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# Occurrence of pathogenic bacteria in surface water of an urban river in Argentina (Reconquista River, Buenos Aires)

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**Abstract:** Buenos Aires city and surroundings is one of the largest 20 urban agglomerations in the World. It is crossed by the Reconquista River, a lowland course directly associated with millions of people. The present study deals with an assessment of microbial populations from surface water along the main course and its tributaries over a period of three years. These watercourses showed high levels of total bacteria and coliform densities. The following seventeen bacterial species were identified: *Acinetobacter baumannii, Aeromonas hydrophila, Burkholderia cepacia, Chromobacterium violaceum, Citrobacter freundii, Cronobacter sakazakii, Erwinia carotovora, Escherichia coli, Photobacterium damselae, Providencia rettgeri, Pseudomonas aeruginosa, P. luteola, P. oryzihabitans, Serratia fonticola, S. marcescens, S. odorifera* and *Vibrio fluvialis* These include pathogens related with nosocomial infections. Their implications for human health as well as for the aquatic ecosystems are analyzed.

**Key words:** Aquatic bacteria; urban river ecology; river pollution; Reconquista River basin (Argentina)

## Introduction

The presence of bacteria in watercourses is diverse and constitutes a complicated subject. Many species are autochthonous and play an important role in the aquatic ecosystems while many others arise from untreated or poorly treated waste from industrial and domestic sources. Bacterial counts are usually employed to assess the degree and impact of sewage pollution, while isolation and taxonomical identification provides valuable information, especially in the case of species that can cause diseases (Prescott, 2005).

Pathogenic bacteria have been detected in many rivers associated with large cities worldwide (Abraham, 2011). The occurrence of emerging or reemerging pathogens in waters was stressed as a problem of great significance (Merlani and Francioli, 2003; Nwachcuku and Gerba, 2004), but water pollution investigations frequently do not focused on species level.

Buenos Aires city and surroundings is one of the largest 20 agglomerations in the World and the second most populated in South America. It is crossed by the Reconquista River, a lowland course directly associated with millions of people and thousands of industries. Physicochemical and toxicological studies indicate that the main course and its tributaries are strongly polluted, showing high levels of phosphates (5.2 mg/l), phenols (1.5 mg/l), chemical oxygen demand (1600 mg/l) and heavy metals such as zinc (2.94 mg/l), chromium (1.91 mg/l), nickel (0.20 mg/l) and lead (0.11 mg/l) (Topalián *et al*., 1999; Rovedatti *et al*., 2001; Castañé *et al*., 2006; Kuczynski 2007; García-Reiriz *et al*., 2011; Nader *et al*., 2013; Gil-Cardeza *et al*., 2014), as well as ecotoxic effects in amphibian embryos (Herkovits *et al*., 1996 and 2002).

One of its tributaries (the Morón stream) is considered the second most polluted watercourse of Argentina and one of the most polluted of South America. Earlier studies show very high levels of organic matter and bacterial densities in this stream (Kuczynski, 1994; Mondino, 2007).

Several previous papers have been reported large amounts of coliform bacteria ($10^6$ to $10^7$ MPN/100 ml)

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in Reconquista waters (Martínez and Salibián, 1995; López *et al*., 2013). The present study deals with an assessment of bacterial populations along the river and its tributaries revealing the occurrence of species that have been referenced by numerous studies showing pathogenic effects both in animals and humans, with subsequent implications for public health as well for the aquatic ecosystem.

## Material and methods

### Study area

Reconquista River basin covers a surface area of 1,700 km$^2$ in an extensive plain divided geographically into upper, middle and lower basins (Fig. 1). The upper section includes the drainage area of streams which unite to form the main course. At their junction point a dam was built to control increasing floods that were occurring as new settlements were installed along the river.

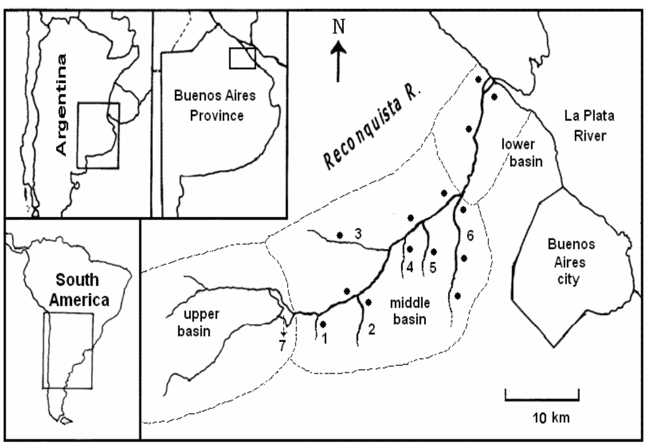


**Fig. 1: Map of the study area of the Reconquista River basin (Argentina) with indication of the sampling stations and limits between upper, middle and lower basin. 1: Torres stream; 2: Salguero stream; 3: Las Catonas stream; 4: Forletti stream; 5: Soto stream; 6: Morón stream; 7. Roggero dam.**

Nearby the middle part of its distance the main course receives at its right margin waters from the Morón stream, which runs perpendicular from south to north. The confluence site is considered as the limit between middle and lower basins. This point is also the limit of the influence of the tides of the immense Plata River, which allows the presence in the lower Reconquista of faunistic components of Plata River basin origin. The study area was divided into the four following sections:

1) The middle Reconquista (the main course after emerging from the Roggero dam to the confluence of the Morón stream).
2) The lower Reconquista (the main course from its confluence with the Morón stream to its mouth).
3) The Morón stream.
4) Other tributary streams from the middle basin (namely Forletti, Soto, Salguero, Torres, Las Catonas streams).

### Sample collection and analysis

Sampling was performed monthly or twice a month over a period of three years, from March 2012 to December 2014. Samples were taken on surface water at 14 sites along the main course and tributaries (Fig. 1) in sterile screw capped plastic bottles (250 ml) and immediately transferred to laboratory. Total bacterial count, total coliforms and bacterial isolation were determined according to standard methods (APHA, 2005). For taxonomical analysis and species confirmation the bioMérieux API® biochemical test systems were employed.

## Results

Table 1 shows the range and the mean number of bacterial counts. High levels of both total bacteria and coliforms were present at all sampling stations. In the middle basin section total bacteria ranged from $5.8\times10^4$ to $3.6\times10^6$ cfu/ml while in the lower basin they ranged from $1.9\times10^4$ to $1.1\times10^6$ cfu/ml. Total coliform counts in the middle basin ranged from $3.2\times10^5$ to $1.1\times10^7$ MPN/100 ml while in the lower basin values ranged from $1.05\times10^5$ to $8.4\times10^6$ MPN/100 ml.

**Tab. 1: Range and mean ± SD of bacterial counts in surface water samples from the study area.**

| | Total aerobic bacteria (CFU/ml) | Total coliforms (MPN/100 ml) |
|---|---|---|
| **Middle basin** | $5.8\times10^4 – 3.6\times10^6$<br>$7.5\times10^5 \pm 8.4\times10^5$ | $3.2\times10^5 – 1.1\times10^7$<br>$1.6\times10^6 \pm 2.06\times10^6$ |
| **Lower basin** | $1.9\times10^4 – 1.1\times10^6$<br>$7.0\times10^5 \pm 3.2\times10^5$ | $1.05\times10^5 – 8.4\times10^6$<br>$2.2\times10^6 \pm 1.4\times10^6$ |
| **Morón stream** | $2.7\times10^5 – 1.4\times10^7$<br>$2.6\times10^6 \pm 3.7\times10^6$ | $2.8\times10^5 – 3.5\times10^7$<br>$3.9\times10^6 \pm 5.4\times10^6$ |
| **Other tributary streams** | $1.2\times10^3 – 2.3\times10^6$<br>$3.2\times10^5 \pm 3.4\times10^5$ | $3.6\times10^3 – 4.2\times10^6$<br>$1.1\times10^6 \pm 6{,}7\times10^5$ |

In the samples from the Morón stream total bacterial counts ranged from $2.7\times10^5$ to $1.4\times10^7$ cfu/ml and coliform numbers ranged from $2.8\times10^5$ to $3.5\times10^7$ MNP/100 ml. In the other tributary streams total bacterial counts ranged from $1.2\times10^3$ to $2.3\times10^6$ cfu/ml and coliforms from $3.6\times10^3$ to $4.2\times10^6$ MNP/100 ml.

The following seventeen bacterial species were identified: *Acinetobacter baumannii, Aeromonas hydrophila, Burkholderia cepacia, Chromobacterium violaceum, Citrobacter freundii, Cronobacter sakazakii, Erwinia carotovora, Escherichia coli, Photobacterium damselae, Providencia rettgeri, Pseudomonas aeruginosa, P. luteola, P. oryzihabitans, Serratia fonticola, S. marcescens, S. odorifera* and *Vibrio fluvialis.* These include opportunistic species as well as pathogens of great sanitary significance. Frequency of occurrence of isolated taxa in the different sections of the study area is shown in Table 2.

**Tab. 2: Frequency of occurrence (%) of isolated bacterial taxa in water samples from Reconquista River and its tributaries.**

| Species | Middle basin | Lower basin | Morón stream | Other tributary streams |
|---|---|---|---|---|
| *Acinetobacter baumannii* | 3.8 | - | - | 5.4 |
| *Aeromonas hydrophila* | 5.1 | 2.6 | 3.7 | 6.2 |
| *Burkholderia cepacia* | 7.5 | 2.6 | - | 5.1 |
| *Chromobacterium violaceum* | 3.8 | 9.0 | - | 2.3 |
| *Citrobacter freundii* | 12.0 | 9.0 | 23.7 | 18.5 |
| *Cronobacter sakazakii* | 5,0 | 8.2 | - | - |
| *Erwinia carotovora* | 7.4 | 5.1 | - | 3.3 |
| *Escherichia coli* | 56.0 | 61.0 | 87.6 | 37.0 |
| *Photobacterium damselae* | 5.1 | - | - | 3.7 |
| *Providencia rettgeri* | 7.5 | 3.8 | - | 3.0 |
| *Pseudomonas aeruginosa* | 3.8 | 2.5 | 5.6 | 7.5 |
| *Pseudomonas luteola* | - | 8.0 | - | - |
| *Pseudomonas oryzihabitans* | 3.8 | - | 2.8 | 6.2 |
| *Serratia fonticola* | 5.2 | 4.8 | 6.0 | 14.4 |
| *Serratia marcescens* | - | 3.8 | - | - |
| *Serratia odorifera* | 4.8 | 3.8 | - | 8.6 |
| *Vibrio fluvialis* | 9.0 | 5.0 | 2.5 | 5.0 |

## Discussion

Bacterial levels were very high and confirm the marked deterioration in water quality. Highest values in the tributary streams ($2.3\times10^6$ CFU/ml) were similar to the ones in the main course of the river ($3.6\times10^6$ CFU/ml). The Morón stream, which is surrounded by several informal settlements, seems to be more polluted than the other analyzed streams, in accordance with previous reports (Mondino, 2007; Nader *et al*., 2013). Pathogens occurred in most samples both in the main course and in all tributaries.

Bacterial counts were similar in ranges to those reported from other urban rivers located in developing countries as Nigeria (Olayemi, 1994; Agbabiaka and Oyeyiola, 2012), South Africa (Paulse *et al*., 2009; Britz *et al*., 2013) or India (Hamner *et al*., 2006).

The results of bacterial identification showed that most of them are of sanitary importance. Bibliography provides a great number of references about diseases and infections produced by the isolated bacteria.

*Citrobacter freundii* is commonly found in the environment and in aquatic ecosystems plays an important ecological role in the nitrogen cycle. It is considered as an opportunistic pathogen that can produce diverse types of infections of the respiratory tract, urinary tract, brain abscesses, neonatal sepsis, meningitis and pneumonia, among others (Hodges *et al*., 1978; Drelichman and Band, 1985; Flegg and Mandal, 1989; Samonis *et al*., 1991; Raman *et al*., 1993; Chen *et al*., 2002). The species has been found in urban river waters used for drinking and bathing purposes in India (Antony and Renuga, 2012) and South Africa (Paulse *et al.,* 2012).

*Cronobacter sakazakii* is an emerging pathogen that causes enterocolitis, sepsis and meningitis (Farber and Forsythe, 2008). It is considered an opportunistic pathogen associated with the consumption of commercially prepared non-sterile infant formula. It poses high tolerance to environmental factors such as osmotic stress, elevated temperature and decontamination processes and has a broad antibiotic resistance (Breeuwer *et al*., 2003; Fakruddin *et al.*, 2013). The natural habitat of *C. sakazakii* is currently unknown. It was reported from Narwada and Yamuna rivers in India (Raghav and Aggarwal, 2007; Sharma *et al.*, 2012).

*Aeromonas hydrophila* is a ubiquitous species frequently isolated from food and aquatic environments (Daskalov 2006), which is able to survive a wide range of temperatures and other physicochemical parameters (Janda and Abbott, 2010). It was reported to cause disease in fishes, amphibians, reptiles and mammals (Janda *et al*., 1995). In humans it has been implicated as a potential agent of gastroenteritis, septicemia, meningitis and wound infections (Merino *et al*., 1995; Ko *et al.*, 2000; Janda and Abbott, 2010). The bacterium showed resistance to standard water treatments, probably due to its aptitude to growth in biofilms (Chauret *et al.*, 2001; Lynch *et al.*, 2002).

*Erwinia carotovora* is a widespread species that was frequently isolated from samples of surface water from rivers, streams, lakes and ponds (Harrison *et al*., 1987). The bacterium is a plant pathogen with a diverse host range, including many agriculturally important species (Perombelon, 2002). *E. carotovora* causes big injuries in fruits, vegetables and other sources of food of massive consumption.

*Serratia* spp. are considered opportunistic pathogens of worldwide distribution and one of the most common causes of noscomial infections. They are responsible for a variety of infections, including bacteremia, pneumonia, osteomyelitis and endocarditis, among others (Van Houdt *et al*., 2007; Laupland *et al*., 2008; Engel *et al*., 2009). Many plants and animals have been found to be hosts to *Serratia* (Grimont and Grimont, 2006).

*Serratia marcescens* was largely isolated from water samples, small mammals and hospitalized patients (Grimont and Grimont 2006). It has been reported by medical literature to cause nosocomial infections, especially in infants.

*Serratia fonticola* is an opportunistic pathogen that can cause infectious diseases both in animals (García *et al.*, 2008) and humans (Bollet *et al.*, 1991). It was commonly found in samples from Reconquista River.

*Serratia odorifera* was considered to have been responsible for invasive infections (Chmel, 1988; Mermel and Spiegel, 1992). This bacterium was also reported as a stable component of the gut flora of *Aedes aegypti,* showing enhancement in mosquito's susceptibility for dengue and chikungunya viruses (Apte-Deshpande *et al*., 2012 and 2014).

*Burkholderia cepacia* is a very adaptable bacterium with a great ability to survive in hostile conditions, including antibiotics and disinfectants. It is widely present in water and soil, especially around the roots of plants. It is an important pathogen for individuals with cystic fibrosis or immunocompromised patients (Pallent *et al.*, 1983; Coenye and Vandamme, 2003¸ Lipuma, 2005).

*Pseudomonas aeruginosa* is an opportunistic pathogen commonly found in aquatic ecosystems worldwide (Mena and Gerba, 2009). It has also been reported for many anthropic water environments such as swimming pools, drains and even antiseptic solutions. The bacterium can infect burns, wounds and different organs of the body but typically attacks the respiratory tract causing bacterial pneumonia. Furthermore, medical treatments are impeded because the species shows multiple antibiotic resistances (Van Eldere, 2003; Driscoll *et al.*, 2007). A positive relationship between the extent of pollution and the prevalence of *P. aeruginosa* was indicated for the Woluwe River in Belgium (Pirnay *et al*., 2005), concluding that river water constitutes a reservoir of potentially pathogenic bacteria.

*Pseudomonas luteola* is also an opportunistic pathogen that can cause bacteremia, meningitis, peritonis and other infections (Chihab *et al.*, 2004; Dalamaga *et al*., 2004; Arnold *et al*., 2005; Ngoh *et al*., 2011). This species showed the ability to absorb certain heavy metals associated with industrial wastewaters like chromium and aluminium (Ozdemir and Baysal, 2004).

*Pseudomonas oryzihabitans* was first isolated from rice paddies and found in both natural and hospital moist environments. Main pathogenic effects seem to be similar than those produced by *P. luteola*. It was reported that it caused bacteremia, peritonitis and endophthalmitis (Freeney *et al.*, 1988; Yu and Foster, 2002; Lejbkowicz *et al*., 2003). As occur for other pathogens, *Pseudomonas oryzihabitans* can survive in biofilms where is protected from chlorine disinfection, allowing it to persist after conventional drinking water treatments.

*Providencia rettgeri* is an emerging pathogen that can cause urinary tract infections, enteritis and traveler's diarrhea. It was found in soil, sewage and water samples as well as in multiple animal reservoirs including cats, dogs, birds and many reptiles. It was particularly associated with septicemia and meningitis in crocodilians (Ladds *et al*., 1996; Camus and Hawke, 2002). The bacterium is of increasing relevance in ophthalmology because it has been implicated in keratitis, endophthalmitis, conjunctivitis and other ocular infections (Koreishi *et al*., 2006).

*Acinetobacter baumannii* was isolated from soil and water samples as well as from hospital environments worldwide (Seifert *et al*., 1993; Cisneros and Rodríguez-Baño, 2002; Allen and Hartman, 2010). The ability to form biofilms allows it to survive in both abiotic and biotic surfaces, including epithelial cells and components of human microbiota than act as a permanent reservoir for future infections (Espinal *et al*., 2012; Worthington *et al*., 2012). *A. baumannii* infections are associated with high mortality rates, in part due to the bacterium shows a remarkable multidrug resistance (Dijkshoorn *et al*., 2007; Rice, 2008; Sullivan *et al*., 2010; Patel *et al*., 2011). Most significant infections include skin, soft tissues, surgical sites, bacteremia, pneumonia, osteomyelitis, endocarditis and meningitis.

*Chromobacterium violaceum* was found in natural environments in water and soil samples from hot climate regions. It is infrequent in human infections, but in these cases it showed a fulminant septicemia that may be fatal (Ray *et al*., 2004; Teoh *et al*., 2006; Baker *et al*., 2008; Jitmuang, 2008).

*Photobacterium damselae* was typically reported from estuarine and marine waters. Its occurrence in Reconquista River means a possible anthropic source. Our isolated belongs to the subspecies *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*), a pathogen for a wide range of fishes species and other aquatic animals, including mammals (Ghinsberg *et al*., 1995; Rivas *et al*., 2013). Besides showing a great relevance for aquaculture as an emerging pathogen, the bacterium constitutes a serious sanitary risk. It can cause hemorrhagic septicemia, wound infections and severe necrotizing lesions with possible rapid fatal progression (Perez-Tirse *et al*., 1993; Shin *et al*., 1996; Yamane *et al*., 2004; among others). A toxin released by the bacterium was suggested for understanding the rapid propagation of virulence.

*Vibrio fluvialis* is considered as an emerging or reemerging pathogen worldwide distributed of increasing public health concern (Ramamurthy *et al*., 2014). The species has been the subject of intensive study for the last decades that provided a great number of medical and environmental references. Its occurrence means a very high risk and a challenge for sanitation programs, especially in polluted rivers associated with populated areas (De *et al*., 1993; Igbinosa and Okoh, 2010). It can cause gastroenteritis, fever, tissue necrosis and a variety of infections. Enteric diseases attributed to *V. fluvialis* are similar to those caused by *Vibrio cholera*. The bacterium produces several toxins of high clinical importance. In the Reconquista River basin *V. fluvialis* was isolated both in the main course and in all the analyzed streams. Discharges of wastewater without treatment or with insufficient treatment are possibly the major source of *V. fluvialis* in surface waters.

The presence of *E. coli* is indicative of human or animal faecal pollution sources. *E. coli* was always present in most of the sampling stations. A monitoring study on the upper basin and one site located on the middle basin of the river (López *et al*., 2013) showed that 100 % of the coliforms were represented by faecal coliforms in many of the cases, meaning a sewage origin. The high coliforms counts in our samples indicate possible continuous sewage discharges into the middle and lower basins. Poorly treated effluents from several slaughterhouses and meat processing industries located in the area possibly contribute to the high bacterial counts.

Domestic and industrial discharges have been described as the main causes of Reconquista River pollution by previous studies (Mondino, 2007; García-Reiriz, 2011; Nader, 2013) but no references to pathogenic bacteria were found. The source in surface waters of pathogenic species related to nosocomial infections should be discussed. On the one hand, bacteria can live in any environment. Many pathogenic bacteria are waterborne and their occurrence always presents a potential risk to human health (Abraham, 2011). They show a wide tolerance to environmental factors and can survive in water and soil.

On the other hand, many health care facilities such as public hospitals, clinics, medical and biochemistry laboratories as well as several laboratories for biological research are located in the river basin. Despite the existence of strict laws and official environmental policy to control medical waste and water discharges from health care centers, inspections appear to be insufficient to assure an adequate control of dozens of major hospitals and hundreds of laboratories, showing a possible anthropic origin for the isolated pathogenic bacteria. Even if there are no reliable statistics, private inquiries indicate that at least forty per cent of medical waste generated in Buenos Aires city and surroundings have an uncertain fate (MAE, 1998).

Besides showing a great tolerance to a variety of factors, many planktonic bacteria can live in several microhabitats as biofilms. Biofilm formation represents a protected mode of growth that allows cells to survive in hostile environments (Hall-Stoodley *et al*., 2004). Particular attention has recently been paid to the role of biofilms in the environment. Microorganisms form biofilm on any biotic and abiotic surfaces which creates serious problems in human health (Abraham, 2011; Romanova and Gintsburg, 2011). Protozoans and multicellular organisms like amoebae and nematodes also constitute useful niches for pathogen protection (Bichai *et al*., 2008). It should be noted that in plankton samples from Reconquista River both free-living amoebae and nematodes have been commonly found (unpublished data).

Although densities of pathogenic bacteria can dilute into river waters and decrease with the distance to the sites of discharge, they also constitute a potential sanitary risk because of increasing resistance against antibiotics (Abraham, 2011). Moreover, aquatic pollution makes physicochemical changes that result more favorable for the development and persistence of pathogenic microorganisms. Observations suggest that multiple sources are involved in the deterioration of water quality. These data indicate that more attention needs to be paid to understand the dynamic source and fate of bacterial species that have been found in the Reconquista River basin.

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