

AEROMONAS PRESENCE IN DRINKING WATER FROM COLLECTIVE RESERVOIRS AND WELLS IN PERI-URBAN AREA IN BRAZIL

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ABSTRACT

Aeromonas genus is considered an emerging pathogen and its presence in drinking water supplies is a reason to public health concern. This study investigated the occurrence of *Aeromonas* in samples from collective reservoirs and wells used as drinking water sources in a peri-urban area. A total of 35 water samples were collected from collective reservoirs and 32 from wells bimonthly, from September 2007 to September 2008. *Aeromonas* spp determination was carried out using a Multiple-Tube Technique. Samples were inoculated into alkaline peptone water and the superficial film formed was transferred to blood agar plates amended with ampicillin. Typical *Aeromonas* colonies were submitted to a biochemical screening and then to biochemical tests for species differentiation. *Aeromonas* was detected in 13 (19%) of the 69 samples examined (6 from collective reservoirs and 7 from wells). Concentrations of *Aeromonas* in collective reservoirs ranged from <0.3 to 1.2 x10²MPN/100mL and, in wells, from <0.3 to 2.4 x10²MPN/100mL. The most frequent specie in the collective reservoir samples was *Aeromonas* spp (68%), followed by *A. encheleia* (14%) and *A. allosaccharophila* (8%) and *A. hydrophila* (8%). *Aeromonas* spp (87%) was the most frequent specie isolated from well samples, followed by *A. allosacchariphila* (8%), *A. encheleia* (2%) and *A. jandaei* (5%). These data show the presence and diversity of *Aeromonas* genus in the samples analyzed and highlight that its presence in drinking water poses a significant public health concern.

Key words: *Aeromonas*, peri-urban area, water quality

INTRODUCTION

Aeromonas genus is considered an emerging pathogen (26, 27) and responsible for gastrointestinal disturbances (19), septicemia, endocarditis, conjunctivitis and wound infections (8, 9, 13, 16, 18). These organisms are largely distributed in

several water sources such as rivers, lakes, wells, sewage, wastewaters and drinking water supplies (19, 24).

The presence of these organisms in drinking water supplies, including in those chlorinated, is a reason to public health concern due to their capacity to produce toxins (8,20,21), to colonize biofilms (5,20) and to resist to chlorine

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disinfection (3,24). Ghenghesh *et al.* (10) analyzed 1000 samples of water collected from wells (980) and miscellaneous sources (120) and *Aeromonas* species were isolated in 48.7% of them. They carried out *Aeromonas* speciation in 381 isolated strains and found 225 (59%) *A. hydrophila*, 103 (27%) *A. caviae*, 42 (11%) *A. sobria* and 11 (3%) atypical *Aeromonas*. Occurrence of *Aeromonas hydrophila* was detected by Fernández *et al.* (7) in chlorinated treated water in Argentina when the maintenance disinfection program failed. In the same way, Ivanova *et al.* (14) isolated *Aeromonas* from drinking water reservoirs and observed high incidence of *A. sobria* and *A. poppoffi*, which are more frequent in water without fecal pollution. Study carried out by Villarruel-López *et al.* (24) detected *Aeromonas* in 31% of the samples from drinking water plants in Mexico City. Razzolini *et al.* (19) detected *Aeromonas* in chlorinated water reservoirs and drinking water fountains in the city of São Paulo.

In view of this, the objective of this study was to investigate the occurrence of *Aeromonas* genus in chlorinated water from reservoirs and wells that are used as drinking water sources in a peri-urban area of the Metropolitan Region of São Paulo, Brazil.

MATERIALS AND METHODS

Study area

Three peri-urban areas irregularly established in Protection Water Catchments of Suzano, one of the cities of the Metropolitan Region of São Paulo, were identified.

The population in these areas is about 2,000 inhabitants. Drinking water supply has been done by the transport of treated water in truck-tanks and transference to collective reservoirs with capacity of 5m³. These reservoirs are disposed in public areas and the water withdrawn have been done through hoses, storage in vessels or other recipients such as buckets which are considered inappropriate. The cleaning of the collective reservoirs does not have a fixed frequency and the community is in charge of this task. As water sources are scarce, people

look for an alternative water source such as digging well in their backyard. It is important to highlight that the construction of the wells and septic tanks are done without any criterion.

Sampling

A total of 35 water samples were collected from collective reservoirs and 32 from wells. The water samples were collected bimonthly from September 2007 to September 2008. The samples were collected according to the APHA Standard Methods (1) in sterile disposable bottles, chilled for transportation and examined within a 24-hour period. Residual chlorine was measured in samples from the collective reservoirs by colorimetric method using Free-chlorine Analyser Policontrol®. Water temperature was taken using mercury column and the pH value using universal paper pH indicator. The occurrence of rainfall in the last 24 hours was recorded.

Aeromonas spp determination was carried out using a Multiple-Tube Technique as recommended by the APHA (1). In brief, serial dilutions were done with sterile 0.85% sodium chloride solution, 1mL of diluted samples were inoculated into alkaline peptone water, pH 8.6, and incubated 24h at 35°C. The superficial film formed in each tube was transferred to triplicates blood agar plates amended with 10mg/mL ampicillin and incubated for 24h at 35°C. Typical *Aeromonas* colonies, with 2-3mm diameter, convex, producing haemolysis or not, were screened for cytochrome oxydase, indole and gas production, lactose acidification and glucose fermentation.

The strains presenting positive results for the *Aeromonas* genus were submitted to complementary biochemical tests for the determination of the species as follow: nitrate reduction; arginine dihydrolase; lysine and ornithine decarboxylase; growth in peptone broth with 6% NaCl and without sodium chloride; acid production from arabinose, manose, mannitol, sucrose and esculin hydrolysis. Identification to the species level was carried out by comparison of the biochemical results with Table 1. Production of hemolysin was performed in Tryptic Soy Agar (TSA) plates with 5% of sheep blood incubated at 35°C ± 0.5°C for 24 and 48 h.

Table 1. Biochemical characteristics of different species of *Aeromonas*

Species	NO ₃ ⁻ → NO ₂ ⁻	D-Glucose, gas	Indole	Arginine dihydrolase	Lysine decarboxylase	Ornithine decarboxylase	Esculin hydrolysis	NaCl tolerance 0%	NaCl tolerance 6%	Sucrose	D-Manitol	L-Arabinose	Salicin	Manose	β-hemolysis	Resistance to ampicilin ^a
<i>Aeromonas hydrophila</i>	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
<i>Aeromonas salmonicida</i>	+	+	+	V	V	-	+	+	-	+	+	+	-	+	V	+
<i>Aeromonas media</i>	+	-	+	+	-	-	+	+	-	+	+	+	V	+	V	+
<i>Aeromonas encheleia</i>	+	V	+	+	-	-	+	+	-	+	+	-	+	+	V	+
<i>Aeromonas allosaccharophila</i>	+	V	+	+	+	-	V	+	-	+	+	V	-	+	V	+
<i>Aeromonas eucrenophila</i>	+	+	+	+	-	-	+	+	-	V	+	+	+	+	V	+
<i>Aeromonas bestiarium</i>	+	V	+	+	+	-	+	+	-	+	+	+	V	+	V	+
<i>Aeromonas caviae</i>	+	-	+	+	-	-	+	+	-	+	+	+	+	V	V	+
<i>Aeromonas veronii sobria</i>	+	V	+	+	+	-	-	+	-	+	+	-	-	+	+	+
<i>Aeromonas veronii veronii</i>	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+
<i>Aeromonas schubertii</i>	+	-	-	+	+	-	-	+	-	-	-	-	-	+	V	+
<i>Aeromonas jandaei</i>	+	+	+	+	+	-	-	+	-	-	+	-	-	+	+	+
<i>Aeromonas trota</i>	+	V	+	+	+	-	-	+	-	V	V	-	-	+	V	-
<i>Aeromonas popoffii</i>	+	+	V	+	-	-	-	+	-	-	+	V	-	+	-	+
<i>Aeromonas culicicola</i>	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+
<i>Aeromonas simiae</i>	+	-	-	+	+	-	V	+	-	+	-	-	-	+	-	+
<i>Aeromonas molluscorum</i>	+	-	-	+	-	-	+	+	-	+	+	+	+	+	nd	+
<i>Aeromonas sobria</i>	+	V	+	-	+	-	-	+	-	+	+	-	-	+	V	V

Source: Pidiyar and col., 2002; Abbott and col., 2003; Harf-Monteil and col., 2004
 Note: (+) = .85% of positive strains; (-) = > 85% of negative strains; (V) = 50%; nd = not determined

RESULTS AND DISCUSSION

The presence of *Aeromonas* was detected in 13 (18.8%) of the

69 samples examined. *Aeromonas* were found in 6 (17.2%) collective reservoir samples and 7 (21.9%) well samples, as shown in Figure 1.

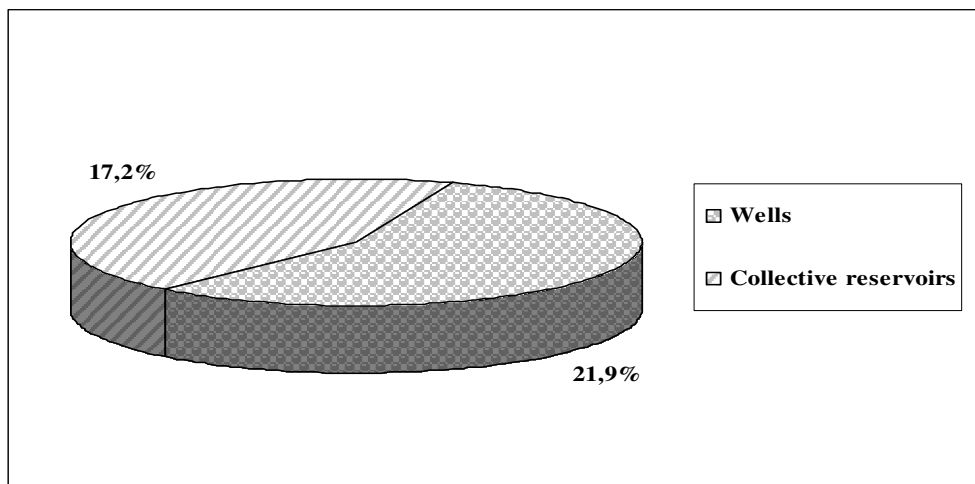


Figure 1. Percentage of positive samples for *Aeromonas* spp according to the type of source of water.

Our findings can be compared with study carried out by Di Bari *et al.* (6) which detected the presence of *Aeromonas* in 37% of non treated water samples collected from wells, springs, fountains and mineral waters. Out of 91 samples from wells, 31 (28%) were positive to *Aeromonas*. Massa *et al.* (15) isolated *Aeromonas* in 25% of well water samples in Lecce, Italy. However, other authors, as Ghenghesh *et al.* (10), found frequency of *Aeromonas* as high as 48.7% in water samples collected from wells (980) and miscellaneous sources (120).

It was observed that the mean value of residual chlorine in the collective reservoirs did not meet the standard established by the Brazilian drinking water legislation (0.2 mg/L), except in some samples analyzed, but *Aeromonas* were not isolated. These results reinforce the findings of Brandi *et al.* (2),

Fernández *et al.* (6) and Villarruel-López *et al.* (19).

Concentration of *Aeromonas* in the collective reservoirs ranged from <0.3 to 1.2 x10² MPN/100mL and, in wells, from <0.3 to 2.4 x10² MPN/100mL. Di Bari *et al.* (5) found in water samples from wells a maximum value of >200 CFU/100mL and <1 CFU/100mL as the minimum value and the geometric mean was 3.9 CFU/100mL. Massa *et al.* (13), in Italy, reported counts ranging from 26 to 1609 CFU/250mL.

The species identified in the current study are presented in Table 2. Out of 37 isolations from collective reservoirs, *Aeromonas* spp. (67.6%) were the most frequent followed by *Aeromonas encheleia* (13.5%) and *A. allosaccharophila* (8.1%) and *A. hydrophila* (8.1%).

A. hydrophila has also been found in drinking water in

Table 2. Frequency of *Aeromonas* species isolated from samples of the collective reservoirs and wells

	Collective reservoirs		Wells	
	Strains (n)	%	Strains (n)	%
<i>Aeromonas</i> spp.	25	67.6	52	86.6
<i>Aeromonas allosaccharophila</i>	3	8.1	5	8.3
<i>Aeromonas bestiarium</i>	1	2.7	nd	nd
<i>Aeromonas encheleia</i>	5	13.5	1	1.6
<i>Aeromonas hydrophila</i>	3	8.1	nd	nd
<i>Aeromonas jandaei</i>	nd	nd	2	3.3
Total	37	100	60	100

nd = not detected

other studies Havelaar *et al.* (12), Chauret *et al.* (5), Sen & Rodgers (21) and Razzolini *et al.* (19). *Aeromonas* spp. (86.6%) was the most frequent specie isolated from well samples followed by *A. allosacchariphila* (8.3%). Different results were found by Massa *et al.* (15) who isolated *A. hydrophila*, *A. sobria* and *A. caviae* from well samples in Italy. Ivanova *et al.* (14) reported that the most frequent isolation from drinking

water samples was *A. sobria* and Razzolini *et al.* (19) found *A. caviae* as the commonest specie in chlorinated drinking water. These findings show the diversity of aeromonads in water sources.

It is worth mentioning that while *A. hydrophila*, *A. bestiarum* and *A. encheleia* occurred only in samples from collective reservoirs, *A. jandaei* was only isolated from well

waters.

The hemolysin production was observed in 39 isolated strains (40.2%), being 21 strains from wells and 18 from collective reservoirs. The species that showed hemolytic activity from collective reservoirs samples were *A. encheleia*, *A. allosaccharophila* and *Aeromonas* sp and from wells samples were *Aeromonas* sp and *A. allosaccharophila*. Absence of hemolytic activity in *A. hydrophila* strains was something unexpected. Production of exotoxin (α and β -hemolysin) is associated with enteric disturbances, as reported by Burke *et al* (4), Monfort and Baleux (17), Singh and Sanyal (22,23), Pin *et al* (18), Handfield *et al.*(11) and Razzolini *et al* (19). These results highlight the pathogenic potential of *Aeromonas* species and pose a public health concern. Takahashi *et al* (25) detected and purified a hemolytic toxin from *Aeromonas sobria* (ASH), which promotes fluid accumulation in the mouse intestinal loop. This work puts in evidence a novel insight into correlation between ion HCO_3^- and secretory diarrhea induced by bacterial infections, considering that ASH actively induces the secretion of HCO_3^- .

The occurrence of *Aeromonas* genus in stored waters is a human health concern because of its ability to produce toxins, re-growth in low-nutrient environment and survive in chlorinated drinking water. Thus, some authors consider that these organisms should be a sanitary quality indicator (7, 19, 21, 24). Moreover, *Aeromonas hydrophila* is listed in the first and second Contaminant Candidate List (CCL1 and CCL2) by the US Environmental Protection Agency – USEPA (26), linking these organisms to public health adverse outcomes.

The findings of this investigation confirm the presence of *Aeromonas* genus in drinking water samples from collective reservoirs and wells in the study area. As *Aeromonas* is considered an emerging pathogen and can cause health disturbances, including serious illnesses, its presence in drinking water poses a significant public health concern.

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