Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Comparing different methods for fast screening of microbiological quality of beach sand aimed at rapid-response remediation

Renan C. Testolin [a], Tito C.M. Almeida [b], Marcus Polette [c], Joaquim O. Branco [d], Larissa L. Fischer [a], Guilherme Niero [a], Gabriel Poyer-Radetski [e], Valéria C. Silva [e], Cleder A. Somensi [f], Albertina X.R. Corrêa [a], Rogério Corrêa [a], Leonardo R. Rörig [g], Ana Gabriela Itokazu [g], Jean-François Férard [h], Sylvie Cotelle [h], Claudemir M. Radetski [a,*]

[a] Universidade do Vale do Itajaí, Laboratório de Remediação Ambiental, Rua Uruguai, 458, Itajaí, SC 88302-202, Brazil
[b] Universidade do Vale do Itajaí, Laboratório de Ecologia de Comunidades, Rua Uruguai, 458, Itajaí, SC 88302-202, Brazil
[c] Universidade do Vale do Itajaí, Laboratório de Conservação, Gestão e Governança Costeira, Rua Uruguai, 458, Itajaí, SC 88302-202, Brazil
[d] Universidade do Vale do Itajaí, Laboratório de Ecologia de Comunidades e Biodiversidade, Rua Uruguai, 458, Itajaí, SC 88302-202, Brazil
[e] Instituto Federal de Santa Catarina, Curso Técnico de Saneamento, Av. Mauro Ramos, 950, Florianópolis, SC, 88020-300, Brazil
[f] Instituto Federal Catarinense, Laboratório de Microbiologia, Rodovia BR 280, km 27, Araquari, SC 89245-000, Brazil
[g] Universidade Federal de Santa Catarina, Laboratório de Ficologia, Campus Trindade, Florianópolis, SC 88040-970, Brazil
[h] Université de Lorraine, Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC), CNRS UMR, 7360, Rue du General Delestraint, Metz F-57070, France

ARTICLE INFO

Article history:
Received 14 December 2016
Received in revised form 15 February 2017
Accepted 24 February 2017
Available online xxxx

Keywords:
Beach sand quality
Microbiological sand contamination
Colorimetric method
Fluorescein
Tetrazolium salt

ABSTRACT

There is scientific evidence that beach sands are a significant contributor to the pathogen load to which visitors are exposed. To develop beach quality guidelines all beach zones must be included in microbiological evaluations, but monitoring methods for beach sand quality are relatively longstanding, expensive, laborious and require moderate laboratory infrastructure. This paper aimed to evaluate the microorganism activity in different beach zones applying and comparing a classical method of membrane filtration (MF) with two colorimetric screening methods based on fluorescein (FDA) and tetrazolium (TTC) salt biotransformation to evaluate a new rapid and low-cost method for beach sand microbiological contamination assessments. The colorimetric results can help beach managers to evaluate rapidly and at low cost the microbiological quality of different beach zones in order to decide whether remedial actions need to be adopted to prevent exposure of the public to microbes due to beach sand and/or water contamination.

1. Introduction

Due to intensive land use in the coastal zone and to the economic importance of beaches, concern regarding environmental and public health has increased considerably in recent years. To address this issue, actions have emerged internationally to improve the sustainable recreational use of beaches, such as the Blue Flag program, aimed at connecting the public with their surroundings and encouraging them to learn more about their environment (ABAE, 2011). With respect to beach sand quality, it is well established that microorganisms can be naturally present (indigenous biota) or originate from anthropogenic contamination (sanitary discharges, runoff). There are many different types of microorganisms that humans can be exposed to through contact with water or sand, such as bacteria, viruses, fungi, protozoans and trematodes (WHO, 2003; Whitman et al., 2014; Heaney et al., 2012; Solo-Gabriele et al., 2016).

Unfortunately, in the microbiological monitoring of beaches worldwide only water quality is considered, despite several authors and organizations highlighting the urgent need for the assessment of beach sand quality (Mendes et al., 1993; WHO, 2003; Sabino et al., 2014; Whitman et al., 2014; Solo-Gabriele et al., 2016). For instance, there are no public policies associating epidemiological data with the health problems caused by primary skin contact with contaminated sands. However, correlations between beach sand exposure and infectious disease have been identified (Phillips et al., 2011; Heaney et al., 2012; Solo-Gabriele et al., 2016), and it should be emphasized that microbiologically contaminated water can contaminate the sand where children often spend most of their leisure time on the beach (Wade et al., 2010; Solo-Gabriele et al., 2016). An additional issue is that only a few studies have investigated a relationship between the water quality and the quality of the wet sand (intertidal zone) or dry sand (supratidal zone), and some of these have shown contradictory results (Chabasse et al.,

* Corresponding author.
E-mail address: radetski@univali.br (C.M. Radetski).

http://dx.doi.org/10.1016/j.marpolbul.2017.02.069
0025-326X/

1986; Aulicino et al., 1985; Roses-Codinachs et al., 1988; Sato et al., 2005).

It should be noted that sand quality is not dependent solely on water quality. The use of the sandy beach zones by a large number of visitors can lead to the proliferation of microorganisms nourished by food waste, which can be aggravated by the lack of good personal hygiene habits and sometimes by the presence of domestic animals (Mancini et al., 2005; Roca et al., 2009). Also, it is well known that floating solid waste can be brought to the beach by the seawater, affecting the visitors' perception of the beach quality (Santos et al., 2009; Díaz-Mendoza et al., 2014). In this regard, WHO have provided scientific evidence that beach sands can contribute significantly to the exposure of visitors to microbes through direct contact between beach sand and human skin, which can lead to health problems (Sabino et al., 2014; Lamparelli et al., 2015).

From the management point of view, very few initiatives have been adopted internationally in relation to beach sand quality. In Brazil, Rio de Janeiro is the only city that has embraced a classification for recreational sands with primary contact based on the results of the densities of total coliforms and *Escherichia coli* (SMAS, 2010). Furthermore, the methods currently available for monitoring the microbiological quality of beach sand are relatively time-consuming (e.g., microorganism cultivation in Petri dishes) and expensive (e.g., quantitative polymerase chain reaction (qPCR)). In this regard, microorganism communities in beach sand are heterogeneous and include bacteria, viruses, protozoa, helminthes (worms) and fungi, which require, in some cases, specific procedures and at least moderate laboratory infrastructure for their identification and/or quantification (WHO, 2003; Solo-Gabriele et al., 2016).

It thus seems reasonable to propose a tiered approach to beach management, starting with a screening method for microbiological contamination prior to applying a method that considers a direct connection with human health. Thus, to ensure effective beach management, municipal managers should consider applying a sand cleaning process at the end of the day on highly frequented beaches. This could involve simply sweeping the sand and/or turning it over. This is pertinent with regard to the situation in Santa Catarina State (Southern Brazil, Lat. 25°58′ S to 29°19′S), which has 36 coastal touristic municipalities, encompassing around 600 km of coastline. There are hundreds of beaches with different morphodynamics, and the intensity of visitors varies considerably (Polette and Vianna, 2006). Every year, the beach towns together receive >7 million visitors during the summer season, which is economically important for the coastal region (Polette and Vianna, 2006). In 2015–2016, two beaches located in Santa Catarina State had the Blue Flag certification granted by the Foundation for Environmental Education (http://www.blueflag.global/), and a third is in the pilot stage of the certification process. In addition, the State Environmental Protection Agency (FATMA-SC) carry out weekly microbial monitoring during the summer season to assess the water quality of beaches in 27 municipalities, where 211 sampling points are evaluated. However, a rapid and simple test could also be conducted to determine if sand zones are microbiologically contaminated, requiring a more powerful sand remediation process. In this context, the goal of this study was to evaluate the microorganism activity in different beach zones (water, wet sand and dry sand) applying and comparing two colorimetric screening methods based on fluorescein and tetrazolium salt biotransformation, using as a reference the classical method of membrane filtration employing Colilert® as a chromogenic substrate. To achieve this goal, 40 sites were tested with the colorimetric and classical methods. It was ensured that these sites had different characteristics: 20 had passed and 20 had failed recent government microbial contamination tests to determine bathing water quality. Each group of 20 sites was subdivided into two groups of 10 sites according to the level of frequency by visitors. A rapid and low-cost colorimetric method was developed, which could help beach managers to evaluate microbial contamination levels as well as to make decisions regarding the need to undertake remedial actions.

2. Material and methods

2.1. Beach locations and water/sand microbiological classification

Water, wet sand (intertidal beach zone) and dry sand (supratidal beach zone) samples were collected from 40 sites located on 36 beaches along the Santa Catarina coast. Table 1 shows the georeferenced data (WGS84 *datum*) and the bathing quality according to the Brazilian Resolution CONAMA 274/2000. The sand samples from the 36 beaches showed similar granulometric characteristics, being comprised of fine sand without rocks, stones or agglomerated organic matter. Of the 40 sites, 20 had failed recent government microbial contamination tests carried out to determine bathing water quality. Of these, 10 sites were not frequented by visitors (sites 1 to 10, denominated CNF) and 10 sites received a low frequency of visitors (sites 11 to 20, denominated CLF). At the other 20 sites, the water had passed recent microbial contamination tests and of these 10 received a low frequency of visitors (sites 21 to 30, denominated ULF) and 10 sites were highly frequented by visitors (sites 31 to 40, denominated UHF). The same sample mass (solid or water) was used in all analysis to ensure an appropriate comparison. Recent beach monitoring data published by the State Environmental Protection Agency (FATMA; http://www.fatma.sc.gov.br/laboratorio/dlg_balneabilidade2.php) was used to select the sites used in this study based on whether they had passed or failed bathing water quality tests.

Water quality is generally classified according to the nature of the water usage. Brazilian legislation, such as CONAMA Resolution 274/2000, determines that seawater is appropriate for recreational use if 80% or more of the samples collected at the same location for the five consecutive weeks contain <1000 thermotolerant coliforms on average, or <800 *E. coli* per mL, or <100 *Enterococcus* per 100 mL. Seawater is considered inappropriate (representing a health risk) if this requirement is not met or if the last sample collected contains over 2500 thermotolerant coliforms, or >2000 *E. coli* per mL, or >400 *Enterococcus* per 100 mL (CONAMA, 2000). The reasoning behind the setting of particular guideline levels by Brazilian regulators has a historical origin. Prior to the creation of Brazilian environmental agencies, marine pollution projects considered the "California standard" (1940s) of 1000 total coliforms per 100 mL. Later, Brazilian regulators adopted this guideline value in the CONAMA Resolutions (including 274/2000). It seems that this value originated from strictly esthetic considerations. Subsequent studies in the USA on *Enterococci* and *E. coli* bacteria present in recreational waters led CONAMA to adopt these parameters in their resolutions as additional indicators of recreational water quality.

With respect to the microbiological classification of beach sands, to the best of our knowledge, despite proposals put forward by researchers (e.g., Mendes et al., 1993; ABAE, 2011), Rio de Janeiro is the only Brazilian city which has a Municipal Resolution (SMAC Resolution 468/2010) related to this issue (SMAC, 2010). Table 2 shows the numeric values for the microbiological classification of beach sand. The guideline values established by this Resolution are based on a study on the sanitary quality of beach sands in Portugal (Azores archipelago), which was carried out in 1993/1995 by Mendes et al. (1993), and also on a pilot project carried out previously by SMAC, which established the colimetric standards based on the analysis of sand on beaches subjected to low levels of anthropogenic impact. It should be noted that the values proposed by Mendes were also adopted by the European Blue Flag Association (ABAE, 2011). In addition, this Municipal Resolution recommends no primary contact with sand that has signs of pollution (perceived via odors or visually).

2.2. Water and sand sampling

Water samples were collected according to the standard procedure described in APHA et al. (2012). Sand samples, both wet and dry, were collected according to the SMAC (2010) protocol, which is summarized

Please cite this article as: Testolin, R.C., et al., Comparing different methods for fast screening of microbiological quality of beach sand aimed at rapid-response remediation, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.02.069