RESEARCH ARTICLE

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Angela M. S. Cardonha[1]
Regine H. S. F. Vieira[2]*
Dália P. Rodrigues[3]
Andrew Macrae[4]
Gizele Peirano[3]
Grace N. D. Teophilo[3]

[1]Department of Nutrition, PGCS, Federal Univ. of Rio Grande do Norte, RN, Brazil
[2]Institute of Marine Sciences, Federal University of Ceará, Fortaleza, CE, Brazil
[3]Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
[4]Institute of Microbiology Prof. Paulo de Goes, Federal University of Rio de Janeiro, RJ, Brazil

Fecal pollution in water from storm sewers and adjacent seashores in Natal, Rio Grande do Norte, Brazil

Summary. A study on the distribution patterns of enteropathogenic bacteria polluting the shoreline in Natal, Rio Grande do Norte, Brazil, was carried out based on 72 samples obtained from three storm sewers and adjoining beach locations, Praia do Meio (PM), Areia Preta (AP) and Ponta Negra (PN). From each location, 12 water samples were taken and analyzed for fecal coliforms (FC) and *Escherichia coli*. In AP, two (16.7%) of the seawater samples and five (41.7%) of the storm sewer samples yielded values above 1.1×10^7 FC/100 ml, whereas only one (8.3%) of the samples from PM reached this level. There was no correlation ($p > 0.05$) between rainfall indeces and FC values. A total of 64 *E. coli* isolates were obtained: 37 from the storm sewer samples and 27 from the seawater samples. Of these isolates, four (O143, two O112ac, and O124) were enteroinvasive and two (O111 and O125) were enteropathogenic. Resistance to antibiotics and to heavy metals was also analyzed. Almost 36% of the *E. coli* strains isolated were resistant to more than one antibiotic. All strains were resistant to zinc and copper at the highest concentration tested (250 μg/ml), and several (23.4%) were resistant to mercury at 50 μg/ml. Our results agreed with previous reports that antibiotic resistance is commonly associated with heavy-metal resistance in pathogens. [**Int Microbiol** 2004; 7(3):213–218]

Introduction

Exposure to contaminated water, by ingestion as drinking water or from recreational activities, is a significant pathway for the transmission of gastrointestinal pathogens. Swimming or bathing in contaminated waters has often been associated with a higher risk of gastrointestinal disease. The WHO estimates that 2.1 million people die annually from diarrheal diseases and that 10% of the population of the less-industrialized world suffers from parasitic worm infections related to improper waste and excreta management. In the northeastern region of Brazil alone, about 17,300 children die every year

from infectious diseases related to poor water and food sanitation [http://www.who.int/water_sanitation_health]. Currently, *Escherichia coli* is one of the main pathogens responsible for gastroenteritis in children in Brazil [16], where several pathogenic categories of the bacterium have been recognized, including enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteropathogenic (EPEC) enteroaggregative (EAggEC) and enteroadhesive (DAEC) [10,16,17].

Sources of contamination of surface water include urban and farm runoff, discharges from wastewater treatment facilities, wildlife, and storm sewers. Controlling the quality of water discharged from urban areas into water along the coast-

line is a priority. Research is essential in order to identify pollution sources and types, and to assess risk, which involves integrating scientific data regarding an environmental hazard into a framework that addresses the risk of exposure and the potential health impacts [18]. Armed with this information, advice can be transmitted to the general public and when required, prevent beach-goers and bathers from being exposed to elevated levels of enteric contaminants and other pathogens capable of causing intestinal, skin, ear and throat infections.

The objective of this work was to establish and characterize the pollution distribution patterns of pathogens reaching Natal's coastline from storm sewers in order to provide baseline data for better city sanitation. To do so, the concentrations of fecal coliform bacteria in samples drawn from storm sewers and their receiving waters on the eastern waterfront of the city of Natal were estimated and correlated with rainfall indices on the days prior to sampling. Furthermore, *E. coli* strains were isolated and characterized with respect to serogroup, resistance to heavy metals and resistance to antibiotics in order to assess their pathogenic diversity and dynamics.

Materials and methods

Sampling location. Three beach locations along the waterfront of Natal, Rio Grande do Norte, Brazil were selected on the basis of their popularity and intense leisure use and because they receive large amounts of runoff water from the city's storm sewers. The sites investigated were Praia do Meio (PM), Areia Preta (AP) and Ponta Negra (PN) (Fig.1).

Sampling. A total of 72 samples—12 from each of the three storm sewers and adjoining beach locations (PM, AP and PN)—were collected during the period January-June, 2002. Sampling was carried out weekly, and randomly with respect to tides, which were registered only as flooding or ebbing. The water samples were placed in sterile 1000-ml amber-colored bottles and transported in iceboxes to the laboratory for immediate processing.

Isolation of fecal coliforms. Fecal coliforms from the samples were determined by standard multiple-tube fermentation methods [1]. The strains were identified biochemically according to the method of Hitchins et al. [12]. Strains classified as *E. coli* were maintained on trypticase soy agar (TSA) (Difco, Detroit, MI).

Antigen analysis. The antigenic characteristics of purified isolates were evaluated using polyvalent and monovalent somatic antisera for the enteropathogenic group (O18, O25, O26, O44, O55, O86, O91, O111, O112ab, O119, O124, O125, O126, O127, O128, and O158) and the enteroinvasive group (O28, O29, O112ac, O124, O143, O144, O152 and O164). The antisera were prepared by the Oswaldo Cruz Foundation using the rapid slide seroagglutination technique [8].

Resistance to antibiotics. Serogroup strains were tested for resistance to nalidixic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (300 µg), sulfametoxazol-trimethoprim (23.75/1.25 µg) and tetracycline (30 µg) by using the disk diffusion method of Bauer et al. [3] and following the recommendations outlined in NCCLS [13]. The standard control strains were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, the acceptable inhibition zone variations for which may be found in NCCLS [13].

Resistance to metals. Using the plate dilution technique described by Elek and Higney [7] and Dias et al. [6], aqueous solutions of $CuSO_4$ and $ZnSO_4$ were incorporated in Mueller Hinton agar at concentrations 100, 150, 200 and 250 µg/ml. $HgCl_2$ was incorporated at concentrations 5, 10, 20, 30, 40, and 50 µg/ml. Metal-free control plates and the control strain *Escherichia coli* ATCC 25922, which is susceptible to Cu, Zn and Hg, were used to evaluate the viability of the strains and culture media.
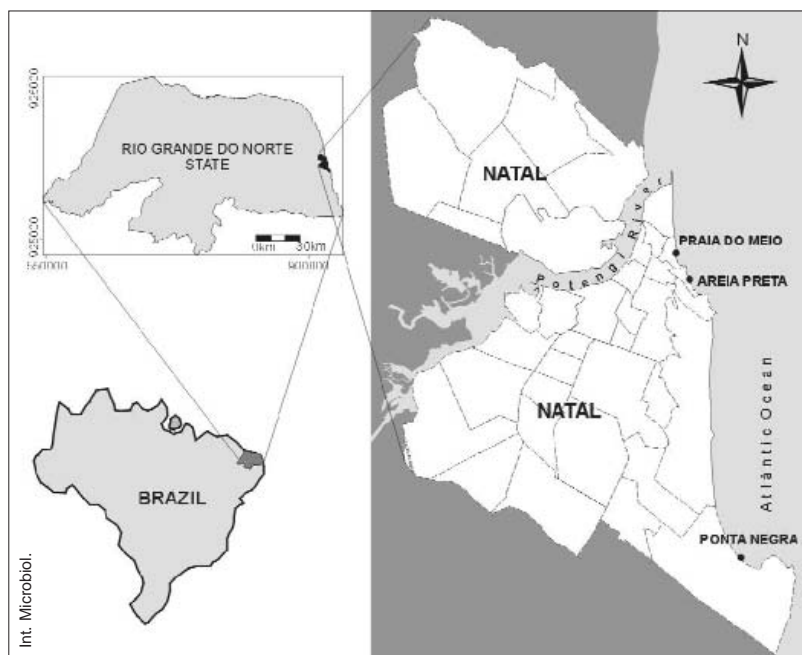
Fig. 1. Map of sampling locations (Praia do Meio, Areia Preta and Ponta Negra) for seawater and storm-sewer water samples on the eastern seashore in Natal, Rio Grande do Norte, Brazil.

Table 1. Rainfall indices and most probable number (MPN) of fecal coliforms in water samples collected from three storm sewers and adjacent beach locations in Natal, Rio do Norte, Brazil, from January to June 2002

Sample	Praia do Meio		Areia Preta		Ponta Negra		Rainfall index (mm)
	Str (MPN/100 ml)	Sea (MPN/100 ml)	Str (MPN/100 ml)	Sea (MPN/100 ml)	Str (MPN/100 ml)	Sea (MPN/100 ml)	
1	1.1×10^7	4.3×10^3	9.3×10^5	9.3×10^4	4.3×10^5	9.3×10^2	8.8
2	4.6×10^6	9.3×10	$>1.1 \times 10^7$	9.3×10^4	1.1×10^7	9.3×10	0.0
3	9.3×10^3	9.3×10	4.3×10^5	4.3×10^5	9.3×10^5	2.3×10	0.8
4	2.4×10^6	1.5×10^4	$>1.1 \times 10^7$	4.6×10^6	4.3×10^4	2.1×10^4	0.0
5	9.3×10^3	2.3×10^5	2.4×10^6	4.6×10^6	2.4×10^6	0.74×10	0.0
6	$>1.1 \times 10^7$	1.2×10^5	$>1.1 \times 10^7$	$>1.1 \times 10^7$	9.3×10^2	4.3×10^3	0.3
7	1.1×10^7	4.3×10^5	$>1.1 \times 10^7$	1.5×10^5	4.6×10^6	9.3×10^3	36.0
8	2.3×10^3	2.3×10^3	1.1×10^7	1.1×10^7	2.4×10^6	9.3×10^2	1.2
9	4.3×10^4	7.5×10^4	4.6×10^6	4.3×10^4	4.3×10^3	9.3×10	14.3
10	2.4×10^6	1.2×10^3	1.1×10^7	2.3×10^5	2.4×10^5	4.3×10^3	4.6
11	1.1×10^7	4.3×10^3	2.4×10^6	2.1×10^3	9.3×10^4	9.3×10^2	25.0
12	4.3×10^5	9.3×10^2	$>1.1 \times 10^7$	$>1.1 \times 10^7$	2.1×10^5	2.4×10^2	29.0

Abbreviations: Str, storm sewer; Sea, seashore.

Rainfall indices. Data on rainfall indices from the day preceding each sampling were retrieved from the Meteorological Service of the Federal University of Rio Grande do Norte.

Statistical analysis. The levels of contamination in the water samples collected from storm sewers and adjacent seashores were compared by analysis of variance (ANOVA). Average values obtained were compared by the Tukey test at $p < 0.05$. The correlation between rainfall and fecal coliform counts in the samples was measured by the Pearson correlation test ($p < 0.05$). Statistical analyses were done using the software Statistics for Windows [Statsoft Inc., Tulsa, OK, USA, 1996].

Results and Discussion

The water samples from the storm sewer and adjacent seashore of the sampling location at AP were the most polluted. Levels of fecal coliform contamination remained high throughout the study period and differed significantly ($p < 0.05$) from those of the other two locations (Table 1 and Fig. 2). Of the 12 samples collected from the storm sewer at AP, five (41.7%) yielded values higher than 1.1×10^7 FC/100 ml, whereas the values obtained for the remaining samples ranged from 4.3×10^5 to 1.1×10^7 FC/100 ml. The discharge pipe of the storm sewer at AP is fitted into a 3-m high cement wall on the beach and produces a continuous flow of wastewater in both dry and wet seasons, with clear visual and olfactory signs of pollution. Of the 12 seawater samples collected in front of the discharge point at AP, two (16.6%) also yielded coliform numbers higher than 1.1×10^7 FC/100 ml. Children often play and bathe in a depression at this location, where seawater is trapped during low tide and mixes with highly contaminated wastewater. Only one (8.3%) of the 12 samples from the storm sewer discharging at PM yielded values of the most probable number (MPN) above 1.1×10^7

FC/100 ml. At this sampling site, in the sand, there was a large puddle of dark, stinking contaminated water. Seawater samples collected in front of this discharge point yielded coliform counts that ranged from 9.3×10 to 4.3×10^5 FC/100 ml (Table 1). At PN, a clandestine 20-cm-wide polyvinylchloride (PVC) wastewater pipe had been connected to the outlet of the storm sewer. The alignment of this structure suggested that it came from a nearby hotel. The flow from that pipe was continuous and formed a rivulet running towards the sea (see Fig. 1). Of the 36 seawater samples tested from the three
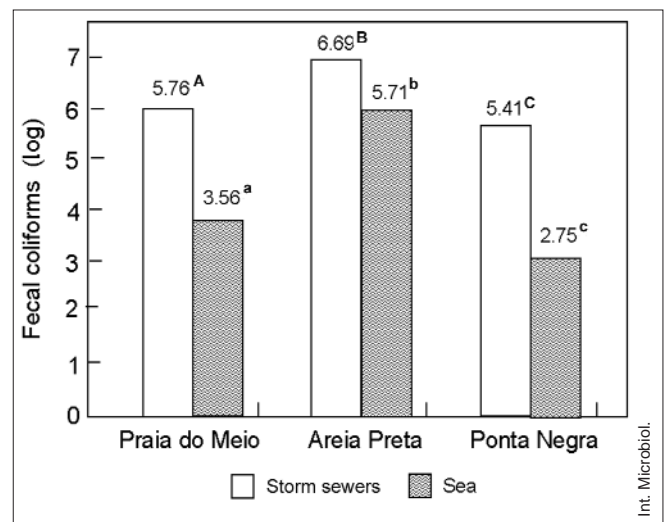
Fig. 2. Average levels of contamination by fecal coliforms in water samples from three storm sewers and adjacent beaches in the city of Natal, Rio Grande do Norte, Brazil, from January to June 2002. ([ABC/abc]: Average values are annotated with letters; significant differences between averages are shown by different letters. Small letters: comparison between beach locations.)

locations (PM, AP and PN), 25 (69.4%) had fecal coliform counts above the legally permitted amount, which implied that those beaches were unsuitable for bathing.

Storm water runoff from urban areas is a major source of microbial contamination of surface waters and accounts for a significant share of the pollution occurring in coastal waters. Fecal material and other residues present on impervious surfaces, including tarmac, roofs, sidewalks, patios, bedrock outcrops and compact soil, are washed off and carried along with rainwater to the nearest body of water [2,15]. For example, more than half of the beach-water quality failures in Santa Monica Bay, California, USA, are associated with rainfall events, even though it usually rains less than 15 days per year [20]. A study of the Atlantic shoreline of Concord, New Hampshire, USA, showed that concentrations of *E. coli* in standing waters increased after rainfall and the arrival of storm water, and returned to baseline levels soon after the storm flow had ceased [5]. In Natal, however, there was no correlation ($p > 0.05$) between rainfall indices and FC counts at the three beach locations investigated. In fact, there were high levels of contamination on both rainy and dry days (see Table 1). Thus, our results suggest an irregular use of the storm sewers.

A total of 64 isolates were identified as *E. coli*; 37 from storm sewer samples and 27 from seawater samples. Of these, four were characterized as EIEC and two as EPEC. Of the four EIEC samples, two came from storm sewer samples and belonged to serogroup O112ac, one came from the shore of PM and was identified as serogroup O124, and one, which we labeled serogroup O143, came from seawater collected at AP. The two EPEC isolates belonged to serogroups O111 and O125 and were detected in storm sewer water samples collected at PN. Similar serogroups had been previously isolated from water samples collected at several seaside resorts in Brazil [11,17,24]. Only a few strains were identified as EPEC and EIEC, and the identification process was restricted to

serogroups. However, the relevance of this finding should not be underestimated. Trabulsi et al. [22] described the division of EPEC strains into typical and atypical. The two groups differed from each other with respect to several characteristics. Typical EPEC strains are a leading cause of infantile diarrhea in developing countries, whereas atypical EPEC strains are becoming an increasingly frequent cause of diarrhea in industrialized countries. The same shift might be occurring in Brazil. Humans are the only reservoir for typical EPEC, whereas both humans and other animals can serve as reservoirs of atypical EPEC. In addition to the geographical differences in their occurrence, typical and atypical EPEC also differ in their genetic characteristics, serotypes, and virulence. Atypical EPEC are more closely related to Shiga-toxin-producing *E. coli*.

Table 2 summarizes the response of the 64 isolates when challenged with drugs of common therapeutic use. As many as 35.9% of the strains were resistant to one or more of the antibiotics tested. The highest rates of resistance were towards ampicillin (21.8%), followed by cephalothin and sulfametoxazol-trimethoprim (18.7%) and tetracycline (14.1%). By contrast, resistance rates were low towards nitrofurantoin (3.1%), nalidixic acid (4.7%) and chloramphenicol (1.6%). All the strains tested were found to be susceptible to cefoxitin, ceftriaxone, ciprofloxacin, gentamicin, and imipenem. *E. coli* strains isolated from beaches in Fortaleza in 1996 and identified as serogroups O55 and O125 were also shown to be resistant to cephalothin and ampicillin [23]. Antibiotic-resistance analysis of fecal coliforms may be used to determine sources of fecal contamination, and aid in water-quality improvement [4]. The increase in microbial resistance to antibiotics has made the management of infections more difficult and has contributed to the growing cost of public health care.

Table 3 shows that all strains were resistant to Cu and Zn at concentrations of 250 µg/ml, whereas 23.4% of the isolates

Table 2. Frequency of antibiotic resistance by sampling location at three beaches in Natal, Rio Grande do Norte, Brazil

Sampling location	Total no. of samples	No. of resistant samples						
		AMP	F	KF	NA	SXT	TE	C
Praia do Meio – Str	13	1	–	–	–	1	–	–
Praia do Meio – Sea	9	–	–	1	1	1	1	–
Areia Preta – Str	12	1	–	3	–	–	3	–
Areia Preta – Sea	12	5	–	3	1	5	3	1
Ponta Negra – Str	12	3	–	2	–	1	–	–
Ponta Negra – Sea	6	4	2	3	1	4	2	–
Total (%)	64	14 (21.8)	2 (3.1)	12 (18.7)	3 (4.7)	12 (18.7)	9 (14.1)	1 (1.6)

Abbreviations: Str, storm sewer; Sea, seashore; AMP, ampicillin; F, nitrofurantoin; KF, cephalothin; NA, nalidixic acid; STX, sulfametoxazol-trimethoprim; TE, tetracycline; C, chloramphenicol.

Table 3. Frequency of resistance to heavy metals and distribution of markers in the highest concentrations tested on *Escherichia coli* samples from three beaches in Natal, RN, Brazil

Sampling location	No. of strains tested	Metal resistance		
		Cu (250 µg/ml)	Zn (250 µg/ml)	Hg (50 µg/ml)
Praia do Meio – Str	13	13	13	3
Praia do Meio – Sea	9	9	9	4
Areia Preta – Str	12	12	12	2
Areia Preta – Sea	12	12	12	2
Ponta Negra – Str	12	12	12	3
Ponta Negra – Sea	6	6	6	1
Total (%)	64	64 (100)	64 (100)	15 (23.4)

Abbreviations: Str, storm sewer; Sea, seashore.

were resistant to 50 µg Hg/ml. According to Nies [14], mercury is much more toxic to *E. coli* than zinc, whose action resembles that of other elements of lower toxicity, such as copper, nickel and cobalt. In general, the isolated strains were highly resistant to heavy metals at the concentrations tested. The association between heavy-metal resistance and antibiotic resistance was marked. Of the 64 *E. coli* strains investigated, 24 (37.5%) were resistant to at least one antibiotic in combination with two or three metals. Our results therefore agree with the findings of Filali et al. [9].

Despite the extensive sanitation programs carried out by local authorities in Natal, similar to those undertaken in other geographical regions, pollution continues to be a cause of concern. Identifying the sources of fecal pollution in water is paramount in assessing the potential human health risk involved as well as in prescribing necessary remedial action. New methodologies are now being employed to identify the pathogens and their sources [19,21]. The application of molecular biology techniques to address questions concerning water quality is very promising and may offer the next generation of tools for researchers and regulators to continue their fight for better sanitation.

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Contaminación fecal en el agua de colectores pluviales y en la costa adyacente en Natal (Rio Grande do Norte, Brasil)

Resumen. Se realizó un estudio sobre la distribución de las bacterias enteropatógenas que contaminan la costa en Natal (Rio Grande do Norte, Brasil), a partir de 72 muestras obtenidas en tres colectores pluviales (*storm sewers*) y en unas playas adyacentes a los mismos, Praia do Meio (PM), Areia Preta (AP) y Ponta Negra (PN). En cada punto se tomaron 12 muestras, que se analizaron en busca de coliformes fecales (CF) y de *Escherichia coli*. En AP, dos (16,7%) de las muestras de agua de mar y cinco (41,7%) de las de agua del colector dieron valores superiores a $1,1 \times 10^7$ CF/100 ml, mientras que sólo una (8,3%) de las muestras de PM alcanzó este nivel. No se encontró correlación ($p > 0,05$) entre los índices de pluviosidad y los valores de CF. Se obtuvo un total de 64 aislados de *E. coli:* 37 a partir de muestras de colectores y 27 a partir de muestras de agua de mar. De estos aislados, cuatro (un O143, dos O112ac y un O124) eran enteroinvasivos y dos (O111 y O125) eran enteropatógenos. Se analizó también la resistencia a antibióticos y a metales pesados. Casi el 36% de las cepas de *E. coli* aisladas eran resistentes a más de un antibiótico. Todas las cepas eran resistentes al zinc y al cobre a la mayor concentración probada (250 µg/ml), y varias cepas (23.4%) eran resistentes al mercurio a 50 µg/ml. Nuestros resultados coinciden con trabajos anteriores que indican que en bacterias patógenas la resistencia a antibióticos suele ir asociada a la resistencia a metales pesados. [**Int Microbiol** 2004; **7**(3):213–218]

Palabras clave: colectores pluviales · contaminación fecal · metales pesados · resistencia a antibióticos

Poluição fecal em águas de galerias pluviais e áreas adjacentes em Natal (Rio Grande do Norte, Brasil)

Resumo. Foi realizado um estudo sobre a distribuição de bactérias enteropatogênicas que contaminam a costa de Natal (Rio Grande do Norte, Brasil) a partir de 72 amostras obtidas em três galerias pluviais (*storm sewers*) e nas praias adjacentes às mesmas: Praia do Meio (PM), Areia Preta(AP) e Ponta Negra (PN). Em cada ponto foram tomadas 12 amostras nas quais foram investigadas a presença de coliformes fecais (CF) e *Escherichia coli*. Na AP duas (16,7%) das amostras de água do mar e cinco (41,7%) das águas das galerias apresentaram valores superiores a $1,1 \times 10^7$ CF/100 ml enquanto somente uma (8,3%) das amostras da PM alcançou este nível. Não foi encontrada correlação ($p > 0,05$) entre os índices pluviométricos e os valores de CF. Obteve-se um total de 64 isolados de *E. coli*: 37 a partir de amostras das galerias e 27 a partir de amostras de água do mar. Destes isolados, quatro (um O143, dois O112ac e um O124) eram enteroinvasivos e dois (O111 e O125) eram enteropatógenos. Foi analisada também a resistência a antibióticos e a metais pesados. Quase 36% das cepas de *E. coli* isoladas eram resistentes a mais de um antibiótico. Todas as cepas eram resistentes a zinco e a cobre na maior concentração testada (250 µg/ml), e várias cepas (23.4%) eram resistentes a mercúrio (50 µg/ml). Os resultados coincidem com trabalhos anteriores que indicam que em bactérias patógenas a resistência a antibióticos está associada à resistência a metais pesados. [**Int Microbiol** 2004; 7(3):213–218]

Palavras chave: galerias pluviais · contaminação fecal · metais pesados · resistência a antibióticos