

Regine H.S.F. Vieira¹
Dália P. Rodrigues²
Norma S.S. Evangelista¹
Grace N.D. Theophilo²
Eliane M.F. Reis²

¹Laboratório de Ciências do Mar, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil
²Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

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Correspondence to:
Regine H. S. F. Vieira. Laboratório de Ciências do Mar. Universidade Federal do Ceará. Av. da Abolição 3207. Meireles. 60165-081 Fortaleza. Ceará. Brazil.
Tel.: +55-852426422 ext. 221.
Fax: +55-852428355.
E-mail: regpoema@labomar.ufc.br

Colimetry of marine waters off Fortaleza (Ceará State, Brazil) and detection of enteropathogenic *Escherichia coli* strains

Summary Bacteriological analyses of seawater from three main beaches in Fortaleza, Brazil were performed during 1997. Thirty-six samples per beach were collected for a total of 108 samples. For Meireles Beach, 44% of the samples had MPN total coliforms values of at least 1100 or over 2400/100 ml, followed by Formosa and Diários beaches showing lower counts. For fecal coliforms the highest numbers were demonstrated for Formosa, followed by Meireles and Diários beaches in this descending order: 13.0%, 11.1% and 8.3%, respectively. *Escherichia coli* strains were identified in 76.8% of the 108 samples. Among 295 strains of *E. coli*, 21 belonged to serogroups O25, O26, O91, O112, O119, O158 and O164. Strains from serogroup O26 were tested using PCR, ELISA and Vero cells to detect Verotoxins VT1 and VT2 and all strains were negative. No LT and ST, as determined by ELISA and suckling mice assays, were detected among the 295 strains. All strains of *E. coli* were sensitive to ampicillin, cephalothin, gentamicin, tetracycline, sulfametox-trimethoprim, chloramphenicol and ciprofloxacin. Although the *E. coli* strains were not toxigenic, their presence in high numbers could be of public health significance.

Key words *Escherichia coli* · Enteropathogenic serogroups · Marine waters · Sewage disposal · Public health

Introduction

The city of Fortaleza (Ceará State, Brazil), with 2.2 million inhabitants, is currently disposing of its untreated sewage directly into the ocean. As municipal sewage invariably contains pathogenic bacteria, its collection and direct disposal into the ocean via dispersal pipes represents a health hazard to the population extensively using the coastal zone for recreation.

Prior to constructing a reasonably safe sewage ocean dispersal system, reliable studies of marine currents and their movements are mandatory. Constant monitoring of the operating dispersal system is necessary to avoid its damage and to establish the impact of continuous raw sewage discharge on the marine environment. In the recent local studies [7, 16], high Most Probable Number (MPN) values for total coliforms and fecal coliforms on the Fortaleza beaches close to the sewage discharge into the ocean were detected. Enteropathogenic *Escherichia coli* isolated and classified in these studies indicate the health hazard to swimmers off the beaches.

In the present research we followed the legislation of the (Brazilian) National Council for the Environment (CONAMA) in order to monitor the beaches through the evaluation of the MPN of total and fecal coliforms and to estimate the balneability grade of the beach seaside environment. Some strains of *E. coli* were isolated and enteropathogenic strains serotyped, checking their toxicity through the use of biochemical and molecular techniques.

Materials and methods

Bacteriological analyses of seawater from three main beaches in Fortaleza (Formosa, Meireles and Diários) (Fig. 1) were performed covering the period of nine months, from July 1996 to March 1997. Thirty-six samples per beach were collected for a total of 108 samples. The MPN of total and fecal coliforms per 100 ml of seawater was determined in each sample. Isolated strains were cultured on EMB agar and identified by conventional methods [1, 6, 13].

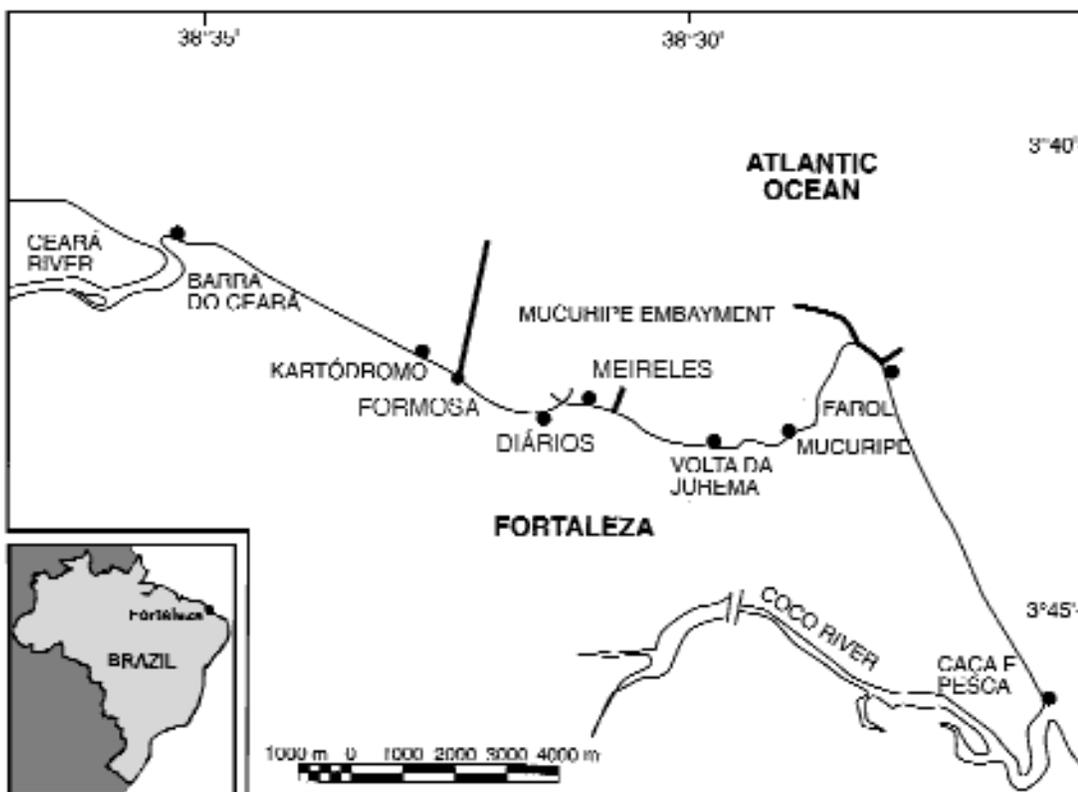


Fig. 1 Collection stations off Formosa, Diários and Meireles beaches (Fortaleza, Ceará, Brazil)

Subsequently, strains identified as *E. coli* were serotyped with regard to enteropathogenic groups, by using tube agglutination with specific antisera [2]. Those identified to belong to serogroup O26 were tested through the ELISA assay utilizing the reactive PREMIER VT test (Meridian Diagnostics) and cytotoxin effect on monolayers of Verocells grown in tissue cultures. In both assays the strains were grown on Trypticase Soy Broth (Difco) and filtrates of culture supernatants were used for the toxin assays [11]. The production of heat labile toxin (LT) and heat stable toxin (ST) was analysed in all isolated *E. coli* strains using ELISA [17] and suckling mice [3] assays, as well as assaying the toxin effects on monolayers of Vero cells. The PCR technique was used to detect verotoxins VT1 and VT2.

Standard procedures [9] were carried out in order to test the sensitivity of the isolated strains to the following antibiotics: ampicillin, cephalothin, gentamicin, tetracycline, sulfametox-trimetropin, chloramphenicol, ciprofloxacin.

Results and Discussion

The highest values for the MPN of total coliforms resulted for Meireles beach which yielded 16 positive samples (44.0% of the total) containing counts between 1100 and 2400/100 ml, followed by Formosa and Diários beaches showing lower counts. For fecal coliforms the highest numbers were demonstrated for Formosa, followed by Meireles and Diários beaches, in this descending order. Correspondingly, the number of samples

containing between 1100 and 2400 /100 ml of fecal coliforms for those beaches were 13.0%; 11.1% and 8.3% , respectively.

According to Number 20 Resolution of the Brazilian National Council for the Environment, from June 18, 1986, for coastal waters, a beach will be considered IMPROPER when 80% or more samples from a set collected at the same place in each of the last five weeks show the maximum of 1000 fecal coliforms/100 ml. These limits were exceeded at Formosa beach the fourth to the eighth weekly collection (Fig. 2). From the second month on, August, this beach could be considered at least SATISFACTORY.

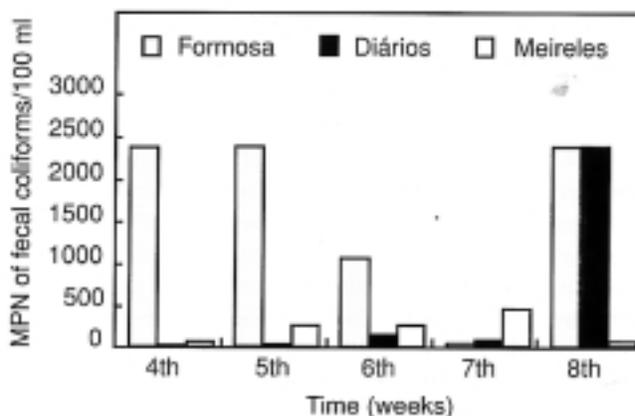


Fig. 2 Variation of the MPN of fecal coliforms per 100 ml in weekly samples of sea water collected off three beaches in Fortaleza (Ceará, Brazil)

Melo et al. [7] indicated a very polluted beach next to Formosa beach (named Kartodromo) that yielded a fecal coliform MPN of 4.3×10^6 /100 ml in March and 4.3×10^3 /100 ml in September. They related this level of contamination to the highly polluted discharge of the Jacarecanga river, also observed by Vasconcelos [15]. In addition to the submarine pipeline sewage dispersal system located close to Formosa beach, the beach also suffers the influence of the Jacarecanga River discharge, since it is located next to Kartodromo beach. In the same article, Vasconcelos [15] also reported that the Ceará River is more polluted during dry periods than during rainy periods. This observation reinforces our findings since the highest numbers for fecal coliforms were obtained in July and August, the rain-free months. The other beaches, Diários and Meireles, have pluvial systems linked to their waters which represent an additional factor contributing to the pollution of these beaches.

From the total of 108 seawater samples collected during the present work, 295 *Escherichia coli* strains were isolated. These strains were isolated from 84 seawater samples from the three beaches, which represent 77.8% occurrence of this fecal bacterium in the samples. Considering that members of the *Escherichia coli* species have a very low tolerance of seawater [4], their presence in the tested waters means a constant discharge of faeces. This is an indication of the beach environment contamination by other fecal-origin pathogenic bacteria such as *Salmonella*, *Shigella* and *Vibrio cholerae*, among others.

Among the 295 *E. coli* strains isolated from our seawaters samples 21 belonged to enteropathogenic groups O25, O26, O112, O119, O158 and O164. According to Nataro and Kaper [8] *E. coli* strains identified as an enteropathogenic agent belong to five distinct groups: enteropathogenic *E. coli* (EPEC); enteroaggregative *E. coli* (EAEC); enteroinvasive *E. coli* (EIEC); enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC). Despite our findings showing that the isolated strains belonged to enteropathogenic groups, tests by ELISA and suckling mice assays applied posteriorly on these strains presented a lack of virulence factors. No LT or ST was determined through those tests. Epidemiological investigations have implicated fecal contamination of water and food sources as the main reason for the high incidence of ETEC especially in developing countries [5]. Valentini et al. [14] also noted the absence of virulence factors in 38 *E. coli* strains, all of them belonging to the enteropathogenic group, isolated from 208 water samples.

Among the diarrheagenic *E. coli*, the EHEC group is a distinct class comprising over 200 serotypes that can express a potent cytotoxin and potential virulence factors encoded in the chromosome and approximately 60-MDa plasmids located in all EHEC strains. The most common non O157:H7 serotypes associated with human diseases include O26:H11, O103:H2, O111:NM and O113:H21 that lead to death and many different symptoms in patients infected with EHEC strains. In the present

study, the ability of the isolated strains from the serotype O26 to produce VT toxin was tested by ELISA and Vero cells culture techniques. Table 1 lists the diarrheagenic *E. coli* strains isolated from all the samples collected to this research. Most of them belonged to serogroup O26, most frequently occurring on Meireles beach. This serogroup was also identified among the strains isolated on Formosa beach. None of the isolated *E. coli* strains showed positive results in both tests used. This observation is in agreement with those reported for elsewhere in Brazil [10, 12] and suggests that the production of cytotoxin is not a common feature among *E. coli* O26 isolated in Brazil.

Table 1 Serogroups belonging to *Escherichia coli* enteropathogenic strains isolated from seawater samples collected off Formosa (A), Diários (B) and Meireles (C) beaches (Fortaleza, Ceará, Brazil)

Day of sampling	Samples*	Beach	Serogroups
24/05/96	2-1B	Diários	O91
22/08/96	8-3B	Diários	O112
22/08/96	8-1C	Meireles	O126
11/09/96	9-3B	Diários	O25
28/09/96	10-1A	Formosa	O164
28/09/96	10-3A	Formosa	O164
28/09/96	10-2C	Meireles	O158
05/10/96	11-1A	Formosa	O164
05/10/96	11-2A	Formosa	O164
05/10/96	11-3A	Formosa	O164
15/10/96	13-1C	Meireles	O26
15/10/96	13-2C	Meireles	O26
15/10/96	13-3C	Meireles	O26
09/11/96	16-1B	Diários	O112
10/12/96	19-3A	Formosa	O26
22/03/97	25-1C	Meireles	O26
22/03/97	25-3C	Meireles	O26
22/03/97	25-3C	Meireles	O119
07/06/97	35-1C	Meireles	O26
07/06/97	35-2C	Meireles	O26
07/06/97	35-3C	Meireles	O26

*A sample is identified as follow: the first figure stands for the weekly collection; the second figure stands for one of the three strains isolated from each beach; the letter indicates the beach.

All 295 *E. coli* strains isolated from the three beaches in this study were sensitive to ampicillin, cephalothin, gentamicin, tetracycline, sulfametox-trimethoprim, chloramphenicol and ciprofloxacin. Although these *E. coli* strains were not toxigenic, their presence in high numbers could be of public health significance, since beaches and other recreational areas affected by fecal effluents cannot be used for water-contact sports because of disease hazards. Apart from crude primary solids removal by grating, Fortaleza sewage is subjected to no significant treatment to prevent health hazards associated with raw sewage disposal into the marine environment. The resulting ocean pollution jeopardizes the recreational use of beautiful beaches, representing a threat to swimmers, surfers and people dependent on the exploitation of ocean resources.

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References

- Barnard RJ, McClure FD (1984) Most probable number determination. In: Bacteriological Analytical Manual (6th edn). Arlington, VI: Association of Official Analytical Chemists, pp III-06
- Ewing WH (1986) Edwards and Ewing's Identification of Enterobacteriaceae (4th edn) New York: Elsevier Science Publishers
- Dean AG, Ching YC, Williams RG, Harden LR (1972) Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. *J Infect Dis* 125:407–411
- Hagler AN, Mendonça-Hagler LC (1998) Indicadores microbiológicos de qualidade sanitária. In: Roitman I, Travassos LR, Azevedo JL (eds) Tratado de Microbiologia. São Paulo: Manole, pp 88–96
- Long KZ, Wood JW, Gariby JV, Weiss KM, Mathewson FJ, Wilson RA (1994) Proportional hazard analysis of diarrhea due to enterotoxigenic *Escherichia coli* and breast feeding in a cohort of urban Mexican children. *Am J Epidemiol* 139:193–205
- Mehlman IJ, Andrews WH, Wentz BA (1984) Coliform bacteria. In: Jackson GJ (ed) Bacteriological Analytical Manual of the Division of Microbiology. Arlington: Association of Official Analytical Chemists, pp 5.01–5.07
- Melo TDM, Vieira RHSE, Saker-Sampaio S, Hofer E (1997) Coliforms and *Salmonella* in seawater near to domestic sewage sources in Fortaleza (Ceará, Brazil). *Microbiologia SEM* 13:463–470
- Nataro JP, Kaper JB (1988) Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 11:142–201
- NCCLS – National Committee for Clinical Laboratory Standards (1988) Performance Standards for Antimicrobiol. Disk Susceptibility Tests (8th edn). Approved Standards. Villanova. 18:M100-58
- Saridakis HQ (1994) Non production of Shiga-like toxins by *Escherichia coli* serogroup O26. *Ver Microbiol* 25:154–155
- Scotland SM, Willshaw GA, Smith HR, Rowe B (1987) Properties of strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Vero cytotoxins VT1 and VT2. *Epidemiol Infection* 99:613–624
- Silva MLM, Scaletsky ICA, Viotto LH (1983) Non production of cytotoxin among enteropathogenic strains of *Escherichia coli* isolated in São Paulo. *Ver Microbiol* 14:161–162
- Simmonds JS (1926) A culture medium for differentiating organisms of typhoid-colon aero-genes groups and for isolation of certain fungi. *J Infect Dis* 39:209–214
- Valentini SR, Gomes TAT, Falcão DP (1992) Lack of virulence factors in *Escherichia coli* strains of enteropathogenic serogroup isolated from water. *Appl Environ Microbiol* 58:412–414
- Vasconcelos FP (1985) Aspectos da poluição nas praias do município de Fortaleza, Estado do Ceará, Brasil. *Bol Ciênc Mar* 41:1–8
- Vieira RHSE, Evangelista NSS, Rodrigues DP (1996) Colimetria das águas marinhas de Fortaleza (Ceará, Brasil) e detecção de cepas de *Escherichia coli* enteroinvasora (EIEC) e enteropatogênica clássica (EPEC). *Arq Ciênc Mar* 30:27–31
- Yolken RH, Greenberg HB, Merson MH, Sack RB, Kapikian AZ (1977) Enzyme linked immunosorbent assay for detection of *Escherichia coli* heat-labile enterotoxin. *J Clin Microbiol* 5:439–444