



Population dynamics and antimicrobial susceptibility of *Aeromonas* spp. along a salinity gradient in an urban estuary in Northeastern Brazil



Camila Magalhães Silva^{a,*}, Norma Suely Evangelista-Barreto^c, Regine Helena Silva dos Fernandes Vieira^b, Kamila Vieira Mendonça^b, Oscarina Viana de Sousa^b

^a Federal University of Ceara, Pos-Graduate Program of Engineering of Fish, Av. Mister Hull, s/n, Campus do Pici, Bloco 848, 60021-970 Fortaleza, CE, Brazil

^b Institute of Marine Science, Av. da Abolição, 3207, Meireles, 60165-081 Fortaleza, CE, Brazil

^c Federal University of Recôncavo Baiano CCAAB – Center for Research on Fisheries and Aquaculture (NEPA), Campus Universitário, 44380-000 Cruz das Almas, BA, Brazil

ARTICLE INFO

Article history:

Available online 4 November 2014

Key words:

Aeromonas
Aquatic environments
Physicochemical parameters
Plasmid curing
Resistance
Health risks

ABSTRACT

The main objective of this study was to quantify population and identify culturable species of *Aeromonas* in sediment and surface water collected along a salinity gradient in an urban estuary in Northeastern Brazil. Thirty sediment samples and 30 water samples were collected from 3 sampling locations (A, B and C) between October 2007 and April 2008. The *Aeromonas* count was 10–7050 CFU/mL (A), 25–38,500 CFU/mL (B) and <10 CFU/mL (C) for water samples, and ~100–37,500 CFU/g (A), 1200–43,500 CFU/g (B) and <10 CFU/g (C) for sediment samples. Five species (*Aeromonas caviae*, *A. sobria*, *A. trola*, *A. salmonicida* and *A. allosaccharophila*) were identified among 41 isolates. All strains were sensitive to chloramphenicol and ceftriaxone, whereas 33 (80, 4%) strains were resistant to at least 2 of the 9 antibiotics tested. Resistance to erythromycin was mostly plasmidial. In conclusion, due to pollution, the Cocó River is contaminated by pathogenic strains of *Aeromonas* spp. with a high incidence of antibacterial resistance, posing a serious risk to human health.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Aquatic environments are impacted by a number of anthropic activities, including aquaculture. Over the past decades, the coastal regions of Northeastern Brazil have been the object of intensive urban development and commercial exploitation associated with an increased risk of chemical and biological contamination (Holland et al., 2004). As a consequence, many local ecosystems, especially estuaries, have been seriously degraded by domestic and industrial effluents.

Like most Brazilian rivers, the Cocó river—an important aquatic ecosystem running through Fortaleza (a state capital in Northeastern Brazil with roughly 3 million inhabitants)—has been subject to extensive microbiological impacts from human activity.

Environmental contamination from discharged organic matter and domestic sewage exposes the local population to health risks such as gastroenteritis and wound infection caused mostly by autochthonous bacteria (Chopra and Roberts, 2001).

Aeromonas bacteria are ubiquitous and can be isolated from every environmental niche where bacterial ecosystems exist

(Janda and Abbot, 2010). The members of this genus are able to inhabit the surface of rivers and lakes, water contaminated by sewage or animal (Sepe et al., 2008), drinking water (Figueras, 2005), thermal water, sea water (Biscardi et al., 2002; Maalej et al., 2003; Pablos et al., 2009) and natural soils, although extensive investigations on the latter subject are lacking (Janda and Abbot, 2010).

According to Albert et al. (2000), infections are often caused by a combination of *Aeromonas* and other pathogens. In fact, *Aeromonas* has been detected in samples of water and soil from a range of aquatic environments, gardens and recreation areas in Brazil (Gibotti et al., 2000). Though an increasingly common cause of outbreaks of gastroenteritis, the presence of *Aeromonas* in the environment has so far received little attention from researchers and health authorities (Marcel et al., 2002).

In an analysis of water and sediment from the Potomac (Washington D.C.), Seidler et al. (2001) observed that samples collected during the warmest months presented the highest counts of *Aeromonas* (4.0×10^5 CFU/g for sediments; 300 CFU/mL for water).

Both water and soil may serve as a reservoir for pathogenic strains of *Aeromonas*. Brandi et al. (1996) reported prolonged survival of *Aeromonas* in soil in the presence or absence of indigenous microbiota, with preservation of the virulence profile and the ability to multiply, indicating soil plays an important role in the epidemiology of human infections associated with *Aeromonas* spp.

* Corresponding author at: Av. da Abolição, 3207, Meireles, 60165-081 Fortaleza, CE, Brazil. Tel.: +55 (85) 33667027.

E-mail address: camilapesca@hotmail.com (C.M. Silva).

In recent years, several outbreaks of infectious diseases caused by microorganisms resistant to multiple antibiotics occurred in several countries (Warren, 2010), and the *Aeromonas* bacteria considered reservoir of antimicrobial resistance genes in aquatic environment, like suggested by Cattoir et al. (2008) who detected the *qnr* gene capable of protecting DNA gyrase from the action of the quinolone antibiotics.

Libisch et al. (2008) and Chang et al. (2007) argue that resistance to multiple antibiotics in *Aeromonas* is mainly due to the presence of multiple determinants of resistance genes associated with mobile DNA elements that contribute to the spread of genetic determinants of antimicrobial resistance by horizontal gene transfer.

According to Maalej et al. (2003) the survival of these bacteria in the environment is not solely dependent on environmental factors such as temperature, salinity and pH, but also the incidence of ultraviolet radiation and nutrient availability.

The objective of this study was to identify and quantify culturable species of *Aeromonas* in sediment and surface water samples collected along a salinity gradient in the Cocó river, an urban estuary in Northeastern Brazil.

2. Materials and methods

2.1. Sampling

A total of 30 sediment samples and 30 water samples were collected from 3 sampling locations along the estuary of the Cocó river – 3°45'28" S, 38°30'07" W; 3°45'13" S, 38°28'59" W; 3°46'16" S, 38°26'08" W- locations A, B and C, respectively (Fig. 1),

on 10 different occasions between October 2007 and April 2008. The water samples were collected approximately 30 cm below the surface using previously sterilized 1L amber vials. The sediment samples were accommodated in polyethylene bags.

Sampling was performed in the morning at varying tides, registering the temperature with a mercury-in-glass thermometer (Incoterm). The samples were transported in ice-cooled isothermal boxes to the Laboratory of Seafood and Environmental Microbiology (LABOMAR/UFC) for immediate analysis and measurement of pH and salinity.

2.2. Sample preparation

The sampled water was diluted in Alkaline Peptone Water (APW) in the proportion 1:9. Serial dilutions from 10^{-1} to 10^{-4} were prepared from which 100 μ L aliquots were drawn and added to 20 μ g/mL ampicillin, followed by spread plating on gelatin phosphate salt agar (Merck) and incubation at 28 °C for 24 h (Hughes and Ribas, 1991). Plates with 25–250 colonies on standard plate count (SPC) were separated (Downes and Ito, 2001). Two or three typical *Aeromonas* colonies (identified by the yellow color induced by the production of amylase and clear halo measuring 2–5 mm) were selected for further culture in inclined tubes containing Tryptone Soy Agar (TSA/Difco), followed by incubation at 28 °C for 24 h.

Sediment samples were collected at 15 cm depth of each sampling point (Van Elsas et al., 1997). The microbial fraction of the sediment was rescued after stirring 25 g step pellet in an Erlenmeyer flask containing 225 ml of peptone water. After a period of 30 min at room temperature, an aliquot of 1 mL (10^{-1} dilution) was removed from this material to initiate necessary for processing

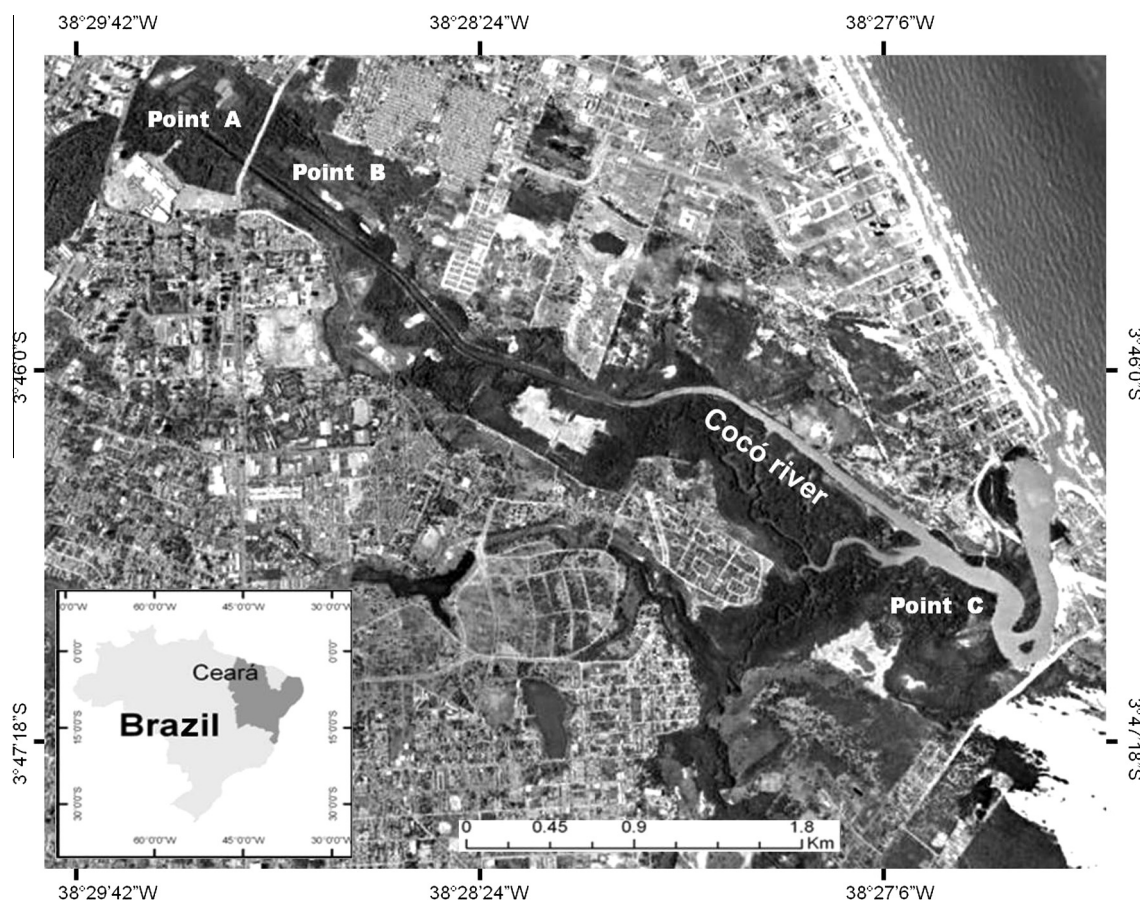


Fig. 1. Satellite image of the Rio Coco, in Fortaleza with a description of the sampling points of the research. The point A (Rio Coco Park); Point B (Bridge on the River Coco near the Shopping Iguatemi) and Point C (Foz do Rio Coco).

the samples dilutions. The standard plate counts (SPC) and the isolation of microorganisms were performed as described above for water samples.

The SPC were performed by the method described by Downes and Ito (2001) from decimal dilutions from 10^{-1} to 10^{-4} of the homogenised material plates that showed no microbial growth were represented by <10 CFU.

2.3. Determination of physico-chemical parameters

At collection was measured the temperature of the water samples with the aid of a thermometer (INCOTERM) and in the laboratory pH were measured using a potentiometer (MARCONI – PA 200P) and the through a salinity refractometer brand ATAGO S/MILL.

2.4. Statistical analysis

The data were analyzed by Pearson correlation using the Statistical Package for the Social Sciences (SPSS). Multiple correlations were used to determine the relationship between the different physico-chemical parameters measured in surface waters of the sampling points in Rio Coco (CFU/mL) and the *Aeromonas* spp. counting.

2.5. Identification of bacteria

Suspected *Aeromonas* colonies were seeded in inclined tubes containing TSA and incubated at 28 °C for 24 h. The microorganisms were identified biochemically using the method described by Palumbo et al. (2001). The strain *Aeromonas* ATCC 7966 was used as control.

2.6. Antibacterial susceptibility test

The antimicrobial susceptibility of the *Aeromonas* isolates was determined by the disc diffusion method on Mueller Hinton Agar (Kirby test) (Bauer et al., 1966) as described by the Clinical and Laboratory Standards Institutes (CLSI, 2009). The antibiotic discs used in this study were purchased from Laborclin. The following antibiotics were tested: ampicillin (AMP; 10 µg), tetracycline (TCY; 30 µg), penicillin (PEN; 10 UI), cephalotin (CET; 30 µg), ceftriaxone (CRO; 30 µg), nalidixic acid (NAL; 30 µg), chloramphenicol (30 µg; CHO), erythromycin (ERY; 15 µg) and vancomycin (VAN; 30 µg).

2.7. Plasmid curing

In order to identify the phenotypical mechanism of resistance, resistant strains were submitted to plasmid curing, as described by Molina-Aja et al. (2002), using Luria–Bertani broth (LB) supplemented with 100 µg/mL orange acridine dye (Sigma). After incubation under shaking at 30 °C for 24 h, the strains were submitted to a second antibiogram test to observe changes in the resistance profile.

3. Theory/calculation

Environmental contamination from discharged organic matter and domestic sewage exposes the local population to health risks, and infections are often caused by a combination of *Aeromonas* and other pathogens. In this study, the dominant cultivable species identified were *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas trota*, *Aeromonas salmonicida* and *Aeromonas allosaccharophila*. However *A. caviae* was the most frequently isolated from samples. All cultivable *Aeromonas* spp. were resistant to ampicillin. The presence of *Aeromonas* strains, as reported in this study, suggest that the aquatic environment could be involved in the transport

and dissemination of this pathogen posing serious health risks to residents, fishermen and bathers.

4. Results

Table 1 shows the physicochemical parameters for surface water samples from the Cocó river. The pH ranged from 7.0 to 8.2, with a tendency towards alkalinity. The highest and lowest pH values were registered in October and April, respectively. The temperature variation observed throughout the study period was not statistically significant. The same was true for the monthly average salinity, despite oscillations from 0‰ to 39‰ due to variations in environmental parameters, especially river discharge, rainfall, evaporation and tides. Salinity was lowest at sampling locations A and B (near the city, far from the river mouth) and highest at sampling location C (near the river mouth, subject to the influence of the tides).

The culturable *Aeromonas* plate counts were higher in sediment samples in points A and B whereas in point C not were detected culturable bacterial cells (Table 2). The SPC of *Aeromonas* spp. in the water samples was 10–7,050 CFU/mL at sampling location A, 25–38,500 CFU/mL at sampling location B, and < 10 CFU/mL at sampling location C. The corresponding ranges for sediment samples were ~100–37,500 CFU/g at sampling location A, 200–43,500 CFU/g at sampling location B, and < 10 CFU/g at sampling location C.

Salinity was the determining factor in the ability of growing bacterial cells. Analyzing the data totals matched, we can see a strong negative correlation between salinity recorded at collection points and scores of *Aeromonas* spp. temperature and pH moderately correlated with scores (Table 3).

Forty-one (34.1%) of the 120 suspected *Aeromonas* strains were identified to the level of species. At sampling point A, 11 strains (26.8%) were isolated from water and 6 strains (14.6%) were isolated from sediments. The corresponding figures were 18 (43.9%) and 6 (14.6%) at sampling point B, and 0 (0%) and 0 (0%) at sampling point C. Five species were identified (*Aeromonas caviae*, *A. sobria*, *A. trota*, *A. salmonicida* and *A. allosaccharophila*), the first of which was the most frequently observed in both water samples (46.6%) and sediment samples (29.2%).

The antibiogram testing revealed that the *Aeromonas* strains isolated from water and sediment samples collected in the estuary of the Cocó river were resistant to AMP ($n = 41$; 100%), CET ($n = 28$; 68%), VAN ($n = 26$; 63%), NAL ($n = 11$; 26%) and TCY ($n = 3$; 7%). None of the isolates were resistant to CHO or CRO.

Between the isolates showing resistance, 33 (80.5%) out of 41 strains were resistant to at least two antibiotics of different classes (Table 4).

Only two of the 33 multiresistant strains identified in the study displayed plasmidial resistance to ERY. Thus, transmission of resistance was vertical (from generation to generation) in most of the resistant strains ($n = 31$), suggesting that the population residing or pursuing activities along the estuary of the Cocó river is at increasing risk of infections caused by *Aeromonas*.

In this study, only one of the five isolated strains of *A. salmonicida* (sampling point B) lost resistance to TCY through plasmid curing. The other four strains remained resistant to TCY and all the other antibiotics tested, indicating resistance was non-plasmidial.

5. Discussion

The high *Aeromonas* counts observed for the last batch of samples (Table 2) may be due to rainfalls towards the end of April 2008. Rain tends to increase the availability of organic matter

Table 1Mean values ± 1 standard deviation, minimum and maximum values for the environmental variables measured in the three sampling stations along Coco river (Ceara, Brazil).

Environment parameters	A		B		C	
	Media \pm SD	Min–max	Media \pm SD	Min–max	Media \pm SD	Min–max
pH	7.52 \pm 0.2	7.0–7.6	7.4 \pm 0.2	7.1–7.6	7.9 \pm 0.2	7.5–8.2
Salinity	0.1 \pm 0.1	0.0–0.3	0.23 \pm 0.1	0.0–0.4	35.5 \pm 1.8	32.0–39.0
Temperature ($^{\circ}$ C)	27.2 \pm 1.0	26.0–29.0	27.27 \pm 1.0	26.0–28.5	28.1 \pm 1.4	27.0–31.0

Table 2Count *Aeromonas* spp. in environmental samples from the three sampling stations along Coco river (Ceara, Brazil).

Sampling	A		B		C	
	Water (CFU/mL)	Sediment (CFU/g)	Water (CFU/mL)	Sediment (CFU/g)	Water (CFU/mL)	Sediment (CFU/g)
1 ^a	90	5500	95	11,500	<10 ^a	<10 ^a
2 ^a	3900	750	285	24,500	<10 ^a	<10 ^a
3 ^a	10	1500	25	<10	<10 ^a	<10 ^a
4 ^a	815	100	490	200	<10 ^a	<10 ^a
5 ^a	40	500	44	<10	<10 ^a	<10 ^a
6 ^a	365	9,750	1180	4750	<10 ^a	<10 ^a
7 ^a	25	5,600	160	1200	<10 ^a	<10 ^a
8 ^a	335	14,000	235	25,000	<10 ^a	<10 ^a
9 ^a	565	3000	435	2500	<10 ^a	<10 ^a
10 ^a	7050	37,500	38,500	43,500	<10 ^a	<10 ^a

^a No colonies detected.**Table 3**Pearson correlation between *Aeromonas* spp. concentrations and physico-chemical parameters in superficial water.

Water parameters	CFU of <i>Aeromonas</i>	
	Water	Sediment
pH	–0.42	–0.58
Salinity	–0.19	–0.40
Temperature	–0.14	–0.36

through leaching and reduces salinity (a limiting factor for *Aeromonas* proliferation). Not surprisingly, the low *Aeromonas* counts observed in the third and fourth batch of samples coincided with dry weather and slightly higher temperatures and salinity.

Generally, *Aeromonas* spp. cannot survive in NaCl concentrations above 5%, despite reports of strains tolerating 6% (Knochel and Jeppesen, 1990). In a study by Marcel et al. (2002) on the incidence of *Aeromonas* in a tropical eutrophic estuary, *Aeromonas* was isolated most frequently (82.53%) from samples collected in the urban part of the estuary and during the rainy season when salinity was below 10‰. A recent study surveying the *Aeromonas* microbiota of the Cocó river (Evangelista-Barreto et al., 2010) also registered low average salinity levels in samples collected along the most urban stretch of the river. The salinity range observed (~0–32‰) is well tolerated by *Aeromonas*, explaining the high percentage of isolation (77%) at this sampling location.

Sautour et al. (2003) evaluated the effect of temperature, water activity and pH on the growth and survival in water of *Aeromonas hydrophila* and found temperature to be the most important factor. In fact, according to Sinton et al. (2007), high temperatures greatly favor the multiplication of aquatic microorganisms, even when nutrients are scarce. In our study, when taken together, the temperature, pH and salinity at sampling locations A and B represent very favorable environmental conditions for the multiplication of *Aeromonas*. Matté et al. (2010) reported that pH values between 5.5 and 9.0 favor the survival and multiplication of *Aeromonas*. The pH values observed in our study (7.0–8.2) lie comfortably within this range. Growth and proliferation of *Aeromonas* require temperatures between 4 $^{\circ}$ C and 45 $^{\circ}$ C (optimal: 30 $^{\circ}$ C) (Martin-Carnahan and Joseph, 2005).

In this study species of *Aeromonas* isolations were more frequent in A and B sampling points (90% of isolates). This points are the closest of urban center with greater anthropogenic influence and where there was a salinity of less than 5‰. This salinity is within the favorable range to *Aeromonas* growth. The point C where it reported high salinities, the counting were less than 10 CFU for water and sediment samples and it was not possible isolate *Aeromonas* strains.

According to Turnbull et al. (1984) and Araújo et al. (1991), *A. caviae* is the most abundant species in fresh water. Nevertheless, in 64 surface water samples and 24 sediment samples collected by Matté et al. (2010) by the dam at Guarapiranga (São Paulo, Brazil), *Aeromonas jandaei* accounted for 76.6%, followed by *A. sobria* (43.7%), *A. caviae* (31.2%) and *A. hydrophila* (18.7%), isolated from different species of Cocó River, were *A. caviae*, *A. sobria*, *A. trota*, *A. salmonicida* and *A. allosaccharophila*.

Studies published by Brandi et al. (1996) show that soil can be an important reservoir for *A. hydrophila*, *A. caviae* and *A. sobria*. This was borne out by our findings.

A. hydrophila is generally considered the most pathogenic member of the genus, but *A. caviae* can produce factors of virulence as well. The latter was isolated from all our samples and is a major cause of infection in humans (Buchanan and Palumbo, 1985).

Our isolates of *Aeromonas* spp. were without exception sensitive to CHO and CRO. Similar results were published in Queensland, Australia, by Koehler and Ashdown (2003) who submitted *Aeromonas* strains from human feces, blood and wounds to antibiogram testing with 22 antibiotics and found all strains to be susceptible to CHO and CRO.

CHO is a bacteriostatic broad-spectrum antibiotic effective against a wide variety of Gram-positive and Gram-negative bacteria by inhibiting protein synthesis. The most common resistance mechanism against CHO is enzyme inactivation by bacterial acetyltransferase (Hancock, 2005).

In a study on *Aeromonas*, Vila et al. (2003) found variable levels of resistance to CHO and TCY, attributed to the extensive use of these antibacterials in industrialized countries. CHO prevents the linking of amino acids by inhibiting peptidyl transferase whereas TCY acts by inhibiting protein synthesis (Hancock, 2005).

The possibility of transference of resistance genes from potentially pathogenic bacteria to previously harmless environmental

Table 4
Antibacterial resistance profile before and after plasmid curing of *Aeromonas* strains isolated from water and sediment samples collected in the Cocó river (Fortaleza, Ceará, Brazil) between October 2007 and April 2008.

Location	Species	Resistance profile	
		Before plasmid curing	After plasmid curing
A	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. trota</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. caviae</i>	AMP; NAL; VAN; ERY; CET; PEN	NAL; VAN; CET; PEN
	<i>A. caviae</i>	AMP; ERY; CET; PEN	CET; PEN
	<i>A. caviae</i>	AMP; ERY; CET; PEN	CET; PEN
	<i>A. caviae</i>	AMP; ERY; CET; PEN	CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. caviae</i>	ERY; CET; PEN	CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. salmonicida</i>	AMP; TCY; VAN; ERY; CET; PEN	TCY; CET; PEN
	<i>A. sobria</i>	AMP; VAN; ERY; CET; PEN	CET; PEN
	<i>A. salmonicida</i>	AMP; VAN; ERY; PEN	VAN; PEN
	<i>A. salmonicida</i>	AMP; TCY; ERY; CET; PEN	TCY
	<i>A. caviae</i>	AMP; NAL; ERY; CET; PEN	-
	<i>A. caviae</i>	AMP; NAL; ERY; CET; PEN	-
B	<i>A. caviae</i>	AMP; TCY; NAL; VAN; ERY; CET; PEN	AMP; TCY; NAL; VAN; CET; PEN
	<i>A. caviae</i>	AMP; TCY; NAL; VAN; ERY; CET; PEN	AMP; TCY; NAL; VAN; CET; PEN
	<i>A. salmonicida</i>	AMP; TCY; NAL; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. sobria</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. caviae</i>	ERY; CET; PEN	CET; PEN
	<i>A. sobria</i>	AMP; NAL; ERY; CET; PEN	NAL; CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	VAN; ERY; CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	ERY; CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	ERY; CET; PEN
	<i>A. sobria</i>	AMP; VAN; ERY; CET; PEN	VAN; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; PEN	PEN
	<i>A. caviae</i>	AMP; TCY; VAN; ERY; PEN	VAN; PEN
	<i>A. caviae</i>	AMP; TCY; VAN; ERY; CET; PEN	TCY; CET; PEN
	<i>A. caviae</i>	AMP; NAL; VAN; ERY; CET; PEN	NAL; VAN; CET; PEN
	<i>A. caviae</i>	AMP; VAN; ERY; CET; PEN	VAN; CET; PEN
	<i>A. salmonicida</i>	AMP; TCY; NAL; VAN; ERY; CET; PEN	TCY
	<i>A. salmonicida</i>	AMP; TCY; VAN; ERY; PEN	TCY; VAN; PEN
	<i>A. caviae</i>	AMP; ERY; PEN	-

AMP – ampicillin, TCY – tetracycline, PEN – penicillin, CET – cephalotin, CRO – ceftriaxone, NAL – nalidixic acid, CHO – chloramphenicol, ERY – erythromycin, VAN – vancomycin.

bacteria is a growing concern in public health, especially in view of the evidence that the use of antibiotics in livestock can lead to increased resistance in human pathogens (Teuber, 2001; Sorum and L'Abée-Lund, 2002). As shown by Kemper (2008), many R plasmids carry two or more determinants flanked by homologous insertions. These insertions are responsible for the rapid evolution of bacterial plasmids transporting genes conferring multiple resistances against antibacterials.

In a study on antibacterial resistance in *Aeromonas* isolated from fish and aquatic environments, Hirsch et al. (2006) found resistance to quinolones to be non-plasmidial, a finding supported by the present study. Other researchers believe *Aeromonas* spp. display an intrinsic resistance to ERY (Kämper et al., 1999).

In general, bacteria become resistant to TCY by acquiring R plasmids. These contain genes encoding proteinaceous transporters (Group A, B, C, and D proteins) or “efflux pumps” in the cytoplasmic membrane capable of expelling antibiotics and other unwanted toxic substances from the cell (Trabulsi and Alterthum, 2005). The association or mobilization of plasmids carrying resistance genes to TCY (alone or in combination with other drugs), favoring plasmid dissemination, has previously been described in the literature (Schmidt et al., 2000; Sorum and L'Abée-Lund, 2002). Tetracyclines are broad-spectrum antibiotics used against Gram-positive and negative bacteria, aerobes and anaerobes (Chopra and Roberts, 2001) in which they inhibit the protein synthesis mainly by binding to the 30S ribosomal subunit (Chopra and Houston, 1999). Efflux pumps are the main mechanism of bacterial resistance to this family of antibiotics.

Palú et al. (2006) were the first Brazilian researchers to report the presence of R plasmids in *Aeromonas*. Working on plant

samples, they isolated three plasmids with the ability to carry resistance genes. When transferred to human pathogens, R plasmids can compromise antibiotic treatment of *Aeromonas* infections.

The presence of extrachromosomal elements in *Aeromonas* strains, as reported in this study, indicates that, regardless of sampling location, the Cocó river is a source of pathogenic bacteria (such as *Aeromonas*) as well as of bacteria capable of transferring resistance factors and adaptive strategies to other microorganisms in the environment.

6. Conclusion

The density of cultivable *Aeromonas* spp. was influenced by salinity along the estuary. According to the findings of this study Coco River, at the three points can be considered a reservoir of various *Aeromonas* species resistant to many antibiotics, which deserves further attention to this environment. The presence of species *Aeromonas* pathogenic in the estuary of the Cocó River with high incidence of antibacterial resistance, posing serious health risks to residents, fishermen and bathers.

References

- Albert, M.J., Ansaruzzaman, M., Talukder, K.A., Chopra, A.K., Kuhn, I., Rahman, M., Faruque, A.S., Islam, M.S., Sack, R.B., Mollby, R., 2000. Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. *J. Clin. Microbiol.* 38, 3785–3790.
- Araújo, R.M., Arribas, R.M., Lucena, F., Peres, R., 1991. Relations between *Aeromonas* and faecal coliforms in fresh waters. *J. Appl. Bacteriol.* 67, 213–217.

- Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45, 493–496.
- Biscardi, D., Castaldo, A., Gualillo, O., Fuscus, R., 2002. The occurrence of cytotoxic *Aeromonas hydrophila* strains in Italian mineral and thermal waters. *Sci. Total Environ.* 292, 255–263.
- Brandi, G., Sisti, M., Schiavano, G.F., Salvaggio, L., Albano, A., 1996. Survival of *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* in soil. *J. Appl. Bacteriol.* 81, 439–444.
- Buchanan, R.L., Palumbo, S.A., 1985. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: a review. *J. Food Safety* 7, 15–29.
- Chang, Y.-C., Wang, J.Y., Selvam, A., Kao, S.C., Yang, S.S., Shih, D.Y.C., 2007. Multiplex PCR detection of enterotoxin genes in *Aeromonas* spp. from suspect food samples in Northern Taiwan. *J. Food Prot.* 71, 2094–2099.
- Chopra, A.K., Houston, C.W., 1999. Enterotoxins in *Aeromonas* associated gastroenteritis. *Microbes Infect.* 1, 1129–1137.
- Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of the action, application, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65, 232–260.
- Clinical and Laboratory Standards Institute, 2009. Performance Standards for antimicrobial susceptibility testing: 19th informational supplement. CLSI document M100–S19. CLSI, Wayne, PA.
- Downes, F.P., Ito, K., 2001. *Compendium of Methods for the Microbiological Examination of Foods*. 4th ed. ASM, Washington, DC.
- Evangelista-Barreto, N.S., Carvalho, F.C.T., Vieira, R.H.S.F., Reis, C.M.F., Macrae, A., Rodrigues, D.P., 2010. Characterization of *Aeromonas* species isolated from an estuarine environment. *Braz. J. Microbiol.* 41, 452–460.
- Figuera, M.J., 2005. Clinical relevance of *Aeromonas* sM503. *Rev. Med. Microbiol.* 16, 145–153.
- Gibotti, A., Saridakis, H.O., Pelayo, J.S., Tagliari, K.C., Falcao, D.P., 2000. Prevalence and virulence properties of *Vibrio cholerae* non-O1, *Aeromonas* spp. and *Plesiomonas shigelloides* isolated from Cambe Stream (State of Parana Brazil). *J. Appl. Microbiol.* 89, 70–75.
- Hancock, R.E.W., 2005. Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Infections Diseases* 5, 209–218.
- Hirsch, D., Junior, D.J.P., Logato, P.V.R., Piccoli, R.H., Figueiredo, H.C.P., 2006. Identification and antimicrobial resistance of motile *Aeromonas* isolated from fish and aquatic environment. *Cienc. Agrotech.* 30, 1211–1217.
- Holland, A.F., Sanger, D.M., Gawle, C.P., Lerberg, S.B., Santiago, M.S., Riekerk, H.M., Zimmerman, L.E., Scott, G.I., 2004. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *J. Exp. Mar. Biol. Ecol.* 298, 151–178.
- Hughes, J.M., Ribas, F., 1991. SGAP-10C agar for the isolation and quantification of *Aeromonas* from water. *J. Appl. Bacteriol.* 70, 81–88.
- Janda, J.M., Abbott, S.L., 2010. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.* 23, 35–73.
- Kämper, P., Christmann, C., Swings, J., Huys, G., 1999. *In vitro* susceptibilities of *Aeromonas* genomic species to 69 antimicrobial agents. *Syst. Appl. Microbiol.* 22, 662–669.
- Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Ind.* 8, 1–13.
- Knochel, S., Jeppesen, C., 1990. Distribution and characteristics of *Aeromonas* in food and drinking water in Denmark. *Int. J. Food Microbiol.* 10, 317–322.
- Koehler, J.M., Ashdown, L.R., 2003. *In vitro* susceptibilities of tropical strains of *Aeromonas* species from Queensland, Australia, to 22 antimicrobial agents. *Antimicrob. Agents Chemother.* 37, 905–907.
- Libisch, B., Giske, C.G., Kovács, B., Tóth, T.G., Füzi, M., 2008. Identification of the first VIM metallo- β -lactamase-producing multidrug-resistant *Aeromonas hydrophila* strain. *J. Clin. Microbiol.* 46, 1878–1880.
- Maalej, S., Mahjoubi, A., Elazri, C., Dukan, S., 2003. Simultaneous effects of environmental factors on motile *Aeromonas* dynamics in an urban effluent and in the natural seawater. *Water Res.* 37, 2865–2874.
- Marcel, K.A., Antoinette, A.A., Mireille, D., 2002. Isolation and characterization of *Aeromonas* species from an eutrophic tropical estuary. *Mar. Pollut. Bull.* 44, 1341–1344.
- Martin-Carnahan, A., Joseph, S.W., 2005. *Aeromonadaceae*. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garit, G.M. (Eds.) *Bergey's Manual of Systematic Bacteriology, The Proteobacteria, Part B*, vol. 2, second ed., Springer-Verlag, New York, USA, pp. 556–578.
- Matté, M.H., Martone-Rocha, S., Piveli, R.P., Matté, G.R., Dória, M.C., Dropa, M., Morita, M., Peternella, F.A., 2010. Dynamics of *Aeromonas* species isolated from wastewater treatment system. *J. Water Health* 8, 703–711.
- Molina-Aja, A., García-Gasca, A., Abreu-Grobois, A., Bolán-Mejía, C., Roque, A., Gomes-Gil, B., 2002. Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. *FEMS Microbiol. Lett.* 213, 7–12.
- Pablos, M., Rodríguez-Calleja, J.M., Santos, J.A., Otero, A., García-López, M.L., 2009. Occurrence of motile *Aeromonas* in municipal drinking water and distribution of genes encoding virulence factors. *Int. J. Food Microbiol.* 135, 158–164.
- Palú, A.P., Gomes, L.M., Miguel, M.A.L., Balassiano, I.T., Queiroz, M.L.P., Freitas-Almeida, A.C., Oliveira, S.S., 2006. Antimicrobial resistance in food and clinical *Aeromonas* isolates. *Food Microbiol.* 23, 504–509.
- Palumbo, S., Abeyta, C., Stelma, G., Wesley, I.W., Wei, C., Koberger, J.A., Franklin, S.K., Tucker, L.S., Murano, E.A., 2001. *Aeromonas*, *Arcobacter* and *Plesiomonas*. In: *Compendium of Methods for the Microbiological Examination of Foods*, third ed., APHA, Washington, 2001, pp. 283–290 (Chapter 30).
- Sautour, M., Mary, P., Chihib, N.E., Hornez, J.P., 2003. The effects of temperature, water activity and pH on the growth of *Aeromonas hydrophila* and on its subsequent survival in microcosm water. *J. Appl. Microbiol.* 95, 807–813.
- Schmidt, A.S., Morten, S.B., Dalsgaard, K.P., 2000. Occurrence of antimicrobial resistance in fish pathogenic and environmental bacteria associated with four Danish rainbow trout farms. *Appl. Environ. Microbiol.* 66, 4908–4915.
- Seidler, R.J., Allen, D.A., Lockmann, H., Colwell, R.R., Joseph, S.W., Daily, O.P., 2001. Isolation, enumeration and characterization of *Aeromonas* from polluted waters encountered in diving operations. *Appl. Environ. Microbiol.* 39, 1010–1018.
- Sepe, A., Barbieri, P., Peduzzi, R., Demarta, A., 2008. Evaluation of *recA* sequencing for the classification of *Aeromonas* strains at the genotype level. *Lett. Appl. Microbiol.* 46, 439–444.
- Sinton, L., Hall, C., Braithwaite, R., 2007. Sunlight inactivation of *Campylobacter jejuni* and *Salmonella enterica*, compared with *Escherichia coli*, in seawater and river water. *J. Water Health* 5, 357–365.
- Sorum, H., L'abée-Lund, T.M., 2002. Antibiotic resistance in food-related bacteria – a result of interfering with the global web of bacterial genetics. *Int. J. Food Microbiol.* 78, 43–56.
- Teuber, M., 2001. Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol.* 4, 493–499.
- Trabulsi, R.L., Alterthum, F., 2005. *Microbiology*, fourth ed. Atheneu, São Paulo, p. 718.
- Turnbull, P.C.B., Lee, J.V., Miliotis, M.D., Van de Walle, S., Koornhof, H.J., Jeffery, L.J., Bryant, T.N., 1984. Enterotoxin production in relation to taxonomic grouping and source of isolation of *Aeromonas* species. *J. Clin. Microbiol.* 19, 175–180.
- Van Elsas, J.D., Duarte, G.E., Rosado, A.S., Smalla, K., 1997. Microbiological and molecular methods for monitoring members of soil microbial communities. *J. Microbiol. Methods* 32, 133–154.
- Vila, J., Marco, F., Soler, L., Chacon, M., Figueras, M.J., 2003. *In vitro* antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype *sobria*. *J. Antimicrob. Chemother.* 49, 697–702.
- Warren, L., 2010. *Medical Microbiology and Immunology*, tenth ed. Electronic book text, p. 620.