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Enterococcus in Water, Sediment and Clams in a Tropical Environment, Maracaibo Lake, Venezuela

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Abstract

The genus *Enterococcus* has widespread extra enteric sources and reservoirs. It has been suggested that coastal and Great Lakes States adopt enterococci as an alternative indicator for the monitoring of recreational water quality. Limited information, however, is available about the presence of enterococci in Lake Maracaibo, which is an important estuary in Venezuela, with the income and interchange of the Caribbean Sea, and is used by the people for recreational purposes and the culture of marine organisms. In this study, the density and species composition of enterococci in sediment, clams and water were examined at Lake Maracaibo.Enterococci was enumerated by the Most Probable Number Technique (MPN), and isolated by standard methods. Results obtained by MPN analyses indicated that enterococci were present in all samples, and their densities were generally higher in clams than sediment and water with means of $1.0x10^6$ MPN/100 g, $2.1x10^3$ MPN/100 g, and $6.0x10^1$ MPN/100 ml, respectively. Dominant *Enterococcus* species were *E. faecalis* (65%), *E. casseliflavus* (20%), *E. sanguinicola* (5%), *E. faecium* (5%), and unidentified strains (5%). Results suggest that some enterococci are able to persist in Lake Maracaibo, especially in clams and sediment, for a prolonged amount of time after being introduced.

Keywords: *Enterococcus*; Water sediment; Clams; Tropical environment

Introduction

Enterococci are spherical or ovoid cells arranged in pairs or chains, Gram positive non-spore-forming, obligately fermentative chemoorganotrophs. They are important members of gut communities in many animals, and opportunistic pathogens that cause millions of infections annually. They can be found in soil, water, and plants [1]. When they are found in water it is associated with fecal matter. In the last two decades, the genus *Enterococcus* has been believed to be one of the most common causative agents of nosocomial diseases in humans following staphylococci [2-4]. Additionally, some vancomycin-resistant enterococci belonging to the group of zoonoses (Slovak Regulation No.626/2004) can be transmitted to humans via food chain, and are employed as vectors in dissemination of resistance genes inside as well as outside of genus. This feature shifts them to the group of potential reservoirs of resistance genes with significant risk of resistance transfer to obligate pathogens, such a *Staphylococcus* spp. or *Listeria* spp. [5-7]. Also, *Enterococcus* has been proposed as fecal indicator in some countries, especially in recreational waters. The vast majority of clinical enterococcal infections in humans are caused by *Enterococcus faecalis* (around 80% of clinical isolates) and *Enterococcus faecium* (most of the remainder), with occasional infections being caused by *E. durans, E. gallinarum, E. casseliflavus, E. avium, E. hirae, E. mundtii, and E. raffinosus* [8,9]. The emergence of *Enterococcus faecalis* and *Enterococcus faecium* was paralleled by increases in glycopeptide and high-level aminoglycoside resistance, both important compounds for the treatment of human infections [10]. Although *Enterococcus faecalis* is the causative agent in most enterococcal infections, a partial replacement of *Enterococcus faecalis* by *Enterococcus faecium* has been noted in the last years, and presently, up to one-third of enterococcal infections in some countries is attributed to this species [11,12].

The presence of enterococci as an indicator of fecal contamination has been used in management of recreational water quality standards as it correlates best with the incidence of swimming-related illnesses [13,14].

Studies conducted by EPA to determine the correlation between different bacterial indicators and the occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. For salt water, enterococci are the best [15].

However, little is known about the behavior of FIB in tropical environments, particularly in estuaries, since most studies have been performed in temperate to cold locations [16].

Although some countries required enterococci test for recreational bacterial water quality standards, the requirement in Venezuela are only total and fecal coliforms [17]. Previous studies in Venezuela have been showed the presence of enterococci in the environment but not much is available about the presence of enterococci in Lake Maracaibo [18]. The objective of this research was to analyze and compare the density and species composition of enterococci in water, sediment and clams at Curarire beach, located at Lake Maracaibo, Venezuela.

Materials and Methods

Study area. Lake Maracaibo is a tropical lake and remains warm (28-32.5°C; mean 30°C) throughout the annual seasonal cycle; it is the largest lake in South America [19], located at Zulia State, Venezuela. This estuarine lake is connected to the Gulf of Venezuela via the Strait of Maracaibo and El Tablazo Bay, and is brackish due to intrusion of salty water from the strait [19]. Lake Maracaibo receives large loads of nutrients from tributaries, sewage discharges, and agricultural sources [20].

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Samples collection

Samples were collected from three different points at Curarire Beach, during a period of a year (October 2008 to 0ctober 2009). Water, sediment and clams samples were, using a sterile 1 L screw-top bottle or sterile Whir-Pak bags (Nasco), respectively. Water samples were collected from 0.3 m below the surface of the water. Samples were transported in a cooler on ice packs to the laboratory and tested within 6 h of collection [21].

Samples analysis

The seafood and sediment samples were prepared for testing by blending 25 g in 225 mL of 0.1% sterile peptone water for 2 minutes [21,22].

Microbial density: Enterococci in water, sediment, and clams homogenate, were enumerated using the standard Most Probable Number Technique using Azide dextrose broth (Himedia, India) and KF-enterococcus agar (Himedia, India). Strains grown on KF-streptococcus agar were identified as *Enterococcus* with the following biochemical tests: Gram stain, catalase, growth with 6.5% of NaCL, growth at 45ºC, bile esculine hydrolysis.

Species identification: Enterococcal strains were further identifies to the species level by using conventional physiological tests which are based on carbohydrate fermentation using 1% solution of the following sugars: manitol, sorbitol, sucrose, raffinose, and arabinose; by arginine descarboxilation in Moellers descarboxylase broth; tellurite; motility test, pigment production detected on tryptic soy agar (TSA) (Difco, USA) [23].

Physicochemical parameters: Temperature, pH, Turbidity and salinity were measures in the water using convectional techniques [21].

Results and Discussion

Water, sediment and clam samples were taken at Curarire Beach, Lake Maracaibo. The water temperature ranged between 23.0ºC to 35.0ºC, with a mean of 30.6ºC; pH 8.74 to 10.5 (mean 9.42); salinity 2 PSU to 6 PSU (mean 3.85), and turbidity 20.7 NTU to 94.7 NTU (mean 44.48). *Enterococcus* densities in water, sediment and clams ranged from <2-50 MPN 100 mL^{-1}, <2-130 MPN 100 g^{-1}, and <2-70 MPN 100 g^{-1}, respectively with a geometric mean between 2 and 130 (Figure 1). *Enterococcus* prevalence rate was 71%. One of the water sample exceeded EPA guidelines, being the sample with the highest fecal coliform value (700 MPN 100 mL^{-1}) (data not shown). Previous studies have shown the highest value of fecal coliforms in shellfish *Rangiacuneata* evaluated from this environment exceeding the Venezuelan guidelines [24].

Densities were generally higher in sediment than clams and water with means of 25.9 MPN/100 g, 11.4 MPN/100 g, and 5.6 MPN/100 mL, respectively. Higher concentration of enterococci in sediment and clams than water has been reported before. LeFevre and Lewis 2003, defined a transport pathway for enterococci that begins with the primary source, followed by deposition to a sink, in which enterococci are temporarily sequestered (e.g., sediments), and eventually it can become a secondary source when organisms reenter the water column following a disturbance [25] and can also be filtered by shellfish as clams. Culturable enterococci cells persist longer in sand than water and some studies have documented growth of this microorganism rather than loss, in beach sand [26].

Survival in sediment may be enhanced relative to water because of protection from sunlight/UV inactivation, buffered temperatures, and availability of nutrients accumulated from algae, debris and plankton [27]. Bacteria may also be protected within biofilms on moist sand grains [28]. In some geographical regions, highly favorable conditions may be encountered outside the host. In relatively warm, nutrient-rich, pristine tropical soils and waters, EC have been found at densities far exceeding the concentrations found in highly-polluted temperate waters [29]. *Enterococcus* densities were higher during November to March related with dry season in Venezuela (Figure 1). Other research reported higher values during summer months.

Sixty strains of enterococci were isolated from ninety samples collected form water, sediment and clams at Curarire beach, Lake Maracaibo. The most frequent species isolated was *E. faecalis* (65%) followed by *E. casseliflavus* (20%) (Figure 2). The water samples obtained from different points, *E. faecium* and *E. faecalis* as the most frequent species isolated followed by *E. gallinarum* and *E. casseliflavus* [30]. *E. faecalis* is the most common specie isolated from clinical samples (80-90%), interacts with many other organisms and has effects on the environment. In the other hand, *E. casseliflavus* has been reported in 20% of beach samples and it is associated with natural source, such as plants [31-33]. This is consistent with the results found in this research.

Results showed the importance of surveillance enterococci in tropical water especially in Venezuela in order to determine the ability to survive in Maracaibo Lake and if it's necessary to include it in the Venezuelan guidelines.

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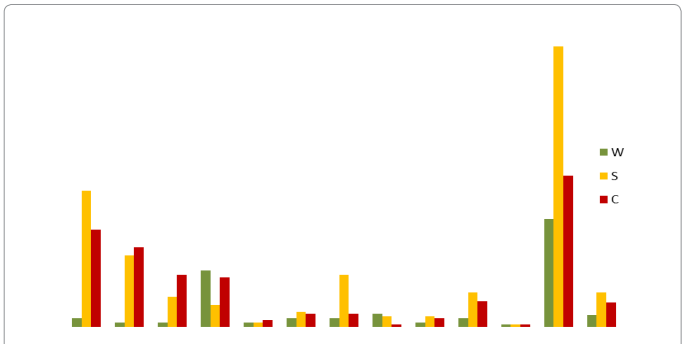

Figure 1: Most probable number (MPN/100 ml or g) of enterococci during sampling period in water (W), sediment (S) and clams (C) from Lake Maracaibo, Venezuela.

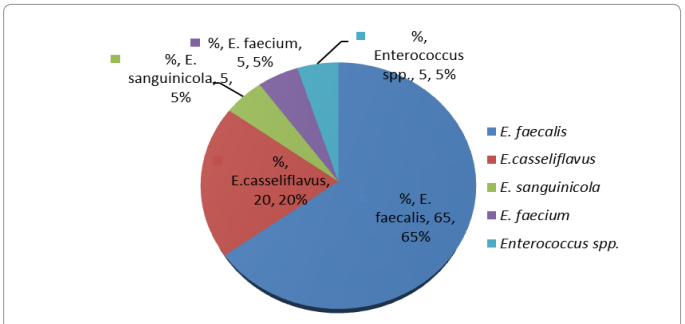

Figure 2: Percentage of different *Enterococcus* species isolated from Water, sediment and clams taken from Curarire Beach, Maracaibo, Venezuela.

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