Microbiological quality assessment of sand and water from three selected beaches of South Coast, São Paulo State, Brazil

ABSTRACT
This study aimed to assess the sanitary quality of water, and wet and dry sand from three beaches located in the South Coast region of São Paulo State, Brazil, selected taking into account the frequency of tourists and the water quality (good, fair and poor). Thirty-six water samples each of wet and dry sand and seawater were collected monthly over a period of one year and analyzed for fecal indicator bacteria (FIB: thermotolerant coliforms, Escherichia coli, and enterococci), presumptive Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and dermatophytes. The results revealed FIB concentrations more elevated in dry sand followed by wet sand and water. P. aeruginosa and presumptive S. aureus were detected with a similar frequency in water and sand samples, but maximum concentrations and geometric means were higher in dry sand. C. albicans was detected only in water samples whereas the dermatophyte Microsporum sp. was isolated exclusively from dry and wet sand samples. This evaluation showed also that the environment had a significant influence on P. aeruginosa but not on presumptive S. aureus concentrations. According to threshold values proposed in the literature for E. coli and enterococci dry sand densities, none of the beaches would be considered of sufficient quality for recreational activities.

Key words | beach sand, fecal indicators, marine recreational waters, pathogens, sanitary quality

INTRODUCTION
Poor quality of coastal recreational seawaters has been associated with swimming-related illness, caused mainly by pathogenic microorganisms from fecal origin. Population exposure to contaminated marine recreational waters may cause 120 million cases of gastrointestinal disturbances and 50 million cases of respiratory diseases per year (Shuval 2003; Yahamara et al. 2009; Abdelzaher et al. 2010). Waterborne gastroenteritis outbreaks in swimmers occur more frequently during summer, when there is an increase of sewage discharge to seawater due to the higher number of tourists in coastal cities where beaches are located.

Marine waters are susceptible to fecal contamination from sewage, polluted rivers that flow into the sea, recreational users and wild and domestic animals. The control of these sources is essential to reduce the densities of pathogenic microorganisms. However, beach sand quality should also be taken into account as recent studies have reported elevated concentrations of fecal indicator bacteria (FIB) as well as pathogenic microorganisms in the sand (Sanchez et al. 1986; WHO 2003; Ghinsberg et al. 2004; Sato et al. 2005; Vantarakis et al. 2005; Bonilla et al. 2007; Vogel et al. 2007; Abdelzaher et al. 2010). Recently, epidemiological studies have also been performed to evaluate the illness risks associated with beach sand contact (Bonilla et al. 2007; Stone et al. 2008; Wade et al. 2008; Heaney et al. 2009). The behavior of bathers may have a direct influence on the quality of sand; therefore environmental education plays a fundamental role to promote a better quality of recreational areas and to protect health. Management actions, such as the cleaning of the sand, are very important too.

Fecal pollution of the São Paulo State coast is of particular concern as this region consists of densely populated
cities which are visited by large numbers of bathers and swimmers during weekends and summer months due to the proximity of the Great São Paulo Metropolitan area. Inadequate sanitation conditions prevail in these urban areas, leading to elevated amounts of sewage launched to the sea. According to SEADE’s estimates, in 2010, tourists represented an increase of 19.4% in the Santos population, 15.5% in São Vicente and 65.5% in Guarujá (SEADE – State Foundation of Data Analysis System 2004).

Inadequate sanitation conditions prevail in these urban areas leading to elevated amounts of sewage launched to the sea. Enterococci have been used by the Environmental Agency of São Paulo State (CETESB), Brazil, to evaluate the recreational water quality of São Paulo State Coast since 2003. According to the criteria established by a Federal Brazilian regulation in 2000, beach water is considered proper for recreational use if 80% of five weekly samples present less than 100 enterococci/100 mL. This regulation also recommends environmental agencies to assess the parasitological and microbiological quality of beach sands in order to gather information for the establishment of future standards.

Previous studies conducted by CETESB (Sanchez et al. 1986; Sato et al. 2005) revealed elevated densities of fecal indicators and parasites in the sand of beaches from North and South Coast of São Paulo. Considering this previous data and the importance of South Coast beaches as recreational areas, a new study was designed to measure the levels of FIB commonly associated with diarrheal diseases (thermotolerant coliforms, Escherichia coli and enterococci) and other microorganisms responsible for skin and mucous membrane infections (Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and dermatophyte fungi) at three beaches located in a densely populated region of South Coast, known as ‘Baixada Santista’.

**METHODS**

**Study sites**

Three beaches located at Baixada Santista (South Coast), 60 km from Greater São Paulo, were studied (Figure 1). The sites were selected taking into account the high frequency of tourists, especially in the summer, and the annual classification of the beaches. Beaches that fail an 80 percentile of 100 enterococci/100 mL (calculated for five samples collected and analyzed for five consecutive weeks) are classified as non-compliant or improper for bathing. The beaches are further evaluated according to the time they remain improper for bathing. Gonzaguinha Beach (A) was improper 100% of the time, whereas Boqueirao Beach (B) and Pitangueiras Beach (C) were proper 52% and 100% of the time, respectively.

**Sampling**

Samples of water, and wet and dry sand were taken monthly, on Sundays, from January to December 2009. Water samples were collected in sterile 2 L wide-mouth plastic bottles according to APHA Standard Methods (APHA 2006a). Samplings were performed on Sundays because Brazilian regulation states that samples should be taken on days of higher presence of people on the beaches. The water sampling was performed at 1 m depth, according to the Brazilian regulation (Brazila 2000). In order to perform a

![Figure 1](https://iwaponline.com/wst/article-pdf/66/11/2475/440984/2475.pdf)
representative sampling, 500 g of sand were collected from a previously delimited area of 2 m², being composed of five cores of 100 g taken from the superficial layer (5 cm) of different regions within this area and pooled in sterile plastic bags. Dry sand was collected in non-flooded areas (above high tide line) and wet sand was sampled in an intermediate area between the dry sand and the seawater (intertidal zone), in sites where bathers could be found. The samples were kept chilled during transportation and analyzed within a 24 h period.

**Bacteria enumeration**

**Water**

The membrane filtration technique was used to determine thermotolerant coliforms, *E. coli*, enterococci and presumptive *S. aureus* densities, using mFC agar (APHA 2005), modified mTEC agar, Baird Park agar (APHA 2007a) and MEI agar (APHA 2007b), respectively. *P. aeruginosa* was also analyzed by the membrane filtration technique using mPA-B agar (Dutka & Kwan 1977).

**Sand**

Sand samples (100 g) were blended with 900 mL of dilution water for 1 min at low speed (APHA 2006a) and analyzed by the multiple tube technique (APHA 2006b) for thermotolerant coliforms and enterococci using A1 medium and azide-dextrose broth, respectively, and for *P. aeruginosa* (APHA 2007b) using asparagine and acetamide broth. Positive cultures from A1 medium were inoculated into EC-MUG tubes in order to obtain the most probable number of *E. coli*. The most probable number of presumptive *S. aureus* was determined in Trypticase Soy Broth containing 10% NaCl and 1% sodium pyruvate (FDA 1998).

**Fungal analyses**

*C. albicans* in the sand and water samples was determined by the membrane filtration technique according to Ghinsberg et al. (1994). Dermatophyte analyses in water samples were performed by membrane filtration (0.45 μm pore size, 47 mm diameter, mixed ester membranes), whereas the spread plate technique was employed for sand samples, using Mycozel Agar for both techniques. After an incubation period of 1–4 weeks at 25–30 °C, typical colonies were cultivated in Sabouraud Dextrose Agar at 25–30 °C for 5–7 days. Fungal colonies were then submitted to a 15-day macroscopic evaluation considering growth velocity, diameter, size, and color, and general characteristics such as border aspect, media pigmentation and consistency of the colony. Microscopic evaluation was done using a colony fragment, microculture in slide (Lacaz et al. 1984). The sand samples were also analyzed by the hair bait technique (Ajello and Georg, 1957 cited in Lacaz et al. 1984): sterilized horsehair was sprinkled on a wet sand portion (approximately 50 g) in a Petri plate. From the visible fungi growth, the horsehair was transferred to Mycozel Agar and submitted to the same macroscopic and microscopic evaluations described above.

**Statistical analysis**

The Lambda Multivariate test of Wilks was used to study the concentrations of microorganisms in relation to four factors: environment (water, dry and wet sand), month of collection, site (three beaches) and the occurrence of rain 24 h prior to collection. The F-Fischer test was used to determine the factors which influenced each microorganism (environment, month of collection, site or rain event). The statistical package used for analysis was SPSS software version 12.

**RESULTS AND DISCUSSION**

Over the course of the one-year study 36 seawater samples and 72 sand samples were collected and analyzed for thermotolerant coliforms, *E. coli*, enterococci, *P. aeruginosa*, presumptive *S. aureus*, *C. albicans* and dermatophyte fungi at three beaches from the South Coast of São Paulo State.

The geometric average, minimum and maximum concentrations of the FIB for water and sand samples in the three beaches are presented in Table 1. The statistical analysis of the results revealed a similar behavior of FIB concentrations in the three environments of the beaches: higher densities were detected in dry sand, followed by wet sand and water. This evaluation showed also that the environment had a significant influence on *P. aeruginosa* ($P = 0.003$) but not on presumptive *S. aureus* concentrations ($P = 0.223$). Beach C, presented the more elevated densities (geometric mean as well maximum concentrations) of FIB in dry sand, compared with the two other beaches. These results can be explained by the existence of a greater number of facilities on this beach, such as malls, public restrooms, and public square, among others. These facilities provide a shelter area for a permanent homeless population.
and a high concentration of floating population during holidays and summer vacations.

Other authors also found similar relationships between FIB densities in seawater and dry sand samples. Sato et al. (2005) reported more elevated concentrations of thermotolerant coliforms ($10^3$–$10^6$ MPN/100 g) and fecal streptococci ($10^3$–$10^5$ MPN/100 g) concentrations in dry sand during summer in 14 beaches from the North and South Coast of São Paulo State. The prevalence and distribution of FIB in Florida beach sand was evaluated by Bonilla et al. (2007). The authors demonstrated that the bacteria were concentrated in 100 g of sand (2–23 fold in wet sand and 30–460 fold in dry sand) compared with 100 mL samples of water. E. coli densities in sand and water samples from five locations in Chicago, Illinois, were assessed by Whitman et al. (2009). They found E. coli densities ranging from $3.3 \times 10^3$ to $1.8 \times 10^5$ MPN/100 g in sand samples and from 17 to $2.2 \times 10^5$ MPN/100 mL in the water.

The occurrence of rain 24 h prior to sample collection influenced FIB and P. aeruginosa concentrations leading to a reduction of these organisms in dry sand and to an increase in the water or in the wet sand. This indicated the transfer of microorganisms from dry sand to wet sand and water. Tides may have a similar influence on the concentrations of microorganisms in these environments. A study conducted by Abdelzaher et al. (2010) at a subtropical recreational marine beach from California detected FIB concentrations in wet and dry sand samples collected at low and high tides, that demonstrated an indicator microbe wash-in from the shoreline. Bonilla et al. (2007) discuss the tide effects on microbial concentrations in water, dry and wet sand and also propose that less predation by macroinvertebrate consumers and larger protozoa should take place in dry sand due to the reduced water content of this environment.

Regarding compliance with recreational water regulations and guidelines for enterococci densities, only Beach C would be considered of suitable quality for bathing (Brazil 2000; WHO 2003; EP 2006). None of these beaches was in compliance with the USA regulation (USEPA 2004), and beaches A and B fell in a category that involves significant risk of high levels of minor illness transmission ($>10\%$ of gastrointestinal illness risk and $>3.9\%$ of acute febrile respiratory illness) according to the World Health Organization (WHO 2003).

Table 2 shows the frequency of positive samples, geometric average, minimum and maximum concentrations of presumptive S. aureus and P. aeruginosa for water and sand collected from the three beaches. The frequency of detection of these bacteria was quite similar for water and sand samples, but maximum concentrations and geometric mean were more elevated in dry sand. Beach A presented the highest percentage of positive seawater samples for
presumptive *S. aureus* and *P. aeruginosa*, a result already expected as this beach had the poorest microbiological quality. *C. albicans* was detected only in water samples from Beach A and B (54.5 and 91.1%, respectively), in concentrations ranging from 1 to 4 CFU/500 mL.

Sanchez et al. (1986) detected few positive samples for *P. aeruginosa* in 155 water samples from eight beaches of São Paulo State South Coast in low concentrations (<10 MPN/100 mL). On the other hand, they reported concentrations exceeded 100 MPN/100 mL for *P. aeruginosa* in 45.6% out of 171 sand samples analyzed, and very high concentrations (>10⁴ MPN/100 g) in 18 of these samples. CETESB (2010) reported high concentration of *P. aeruginosa* (10² CFU/100 mL) in water samples and dry and wet sand samples (10⁵ MPN/100 g) as well. Abdallah et al. (2005) found *P. aeruginosa* concentration in the order of 10⁵ CFU/100 g in wet sand samples.

Ghinsberg et al. (1994) reported a higher frequency of *S. aureus*, *P. aeruginosa* and *C. albicans* positive samples in the sand of Tel Aviv beaches. The authors of the two studies highlight the importance of such results, because these are potentially pathogenic microorganisms and may cause skin and mucous membrane infections for people with prolonged contact with sand.

Dermatophytes were detected in 16.7% of the wet sand samples from the three beaches, and in 25, 16.7 and 8.3% of the dry sand samples from Beach A, B and C, respectively. Seawater samples were negative for these microorganisms. The only genus identified was *Microsporum* sp. in the wet and sand samples from beach A, B and C. It is interesting to add these fungi grew only when the horsehair bait technique was used.

During a five-year study conducted at 33 beaches along the Portuguese coast, Sabino et al. (2011) reported 14.3% of dermatophyte positive samples (the predominant genus was *Trichophyton* sp.) out of 495 sand samples.

The results obtained in the present evaluation are in agreement with previous studies conducted by CETESB (Sanchez et al. 1986; Sato et al. 2005) and also with other authors who reported higher densities of FIB in dry sand compared with wet sand and seawater (Bonilla et al. 2007; Whitman et al. 2009). As many people, especially children, stay longer in the sand, there is a potential health risk of exposure to fecally transmitted pathogens. Whitman et al. (2009) using *E. coli* and MS2 coliphage as surrogates demonstrated the transference of these microorganisms from contaminated sand to the human skin. A large prospective cohort study conducted by Heaney et al. (2009) showed an

### Table 2

<table>
<thead>
<tr>
<th>Beaches</th>
<th>Water Presumptive S. aureus Frequency (%)</th>
<th>CFU/100 mL GM</th>
<th>Min.</th>
<th>Max.</th>
<th>P. aeruginosa Frequency (%)</th>
<th>CFU/100 mL GM</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41.7</td>
<td>5.5</td>
<td>&lt;1</td>
<td>7.0 × 10²</td>
<td>100.0</td>
<td>9.4</td>
<td>&lt;1</td>
<td>23</td>
</tr>
<tr>
<td>B</td>
<td>25.0</td>
<td>2.3</td>
<td>&lt;1</td>
<td>10²</td>
<td>83.3</td>
<td>4.6</td>
<td>&lt;1</td>
<td>28</td>
</tr>
<tr>
<td>C</td>
<td>33.3</td>
<td>1.9</td>
<td>&lt;1</td>
<td>20</td>
<td>83.3</td>
<td>2.8</td>
<td>&lt;1</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td><strong>Beaches Wet sand Presumptive S. aureus Frequency (%)</strong></td>
<td><strong>MPN/100 g GM</strong></td>
<td><strong>Min.</strong></td>
<td><strong>Max.</strong></td>
<td><strong>P. aeruginosa Frequency (%)</strong></td>
<td><strong>MPN/100 g GM</strong></td>
<td><strong>Min.</strong></td>
<td><strong>Max.</strong></td>
</tr>
<tr>
<td>A</td>
<td>25.0</td>
<td>3.6</td>
<td>&lt;1</td>
<td>2.3 × 10³</td>
<td>58.3</td>
<td>8.8</td>
<td>&lt;1</td>
<td>140</td>
</tr>
<tr>
<td>B</td>
<td>25.0</td>
<td>2.2</td>
<td>&lt;1</td>
<td>40</td>
<td>75.0</td>
<td>17.4</td>
<td>&lt;1</td>
<td>150</td>
</tr>
<tr>
<td>C</td>
<td>16.7</td>
<td>1.6</td>
<td>&lt;1</td>
<td>20</td>
<td>41.7</td>
<td>4.3</td>
<td>&lt;1</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td><strong>Beaches Dry sand Presumptive S. aureus Frequency (%)</strong></td>
<td><strong>MPN/100 g GM</strong></td>
<td><strong>Min.</strong></td>
<td><strong>Max.</strong></td>
<td><strong>P. aeruginosa Frequency (%)</strong></td>
<td><strong>MPN/100 g GM</strong></td>
<td><strong>Min.</strong></td>
<td><strong>Max.</strong></td>
</tr>
<tr>
<td>A</td>
<td>41.7</td>
<td>9.9</td>
<td>&lt;1</td>
<td>1.2 × 10³</td>
<td>66.7</td>
<td>21.0</td>
<td>&lt;1</td>
<td>7.0 × 10²</td>
</tr>
<tr>
<td>B</td>
<td>33.3</td>
<td>5.2</td>
<td>&lt;1</td>
<td>1.7 × 10⁴</td>
<td>83.3</td>
<td>69.1</td>
<td>&lt;1</td>
<td>9.4 × 10³</td>
</tr>
<tr>
<td>C</td>
<td>33.3</td>
<td>9.8</td>
<td>&lt;1</td>
<td>7.0 × 10³</td>
<td>83.3</td>
<td>86.6</td>
<td>&lt;1</td>
<td>3.3 × 10³</td>
</tr>
</tbody>
</table>

GM: Geometric mean; Min.: Minimum; Max.: Maximum.
elevated risk of enteric disease for beachgoers who had contact with the sand (defined as digging in the sand and being buried in the sand). The authors could not find an association between sand contact and non-enteric illnesses and report that site-specific factors may play a role in the risk of disease following sand exposure. A pilot epidemiological study conducted by Bonilla et al. (2007) demonstrated that gastrointestinal illness in beach users was associated with water and intertidal sand exposure, whereas upper beach sand contact did not lead to health effects. A cohort prospective epidemiological study conduct by CETESB during the summer of 1999 at beaches of São Paulo Coast showed that the simple contact with the sand was a risk factor for the manifestation of diarrheal symptoms (data not published).

Previous studies (Mendes et al. 1993) as well as a recent regulation from the municipality of Rio de Janeiro (SMAC 2010) have proposed the establishment of sand quality guidelines based on monitoring data. Sabino et al. (2011) also question the lack of bacterial and mycological beach sand limits, as more beachgoers have contact with beach sand than with water.

The approach of Mendes et al. (1993) was to set imperative values for sand quality considering a limit of $10^4$/g, $10^5$/g and $10^6$/g for total coliforms (TC), fecal coliforms and E. coli, respectively, and 1/g for Candida sp. The regulation of Rio de Janeiro city classifies beaches according to TC and E. coli concentrations in sand samples, proposing threshold values of $3 \times 10^4$ MPN/100 g for TC and $3.8 \times 10^5$ MPN/100 g for E. coli. Sabino et al. (2011) suggest P95 limits of 15 CFU/g for yeasts, 17 CFU/g for potential pathogenic fungi, 8 CFU/g for dermatophytes, 25 CFU/g for E. coli and 10 CFU/g for enterococci. Taking into account these E. coli and enterococci limits, the dry sand of beaches A, B and C would not be in compliance, whereas, according to SMAC (2010) limits proposed for E. coli, 67, 33 and 25% of the dry sand samples from beaches A, B and C respectively would be considered ‘regular’ (Table 3). However, according to the results of many monitoring studies quoted by the World Health Organization (WHO 2003) ‘sand contamination is highly variable over short distances, making interpretation of results difficult’. Large variations of enterococci concentrations for sand samples collected over short distances were also reported by Bonilla et al. (2007).

Besides these restrictions to the establishment of such criteria, there is a lack of studies on sand dermal exposure and ingestion rates to allow the assessment of exposure and the estimation of health risk for beachgoers. It should be stressed, however, that the microbiological monitoring of beach sand quality should be periodically performed, even in the absence of quantitative criteria, in order to gather data for supporting characterization studies and estimating the health risk. Such results will also be useful to implement preventive measures or verify their effectiveness, such as the mechanical cleaning of the sand and educational campaigns for beachgoers and local traders. It is worth noting that WHO (2003) does not recommend the disinfection of sand but recommends the proper management of the coast instead.

**CONCLUSIONS**

The results obtained during this study demonstrated poor microbiological quality of the three beaches evaluated, except for water from site C, in which, apparently, sand and water quality are not related. For beach C, the existence of a great number of facilities, such as malls, public restrooms among others, appeared to play a more important role in sand quality. In agreement with other evaluations, higher microorganism concentrations were found in the dry sand regardless of rainfall events, followed by the wet sand, whereas the lowest concentrations were determined in the water. The low percentage of positive samples for

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**Table 3** | Beaches A, B and C FIB sand densities and standards proposed

<table>
<thead>
<tr>
<th>FIB</th>
<th>Limits</th>
<th>Reference</th>
<th>Beaches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>E. coli</td>
<td>P95  = $2.5 \times 10^5$ CFU/100 g</td>
<td>Sabino et al. (2011)</td>
<td>$1.3 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>$3.8 \times 10^5$ MPN/100 g$^a$</td>
<td>SMAC (2010)</td>
<td>67%$^b$</td>
</tr>
<tr>
<td>Enterococci</td>
<td>P95  = $10^5$ CFU/100 g</td>
<td>Sabino et al. (2011)</td>
<td>$2.5 \times 10^4$</td>
</tr>
</tbody>
</table>

$^a$Individual value.

$^b$Percentage of individual values in compliance with the proposed limits.
S. aureus, C. albicans and dermatophytes demonstrates that microorganisms associated with skin illness are not a great concern in these environments. On the other hand, based on the results for FIB and P. aeruginosa, sanitary and educational measures need to be addressed to improve sand quality and to minimize health risks. Although some preliminary studies indicate adverse health effects associated with contaminated sand exposure, the adoption of quality criteria, as proposed by some authors, still faces difficulties.

REFERENCES


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