The occurrence of *Aeromonas* spp in drinking water

Ocorrência de *Aeromonas* spp em água de consumo humano

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RESUMO

A ocorrência de *Aeromonas* spp móveis foi verificada em um total de 730 amostras de água de consumo humano de diferentes origens, sendo 495 da rede de abastecimento público tratada, 208 de soluções alternativas (compreendendo 65 de poços artesianos, 69 de poços rasos, 74 de minas), além dessas foram analisadas 27 amostras de água mineral envasada. Os resultados mostraram que 6,3%, 55,3% e 11,1% das amostras de água da rede de abastecimento público tratada, soluções alternativas e água mineral envasada estavam contaminadas com *Aeromonas* spp, respectivamente. Verificou-se que nem sempre a ocorrência de *Aeromonas* estava associada à presença de indicadores fecais. Das 613 cepas de *Aeromonas* isoladas 75,4% pertenciam a espécie *Aeromonas hydrophila*, seguidos por 9,8% de *Aeromonas caviae* e 0,6% de *Aeromonas sobria*. Estes achados mostram que estudos adicionais são necessários para avaliar possíveis riscos que a presença de *Aeromonas* spp poderia acarretar à saúde pública.

Palavras-Chave. *Aeromonas* spp, água tratada, fontes alternativas, água mineral engarrafada.

ABSTRACT

The presence of motile *Aeromonas* spp was investigated in a total of 730 samples of drinking water from different origins: 495 samples from the treated water distribution system, 208 from alternative water supply (65 from artesian wells, 69 from shallow wells, and 74 from springs)**,** and 27 samples of bottled mineral water. The present study indicated that 6.3%, 55.3% and 11.1% of samples from treated water distribution system, alternative water supply, and bottled mineral water were contaminated with *Aeromonas* spp, respectively. The highest counts of *Aeromonas* spp were not always associated to the presence of faecal indicators. *Aeromonas hydrophila* was the most frequently isolated phenospecies in water, followed by *Aeromonas caviae* (9.8%) and *Aeromonas sobria* (0.6%). These findings show that further studies are necessary to evaluate the possible risks that the occurrence of *Aeromonas* spp may pose to public health.

Key Word. *Aeromonas* spp, treated water, alternative water supply systems, bottled mineral water.

INTRODUCTION

The genus *Aeromonas* currently belongs to the family *Aeromonadaceae*, and is characterized as short, Gram negative, oxidase – positive, rod - shaped bacteria, which metabolise glucose by both the respiratory and fermentative pathways and show resistance to the vibriostatic agent O/ 129[1]. It can be divided into two groups: the first includes the psychotrophic *Aeromonas*, represented by *Aeromonas salmonicida* and the second, by the mesophyllic *Aeromonas*[2].

The taxonomy of *Aeromonas* is under constant development, until the last decade only 14 different species[3] were known. From these, six species are considered to be clinically important: *A. hydrophila, A. caviae, A. veronii, A. sobria, A. jandaei and A. schubertii*[4]. Recently, *A. molluscorum*[5], *A.* culicicola[6] and *A.* simiae[7] were isolated from bivalve mollusks, midgut of *Culex quinquefasciatus* and monkey faeces, respectively. Those organisms cause extra-intestinal (endocarditis, peritonitis, septicaemia) and intestinal infections in children and immunocompromised patients, being considered

as opportunistic pathogens[8]. However, epidemiological studies indicated that *Aeromonas* spp can also act as primary etiological agents of gastroenteritis[9]. Knøchel; Jeppesen[10]; Kühn et al.[11], and Ørmen; Østensvik[12], verified that some species of *Aeromonas* spp are carriers of certain virulence factors, which are responsible for their survival, adhesion and colonization of the intestinal mucous membrane, leading to gastrointestinal disorders.

Aeromonas species are widely spread in nature, especially in aquatic environments. These microorganisms have frequently been isolated from surface[13] and subterranean[14,15] waters and from the water distribution system, both of treated and untreated drinking water[11] and even from biofilms[16]. This observation is a cause of concern, since *Aeromonas* could survive in the biofilms and, under certain conditions, such as low chlorine concentrations, high pH and low temperatures, these organisms multiply and spread through the water distribution network[17], exposing the consumer to health risks. Drinking water is considered to be one of the possible sources of diarrheagenic strains of *Aeromonas*[4,18].

Considering these facts, the evaluation of the presence of these bacteria in human drinking water is an important aspect of public health, since national studies on this theme are scarce. Thus, the objective of this study was to verify the occurrence of *Aeromonas* spp in human drinking water sent to the laboratory by municipal sanitary inspection agencies.

MATERIALS AND METHODS

Samples

In this study, water samples were collected by municipal sanitary inspection agencies attended by laboratories I in Campinas and in Santo André. Laboratory I in Campinas examined 348 water samples, including 212 from the public distribution network, 27 of bottled mineral water and 136 from other water systems (18 artesian wells, 34 shallow wells, 57 springs). Laboratory I in Santo André analysed 382 samples, 283 from the public distribution network and 99 from other water systems (47 artesian wells, 35 shallow wells and 17 springs). Treated water samples were collected aseptically in 500ml sterile flasks containing 0.1 ml of 10% solution of sodium thiosulphate per 100 ml water. All the water samples were delivered to the laboratories in isothermal boxes and analysed within 24 hours after collection. Water from mineral springs was sent to laboratory I in Campinas in various types of packaging, such as: PVC, polyethylene, glass and polycarbonate. These packages were originally used by the bottling unit and had different volumes. Each sample consisted of 5 units with the same filling date. In the laboratory, 100 ml was withdrawn from each unit and poured into a sterile flask and the analysis was carried out on a composite sample, according to Food Microbiology Committee – Instituto Adolfo Lutz[19].

Methods

Determination of water quality indicators.

Coliforms and faecal coliforms were determined for each water sample according to Standard Methods[20], using m - Endo Agar LES (Difco – Detroit - MI) incubated at 35°C and m – FC agar (Difco – Detroit - MI) incubated at 45°C, respectively. All bacteria that produced red colonies with a metallic sheen within 24h incubation on m – Endo Agar LES were considered members of the coliform group. Colonies produced by faecal coliform bacteria on m – FC agar were blue. Determination of the possible presence of *E. coli* was conducted by selecting a well – isolated colony and inoculating into a Bac – tray system (Difco – Detroit – MI).

Procedures for the isolation of *Aeromonas* spp.

The membrane filtration technique proposed by Havelaar et al.[21] was used. The volume of water filtered varied according to the origin of the water. A volume of 100 ml was filtered for waters obtained from the public distribution network, artesian wells and bottled mineral water. For those obtained from shallow wells and springs three different volumes were filtered: 100 ml, 10 ml and 0.1 ml. In order to filter volumes of 10 ml and 0.1 ml, 20 ml of sterile dilution water was added to the filter before filtering the sample. Samples were filtered through cellulose ester membranes with pore size equal to 0.45 μm (Millipore São Paulo) and the membrane then transferred to a Petri dish containing ampicillin-dextrin agar. After incubation at 30°C/24h, brilliant yellow colonies were counted.

Identification of *Aeromonas* spp.

Typical, representative colonies were transferred to trypticase agar (TSA) (Difco/Detroit–MI) and Aeromonas Hydrophila medium (AH)[22] slants for the presumptive identification of *Aeromonas* spp. After incubation at 30°C/24h, cultures presenting an acid butt (yellow or greyish yellow) with an alkaline (purple) band at the slant, positive motility (turbid), production of indol after the addition of Kovacs reagent and positive oxidase test in AH medium, were presumptively considered as *Aeromonas* spp. Using the TSA cultures, suspect strains were then submitted to confirmation using the following tests: Gram stain, sensitivity to the vibriostatic agent O/129, nitrate reduction, acetoin production, oxidative/fermentative metabolism according to Hugh-Leifson, growth at 37°C and in 0%, 3% and 6% sodium chloride solutions, fermentation of sucrose, salicin, arabinose, arginine and lysine, production of gas from glucose, hydrolysis of esculin, Camp test[23] and presence of monotrichous flagellum. Final count was calculated based on the proportion of suspect colonies confirmed as *Aeromonas* spp. and expressed as CFU/100 ml water.

RESULTS AND DISCUSSION

Table 1 shows the data for the occurrence of *Aeromonas* spp. in drinking water from different origins. In treated water

from the public distribution network, 6.3% of the total of 495 samples was contaminated with *Aeromonas* spp., with counts ranging from 1 to 3.0 x 10^2 CFU/100 ml water (Table 2). Krovaceck et al.[24] obtained higher values, equal to 85%, with populations of up to 8.6 x 10^2 CFU/100ml treated water. Other authors, such as Khun et al.[11] and Gavriel et al.[25] have also shown the presence of these microorganisms in treated water.

According to Van Der Koij; Hijinen[26], the frequent isolation of *Aeromonas* from treated water is due to the difficulty in maintaining an adequate level of residual chlorine in the water distribution network. In addition, some studies such as that of Sisti et al.[27], showed that the bactericidal action of chlorine compounds was strongly influenced by temperature: the efficiency of chlorine at 20°C was two to three times lower than that observed at 5°C.

Still in relation to treated water, it was observed that the isolation of *Aeromonas* in this type of water was not necessarily associated with the presence of the traditionally used faecal indicators.

In the present study, of the 495 samples examined, 3.2% contained total coliforms and 0.4% *Escherichia coli* (Table 1). These findings are in agreement with the observations of Burke et al.[28] in Australia and of Legnani et al.[14] in Italy, who isolated *Aeromonas* from treated water in which faecal indicators were absent.

Drinking water samples from the other water systems presented high rates of *Aeromonas* isolation. From 208 samples analysed, 55.3% were contaminated with *Aeromonas* spp. From

Table 1. Frequency of drinking water samples positive for *Aeromonas* spp., total coliforms and *E. coli* in the cities attended by the Laboratories I in Campinas and in Santo André.

Water origin	Laboratories I	No. of samples	Aeromonas *spp*		Total coliforms		E. coli	
			No.	%	No.	%	No.	%
PDN*	Campinas	212	16	7.5	09	4.2	01	0.5
	Santo André	283	15	5.3	07	2.5	01	0.4
Total 1		495	31	6.3	16	3.2	02	0.4
Other water systems								
Artesian wells	Campinas	18	08	44.4	06	33.3	02	11.1
	Santo André	47	19	40.4	08	17.0	02	4.3
Sub-total 1		65	27	41.5	14	21.5	04	6.2
Shallow wells	Campinas	34	27	79.4	24	70.6	02	6.0
	Santo André	35	16	45.7	15	43.0	05	14.3
Sub-total 2		69	43	62.3	39	56.5	07	10.1
Springs	Campinas	57	35	61.4	32	56.1	06	10.5
	Santo André	17	10	59.0	09	53.0	06	35.3
Sub-total 3		74	45	61.0	41	55.4	12	16.0
Total 2		208	115	55.3	94	45.2	23	11.0
Bottled mineral water	Campinas	27	03	11.1	01	3.7	-	-
	Santo André	-	-	-	-	-	-	-
Total 3		27	03	11.1	01	3.7		
Total		730	149	20.4	111	15.2	25	3.4

*PDN = public distribution network

these, 41.5% were from artesian wells, 62.3% from shallow wells and 61.0% from springs (Table 1).

In this study, the minimum counts of *Aeromonas* spp. isolated from water collected from artesian wells, shallow wells and springs was equal to 5 CFU/100 ml water, and the maximum counts obtained were $2.3x10^3$, $5.0x10^4$ and $1.5x10^4$ CFU/100 ml water, respectively (Table 2).

Kipperman et al.[29] and Krovacek et al.[30] investigated water supplies involved in outbreaks of diarrhoea and found *Aeromonas* populations varying from $1.0x10^2 – 6.4x10^4$ CFU/100 ml. The levels found in this study are within this range.

A comparative analysis of the data found in Table 1 for waters from other water systems showed that the highest rates for the isolation of *Aeromonas* were found in water from shallow wells (62.3%) and springs (61.0%). These water supplies are highly susceptible to contamination due to the lack of protection factors, as observed in the majority of systems studied. In addition, shallow wells show a maximum depth of 15 metres, and springs emerge on to the surface, being subsequently piped. In such circumstances, the filtrating power of the soil is limited and water sources are exposed to contamination, especially from waters flowing along the surface and those that infiltrate the soil and reach the watertable. Geldreich[31] stated that water flowing on the surface is the main factor contributing to the deterioration in microbiological quality of subterranean water, making it a consumer health risk.

Table 1 also shows good correlation between the detection of *Aeromonas* and total coliforms, especially for water from shallow wells and springs, although such an association was not observed for *E. coli*. Similarly, Massa et al.[15] did not report such correlation, either.

With respect to the total coliform and *E. coli* populations, both of them varied from 5 to $1.1x10^5$ CFU/100 ml of water from other water systems (Table 2).

As for the legal regulations in effect in Brazil (Regulation 518/GM)[32], that determines the absence of *E. coli* and thermotolerant coliforms in 100 ml water in the public distribution network or other water systems, excluding bottled waters, it was observed that only 0.4% of the network water and 11% from the other water systems could be considered unacceptable for human consumption due to the presence of *E. coli* (Table1). However, 55.3% of the waters from the other water systems

Table 2. Distribution of the minimum and maximum counts of *Aeromonas* species, total coliforms and *E. coli* for positive samples of drinking water in the cities attended by the Laboratories I in Campinas or in Santo André**.

Water origin	*Aeromonas* Counts CFU/100ml		Total coliforms Counts CFU/100ml		*E. coli* Counts CFU/100ml	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
PDN*	1	3.0×10^2	01	1.4×10^2	10	5.0×10
Artesian wells	5	2.3×10^3	05	8.0×10	05	5.0×10
Shallow wells	5	5.0×10^4	10	1.1×10^5	10	1.1×10^5
Springs	5	1.5×10^4	05	1.1×10^4	15	1.1×10^4
Bottled mineral water	2	3.0×10^2	60	6.0×10	0	0

*PDN = public distribution network

** = minimum and maximum counts used correspond, respectively, to the lowest and greatest count obtained in one of the laboratories I (in Campinas or in Santo André)

Table 3. Distribution of the number and percentage of *Aeromonas* species isolated in samples of drinking water in the cities attended by the Laboratories I in Campinas and in Santo André.

Water origin	Strains of *Aeromonas*	*Aeromonas* spp		*Confirmed strains* A . hydrophila		A . caviae		A . sobria	
		n.	%	n.	%	n.	%	n.	%
PDN*	110	13	11.8	78	70.9	19	17.3	-	-
Artesian wells	167	15	9.0	149	89.2	03	1.8	-	-
Shallow wells	185	35	19.0	122	66.0	26	14.0	02	1.0
Springs	148	24	16.2	110	74.3	12	8.1	02	1.4
Bottled mineral water	03	-	-	03	100.0	-	-	-	-
Total	613	87	14.2	462	75.4	60	9.8	04	0.6

*PDN = public distribution network

were contaminated with *Aeromonas* (Table1), the majority of the samples presenting counts of about 10^4 CFU/100 ml water (Table 2).

Since the eighties, scientists in various countries, such as Burke et al.[28], in Australia and LeChevallier et al.[33], in the USA, have questioned the adequacy of water quality control from a bacteriological point of view, based only on faecal indicators. In the USA, *Aeromonas* has been included in the list of emerging pathogens and research on this microorganism has intensified in recent years[34].

In relation to bottled mineral water, 3 (11.1%) of the 27 samples contained *Aeromonas* (Table 1), with counts varying from 2 to $3.0x10^2$ CFU/100ml water (Table2). Researchers from various other countries have isolated *Aeromonas* spp. from bottled mineral water, such as Slade et al.[35], in Saudi Arabia, Gonzalez et al.[36], in Spain and Tsai & Yu[37], in Taiwan. Contamination of mineral water by *Aeromonas* can occur at the source or during bottling. Brandi et al.[38] isolated *Aeromonas* from bottled mineral water samples stored for more than 100 days. According to Kersters et al.[39], *Aeromonas* can multiply in water with low concentrations of nutrients, reaching large population numbers and becoming a health risk to consumer.

Table 1 also shows that only 1 (3.7%) of the 27 samples of bottled mineral water was positive for total coliforms without presenting *E. coli.* Similarly, Alves et al.[40] found only 1 in 18 samples of bottled mineral water contaminated with total coliforms.

The updated and improved Brazilian regulation controlling mineral waters (Resolution RDC 54)[41] still does not include *Aeromonas* among the parameters to be analysed in quality control of this product. Some international legislations for mineral waters have contemplated *Aeromonas hydrophila* amongst the parameters required for their quality control. For example, Italian law (GUDRI)[42] has established maximum numbers for *Aeromonas hydrophila* in the spring and during trade equal to, respectively, 10 and $1.0x10^2$ CFU/100 ml mineral water.

Table 3 shows that *A. hydrophila* predominated amongst the mobile *Aeromonas* species, representing 75.4% of the total number of strains identified as *Aeromonas* spp., followed by *A. caviae* (9.8%) and *A. sobria* (0.6%). The remaining 87 (14.2%) strains classified as *Aeromonas* spp. in this study showed diversified behaviour in some of the biochemical tests. In Holland, different from our results, *A. caviae* was the most frequently isolated biotype, both in faeces and in drinking water[43]. However, in Denmark, Knøchel & Jeppesen[10] also isolated a high percentage of *A. hydrophila* (97%) and only 3% *A. sobria* in drinking water.

Results obtained in this study indicate that drinking waters, particularly those that are not systematically treated, may pose a risk to consumers.

It is recommended that some segments of the population, such as children, the elderly and immunocompromised avoid the consumption of untreated water that come from other systems.

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