PERSISTENCE OF TOTAL AND FECAL COLIFORMS AND *Pseudomonas aeruginosa* IN IN NATURA WATER SAMPLES FROM RIVERS THAT SUPPLY A WATER TREATMENT STATION IN CURITIBA, BRAZIL

Persistência de coliformes fecais e totais e Pseudomonas aeruginosa *em amostras de água in natura de rios que abastecem uma estação de tratamento de água em Curitiba, Brasil*

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Abstract

An unknown parcel of the planet water is contaminated by chemical and/or biological agents. Human and other animals excreta have been associated to many infectious diseases that can be disseminated through contaminated water. Enteric bacteria and other pathogens can cause gastroenteritis, cholera, systemic infections, among others. Once in the environment, these microorganisms can persist for large periods of time and under certain conditions can even replicate, increasing their number. This paper aimed the research and evaluation of survival rates for total coliforms, fecal coliforms (*Escherichia coli*) and *Pseudomonas aeruginosa in natura* water samples from Passaúna and Iraí barrages and Iguaçú River, in Curitiba, Paraná. Colimetric analysis of water samples were performed by the membrane filtration method and *P. aeruginosa* research was assayed using the multiple tubes method in Asparagin and Acetamyde Broth. Confirmatory tests such as catalase presence, citocrome oxidase test and pigment P (pyocyanin) production were also performed. It was observed the occurrence of total coliforms in water samples from Iguaçú River for approximately 50 days, while in Iraí and Passaúna barrages, the periods were 35 and 14 days, respectively. Fecal coliforms were found for about 35 days in samples from Iguaçú River and Iraí barrage, while in Passaúna barrage only for 14 days. *P. aeruginosa* persisted for approximately 63 days in all the water samples assayed. The higher persistence rate observed in *P. aeruginosa* can be related to an antagonism towards total and fecal coliforms, which had their populations decreased.

Keywords: Microbiology. Waters. Rivers. Contamination. Coliforms.

Resumo

*Uma parcela desconhecida de água do planeta encontra-se contaminada por agentes químicos e/ou biológicos. As excretas de humanos e outros animais endotérmicos têm sido associadas a muitas doenças infecciosas que podem ser disseminadas pela água contaminada. Enterobactérias e outros patógenos podem causar gastroenterites, cólera, infecções sistêmicas, entre outras. Uma vez no ambiente, estes micro-organismos persistem por longos períodos de tempo e, sob determinadas circunstâncias, podem, inclusive, aumentar em número. Este trabalho teve como objetivo a pesquisa e a determinação do tempo de sobrevivência de coliformes totais, fecais (*Escherichia coli*) e* Pseudomonas aeruginosa *em amostras de água* in natura *das barragens dos rios Irai, Passaúna e Iguaçú, em Curitiba, Brasil. A análise colimétrica da água foi feita pelo método da membrana filtrante e de* P. aeruginosa *pelo método dos tubos múltiplos, em caldo Asparagina e Acetamida. Testes confirmatórios como presença de catalase, citocromo oxidase e pigmento P (Piocianina) também foram empregados. Observou-se a presença de coliformes totais em amostras do Rio Iguaçú por aproximadamente 50 dias, enquanto nas barragens dos rios Iraí e Passaúna, 35 e 14 dias, respectivamente. Coliformes fecais foram encontrados por 35 dias em amostras do Rio Iguaçú e na barragem Iraí, enquanto na barragem Passaúna apenas por 14 dias.* P. aeruginosa *persistiu por aproximadamente 63 dias em todas as amostras avaliadas. A maior taxa de persistência de* P. aeruginosa *pode estar relacionada ao antagonismo em relação aos coliformes totais e fecais, os quais tiveram suas populações reduzidas.*

***Palavras-chave**: Microbiologia. Águas. Rios. Contaminação. Coliformes.*

INTRODUÇÃO

Although three quarters of Earth were occupied by water, only 2.5% are freshwater that can be used for human and animals. Among these, an unknown parcel of the planet water is contaminated by chemical and/or biological agents, representing a potential risk for human and environmental health (1).

The source for surface water contamination includes municipal wastewater discharges, septic leachate, agricultural or storm runoff, wildlife populations, or nonpoint sources of human and animal waste (2, 3). The feces of animals may contain several pathogenic microorganisms such as *Campylobacter, Salmonella, Shigella, Yersinia, Aeromonas, Pasteurella, Francisella, Leptospira, Vibrio*, some protozoa and several viruses groups that could lead to the transmission of pathogens and, therefore, to waterborne diseases (4). Once in the environment, these microorganisms can persist for large periods of time and under certain conditions can even replicate, increasing their number (5).

Traditionally, indicator microorganisms have been used to suggest the presence of pathogens (6). They are described as resident in the gastrointestinal tracts of humans and animals and used worldwide to assess the microbiological safety of drinking water, recreational waters, and shellfish waters (7).

Coliforms form a heterogeneous group of organisms, many of which were not of fecal origin. In attempt to obtain faster identification, only the four most common genera of coliforms are assessed, which are *Citrobacter, Enterobacter, Klebsiella* and *Escherichia* (5). Agência de Vigilância Sanitária (Anvisa –Brazilian Sanitary Vigilance Agency) and many other entities from other countries recommend *Escherichia coli* as an indicator organism for freshwater, and members of the genus *Enterococcus* for both freshwater and saltwater (8, 9). Presence of *E. coli* in water indicates contamination by fecal material of humans or other warm-blooded animals, and also indicates the potential for the presence of pathogenic organisms (3).

It has become clear that *E. coli,* even having as only habitat the intestinal treat of humans and other warm-blood animals, as well as other indicator fecal bacteria can persist and perhaps replicate in soil and water under certain conditions. These microorganisms are ubiquitous and can persist for long periods of time in tropical and subtropical soils and water (10, 11).

According to Sousa and Silva-Sousa (12), the majority of bacteria present in aquatic environments is originated from soil, being carried through by rain water or as a consequence of human activity. Among those, *Bacillus* sp. and *Pseudomonas* sp. are genera strongly adapted in both freshwater and soil; however, we can also find members from Enterobacteriaceae and the following genera *Flavobacterium, Acinetobacter*, *Moraxella*, *Aeromonas*, *Micrococcus*, *Staphylococcus, Streptococcus*, *Clostridium* and *Pseudomonas.*

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium that grows in soil, marshes and coastal marine habitats, as well as on plant and animal tissues (13-15). It has been considered one of the top three causes of opportunistic human infections, due its intrinsic resistance to antibiotics and disinfectants and is a significant source of bacteremia in burn victims, urinary-tract infections in catheterized patients, and hospital-acquired pneumonia in patients on respirators (16). Although not generally considered a cause of infectious diarrhea, it was the predominant organism isolated from the feces of 23 unrelated, hospital outpatients (17). This species is not infectious when ingested, being associated to skin and respiratory infections, mostly by contact or inhalation of water drops (5).

The capacity for metabolizing over a hundred different organic and inorganic substances, including aromatic compounds, halogen derived and organic residues, and yet, the ability for using nitrate as final electron acceptor, in the absence of oxygen, makes *P. aeruginosa* an extraordinary well adapted microorganism (18, 19).

The search for *P. aeruginosa* in water samples has been questioned due its opportunistic nature and non fecal origin (20, 21). However, it has been observed its antagonism towards bacteria from coliform group, known as universal indicators for potability and contamination of water (22).

In this paper, we aimed to determine the occurrence and persistence of total and fecal coliforms (*E. coli*) and *P. aeruginosa in natura* water samples from a river and two barrages that supply a water treatment station in Curitiba, Brazil.

MATERIAL AND METHODS

Sample obtention: water samples were collected in plastic flasks (5 L) from the Iguaçú River, and the barrages resultant from Iraí and Passaúna Rivers, respectively. These rivers supply the capital of Paraná State, Curitiba, and also cities located in its peripheral areas. The water samples were obtained before entering the treatment station. Collects were performed in October, 2005, and analyzed weekly until December, 2005.

Colimetric analysis and ***P. aeruginosa*** **research**: colimetric analysis of water samples were performed by the membrane filtration method, according to Standard Methods for the Examination of Water and Wastewater (23). *P. aeruginosa* research was assayed using the multiple tubes method in Asparagin and Acetamyde Broth (Merk), respectively. Confirmatory tests such as catalase presence, cytochrome oxidase test and pigment P (pyocyanin) production were also performed. All analyses were conducted in Companhia de Saneamento do Paraná (SANEPAR).

Water samples were diluted (1:10; 1:100; 1:1000) in water added with KH_2PO_4 (0.5 M) and $MgCl_2$ (0.8 M). An aliquot of 1 mL of the diluted samples was inoculated in 15 tubes containing each 9 mL Asparagine Broth and incubated at 36 °C for 96 h. Tubes that showed turbidity and eventually fluorescence were considered positives for bacterial growth and samples from them were inoculated in Acetamyde Broth tubes and incubated at 36 °C for 48h. Positive results were characterized by changes in the medium color, from red to purple. The density units were expressed with the Most

Probable Number (MPN) per 100 mL. Samples from positive tubes were placed in Petri dishes containing Cetrimide Agar, a selective medium for *P. aeruginosa*, and incubated at 36 °C for 48h, according to the Standard Methods for the Examination of Water and Wastewater (23). Samples were concomitantly placed in Petri dishes with Teague Agar (Merk), a selective medium for Gram-negative bacteria. Samples that resulted in growth in both media were then assayed for catalase and oxidase presence. When positives for these biochemical tests, samples were inoculated in P medium, which stimulates pigment P (pyocianyn) production by *P. aeruginosa,* and incubated at 36 °C for 48 h. After, the bacterial isolates that showed pigment production were placed in Burton medium and incubated at 36 °C, for 2 weeks. When an intense green pigment was observed in the medium it was associated to probable *P. aeruginosa* presence. Adding chloroform until the formation of two distinct liquid phases, one of them blue (pyocyanin pigment), confirmed the presence of the species *P. aeruginosa*.

RESULTS

It was observed the occurrence of total coliforms in water samples from Iguaçú River for approximately 50 days, while in Iraí and Passaúna barrages the periods were 35 and 14 days, respectively. Fecal coliforms were found for about 35 days in samples from Iguaçú River and Iraí barrage, while in Passaúna barrage only for 14 days. Water samples from Iguaçú River showed higher contamination levels in comparison with Iraí and Passaúna barrages, probably due its great amount of organic material, which provides nutrient sources for many bacterial species.

Total and fecal coliforms were observed in water samples from Passaúna barrage for only a short period of time, 14 days, and in lower numbers (Figure 1) in comparison with the others water sources.

Pseudomonas aeruginosa persisted for approximately 63 days in all the water samples assayed, while fecal coliforms (*E. coli*) survived for 35 days in Iguaçú River and Iraí barrage samples, and only 14 days in water from Passaúna barrage.

In samples from Iraí barrage and Iguaçú River were observed an increase in the MPN of *P. aeruginosa* in the second week. However, in water samples from Iguaçú River there was low variation in number of *P. aeruginosa* until the ninth week, when it decreased drastically. Figure 1 shows the *P. aeruginosa,* total coliforms and fecal coliforms populations fluctuation over the weeks.

In the fifth week, an increase in MPN of *P. aeruginosa* was observed (Figure 1). In the third and fourth week were observed the production of blue-green pigments, which are characteristic of *P. aeruginosa*. In the subsequent weeks, this production was not detected; however the presence of *P. aeruginosa* had been supported by biochemical tests performed.

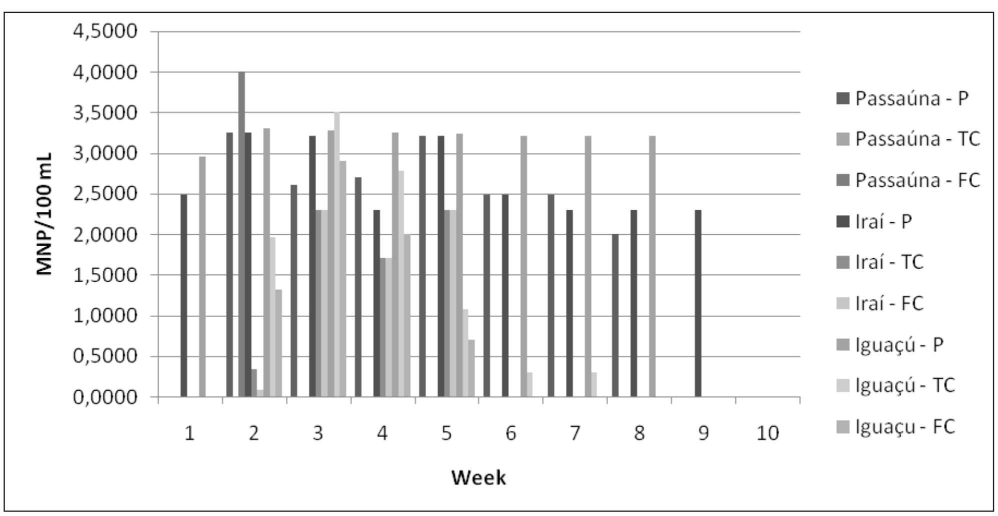

FIGURE 1 - Persistense of *P. aeruginosa,* total coliforms and fecal coliforms in water samples from Iraí and Passaúna barrages and Iguaçú River, Curitiba, Paraná, Brazil

Note: Data transformed for log (x+1). P: *P. aeruginosa;* TC: total coliforms; FC: fecal coliforms.

Source: Author’s elaboration.

DISCUSSION

The microorganisms survival times reported in this paper may vary considerably from survival times in the field (19).

It has been described that *P. aeruginosa* persists in water for a longer time and can reduce the survival rates of *E. coli*, due the competition for nutrients (4). Banning et al. (19) showed that the addition of organic material in the water can increase and even stimulate biofilm development, interfering in some bacteria survival, such as *E. coli*. However, the same event can led to an overgrowth of *P. aeruginosa*, once this species is more competitive, and consequently more successful, than the general members of the aquatic microorganisms. These authors compared survival rates of *P. aeruginosa* and *E. coli* in biofilm, evidencing the highest persistence of *P. aeruginosa*. The antagonistic phenomenon was observed against the coliform group and was evidenced when pyocyanin was present (24, 25, 19, 22).

The fluctuation of populations of *P. aeruginosa,* total coliforms and fecal coliforms over the weeks can be supported by the knowledge that in bacterial lag phase there are minimum cell divisions and the growth only increases after metabolic adaptation. In log phase, characterized by maximum metabolic activity, cells are quite sensitive to changes in the environment. As the natural growth curve advances, metabolites are accumulated and the nutrients are totally consumed by the cells. In the end of the logarithm phase and through the stationary phase, the pyocyanin is released causing death to susceptive cells (15). The decrease in number of bacteria cells observed in the fourth week can be due those events described above, once the diminution in total and fecal coliforms numbers was also spotted in this same period. In the present work, it was observed a reduction in coliforms populations (Figure 1), which was also reported by Vasconcelos (22).

Factors that commonly limit the survival of bacterial pathogens introduced into groundwater include the low level of available nutrients, oxygen levels as well as the competitive, antagonistic and predatory activities of the indigenous microbial population also related to bacteria persistence in the water (19).

An increase in MPN of *P. aeruginosa* was observed in the fifth week (Figure 1). The capacity for metabolizing over a hundred different organic and inorganic substances, including aromatic compounds, halogen derived and organic residues, and yet, the ability for using nitrate as final electron acceptor, in the absence of oxygen, make *P. aeruginosa* an extraordinary well adapted microorganism (18, 19).

Legani et al. (26) observed, in a 5 years study, that *P. aeruginosa* reached the stationary phase in about seven days in mineral waters placed in bottles. These authors also detected high cell numbers for 71 days, corroborating the high capacity for living in environments with low nutrient rates showed by this species.

Sibillea (27) showed that the *P. aeruginosa* dynamics in water treatment systems is complex and is often related to parameters such as organic carbon dissolved, residues, nature and state of pipes and relative free biomass.

The search for *P. aeruginosa* in water samples has been questioned due its opportunistic nature and non fecal origin (20, 21). However, based on its antagonism towards bacteria from coliform group, known as universal indicators for potability and contamination of water (22) suggests that the search for *P. aeruginosa* in water samples should be reconsidered.

The lower numbers of total and fecal coliforms in Passaúna barrage, as well their reduced persistence, may be due the production of pyocyanin by *P. aeruginosa*, a pigment that was detected since the third week. D'Aguilla (28) describes pyocyanin as an inhibitor against bacteria from coliform group.

Probably it wasn't observed decrease in *P. aeruginosa* numbers in the third and forth weeks in samples from Iguaçú River, since this river has large quantities of organic and inorganic material due its pollution. These results differ from those obtained in Iraí and Passaúna barrages, which have not as much organic material and consequently a decreased number of bacterial cells.

The results obtained in this paper reflect the importance of detection and survival rates of total and fecal coliforms, and also *P. aeruginosa* for minimize the occurrence of diseases spread by waters, through the establishment of more efficient control programs by the official entities.

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