

Available at [www.sciencedirect.com](http://www.sciencedirect.com)journal homepage: [www.elsevier.com/locate/watres](http://www.elsevier.com/locate/watres)

## Quantitative evaluation of enterococci and Bacteroidales released by adults and toddlers in marine water

Samir M. Elmir<sup>a,b</sup>, Tomoyuki Shibata<sup>a,c,d</sup>, Helena M. Solo-Gabriele<sup>a,e,\*</sup>,  
Christopher D. Sinigalliano<sup>a,c</sup>, Maribeth L. Gidley<sup>a,c</sup>, Gary Miller<sup>b</sup>, Lisa R.W. Plano<sup>a,f</sup>,  
Jonathan Kish<sup>a,g</sup>, Kelly Withum<sup>a</sup>, Lora E. Fleming<sup>a,b</sup>

<sup>a</sup>NSF-NIEHS Oceans and Human Health Center, University of Miami, Rosenstiel School for Marine and Atmospheric Sciences, 4600 Rickenbacker Causeway, EG 211 Key Biscayne, FL 33149, USA

<sup>b</sup>Miami-Dade County Health Department, 1725 NW 167 Street Miami, FL 33056, USA

<sup>c</sup>NOAA Atlantic Oceanographic and Meteorological Laboratory, Miami, FL, USA

<sup>d</sup>University of Northern Illinois, Public Health and Health Education Programs, DeKalb, IL, USA

<sup>e</sup>University of Miami, Department of Civil, Architectural, and Environmental Engineering, P.O. Box 248294, Coral Gables, FL 33124-0630, USA

<sup>f</sup>Departments of Pediatrics Microbiology and Immunology, University of Miami, Miami, FL 33130, USA

<sup>g</sup>University of Miami, Department of Epidemiology and Public Health, 1120 NW 14th Street, Room 1049, Miller School of Medicine, Miami, FL 33136, USA

### ARTICLE INFO

#### Article history:

Received 20 May 2009

Received in revised form

3 July 2009

Accepted 7 July 2009

Published online 31 July 2009

#### Keywords:

Bather shedding

Enterococci

Bacteroidales

Beach sand

### ABSTRACT

Traditionally, the use of enterococci has been recommended as the fecal indicator bacteria of choice for testing marine recreational water quality, and prior studies have shown that bathers shed large numbers of enterococci into the water. The current study expands upon prior research by evaluating shedding from both toddlers and adults, and by the expansion of measurements to include enterococci shedding via three different methods (membrane filter (MF), chromogenic substrate (CS), and quantitative polymerase chain reaction (qPCR)) and shedding of alternative fecal indicator bacteria (Bacteroidales human markers UCD and HF8 via qPCR). Two sets of experiments were conducted. The first experiment consisted of two groups of 10 adults who bathed together in a large pool. The second study consisted of 14 toddlers who bathed individually in a small pool which allowed for sand recovery. Sand recovery was used to estimate the amount of sand transported on the bodies of toddlers and to estimate the number of fecal indicator bacteria released from this sand. The numbers of estimated enterococci shed per adult ranged from  $1.8 \times 10^4$  to  $2.8 \times 10^6$  CFU, from  $1.9 \times 10^3$  to  $4.5 \times 10^6$  MPN, and from  $3.8 \times 10^5$  to  $5.5 \times 10^6$  GEU based on the MF, CS, and qPCR methods, respectively. The estimated numbers of Bacteroidales human markers ranged from  $1.8 \times 10^4$  to  $1.3 \times 10^6$  for UCD, and ranged from the below detection limit to  $1.6 \times 10^5$  for HF8. The estimated amount of sand transported per toddler ( $n = 14$ ) into the water column after sand exposure was  $8 \pm 6$  g on average. When normalizing the numbers of enterococci shed from toddlers via sand by the 3.9 body surface area ratio, the differences between toddlers and adults were insignificant. Contributions of sands to the total enterococci (MF) shed per toddler was  $3.7 \pm 4.4\%$  on average. Although shedding via beach sand may contribute a small fraction of the

\* Corresponding author at: University of Miami, Department of Civil, Architectural, and Environmental Engineering, P.O. Box 248294, Coral Gables, FL 33124-0630, USA. Tel.: +1 305 284 2908; fax: +1 305 284 3492.

E-mail address: [hmsolo@miami.edu](mailto:hmsolo@miami.edu) (H.M. Solo-Gabriele).

0043-1354/\$ – see front matter © 2009 Elsevier Ltd. All rights reserved.

doi:10.1016/j.watres.2009.07.006

microbial load during initial bathing, it may have a significant role if bathers go to water repetitively after sand exposure.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Beach advisories are issued when water quality exceeds the microbial standards which in the U.S. are based upon *E. coli* and enterococci for fresh and marine waters, respectively (U.S. EPA, 1986). For many advisories (NRDC, 2007), the source of pollution (i.e. humans, animals, and/or environmental) is rarely identified. Humans represent a non-point source of fecal indicator bacteria to recreational waters, and quantifying their bacterial load during bathing can help in developing effective beach management strategies which minimize the number of beach advisories.

Traditionally, measurements of fecal indicator bacteria in recreational waters have relied on common culture-based methods such as membrane filtration (MF). However, more recently measurements have expanded beyond MF to include chromogenic substrate (CS) and quantitative polymerase chain reaction (qPCR) for alternative measurements of fecal indicator bacteria. This expansion was due to the fact that the MF method requires a 24 h incubation period, and the method does not differentiate between bacteria of animal versus human origin. In this study, CS (another culture-based method which has gained considerable use in the regulatory community) and qPCR methods were integrated with the use of the standard MF method for water analysis. Integrating molecular with traditional laboratory techniques could provide data about non-culturable microbes and possible sources (i.e. humans versus animals). While the molecular methodology for the detection of general and human-specific Bacteroidales has not been adopted by the U.S. EPA for routine monitoring, research has shown that it can be potentially used for identifying bacterial contamination from human origin (Gawler et al., 2007; Walters et al., 2007; U.S. EPA, 2007); in addition, the U.S. EPA has included Bacteroidales in its recent epidemiologic studies (Wade et al., 2006), suggesting its potential future use for routine monitoring.

The main objectives of the this study were to measure shedding of enterococci and Bacteroidales using traditional and emerging laboratory methods, and to evaluate shedding from toddlers and adults. The field experimental design for the current study was based upon the prior work of Elmir et al. (2007). The added value of the current study was the evaluation of shedding from toddlers (all prior studies used adult volunteers), and the use of additional methods of fecal indicator bacteria analyses (i.e. enterococci by CS and qPCR, and Bacteroidales by qPCR) as no data are available which directly measure fecal indicator bacteria shedding using these alternate methods. The use of the same field study design allowed for the comparison of the MF method results between the Elmir et al. (2007) study and the current study.

## 2. Materials and methods

Two distinct sets of experiments (“large pool” and “small pool”) were conducted as described earlier by Elmir et al.

(2007). The study was approved by both the Miami Dade Department of Health Internal Review Board (IRB 1491; DOH IRB Number, H07164) and by the University of Miami Internal Review Board (IRB 20070306). Consistent with IRB approval, consent forms were signed by each volunteer (or parent/guardian) and volunteer identity was kept confidential. The experiments took place at the same sub-tropical non-point source recreational marine beach location as described by Elmir et al. (2007). At the time of the current study the measured salinity was 34 ppt, pH was 7.9, and water temperature was 31 °C.

### 2.1. Large pool field study

The Elmir et al. (2007) “large pool” field study protocol was used as the basis to evaluate the numbers of enterococci and Bacteroidales released from the bodies of adult bathers. The same source water, and type and size of inflatable pool were used as in the previous work. In this current study, the “large pool” protocol differed from Elmir et al. (2007) only in that additional microbial parameters were measured (i.e. addition of enterococci by CS and qPCR and analysis of Bacteroidales), and in that the study was repeated two times on the same day using two groups of adult participants (10 per group) instead of only one group. In addition, 5 L samples were collected (versus 100 ml samples in the original study) to allow for the analysis of additional bacterial indicators. As in the prior “large pool” study, each group of 10 adult bathers were subjected to four 15-min bathing cycles where participants were not exposed to beach sand during the first two cycles, and were exposed to beach sand during the last two cycles. The “large pool” field study was conducted on a single day in July 2008, with the first group starting at 9 am and the second group starting at noon; each group participated in the study for a period of approximately 1.5 h.

### 2.2. Small pool field study

The Elmir et al. (2007) “small pool” field study protocol was used to estimate the amount of beach sand transported on the bodies of humans, and to estimate the fecal indicator bacteria released from this sand. The “small pool” experimental protocol used in the current study was identical to the Elmir et al. (2007) study, with the exception that the participants were toddlers in regular diapers (instead of adults), and that water samples were split for analysis of enterococci by MF, CS, and qPCR and for Bacteroidales HF8 and UCD markers. As in the prior study, the sediment was analyzed only by MF because the small sand sample size did not permit for the analysis of additional microbial parameters.

The “small pool” field study was conducted during two different dates in July and August of 2008. In brief, during this “small pool” study, each toddler wearing a bathing suit over diapers spent 15–30 min on the beach sand (e.g. playing, sitting, lying, walking, etc.). Thereafter, each individual

Download English Version:

<https://daneshyari.com/en/article/4485511>

Download Persian Version:

<https://daneshyari.com/article/4485511>

[Daneshyari.com](https://daneshyari.com)