Isolation of Bacteria from Remote High Altitude Andean Lakes Able to Grow in the Presence of Antibiotics

Julián R. Dib*[1], Annika Weiss*[2], Anna Neumann*[2], Omar Ordoñez[1], María C. Estévez[*1] and Maria E. Farías*[1]

*[1]*PROIMI, Planta Piloto de Procesos Industriales Microbiológicos, Av. Belgrano y Pasaje Caseros -(4000) - Tucumán, Argentina. CONICET Consejo Nacional de Investigaciones Científicas y Técnicas,* *[2]*Technische Universität Berlin, Fakultät III, Umweltmikrobiologie Franklinstr. 29, 10587 Berlin, Germany*

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Abstract: High altitude Andean lakes are placed in Puna desert over 4400 above sea level. Completely isolated, they are exposed to extreme environmental factors like high levels of salinity, UV radiation and heavy metals and low concentrations of phosphorus. Nevertheless, they are the habitat of enormous populations of three flamingo species that migrate among these Lakes. Previous reports have determined that bacteria isolated from these environments present high levels of resistance to antibiotics.

The aim of this work was to determine the diversity of antibiotic resistant bacteria in water from Andean Lakes and their connection with flamingo enteric biota. Bacteria from water and birds faeces from high altitude Lakes: Laguna (L.) Aparejos, L. Negra, L. Vilama and L. Azul (all are located between 4,200 and 4,600 m altitude) were isolated by plating in five different Antibiotics (ampicillin, 100 μg ml^{-1}; chloramphenicol, 170 μg ml^{-1}; colistin , 20 μg ml^{-1}; erythromycin, 50 μg $ml^{-1}$and tetracycline 50 μg ml^{-1}). 56 bacteria were isolated and identified by 16 S rDNA sequencing. Antibiotic resistance profiles of isolated bacteria were determined for 22 different antibiotics. All identified bacteria were able to growth in multiple ATBs. Colistin, ceftazidime, ampicillin/sulbactam, cefotaxime, cefepime, cefalotin, ampicillin and erythromycin were the most distributed resistances among the 56 tested bacteria

The current results demonstrated that antibiotic resistance was abundant and diverse in high altitude Lakes. Also the present article indicates some useful patents regarding the isolation of bacteria able to grow in the present of antibiotics.

Keywords: Andean lakes microbiology, antibiotics resistance in environmental bacteria, extreme environments, water birds.

[#]Dedicated to the memory of Dr. Sandra Caziani, a great ornithologist and woman. Her spirit keeps on flying with the birds in the Puna sky.

INTRODUCTION

Extreme environments are interesting resources for microorganisms with exceptional phenotypic and genotypic characteristics. High Altitude Andean Lakes are sites with extreme conditions like high UV incidence, high arsenic concentrations, high salinity and oligotrophy, among others. Microbial communities living in such aquatic ecosystems are tolerant to large fluctuations in environmental factors in addition to steady-state extreme conditions [1-3]. These Lakes have demonstrated to be a source of microbial diversity and strategies that allow microorganisms to survive under severe conditions [4-6]. UV radiation is considered one of the most extreme conditions microorganisms living in these environments have to cope with [5]. In response to this, the cell repair mechanisms increases mutational events. Spontaneous resistance to antibiotics is known to emerge under mutagenic conditions; in fact, bacterial mutagenesis after UV stress has been observed with spontaneous resistance to rifampicin or nalidixic acid [4, 6].

*Address correspondence to this author at the PROIMI, Planta Piloto de Procesos Industriales Microbiológicos, Av. Belgrano y Pasaje Caseros - (4000) - Tucumán, Argentina. CONICET Consejo Nacional de Investigaciones Científicas y Técnicas; Tel: 54-381-4344888 # 24; Fax:54-381-4344-887;E-mail:mefarias@proimi.org.ar; mefarias2001@yahoo.com.ar

*[*1]Authors who contributed equally to this work.*

Bacterial resistance is the ability of microorganisms to continue growing in the presence of a cytotoxic compound**.** Environmental microorganisms are successful creators of new biosynthetic pathways that generate new bioactive compounds (many of them cyto-toxic) while maintaining evolutionary successful ones that occurred millions of years ago [7]. Many of these natural products function as ATBs, or at least generate a molecular response in bacteria that is equivalent to resistance. Therefore, environmental bacteria are a reservoir of resistance to novel ATBs [8-15]. In previous studies at our laboratory we have demonstrated the presence and correlation of resistance to UV-B radiation, arsenic and ATBs (Azithromycin, Erythromycin, Clarithro-mycin, Roxithromycin, Streptomycin, Chloramphenicol, Gentamicin, Kanamycin, Tetracycline and Ampicillin) in two pristine Andean Lakes, L. Azul and L. Vilama, situated at 4,400 and 4,600 m asl, respectively, where ATB selective pressure is supposed to be absent [4-6]. Although these Lakes are quite distant from each other (700 km), the bacteria isolated from both Lakes showed similar ATB resistance patterns. As has been mentioned before, flamingoes are the predominant vertebrate fauna in these Lakes. They feed themselves by filtrating water from these Lakes and they migrate between them. Birds have been postulated as microbial dispersers [10, 11, 16].

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The aim of this paper was to determine whether the ability to grow in the presence of ATBs is an extended characteristic of bacteria that inhabit these environments. Therefore, bacteria were isolated from water from four Lakes: L. Aparejos, L. Negra, L. Vilama and L. Azul (4,200, 4,400, 4,600 and 4,400 m asl respectively). Considering that flamingoes are the principal inhabitant of these environments and the fact that they only feed from water of these Lakes; flamingo feces were also included in the samples to broaden the search for bacterial resistance.

MATERIALS AND METHODS

Description of Sample Sites

General parameters of the environments studied are described in Table **1**.

L. Negra, L. Aparejos and L. Azul are located in the Andes mountains in the Northwest of Argentina. They belong to the 'Salar de la Laguna Verde' in Catamarca province, Argentina (27º 34´ S; 658º 32´ W), a group of shallow lakes and salt flats Fig. (**1**). The area is practically unexplored mainly due to the lack of access roads. The distance among the three lakes is almost 40 km. Two of the highest mountains of the Andes are found in this area: Ojos del Salado (6,885 m) and Nevado Pissis (6,779 m). Rain fall is scarce and glaciers are found above 5,800 m. The water temperature was 5ºC at the moment of sampling (1:30 PM) during a summer day in the southern hemisphere and maximum UV-B irradiance reached 10.78 W m^{-2} at 312 nm (half band with 300 to 325 nm).

L. Vilama is also located in the Argentine *Puna* desert but further north (22°35'S and 66°55'W) at 4,600 m altitude Fig. (**1**). L. Vilama is, inhabited with no access roads. The area is cold and arid and is exposed to strong winds. It endures large daily temperature fluctuations (- 40ºC at night to -20ºC during the day) and high solar radiation levels, especially during summer (8.94 W m^{-2} of UV-B at noon). It is an oligotrophic environment with no detectable phosphorus contents and consequently chlorophyll production is low (2.8 10^{-4} µg L^{-1}). It has hyper saline characteristics (117 ppm) with crystallizer ponds (i.e., ponds where sodium chloride precipitates) and high arsenic contents (11.8 mg L^{-1}), the water is clear and average depth is only 20 cm Table **1**. Gamma-proteo-bacteria, HGC bacteria and Firmicutes were the predominant phylogenetic affiliation of isolates from this lake and most of them, like *Pseudomonas* sp. and *Brachybacterium* sp., presented high UV-B resistance [4].

The four Lakes are the habitat of three sympatric species of South American flamingoes: the Chilean Flamingo (*Phoenicopterus chilensis*), the Andean Flamingo (*Phoenicopterus andinus*) and James' Flamingo (*Phoenicopterus jamesi*) [17]. They feed on zooplankton, primarily composed of copepods and daphnias, and to minor extent algae, mainly diatoms and microbial community [18]. These flamingoes migrate only around these Lakes, which provide their only source of food [17, 18].

Sampling

Surface water samples were collected during early spring 2006 (September) in 10l acid-washed polyethylene bottles, after pre-rinsing the containers with lake water. Water samples were stored at 4ºC until further processing in the laboratory (within approximately 24h after collection), which was located 800 km away from the sampling site. Fresh feces samples were taken from flamengoes and were retained axenic at 4ºC until processing in the lab. Four samples were taken from each Lake (L. Vilama, L. Aparejos, L. Negra and L. Azul). Boyle in WO patent describes the cross-reaction and neutralization of multiple species by isolation of antibiotic [19].

Bacterial Isolation and Culture Media

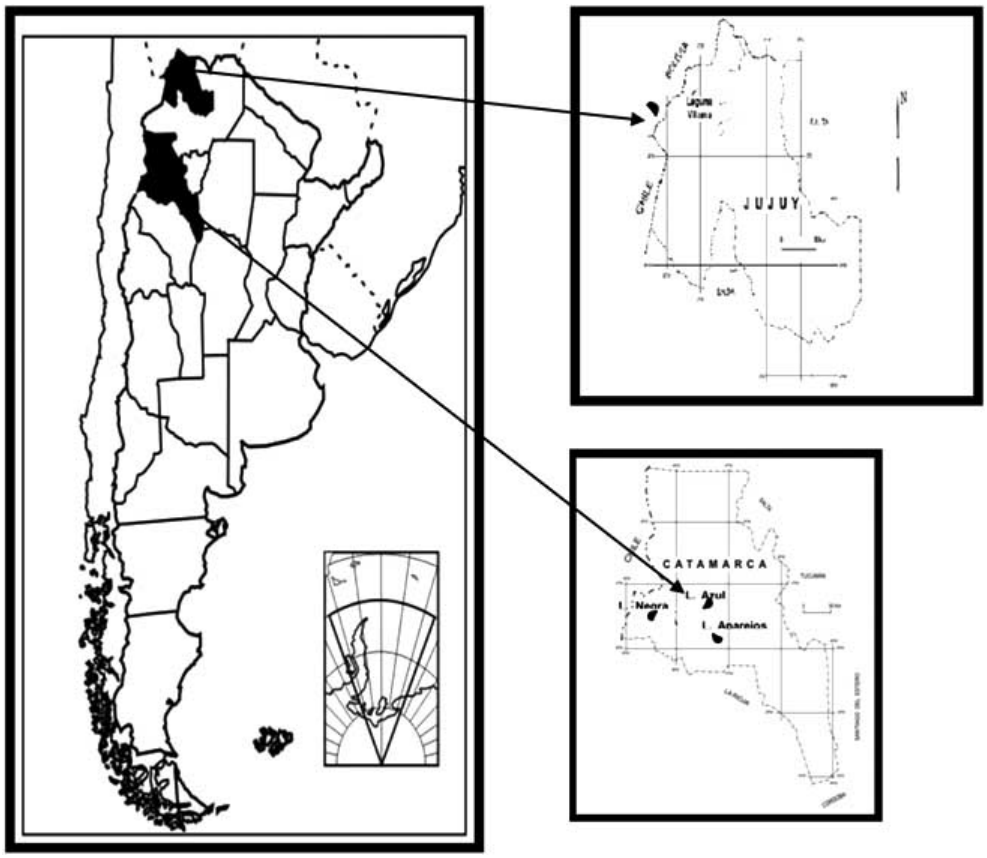
Water and feces samples were plated R2A medium (0.5 g of yeast extract, 0.5 g of proteose peptone, 0.5 g of casamino

Table 1. Characteristics of Four Lakes in Argentina: Laguna Aparejos, L. Negra, L. Azul and L. Vilama

Lake	L. Aparejos	L. Negra	L. Azul	L. Vilama
Geographic position	Catamarca Province	Catamarca Province	Catamarca Province	Jujuy Province
Global position	27º 34´S 68º 23´W	27º40¨S 68º 23´W	27º 38´S 68º 32´W	22º 35´S 66º 55´W
Depth (cm)	10	20	100	20
Altitude (m asl)	4,200	4,400	4,400	4,600
pH	6.5	6.8	7.5	7.1
Arsenic (mg L^{-1})	2.5	3	0.8	11.8
Phosphorus (mg L^{-1})	ND**	< 0,05	< 0.012	ND**
Salinity (ppm)	0,4	32	5	117
Chlorophyll (µg L^{-1})	5.6 10^{-4}	3.0 10^{-4}	1.9 10^{-4}	2.8 10^{-4}
Max UV-B registered (W m^{-2} *in situ* 280-312 nm)	9.8	10.8	10.78	8.94

ND: not determined.

** Below detection limit.



A

B

C

Fig. (1). Locations of the high altitude Andean Lakes: Jujuy province with L. Vilama and Salar de la Laguna Verde, Catamarca province, with L. Negra, L. Aparejos and L. Azul (**A**). View of Laguna Vilama (**B**) and Salar de la Laguna Verde with L. Negra and L. Verde (**C**).

acids, 0.5 g of glucose, 0.5 g of soluble starch, 0.3 g of Na-pyruvate, 0.3 g of K_2HPO_4, 0.05 g of $MgSO_4$ x $7H_2O$/liter; pH 7.2). Media were supplemented with five different ATBs: ampicillin (Amp), 100 μg ml^{-1}; chloramphenicol (Cmp), 170 μg ml^{-1}; colistin (Col), 20 μg ml^{-1}; erythromycin (Ery), 50 μg $ml^{-1}$and tetracycline (Tet) 50 μg ml^{-1}. Colonies were grouped into morphologically identical types; the homogeneity of different colony groups was corroborated by Rep-PCR (data not shown). Isolates were cultured in R2A broth at 20ºC with shaking. Genomic DNA extraction, amplification and sequencing of the partial 16S rDNA gene were carried out as described by Fernández Zenoff *et al.* [5] and resulting sequences were registered at GenBank Table **2**. Chen *et al.* in US patent 7384778 showed the susceptibility of antimicrobial and the devices for the detection of pathogenic microorganisms [20].

Determination of Antibiotic Resistance

Isolates were tested by the disk diffusion method on Mueller-Hinton agar according to the recommendations of

Table 2. Phylogenetic Affiliations and ATB Resistance Profiles of Isolates from ATB Enriched Cultures from Water and Feces from L. Negra, L. Aparejos, L. Azul, and L. Vilama. W: Water without Antibiotics, WAmp: Water with Ampicilin, WCmp: Water with Chloramphenicol, WCol: Water with Colistin, WTet: Water with Tetracycline, WEry: Water with Erythromycin, F: Feces without Antibiotics, FCol: Feces with Colistin, FAmp: Feces with Ampicillin, FCmp: Feces with Chloramphenicol, FEry: Feces with Erythromycin, FTet: Feces with Tetracycline

Phylogenetic Affiliation	Closest Identified Relative (Accession Number)	% of Identity	Source	Color	Resistance to Antibiotics	Resistance to No. of ATBs	Isolation Source
L. Negra							
Actinobacteria							
Ni2	*Dietzia* sp. AM711580	99	W	Salmon/orange	Ctx, Caz, Men, Fep Cet, Col	6	WCol
Firmicutes							
Ni4	*Bacillaceae* AM711582	99	W, F	White	Ams, Ctx, Caz, Fep Col	5	WAmp, FCol FAmp
Ni6	*Exiguobacterium* sp. AM711583	99	W	Transparent	Ams, Ctx, Imp, Caz Men, Taz, Fep, Cet Col, Ery, Tet	11	W, WCol, WEry WTet
Ni14	*Bacillus pumillus* AM712182	99	W, F	White	Caz, Cet, Col	3	W, F
Ni17	*Bacillus thuringiensis* AM712183	100	W, F	Light pink	Ams, Ctx, Tms, Caz Fep, Cef, Cmp, Col	8	WCol, F, FCol, F
Ni19	*Bacillus thuringiensis* AM712184	98	W, F	Orange	Ams, Tms, Imp, Caz Men, Cip, Taz, Fep Cef, Akn, Cmp, Col	12	WCmp, F
Ni21	*Bacillus thuringiensis* AM712185	100	F	White	Ams, Ctx, Tms, Fep, Col	5	F
Alfa Proteobacteria							
Ni3	*Sphingomonas* sp. AM711581	99	W	Yellow	Cip, Cef, Col, Ery Tet	5	W, WAmp WCol, WEry WTet
Ni9	*Sphingomonas* sp. AM711585	99	W	Dark/intense yellow	Men, Cip, Col	3	W, WCol
Ni13	*Caulobacter* sp. AM712181	82	W, F	yellow/beige	Tms, Caz, Cip, Col	4	W, FEry, F
Beta Proteobacteria							
Ni12	*Burkholderia cepacia* AM711586	99	W	Transparent/ yellow	Ams, Ctx, Men, Cef Col, Tet	6	WTet
Gamma Proteobacteria							
Ni7	*Stenotrophomonas maltophilia* AM711584	99	W, F	Orange	Ams, Ctx, Imp, Caz Men, Taz, Fep, Cet Col, Tet, Ery, Amp	12	W, WAmp WCol, WEry WTet, FAmp, F FCol, FEry
L. Aparejos							

(Table 2) Contd….

Phylogenetic Affiliation	Closest Identified Relative (Accession Number)	% of Identity	Source	Color	Resistance to Antibiotics	Resistance to No. of ATBs	Isolation Source
Firmicutes							
Api4	*Bacillus cereus* AM711589	99	W, F	Snow/white	Ams, Ctx, Tms Imp, Caz, Fep, Cef Col, Amp	9	W, WAmp WCol, F
Api7	*Bacillus cereus* AM711591	99	W, F	White	Caz, Men, Cip, Col Cmp, Amp	6	W, F, FAmp FCmp, FCol
Api11	*Bacillus cereus* AM711594	98	F	Orange	Ams, Ctx, Tms Caz, Taz, Fep, Cef Col	8	FCol
Api10	*Staphylococcus* sp. AM711593	100	F	Transparent	Col	1	FCol
Api13	*Brevibacterium* sp. AM711595	99	F	Yellow/orange	Tms, Caz, Fep, Cef Col, Amp	6	FCol, FAmp
Alfa Proteobacteria							
Api5	*Sphingomonas* sp. AM711590	99	W, F	Dark yellow	Ams, Ctx, Tms Caz, Taz, Fep, Cef Col	8	WCol, F
Beta Proteobacteria							
Api8	*Burkholderia* sp. AM711592	99	W	Yellow	Gen, Men, Cet, Col Amp	5	WAmp, WCol
Gamma Proteobacteria							
Api1	*Pseudomonas plecoglossicida* AM711587	99	W, F	Orange	Ams, Men, Cef Cmp, Col, Ery, Tet, Amp	8	WAmp, WCol WCmp, WEry WTet , FCol FAmp, FCmp FEry, FTet
Api3	*Pseudomonas plecoglossicida* AM711588	99	W, F	Transparent/ light orange	Ams, Ctx, Tms Men, Cet, Cmp, Col Amp, Ery	9	FCol, FAmp
Api17	*Pseudomonas plecoglossicida* AM711596	99	W	White	Cmp, Col	2	WCmp
L. Azul							
Actinobacteria							
A5	*Nocardia* sp. DQ112024	99	W	Light Red	Ery, Clm, Azm Rox, Amp	5	W
A12	*Dietzia* sp. AM882683	98	F	Camel	Col	1	FCol
A1	*Micrococcus* sp. AM403127	98	W	Yellow	Ery, Clm, Azm Rox, Sm, Km, Gen Amp, Cmp	9	W

(Table 2) Contd….

Phylogenetic Affiliation	Closest Identified Relative (Accession Number)	% of Identity	Source	Color	Resistance to Antibiotics	Resistance to No. of ATBs	Isolation Source
Firmicutes							
A3	*Staphylococcus saprophyticus* DQ112023	97	W	Orange	Ery, Clm, Azm Rox, Sm, Gen, Amp	7	W
A4	*Bacillus pumilus* DQ217665	99	W	White	Ery, Clm, Azm Rox, Sm, Amp	6	W
A14	*Bacillus pumilus* AM882685	98	F	Cream	Ctx, Caz, Fep	3	F
A16	*Bacillus pumilus* AM882687	98	F	Transparent	Ctx, Caz, Fep, Col	3	FCol
A15	*Bacillus amyloliquefaciens* AM882686	99	F	Cream	Gen	1	F
A13	*Bacillus pumilus* AM882684	98	F	Cream	Caz, Fep, Col	2	FCol
A10	*Paenibacillus polymyxa* AM882681	98	F	Transparent	Col	1	FCol
A8	*Bacillus subtilis* AM882680	98	F	White	No ATB-resistance found	0	F
Beta Proteobacteria							
A11	*Variovorax paradoxus* AM882682	98	F	Cream	Ams, Caz, Cef, Tet	4	FTet
Gamma Proteobacteria							
A2	*Acinetobacter johnsonii* AY963294	99	W	White	Ery, Clm, Azm Rox, Sm, Km, Amp	7	W
L. Vilama							
Actinobacteria							
V2	*Rhodococcus eritropolis* AM236137	97	W	White	Ery, Clm, Azm Rox, Sm, Amp Ams	7	W
V5	*Brachybacterium* sp. AM236138	97	W	Yellow	Ery, Clm, Azm Rox, Km, Amp Ams	7	W
V7	*Micrococcus* sp. AM403126	98	W	Yellow	Ery, Clm, Azm Rox, Sm, Km, Amp Tet, Cmp	9	W
Firmicutes							
V3	*Bacillus vallesmortis* AM235882	100	W	Light orange	Ery, Clm, Azm Rox, Amp, Tet, Cmp	7	W
V8	*Bacillus vallesmortis* AM235883	100	W	Light orange	Ery, Clm, Azm Rox, Tet, Cmp	6	W
V31	*Exiguobacterium* sp. AM882701	97	W	Orange/pink	No ATB-resistance found	0	W

(Table 2) Contd….

Phylogenetic Affiliation	Closest Identified Relative (Accession Number)	% of Identity	Source	Color	Resistance to Antibiotics	Resistance to No. of ATBs	Isolation Source
V29	*Staphyloccocus xilosus* AM882700	98	W	Orange	Caz	1	W
V27	*Bacillus pumilus* AM882697	97	F	Cream	No ATB-resistance found	0	F
V30	*Bacillus pumilus* AM882699	98	F	Cream	Caz, Fep	2	F
V26	*Bacillus pumilus* AM882696	98	F	Cream	Caz, Col	1	FCol
V23	*Bacillus megaterium* AM882693	98	F	Cream	No ATB-resistance found	0	F
V25	*Bacillus* sp. AM882695	98	F	Cream	Caz, Ctx	2	F
V20	*Bacillus* sp. AM882690	97	F	Yellow	Col		FCol
Alpha Proteobacteria							
V28	*Rhodopseudomonas* sp. AM882698	98	W	Transparent	Ams, Tms, Gen Caz, Akn, Mem Cip, Taz, Fep	9	W
V22	*Chelatococcus asaccharovorans* AM882692	97	F	Pink	Caz	1	F
V18	*Sphingomonas paucimobilis* AM882688	97	F	Yellow	No ATB-resistance found	0	F
Beta Proteobacteria							
V21	*Janthinobacterium* sp. AM882691	98	F	White	Ctx	1	F
Gamma Proteobacteria							
V1	*Pseudomonas* sp. AM403128	98	W	Cream	Ery, Clm, Azm Rox, Sm, Amp Ams, Cmp	8	W
V4	*Enterobacter* sp. AM403125	98	W	Cream	Ery, Clm, Azm Rox, Sm, Km, Amp Ams, Tet, Cmp	10	W
V24	*Stenotrophomonas maltophilia* AM882694	97	F	Cream	Ams, Ctx, Imp, Gen Caz, Cef, Akn Mem, Cip, Taz, Fep Amp	11	FAmp
V19	*Stenotrophomonas maltophilia* AM882689	97	F	Yellow	Ams, Imp, Gen Caz, Cef, Akn Mem, Cip, Fep, Ery	9	FEry

the National Committee for Clinical Laboratory Standards (NCCLS) for ATB susceptibility. The following ATBs were assayed: Azm (azithromycin), 50 μg; Ery (erythromycin), 50 μg; Clm (clarithromycin), 50 μg; Rox (roxithromycin), 50 μg; Sm(streptomycin), 20 μg; Gen (gentamycin), 10 μg; Km (kanamycin), 20 μg; Tet (tetracycline), 20 μg; Amp (ampicillin), 100 μg, Imp (imipenem), 10 μg; Caz (ceftazidime), 30 μg; Ams (ampicillin/sulbactam), 10/10 μg; Ctx (cefotaxime), 30 μg; Tms (trimethoprim/sulfa-methoxazole), 1.25/23.75 μg; Mem (meropenem), 10 μg; Cip

(ciprofloxacin), 5 μg; Taz (piperacillin/tazobactam), 100/10 μg; Fep (cefepime), 30 μg; Cef (cefalotin), 30 μg; Akn (amikacin), 30 μg; Cmp (chloramphenicol), 30 μg and Col (colistin), 10 μg.

Olson and Ceri showed the use of plate for antibiotic against biofilm infection [21].

Production of Antimicrobial Substances

Antimicrobial substance producing organisms were identified using a modified agar-well diffusion assay as described by Portrait *et al.* [22] with *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* as control strains.

RESULTS

Isolation of ATB Resistant Bacteria from Water and Feces

Phylogenetic affiliation of isolates, ATB resistance and isolation source (Water (W) or Feces (F)) with different ATBs are summarized in Table **2**. Distribution of antibiotic resistance among the isolates is represented in Fig. (**2**). Members belonging to major phylogenetic groups like Firmicutes, Actinobacteria, and Gamma, Beta and Alfa proteobacteria were isolated from water and feces in all the Lakes assayed. Firmicutes were predominant in all the environments and bacteria resistant to multiple antibiotics were quite common in both water and feces isolates.

L. Negra presented a medium salinity level, high UV exposure and low arsenic contents Table **1**. Twelve bacteria were isolated from this Lake as shown in Table **2**. Firmicutes were predominant (6 isolates) and most of them belonged to the spore-forming genus *Bacillus*. The only non spore-forming isolate was *Exiguobacterium* sp., which, together with *Bacillus thuringensis* Ni19, presented the widest scope of ATB resistance (resistance to 11 and 12 ATBs, respectively). Alpha proteobacteria presented 3 isolates, belonging to *Sphingomonas* sp. and *Caulobacter* sp. The ATB resistance profile for *Sphingomonas* sp. Ni3 and Ni9 differed (resistance to 5 and 3 ATBs, respectively), denoting that they were different strains. Beta and Gamma proteobacteria were represented only by *Stenotrophomonas maltophilia* with resistance to 12 ATBs. All isolates from feces were also present in water, whereas many isolates were exclusively isolated from water.

L. Aparejos presented a low salinity level, high UV exposure and low arsenic contents. Together with L. Negra it had the lowest degree of oligotrophy and both Lakes harbored the largest population of flamingoes Table **1**. Ten isolates were found in L. Aparejos, which are described in Table **2**. As in L. Negra, *Sphingomonas* sp and *Burkolderia cepacia* were representatives of Alpha and Beta proteobacteria with resistance to 8 and 6 ATBs, respectively. Gamma-proteobacteria were represented by *Pseudomonas plecoglossicida* strains, which presented different ATB resistance profiles (2, 8 and 9) and were distributed between water and feces. Firmicutes were represented by *Bacillus cereus* with different ATB resistance profiles and two non spore–forming genera, *Staphylococcus* sp. and *Brevibacterium* sp., with resistance to 1 and 6 ATBs respectively. Beta proteobacteria were exclusively isolated from water and the non spore-forming Firmicutes were exclusively found in feces; the remaining bacteria were common in both environments.

L. Azul presented low salinity, high UV exposure, low arsenic contents and extreme oligotrophic conditions since

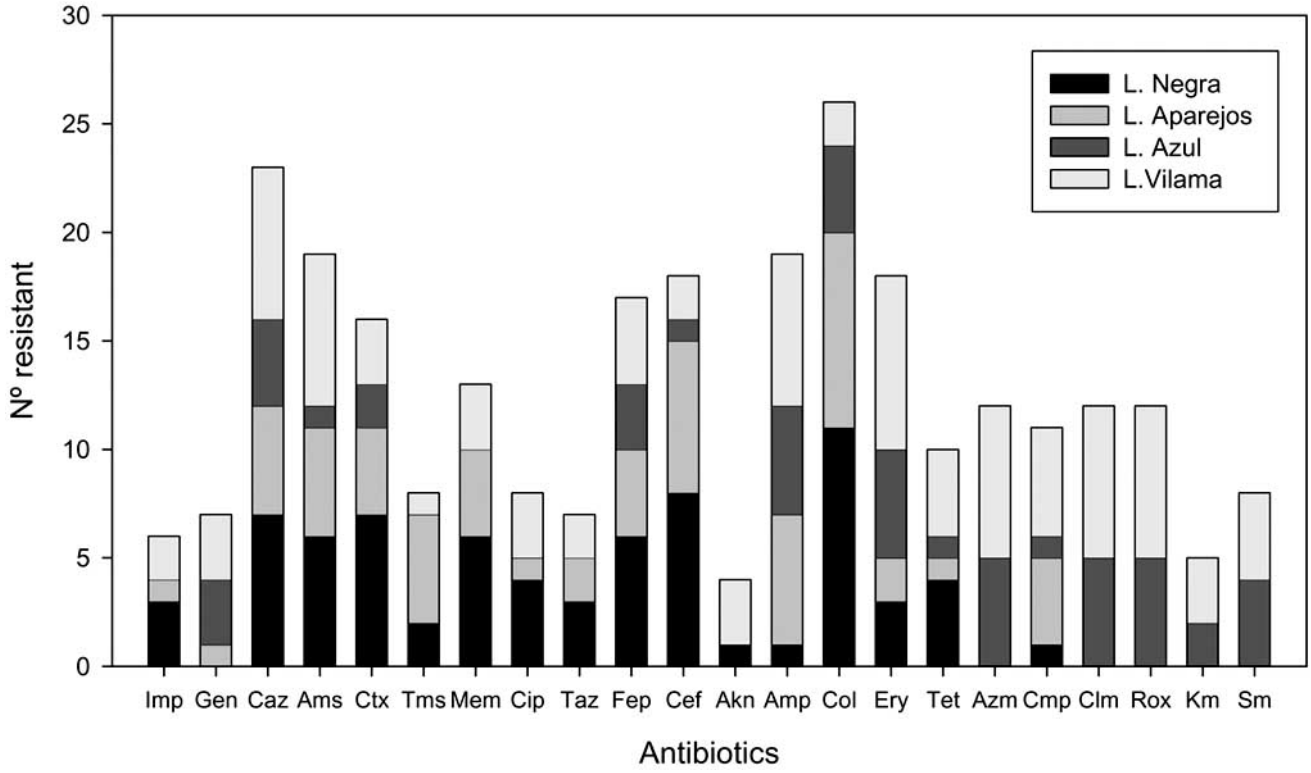


Fig. (2). Number and distribution of resistances to antibiotics of isolated bacteria form L. Negra, L. Aparejos, L. Azul and L. Vilama.

this Lake is located on a stone basin and no sediments were detected Table **1**. The 13 isolates from this Lake are listed in Table **2**. Firmicutes were predominant (8 isolates) with one non spore-forming isolate identified as *Staphylococcus saprophyticus*, which presented resistance to 7 ATBs. Among the spore-forming group, two isolates did not present ATB resistance. Actinobacteria were represented by *Micrococcus* sp. and *Nocardia* sp. with resistance to 9 and 5 ATBs, respectively. *Dietzia* sp. presented no ATB resistance. Gamma and Beta proteobacteria were represented by *Acinetobacter johnsonii* and *Variovorax paradoxus* with resistance to 7 and 4 ATBs, respectively. A low degree of association was found between culturable bacteria from water and feces, because only one isolate was isolated from both feces and water: *Bacillus pumillus* Table **2**.

L. Vilama presented all three extreme conditions simultaneously: high salinity, high UV exposure and high arsenic contents Table **1**. 21 isolates were identified from both water and feces Table **2**. Bacteria belonging to Firmicutes predominated in water and feces (10 isolates). However, apart from *Bacillus vallesmortis*, they showed little ATB resistance compared to other isolates; especially isolates from feces presented resistance to 1 or 2 ATBs or no resistance at all. Three isolates were grouped into Alpha proteobacterias, and those identified as *Rhodopseudomonas* sp. showed resistance to 9 ATBs. Isolates identified as *Sphingomonas paucimobilis* presented no ATB resistance, which highly contrasts with the resistance profile of the same genus isolated from L. Negra. Gamma proteobacterium isolates were represented by *Pseudomonas* sp. and *Enterobacter* sp., exclusively isolated from water, with resistance to 8 and 10 ATBs, respectively, and two strains of *Stenotrophomonas maltophilia* with different ATB resistance profiles (resistance to 11 and 9 ATBs), which were exclusively isolated from feces. Beta proteobacteria were represented by *Janthinobacterium* sp., isolated from feces, and they presented only resistance to one ATB. The three actinobacteria *Rhodococcus eritropolis*, *Brachybacterium* sp. and *Micrococcus* sp., were only isolated from water. The first two strains presented resistance to 7 ATBs and *Micrococcus* sp. to 9 ATBs. The above mentioned bacteria were distributed in water or in feces; there were no common bacteria between both isolation sources.

The most widespread ATB resistances were Col (22 resistances of 18 tested), Caz (16 resistances of 18 tested), Mem (12 resistances of 18 tested), Cet (18 resistances of 18 tested) and Ams and Ctx (13 resistances of 18 tested). Most resistant bacteria were isolated from L. Aparejos and L. Negra. *Exiguobacterium* sp., *Stenotrophomonas maltophila* and *Bacillus thuringiensis* N17 and N19 presented resistance to a high number of ATBs (10, 10, 9 and 12, respectively). N17 and *Stenotrophomonas maltophila* seem to be widely distributed not only in water but also in most bird feces, and consequently, these high ATB resistant bacteria could be spread by birds and therefore require more thorough studies. *Pseudomonas plecoglossicida*, *Bacillus cereus* and *Sphingomonas* sp. showed resistance to the highest number of different ATBs in L. Aparejos. Distribution of ATB resistant bacteria in water and bird feces in this Lake was also widespread.

All isolates were tested for production of antimicrobial substances against Gram positive and Gram negative pathogenic bacteria Table **3**. Seven strains presented production of antimicrobial substances against *E. coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes*.

DISCUSSION

Singer *et al.* [14] proposed that landscape ecology, which links the biotic and abiotic factors of an ecosystem, might help untangle the complexity of antibiotic resistance and improve the interpretation of ecological studies. As an extension of these ideas ATB resistance was studied in an environmental context. Our results have demonstrated that highly irradiated pristine environments are a rich source of bacteria able to grow in the presence of multiple ATBs. To our knowledge this work is without precedent, since this is the first time that ATB resistance has been assessed at such high altitude and in such extreme environments.

ATB Resistant Bacteria are a Widespread Phenomenon in High Altitude Lakes

Bacterial resistance to ATBs was found in the four high altitude environments studied: L. Negra, L. Azul, L. Aparejos and L. Vilama. These results support the preliminary ideas presented in a previous publication by our

Table 3. Production of Antimicrobial Substances Against Three Pathogens: *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes*

Lake	Strain	*E. coli* ATCC 35218	*S. aureus* ATCC 25923	*L. monocytogenes*
L. Aparejos	*Burkholderia cepacia* AM 711592	-	+	-
L. Vilama	*Stenotrophomonas maltophilia* AM882694	-	-	+
L. Vilama	*Bacillus* sp. AM882690	-	+	-
L. Azul	*Bacillus pumilus* AM882687	-	-	+
L. Azul	*Bacillus amyloliquefaciens* AM882686	-	-	+
L. Azul	*Paenibacillus polymyxa* AM882681	+	+	+
L. Azul	*Bacillus subtilis* AM882680	-	+	+

group, which postulated that there exists a correlation between ATB resistance and UV-B radiation in extreme environments like high altitude Lakes [4]. Under extreme UV stress conditions, bacteria are known to increase mutational events as a last resistance mechanism, called error-prone repair [23]. In many cases, spontaneous resistance to ATBs is known to emerge under such mutagenic conditions, as a consequence of mutagenesis modified potential target genes. Other authors established a possible connection between oxidative stress resistance and resistance to ATBs [24]. It is known that UV produces high oxidative stress and consequently, a highly irradiated environment is expected to select oxidative stress resistant bacteria. There could also be a relationship with ATB resistance found in other irradiated environments. The fact that ATB resistance is more common in irradiated environments than in non irradiated ones could support this idea

Special attention should be given to *Stenotrophomonas maltophila*, since this seems to be the most widest spread bacterium, even that it was detected in DGGE bands from water and feces of from L. Aparejos, L. Negra, L. Azul and L. Vilama, the four Lakes and it was isolated from water and feces of from L. Negra, L. Azul and L. Vilama. In all the cases it was the most resistant bacterium to multiples ATBs (unpublished data). This pathogen has been increasingly recognized as an important cause of related to nosocomial hospital infections. Infection occurs principally, but not exclusively, in debilitated and immune-suppressed individuals patients. Management treatment of *S. maltophilia* associated infections is problematic because many strains of the bacterium manifest resistance to multiple antibiotics [25]. DGGE bands corresponding to *Acinetobacter* sp. were detected both in L. Aparejos and L. Vilama, (unpublished data) this genera was reported by our group as high UV resistant bacteria [4]. In this report these microorganisms were also isolated from water and feces from L. Negra, L. Azul and L. Vilama and presented multiples ATB resistance.

CURRENT & FUTURE DEVELOPMENTS

The ubiquity of multiple ATB resistant bacteria in the environments assayed supports the idea that pathogenic bacteria resistant to multiple ATBs are not a phenomenon that is restricted to human-modified environments. Besides, it shows that pristine environments could be considered important reservoirs of multiple ATB resistance in bacteria like *Staphylococcus* sp., *Aeromonas* sp., *Stenotrophomonas maltophila* and a large group of enteric bacteria. These ATB resistant bacteria could be spread by birds whose migratory patterns are not well established yet. In conclusion, from an epidemiological point of view we propose that pristine, UV irradiated environments should receive more attention as reservoirs of multiple ATB resistance. This may become even more important in the context of global warming that could modify the behavior and migratory patterns of birds and bring them and their ATB resistant microbiota in close proximity to human communities.

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CONFLICT OF INTEREST

None

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