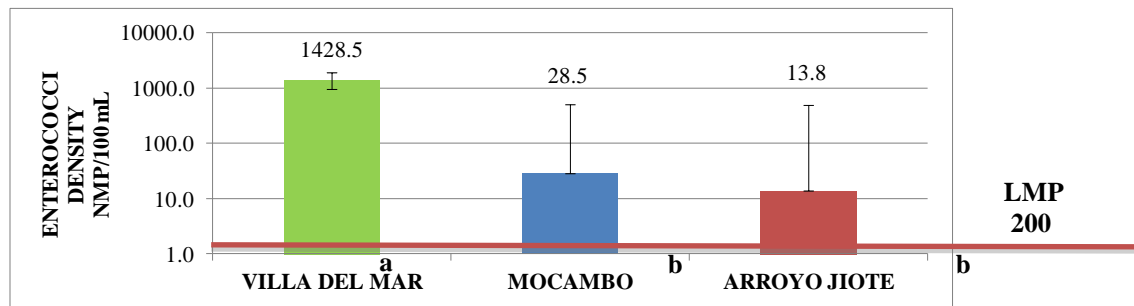


Figure 1. Average density ( $\pm$  standard error) of enterococci in interstitial water on three beaches in the Veracruz-Boca del Rio tourist corridor and levels exceeding the allowable limit for beach water of recreational use. Different letters indicate significant differences ( $p > 0.05$ ).

At the Villa del Mar beach was found the highest density of enterococci, where 44 percent of the samples exceeded the maximum allowable limit for recreational water while Arroyo Jiote was the beach where the lowest concentrations and the lowest average density of enterococci were recorded.

#### 4. Discussion

The results of this study affirm what documented by Wheeler *et al.* (2003) about the presence of *Enterococcus* spp. in sediments at depths of 5 to 10 cm, suggesting that pathogenic fecal bacteria may also be present in the sand. In this regard, Velonakis *et al.* (2014) suggest that this could be due to the sandy beach provides protection to the *Enterococcus* spp. against sunlight and acts as highly saline osmoprotective. Cui *et al.* (2013) suggest that the abundance of enterococci on beach sand appears to be affected by land use, since higher concentrations of microorganism in sand are located on the beaches near population centers and urban use, where they are exposed to receive fecal contamination of municipal discharges, runoff, animal defecation in site, and discharges of organic and inorganic nutrients. This may be happening on the Villa del Mar beach, where the highest concentrations of enterococci were recorded and characterized by abut urban areas and have coastal protection structures. In this regard, microcosm experiments conducted in water and sterile sand suggest that this type of beach promotes an increase in the replication of fecal indicator bacteria (Whitman *et al.*, 2014). Meanwhile, in Mocambo beach intermediate values were recorded, which is to be expected since it is a beach, that while is in contact with the urban area and has the presence of breakwaters, tourist incidence occurs for periods and it is subjected to holidays or vacation days. Besides, there is little or no presence of boats that provide tourist services. Arroyo Jiote is an undeveloped beach, has no protection structures and therefore lower concentrations of enterococci, which is consistent with Cui *et al.*, (2013) who found out that enterococci values did not exceed the permissible limits or were not detected in sandy beaches of rural use.

In a general context, it is not a surprise that the *Enterococcus* spp. is found in the water of the intertidal area beaches of Veracruz-Boca del Rio, as it is a bacterial inhabitant of coastal regions with tropical and temperate climates, which has been widely reported in the world for its high levels on beach sand (Wheeler *et al.*, 2003; Shibata *et al.*, 2004; Yamahara *et al.*, 2012; Cui *et al.*, 2013). However, concentrations of *Enterococcus* spp. in the pore water of the Villa del Mar beach are high and out of levels established for recreational use, which could pose a risk to public health. Especially if there are reports documented recently where a significant statistical correlation of *Enterococcus* spp. was shown with the presence of pathogenic *Vibrio* spp. in the sand (Velonakis *et al.*, 2014). Heaney *et al.*, (2009) in a study conducted in seven US beaches showed a positive correlation between gastroenteritis infections reports of tourists who performed activities in the beach sand and the presence of enterococci.

#### 5. Conclusions

Considering the reports of other scientific studies and the results of this one, where evidence that the genus *Enterococcus* is in the intertidal interstitial water in concentrations that could affect water quality and public health is shown, it is recommended that interstitial water monitoring be included as a measure of predictive microbiological safety of recreational water on sandy beaches.

In future research, it is necessary to know the source of enterococci in pore water, as these could come from a source of fecal pollution, non-Point, transported through wastewater discharges and deposited in the sand by wave and tidal action as suggested in Cui *et al.* (2013), or they may be related to indigenous populations of enterococci in beach sand, as previously reported (Cui *et al.*, 2013, Whitman *et al.*, 2014).

It is important to know the survival of pathogenic bacteria which is introduced into the beach sand and interstitial water by fecal contamination, as they represent a risk to the health of users of the intertidal zone of sandy beaches of the tourist corridor Veracruz-Boca del Río. And also study the role that coastal protection structures play in the presence of pathogenic microorganisms.

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