

MICROBIAL CONTAMINATION OF SAND FROM MAJOR BEACHES IN FORTALEZA, CEARÁ STATE, BRAZIL

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ABSTRACT

The presence of faecal contamination and pathogenic microorganisms in samples of dry and wet sand collected from three major beaches in Fortaleza, Ceará State, Brazil: (Praia do Mucuripe, Praia do Futuro and Praia do Caça e Pesca), during the period of May 1999 to January 2000 was evaluated. Praia do Caça e Pesca had the highest incidence of *E. coli* in dry sand (56%) followed by Praia do Mucuripe (28%) and Praia do Futuro (16%). In wet sand, results were 48%, 28% and 24% for Praia do Caça e Pesca, Praia do Futuro and Praia do Mucuripe, respectively. Only two samples from Praia do Futuro, one from dry sand and another one from wet sand, were positive for *Salmonella*. *V. parahaemolyticus* was isolated from four samples from Praia do Caça e Pesca (two from dry-sand samples and two from wet-sand), one from Praia do Futuro (wet sand), and three and four from Praia do Mucuripe (wet and dry sand, respectively). Yeasts belonged to the *Candida* genus. Dry-sand samples presented higher yeast contaminations level than wet-sand ones. Praia do Futuro had the highest level of yeast contamination (41%), followed by Praia do Caça e Pesca (33%) and Praia do Mucuripe (26%).

Key words: sand contamination, yeasts, faecal coliforms, *Vibrio parahaemolyticus*, *Salmonella*

INTRODUCTION

According to the last census, Fortaleza is the fifth largest Brazilian city in terms of population. The city has approximately 2.2 million inhabitants and c.a. 70% is served by sewage collection system. The sewage is discharged in the ocean through a marine interceptor of 3,300 m length, at 15 m depth, after a "primary" treatment consisting of grating, screening and decantation of the raw sewage. According to data collected *in locus*, only 8% of this material finds its way to adjacent beaches. However, this quantity is sufficient to contaminate these areas (12,28,29).

Sandy beaches are regions of transition between the firm land and the sea, and as such they are subjected to significant influences from both ecosystems. These areas are exposed to sharp variations in the rates of sun exposure, immersion and submersion, rainfall and concentration of nutrients (17,18).

Fortaleza is a busy tourist destination. Local beaches are not only the main attraction for visitors, but also for residents. It is

therefore of a great public health concern that these areas are periodically evaluated in regard to their level of microbial contamination. The present work aimed to determine the microbiological quality of the sand of selected major beaches of Fortaleza. The evaluation focused on *Salmonella*, total and faecal coliforms, *Vibrio parahaemolyticus* and yeasts.

MATERIALS AND METHODS

Thirty samples of dry sand and thirty samples of wet sand were collected from three ocean beaches in Fortaleza (Praia do Futuro, Praia do Mucuripe and Praia do Caça e Pesca) between May 1999 and January 2000. Sampling was carried out on Mondays, in median regions of the beach.

Each sample consisted of 100 g of the surface sand collected at a maximum depth of 2 cm using a sterilised spatula. The material was transferred to a sterile container, transported to the laboratory and tested in less than 2 hours.

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For trials for *Salmonella*, 25 g of sand was weighed into 225 ml of 1% Lactose broth. One ml of this mixture was added to Selenite F and Rappaport medium modified according to Hofer and Kauffmann Tetrathionate broth (27). After 24 hours of incubation at 37°C, inocula were removed with a platinum loop and sown in Hektoen Agar and Holt Harris Teague Agar (Difco). Biochemical identification of typical colonies was made according to Costa and Hofer (3). The antigenic characterization was made through fast binding, employing somatic and flagellar antisera produced by the Department of Bacteriology of the Instituto Oswaldo Cruz, RJ, Brazil.

The ICMSF (8) methodology was adopted in the identification of faecal coliforms, and five colonies grown in EMB agar were taken for further identification. Yeasts were identified by the technique developed by Paula (16). The identification of *Vibrio parahaemolyticus* followed Twedt's procedure (26).

RESULTS AND DISCUSSION

In coastal areas, outbreaks of gastroenteritis, hepatitis, salmonellosis, viral illnesses and occurrence of dermatitis and mycosis during the summer have usually been associated with marine pollution. Recently these factors have also been correlated with the contamination of beach sands, resulting from wastes left by users and from solids deposited by the tides (13).

Among 92 strains isolated from selective media for *Salmonella*, only two were confirmed as *Salmonella*, both serotype *agona*. These originated from Praia do Futuro wet and dry sand samples taken in different days. According to Russ and Yanko (22) and Thomason *et al.* (25), the survival of bacteria in a particular ecosystem is affected by the amount of organic nutrients, the presence of competitive microbiota and physico-chemical factors. The presence of enteric bacteria in the sands of Praia do Futuro can be attributed to the high number of patrons of "Chico do Caranguejo" restaurant where a traditional crab dish is served. The crabs are caught in mangroves and held on the sands of Praia do Futuro prior to processing. It is known that many serotypes of *Salmonella* can survive for periods as long as several months when soil conditions are suitable. These conditions refer mainly to temperature, exposure to sunlight, humidity, pH and the presence of organic matter (20).

Although Sanchez *et al.* (24) were able to isolate *Salmonella* from seawater collected in São Paulo State, Brazil, these authors were not successful in attempts to isolate this enteric bacterium from the sand. On the other hand, Rodrigues *et al.* (21) found *Salmonella* in the water from beaches of Rio de Janeiro, from the inward to the outer area of the Guanabara Bay. The authors identified the *Salmonella agona* serotype at Copacabana and Governador Island which supports the likelihood of the sand-contamination, a finding also confirmed in this work. According to those authors, this serotype is extremely common in the

environment. Hofer and Costa (7) also identified this serotype in the sewage system of Niterói, Rio de Janeiro State.

We believe that the presence of this bacterium in wet sands of Praia do Futuro is associated with seawater contamination. The estuary of Cocó River, which flows through slums where houses are supported on stilts and the inhabitants' excreta are discharged directly into the river, is not very distant from the studied site. These untreated wastes are likely brought to adjacent beaches by a local marine current of an east-west coastal drift (15).

A total of 103 coliform colonies were isolated from the sixty samples analysed. Forty-six were confirmed as being *E. coli*, 53 *Enterobacter* and 4 *Citrobacter*.

The influence of marine currents on the contamination of sand has been proven once more when we analysed data from the remaining sampling sites. Contamination with *Escherichia coli* was detected in both wet- and dry-sand samples collected on all three beaches (Praia do Mucuripe, Praia do Futuro and Praia do Caça e Pesca).

The sands of Praia do Caça e Pesca contained the highest contamination with *E. coli* in both wet-sand and dry-sand samples (Table 1). This site is where the mouth of the estuary of the Cocó River is located. These data do not agree with those reported by Melo *et al.* (12), whose work on total and faecal coliforms on the beaches of Fortaleza showed lower values for this group in waters of Praia do Caça e Pesca. Since in the present work there was no quantification of the total and faecal coliforms, but only their identification, comparisons with Melo *et al.* (12) findings cannot be reliably made. In addition, seawater is toxic to coliforms due to its salinity (6). This was discussed by La Liberte and Grimes (10) who demonstrated that the extensive survival of *E. coli* in sediments can be attributed to fine particles in the soil and the high amount of organic material.

Vibrio parahaemolyticus is another bacterium isolated from the investigated sands. The numbers determined were very small. While many strains of Vibrionaceae were isolated (112), Table 1 shows that the presence of *V. parahaemolyticus* was confirmed in only two wet-sand samples (Mucuripe) and in three dry-sand samples (Mucuripe (2), and Caça e Pesca (1)). *Vibrio parahaemolyticus* is a marine bacterium that requires salt and organic matter to survive. According to Sakazaki *et al.* (23), in the case of contaminated food, the ingestion of at least 10⁶ cells is necessary for the process of gastroenteritis to take place, thus sand should not be a reason for concern. However, infection of open skin wounds would be, since Johnson *et al.* (9) reported a case of infection in the nail of the thumb of a patient who was hurt when opening oysters. Cells of *V. parahaemolyticus* were isolated from this wound.

Castrillón *et al.* (2) reported that yeasts are associated with a variety of terrestrial and aquatic substrates. Sometimes they may belong to specialised habitats and can be considered as indicators of pollution and potential sources of contamination. Lacaz (11) states that the variety of yeasts found in the soil and in seawater

Table 1. Occurrence of *E. coli*, *Salmonella*, *V. parahaemolyticus* and *C. albicans* in sand samples from three beaches in Fortaleza, CE (Mucuripe, Futuro and Caça e Pesca).

Beaches	sand samples (n)	POSITIVITY			
		<i>E. coli</i> n (%)	<i>Salmonella</i> n (%)	<i>V. parahaemolyticus</i> n (%)	<i>C. albicans</i> n (%)
Mucuripe					
Dry sand	10	6(60.0)		2(20.0)	1(10.0)
Wet sand	10	4(40.0)		2(20.0)	1(10.0)
Futuro					
Dry sand	10	3(30.0)	1(10.0)		4(40.0)
Wet sand	10	4(40.0)	1(10.0)		
Caça e Pesca					
Dry sand	10	8(80.0)		1(10.0)	1(10.0)
Wet sand	10	6(60.0)			1(10.0)
Total	60	31	2	5	8

plays an important role in the medical pathology, causing cutaneous mycosis. Correspondingly, many mycosis cases related to beach environments could be directly linked to fungal microbiota. In the samples from the three beaches, the isolated yeast species belonged to the genus *Candida*: *C. tropicalis*, *C. pseudotropicalis*, *C. albicans*, *C. glabrata*, *C. stellatoidea*, *C. parapsilosis* and *C. krusei*.

Of the collections conducted on three beaches, Praia do Futuro was the one that showed the highest percentage of isolated yeasts (41.03%), followed by Praia do Caça e Pesca (33.33%) and Praia do Mucuripe (25.64%). Higher amounts were isolated from dry sands than from wet sands. The yeast *Candida albicans*, the most frequent one in the samples, is considered important in public health since it is significantly more virulent than the other species of *Candida* (19).

Menezes *et al.* (14) investigated the occurrence of yeasts in seawater, and in dry and wet beach sands of the city of Fortaleza. They isolated seven genera of the following microorganisms: *Candida*, *Malassezia*, *Trichosporium*, *Rhodotorula*, *Torulopsis*, *Pichia* and *Hansenula*. The most frequently isolated genus in all samples was *Candida*.

Sanchez *et al.* (24) also isolated *C. albicans* in 32 of 171 samples of sand collected from some beaches in São Paulo. *Candida albicans* is an opportunist pathogen and has been associated with a wide variety of oral, vaginal and cutaneous infections in humans (4,5). Sanchez *et al.* (24) stressed the need of sanitary quality standards for sand of beaches for recreational use.

According to Vieira *et al.* (28), sea pollution results in an increased risk in the recreational use of beautiful beaches since it represents a threat for swimmers, surfers and for the people who depend on ocean resources.

Data in this study (Tables 1 and 2) characterized polluted beaches as those with faecal matter harmful to the swimmers, and mainly to children who play on beach sands. They run the

highest risk of contracting illnesses caused by bacteria and yeasts. Burton *et al.* (1) established considerably higher levels of pathogens in sea sediments than in seawater which amplifies the public health risks since this contamination can persist for a long time.

RESUMO

Contaminação microbiológica da areia de algumas praias de Fortaleza, Ceará, Brasil

No presente trabalho, avaliou-se a presença de contaminação fecal e de microrganismos patogênicos em areia seca e areia úmida de três praias de Fortaleza, Ceará: Praia do Mucuripe, Praia do Futuro e Praia do Caça e Pesca. A praia que apresentou maior índice de contaminação com *E. coli* em areia seca foi a do Caça e Pesca (56%), seguido das praias do Mucuripe (28%) e Futuro (16%). Em areia úmida a ordem decrescente de contaminação por *E. coli* foi Praia da Caça e Pesca, Futuro e Mucuripe com 48%, 28% e 24% de contaminação, respectivamente. *Salmonella* foi isolada de duas amostras da Praia do Futuro, uma de areia seca e outra de areia úmida. *V. parahaemolyticus* foi isolado em 2 amostras de areia seca e 2 de úmida da Praia da Caça e Pesca. Esse patógeno foi isolado de uma amostra de areia úmida da Praia do Futuro e de três de areia úmida e quatro de areia seca da Praia do Mucuripe. As leveduras encontradas em todas as amostras pertenciam ao gênero *Candida*. O índice de contaminação por leveduras foi maior em amostras de areia seca que de areia úmida. A praia que apresentou maior contaminação por leveduras foi a Praia do Futuro (41%), seguida da Praia do Caça e Pesca e (33%) e da Praia do Mucuripe (26%).

Palavras-chave: contaminação de areia, leveduras, coliformes fecais, *Vibrio parahaemolyticus*, *Salmonella*

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