JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S7

Superbugs Contaminating Stagnant and Flowing Freshwater in the Province of Tungurahua, Ecuador

Patricia Paredes Lascano, MD[1], Iván Toapanta Yugcha, MD[2], Leonardo Bravo Paredes, MD[3]

1.Profesora de Pediatría, Universidad Técnica de Ambato Jefa del Servicio de Pediatría, Hospital General Ambato
Email: patricialparedes@uta.edu.ec ORCID: 0000-0002-1029-9558
2.Profesor de Pediatría, Universidad Técnica de Ambato Pediatra, Hospital General Ambato
Email: ig.toapanta@uta.edu.ec ORCID: 0000-0002-6338-3083
3.Postgraduado en Cirugía, Universidad Católica del Ecuador Email: el_alejobravo@hotmail.com
ORCID: 0000-0002-4497-1680

Abstract

Introduction: Water is the vital element that ensures the balance of ecosystems. In recent years, environmental pollution has altered the natural state of water, transforming it into a breeding ground for certain pathogenic bacteria known as superbugs. These bacteria produce extended-spectrum beta-lactamases (ESBLs), which are enzymes that confer multi-resistance to most antibiotics. These enzymes are in bacterial genetic elements such as integrons or inserted into mobile elements such as transposons, plasmids, and efflux pumps, causing therapeutic failures. Currently, these bacteria are found in rivers and pools as community pathogens, causing gastrointestinal, genitourinary infections, and septicemias that are difficult to treat. Objective: To identify ESBL-producing bacteria in 25 pools and 5 rivers in the Province of Tungurahua. **Methodology:** A descriptive field study with a qualitative-quantitative approach, non-probabilistic sampling was conducted to demonstrate the presence of ESBL-producing bacteria and to discriminate sensitivity and resistance to various antibiotics. Results: The strains isolated in the rivers were ESBL-producing E. coli and Klebsiella, showing sensitivity to meropenem, doripenem, ertapenem, imipenem, ciprofloxacin, and gentamicin, resistant to trimethoprim/sulfamethoxazole, ticarcillin/clavulanic acid, and piperacillin/tazobactam. In the pools, ESBL-producing E. coli, Klebsiella, Acinetobacter, and Pseudomonas were isolated, with sensitivity to levofloxacin, ciprofloxacin, gentamicin, and resistance to trimethoprim/sulfamethoxazole, piperacillin/tazobactam, and aztreonam.
Conclusion: The research reveals a significant impact on the contamination in the studied rivers and pools, with the presence of ESBL-producing bacteria resistant to multiple antibiotics. The results highlight issues that challenge current regulations, the lack of consideration of these microorganisms in Ecuadorian regulations underscores the urgent need to review and strengthen water quality standards, incorporating strict microbiological indicators that do not threaten health and biodiversity.
Keywords: river, pool, ESBL-producing bacteria, antibiotic resistance.

1. Introduction

Water is the most abundant component on the planet's surface and a vital element for life, acting as the solvent for all bodily fluids in humans, and as the purifier to expel waste through urine, sweat, and fecal matter. It is involved in digestion, respiration, and blood circulation processes, maintaining homeostasis in living organisms. This natural resource enables the correct functioning of biological processes in ecosystems and ensures the survival of all animal and plant species on

our planet. Water is constantly in motion, and approximately 0.3% of it is found in rivers, lakes, streams, and reservoirs, yet it represents 80% of the renewable surface and groundwater. Thus, the hydrological cycle of this element is crucial for maintaining ecosystems and regulating the climate.

The pollution of rivers throughout history, due to uncontrolled industrialization and the absence of wastewater treatment in poor countries, has turned them into a means of transporting pathogenic bacteria. These bacteria can be opportunistic or part of the normal flora of the human body (Morales et al. 2022). The physical, chemical, and bacteriological analysis of river water (flowing water) is of great importance for identifying microorganisms that can cause diseases in animals and humans, such as otitis, gastroenteritis, amoebiasis, skin and genitourinary infections, conjunctivitis, sepsis, etc. The microorganisms involved that can be transmitted to humans in rivers and recreational pools include Salmonella, Shigella, Klebsiella, Escherichia, Citrobacter, Pseudomonas, Vibrios, Aeromonas, enteroviruses, protozoa, and fungi (Carrasquero Ferrer et al. 2024). Stagnant water (pools) allows for recreation; however, failing to meet the parameters for maintaining water quality turns it into a medium for transmitting pathogens. Populations living near rivers and people who use pools are the most affected, as they use this resource for recreational, tourist, irrigation, and consumption purposes.

In recent decades, the emergence of "superbugs" producing extended-spectrum beta-lactamases (ESBLs) in stagnant and flowing freshwater has posed a challenge for recreational tourism, as these bacteria can cause severe, life-threatening diseases.

Beta-lactamases are enzymes that degrade the beta-lactam ring of penicillin and its derivatives. They were first identified in strains of E. coli in 1940, and these enzymes are used by bacteria to compete for a niche with other microorganisms, making them super-resistant (superbugs).

ESBLs were described in a strain of Klebsiella ozaneae in Germany in 1983, and since then have been reported in epidemic outbreaks in 1988 and 1990 in Europe; they constitute a set of enzymes that cause therapeutic failures by breaking down the beta-lactam ring, a resistance mechanism present in both Gram-negative and Gram-positive bacteria (Santos et al. 2021) (Salazar 2018). These enzymes can be chromosomally encoded in some bacteria or can be horizontally transmitted through genetic material, located in genetic elements such as integrons or inserted into mobile elements such as transposons and plasmids. These beta-lactamases are differentiated by their resistance spectrum; thus, ESBLs confer resistance to penicillin, first, second, third, and fourth generation cephalosporins, and aztreonam, being inhibited by clavulanic acid or other beta-lactamase inhibitors such as tazobactam and sulbactam (Astocondor 2018).

In recent years, the proliferation of the Enterobacteriaceae family producing ESBLs, mainly E. coli and Klebsiella pneumoniae, has rapidly increased worldwide due to the transmission of these bacteria through moving or stagnant water; infected individuals face significant clinical challenges, as delays in starting appropriate antimicrobial therapy and limited treatment options can result in severe consequences such as respiratory tract infections, urinary infections, severe sepsis or septic shock, bacterial meningitis, otitis, eye infections, intra-abdominal infections, vaginitis; particularly severe in the pediatric population (Lenart et al. 2020) (Melzer and Petersen 2007).

E. coli can produce chromosomal or extrachromosomal beta-lactamase enzymes, which are mediated by plasmids. These plasmids encode the ESBLs and carry resistance genes known as transposons to other antimicrobials such as aminoglycosides, tetracyclines, and cotrimoxazole. It is for this reason that cross-resistance occurs, and the treatment of infections caused by these strains is difficult to resolve (Schmiege et al. 2021).

Enterobacteria also display various types of resistance mechanisms (modification of the target site, efflux pumps, porins, etc.) and possess genes that confer resistance to more than one antibiotic. Typically, this resistance is located on the chromosome and in plasmids, which have the characteristic of being mobile genetic elements and can transmit resistance to other antibiotics among bacteria of the same species or different species (Vink et al. 2020) (Vidal et al. 2019).

In this context, it is necessary to explore the epidemiology of ESBLs in these aquatic environments. Moreover, it is essential to discriminate diseases potentially associated with the presence of ESBLs, as well as to determine the magnitude of the risk associated with the use of these recreational water spaces (Flores et al. 2019).

The research identifies physicochemical factors, the relationship between these and the presence of enterobacteria producing extended-spectrum beta-lactamases (ESBLs), quantifies colonies of total and fecal coliforms, and demonstrates the sensitivity and multi-resistance profile to propose targeted therapeutics, as well as prevention and control strategies in pools and rivers to mitigate the impact that these superbugs can have on the health of humans and animals in the province of Tungurahua, Ecuador (Pascual Araoz et al. 2020) (Conejero S 2020).

2. Materials and Methods

This is a descriptive observational field study with a qualitative-quantitative approach aimed at analyzing physicochemical properties and determining the presence of pathogens in five rivers and twenty-five freshwater recreational pools in the province of Tungurahua-Ecuador, during the period from July to December 2023.

Sampling

The sample was selected through non-probabilistic sampling due to the interest in investigating and analyzing the presence of contaminating superbugs found in stagnant, recreational (pools), and flowing (rivers) waters. For the samples, 250 mL amber bottles sterilized by moist heat were used, considering a maximum stability of 6 hours at 4°C. For the samples of recreational water, sodium thiosulfate (100 mg/L) was added prior to sterilization.

The selection of sampling points in the rivers was based on the frequency of use, distance from populations and confluences, the influx of bathers, and the distance from the riverbanks. The populations of Pelileo, Ambato, Patate, Baños, Ulba, and Río Verde were considered.

For the selection of pools, the influx of bathers and tourist preference were considered. Both river and pool samples were spot samples, taken during peak usage hours. In order to safeguard the reputation of the selected pools, the samples were anonymized and collected under informed consent.

Physical analysis of samples in situ and in the laboratory.
At the time of sampling, measurements of ambient temperature and water temperature were taken. Once the sample was transported to the laboratory, determinations of residual chlorine and pH were performed using a calibrated potentiometer.

Determination of CFU concentration (colony-forming units)
Two techniques were used to quantify CFUs in the samples: the most probable number and Petrifilm counting. The former was used for samples with high microbiological load (rivers), where three dilutions (1:2, 1:10, 1:100) in sterilized distilled water were made, and five repetitions of each solution were performed. The dilutions were plated on agar and incubated at 37°C for 24 hours.
For Petrifilm counting, 1 mL of three sample dilutions (1:1, 1:10, and 1:100) was plated, and for samples with a high load of microorganisms, a fourth dilution (1:1000) was performed; the Petrifilms were incubated for 24 hours at 37 °C.

Differentiation of bacterial colonies and Gram staining
In samples that showed growth, a Gram stain was performed and then re-plated on MacConkey agar for 24 hours at 37°C.

Differentiation of colonies resistant to beta-lactams and species differentiation.
In samples plated on MacConkey agar, a visual and microscopic differentiation of the colonies was carried out to distinguish species. Each distinct colony was re-plated on a differential chromagar for gram-negative bacteria resistant to beta-lactam antibiotics; the media were incubated at 37°C for 24 hours.

Determination of minimum inhibitory concentration for beta-lactam antibiotics.
Colonies that grew in the previous test were diluted in sterile water to achieve a 0.5 McFarland standard, 30 µL of this solution was incubated in Mueller Hinton broth. Subsequently, 50 µL of this new solution was deposited in each of the 96 wells of the Sensititre™ GNX2F plate, and incubated for 24 hours at 37°C.

3. Analysis and Results

The physical analysis of the samples showed a high concentration of dissolved chlorine according to the limits proposed by the Unified Text of Environmental Legislation (TULSMA) for recreational use. The pH variation in both the river and pool samples was minimal. The Cutuchi River has a pH of 7.2, while the Pachanlica River shows a pH of 6.5. The Ulba River registers higher levels of residual chlorine with a concentration of 0.04 mg/L. The temperature of the rivers in the province ranged from 14 to 18.2 ºC, with ambient humidity ranging from 36% at the Ulba River to 67% at the Verde River. Regarding the pools, pH levels vary from 6.1 (acidic) at pools T, H, and V, to 7.7 (alkaline) at pool X. Pool S shows high concentrations of residual chlorine with a value of 0.299 mg/L. The temperature of the studied pools varies between 21.3ºC and 36.5 ºC with humidity of 88% at pool I and 24.5% at pool G.

In the Ambato, Pachanlica, and Cutuchi rivers, no bathers were found, but there were foul putrid smells and the presence of wastewater discharges. In contrast, the Ulba and Verde rivers, which were used for recreational and swimming sites, were observed. However, the Ulba River also

shows pollution, as it is near discharge sites from tourist areas. Regarding the pools, there is a considerable influx of bathers at pool T, where the presence of dirt and a high level of chlorine was identified. In contrast, pools H and X, which record a lower influx of bathers, show no residual chlorine in their waters.

Table 1: Physical Analysis (pH, Total Chlorine, Water Temperature, Ambient Tsemperature and Humidity)

No.	ID	Samples	Pool/River	pH	Residual Bleach (mg/L)	Water Temperature(°C)	Ambient Temperature (°C)	Ambient Humidity (%)
1	RV-A	3	Río Verde	6,8	0,01	14	22	67
2	RV-B	3	Río Verde	6,9	0	14	24,6	63
3	RU-A	3	Río Ulba	7,1	0,03	16,5	32,6	39
4	RU4-A	1	Río Ulba	6,8	0,04	15,7	31,3	36
5	RC	3	Río Cutuchi	7,2	0,01	17,6	28,9	44
6	RP	3	Río Pachanlica	6,5	0,02	18,2	25	50
7	RA	3	Río Ambato	6,7	0	16,4	27,5	43
8	M	2	Pool M	7,2	0,229	28,1	12,8	80
9	N	2	Pool N	7,6	0,166	31,9	29,2	44
10	O	2	Pool O	6,5	0,178	23,8	13,5	84
11	P	2	Pool P	6,2	0,1	22,6	18	86
12	Q	2	Pool Q	6,7	0,12	26	36,2	40,5
13	R	2	Pool R	6,8	0,176	32,1	32,7	41,5
14	A	2	Pool A	6,9	0,08	29,7	32,5	39,7
15	S	2	Pool S	6,5	0,299	32	32,6	40,8
16	T	2	Pool T	6,1	0,09	32,2	32,6	42
17	B	2	Pool B	6,8	0,04	25	20	88
18	U	2	Pool U	6,7	0,124	28	24	64
19	C	1	Pool C	6,4	0,031	31,1	16	63,6
20	D	1	Pool D	6,2	0,02	33	16	63,6
21	E	1	Pool E	6,8	0,03	33,5	30,6	56,7
22	F	1	Pool F	6,6	0,09	25,3	31,8	34,6
23	V	1	Pool V	6,1	0,198	31,5	29,7	45,4
24	W	1	Pool W	6,8	0,045	25,8	27,8	64,6

25	X	1	Pool X	7,7	0,14	24,5	30,1	32,9
26	G	1	Pool G	6,4	0,096	32,4	32,4	24,5
27	H	1	Pool H	6,1	0,118	27,7	32	70
28	I	1	Pool I	6,6	0,07	29,9	31,6	88,7
29	Y	1	Pool Y	7,1	0,137	21,3	28	50
30	J	1	Pool J	6,8	0,05	22,2	34,4	59
31	K	1	Pool K	6,5	0,06	27,3	29,6	31
32	L	1	Pool L	7	0,09	26,8	33,3	58

Made by: Paredes, et al.

To quantify colony-forming units (CFUs) in each sample, plating on Petrifilm plates was performed for counting coliforms and enterobacteria. In cases of high CFU concentrations, the sample was diluted with distilled water and quantified using the most probable number methodology. When comparing the results with the national regulations of TULSMA, it was determined that the river samples comply with the limit for fecal and total coliforms, unlike the samples from 4 pools (C, D, G, I) which did not meet the established parameters for recreational water use, exceeding the allowed limit.

Differentiation was performed considering the characteristics of the colonies such as: shape, size, consistency, color, odor, and turbidity. All the analyzed colonies showed a Gram-negative stain, and it was also observed that pools D, E, and G have a higher number of colonies compared to other pools.

Each processed colony was replated on the differential chromogenic medium for enterobacteria producing extended-spectrum beta-lactamases (ESBLs), which also allows for differentiation of enterobacteria species according to the physical characteristics that the colonies exhibit on the medium (table 2). In the 48 processed samples, 2 pools were found where no growth occurred, indicating that the planted enterobacteria are not ESBL producers. Additionally, a high incidence of colonies from the genera Acinetobacter, Pseudomonas, Klebsiella (KEC), and E. coli was found in pool samples, while river samples showed the presence of E. coli and Klebsiella.

Table 2: Isolation and Differentiation of Beta-Lactam Resistant Bacteria

N°	Sample	River/Pool	Growth on Agar	Characteristics	Species	Code
1	RU4-A	Río Ulba	(+)	Azul	*BLEE KEC*	RU-KEC1
2	RC	Río Cutuchi	(+)	Red	*BLEE E. coli*	RC-EC1
3	RC	Río Cutuchi	(+)	Red	*BLEE E. coli*	RC-EC2
4	RC	Río Cutuchi	(+)	Red-Blue	*BLEE KEC*	RC-KEC1
5	RC	Río Cutuchi	(+)	Red	*BLEE E. coli*	RC-EC3

6	RP	Río Pachanlica	(+)	Red	*BLEE E. coli*	RP-EC1
7	RP	Río Pachanlica	(+)	Red	*BLEE E. coli*	RP-EC2
8	RP	Río Pachanlica	(+)	Red	*BLEE E. coli*	RP-EC3
9	RA	Río Ambato	(+)	Red	*BLEE E. coli*	RA-EC1
10	RA	Río Ambato	(+)	Red-Blue	*BLEE KEC*	RA-KEC1
11	RA	Río Ambato	(+)	Red	*BLEE E. coli*	RA-EC2
12	R	Pool R	(-)	(-)	*(-)*	(-)
13	R	Pool R	(-)	(-)	*(-)*	(-)
14	A	Pool A	(+)	Cream	*BLEE Acinetobacter*	A-A1
15	A	Pool A	(+)	Translucent	*BLEE Pseudomonas*	A-P1
16	S	Pool S	(-)	(-)	*(-)*	(-)
17	S	Pool S	(-)	(-)	*(-)*	(-)
18	B	Pool B	(+)	Translucent	*BLEE Pseudomonas*	B-P1
19	B	Pool B	(+)	Translucent	*BLEE Pseudomonas*	B-P2
20	B	Pool B	(+)	Cream	*BLEE Acinetobacter*	B-A1
21	B	Pool B	(+)	Translucent	*BLEE Pseudomonas*	B-P3
22	B	Pool B	(+)	Cream	*BLEE Acinetobacter*	B-A2
23	C	Pool C	(+)	Red-blue	*BLEE KEC*	C-KEC1
24	C	Pool C	(+)	Blue	*BLEE KEC*	C-KEC2
25	C	Pool C	(+)	Cream	*BLEE Acinetobacter*	C-A1
26	D	Pool D	(+)	Cream	*BLEE Acinetobacter*	D-A1
27	D	Pool D	(+)	Cream	*BLEE Acinetobacter*	D-A2
28	D	Pool D	(+)	Red-blue	*BLEE KEC*	D-KEC1
29	E	Pool E	(+)	Cream	*BLEE Acinetobacter*	E-A1
30	E	Pool E	(+)	Red	*BLEE E. coli*	E-EC1
31	E	Pool E	(+)	Red	*BLEE E. coli*	E-EC2
32	E	Pool E	(+)	Red-Blue	*BLEE KEC*	E-KEC1
33	E	Pool E	(+)	Cream	*BLEE Acinetobacter*	E-A2
34	E	Pool E	(+)	Blue	*BLEE KEC*	E-KEC2

35	F	Pool F	(+)	Cream	*BLEE Acinetobacter*	F-A1
36	F	Pool F	(+)	Cream	*BLEE Acinetobacter*	F-A2
37	G	Pool G	(+)	Red	*BLEE E. coli*	G-EC1
38	G	Pool G	(+)	Cream	*BLEE Acinetobacter*	G-A1
39	G	Pool G	(+)	Cream	*BLEE Acinetobacter*	G-A2
40	G	Pool G	(+)	Cream	*BLEE Acinetobacter*	G-A3
41	H	Pool H	(+)	Translucent	*BLEE Pseudomonas*	H-P1
42	I	Pool I	(+)	Cream	*BLEE Acinetobacter*	I-A1
43	I	Pool I	(+)	Cream	*BLEE Acinetobacter*	I-A2
44	J	Pool J	(+)	Cream	*BLEE Acinetobacter*	J-A1
45	K	Pool K	(+)	Cream	*BLEE Acinetobacter*	K-A1
46	K	Pool K	(+)	Cream	*BLEE Acinetobacter*	K-A2
47	L	Pool L	(+)	Cream	*BLEE Acinetobacter*	L-A1
48	L	Pool L	(+)	Cream	*BLEE Acinetobacter*	L-A2

Made by: Paredes, et al

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibiotic that inhibits visible growth of a particular bacterial strain and is measured in micrograms per milliliter (µg/mL).

A heatmap allows complex data to be represented in an intuitive way, identifies patterns of sensitivity or resistance, and proposes treatments more suitable for bacterial infections.

Figure 1. Heatmap of Minimum Inhibitory Concentrations for River

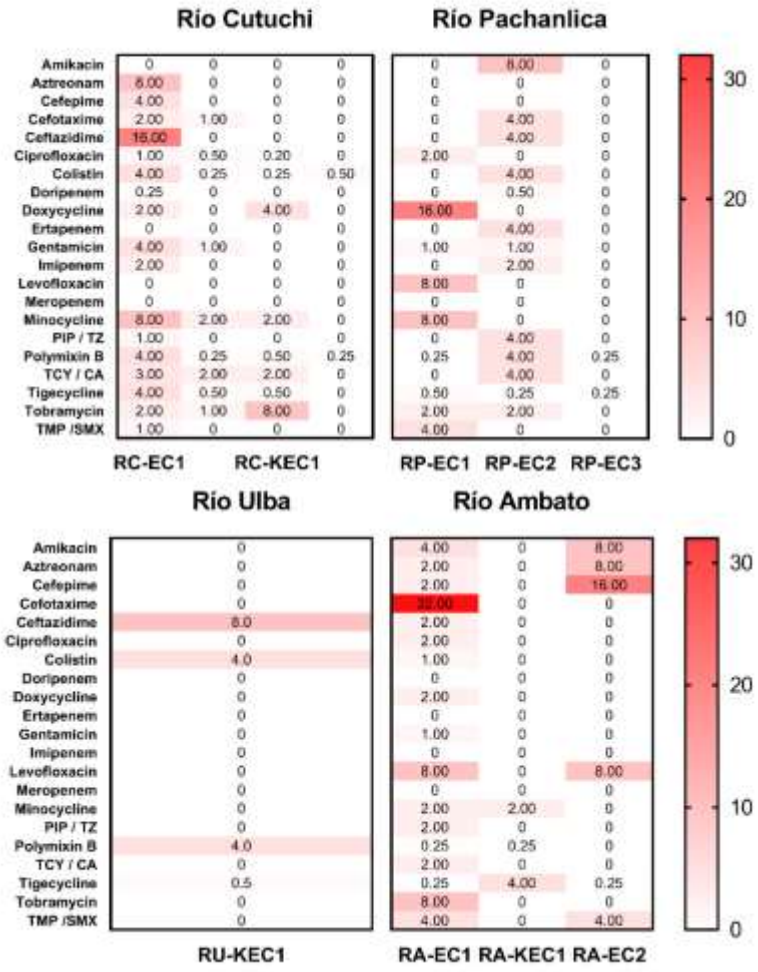


Figure 2. Heatmap of Minimum Inhibitory Concentrations of Bacterial Colonies Found in Pools A-F

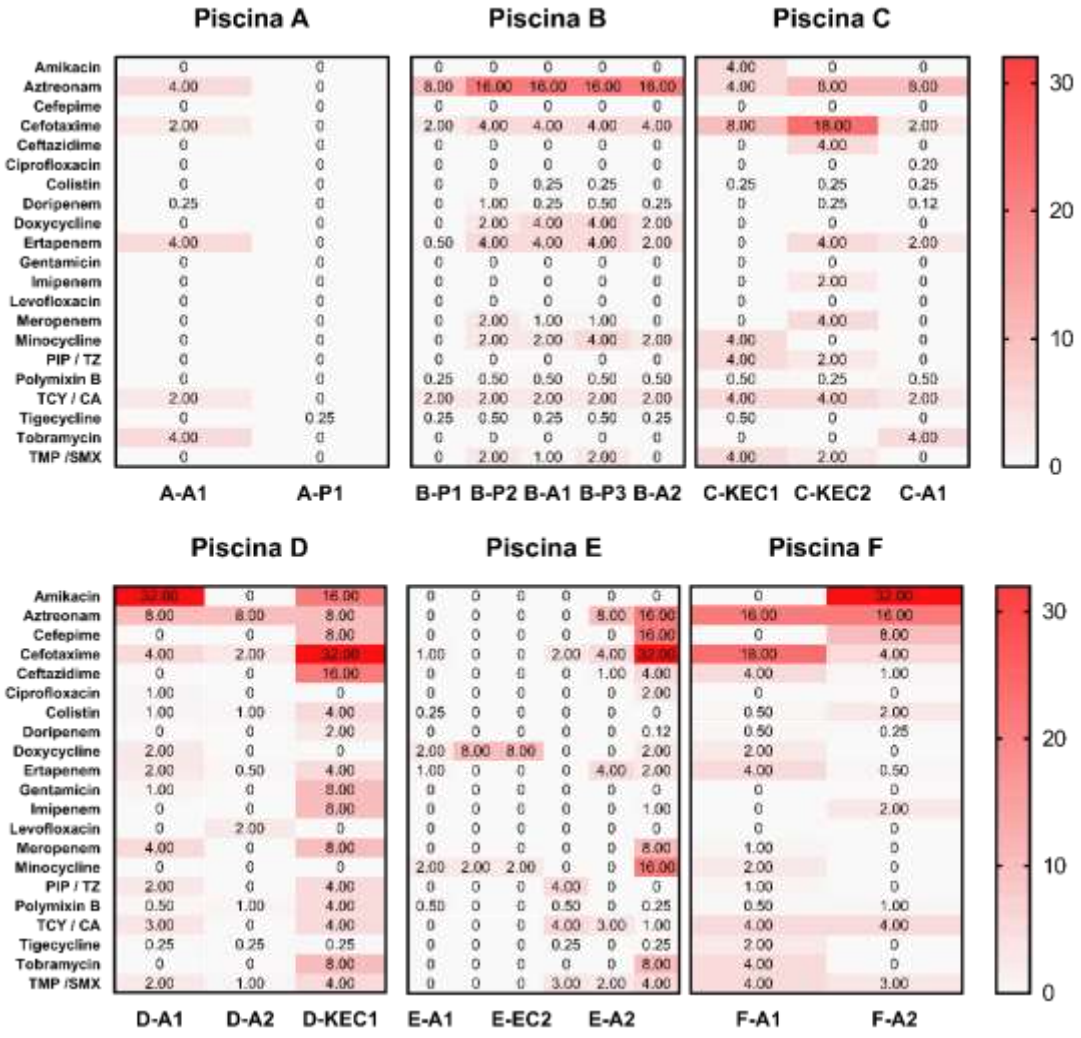
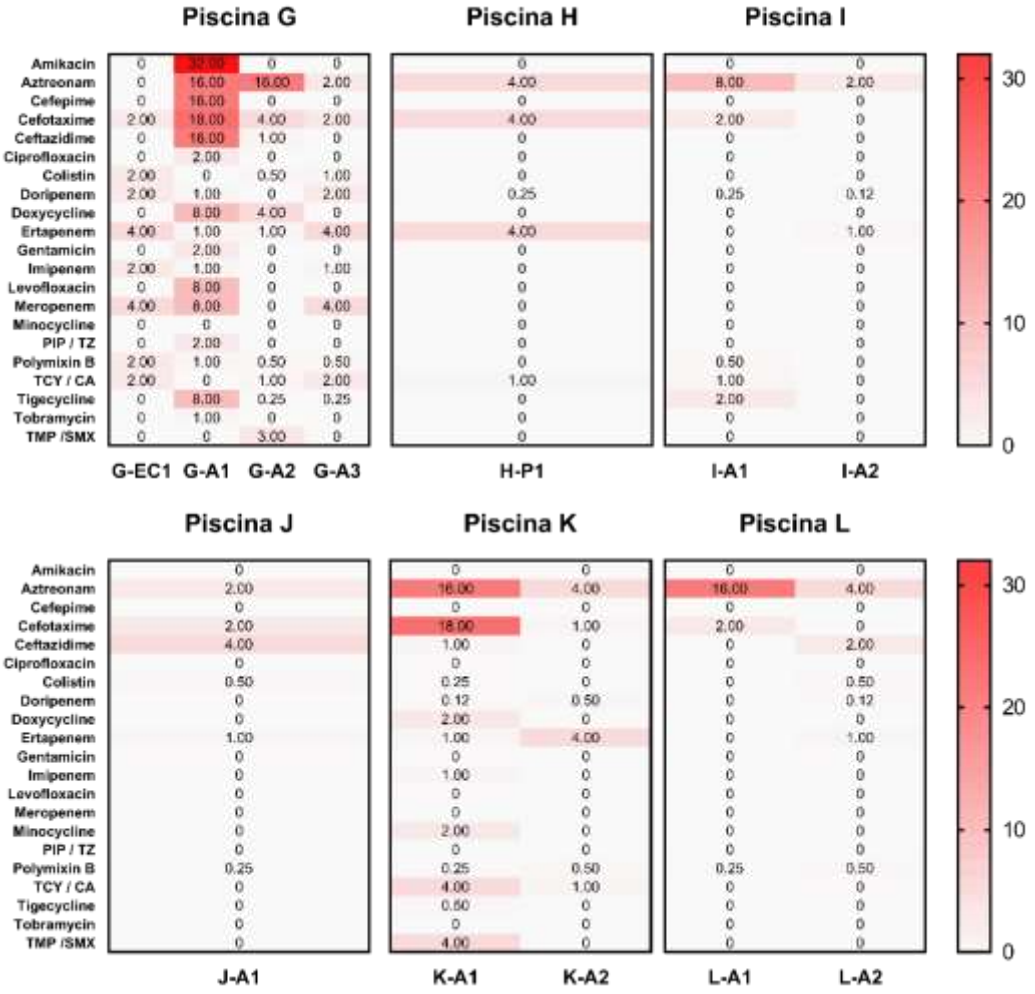


Figure 3: Heatmap of Minimum Inhibitory Concentrations of Bacterial Colonies Found in Pools G-L.



In the heatmaps, intense red coloring indicates high antibiotic resistance, while lighter red coloring denotes partial resistance. Finally, the absence of color indicates no resistance, thus good sensitivity to the antibiotic.

For example, in pool B, the red band indicates high resistance to aztreonam, moderate resistance to ticarcillin/clavulanic acid, and no resistance to Amikacin, Cefepime, Ceftazidime, Ciprofloxacin, Gentamicin, Imipenem, Levofloxacin, Piperacillin/Tazobactam, and Tobramycin.

4. Discussion and Conclusions

According to the WHO, rivers are considered contaminated if there are changes in their composition that could alter the microenvironment (Juliño et al. 2021). In this study, the Ambato, Pachanlica, and Cutuchi rivers showed evidence of contamination. According to Cedeño (2020), Ecuador has an extensive water network that is contaminated by wastewater, solid and liquid (chemical) wastes that are discharged into rivers; however, there are also living rivers thanks to their dynamic ecosystem which maintains a natural balance; this is achieved through the constant flow that disperses pathogens, pollutants, and the action of sunlight (Cedeño 2020).

pH is an indicator of water quality. According to Pérez (2016), acidic pH can cause irritation to mucous membranes, internal organs, and ulcerative processes. On the other hand, an alkaline pH is associated with colored waters, unpleasant odors, and tastes, as observed in the Cutuchi, Pachanlica, and Ambato rivers (Pérez 2016).

Regarding the pH and chlorine levels of the pools, in our study, they varied between 6.1-7.7, and residual chlorine was identified; depending on the pH, the chlorine in pools can generate chloramines that reduce its disinfectant capacity and cause skin lesions. A pH higher than 7.6 could be harmful to users because a high pH transforms chlorine into hypochlorous acid, the real disinfecting agent, which can have harmful effects in amounts exceeding the tolerance range according to regulations, a finding in several pools analyzed (Freixa 1998).

According to the TULSMA regulations, the permissible limit of coliforms in rivers is 200 CFU/100 mL, and 1000 CFU/100 mL for total coliforms; the rivers studied comply with this parameter. However, 4 of the analyzed pools do not meet the regulations for allowable fecal coliforms. Their presence could cause serious infections upon human contact, so these pools should not be used for recreational purposes (Ministry of the Environment 2017).

There are living rivers on the planet; in this study, the Verde and Ulba rivers belong to this category. These rivers do not have contact with human populations; however, the Ulba River ceases to be a living river upon reaching the city, as it is used by a large number of bathers and as a dumping ground for organic waste, which alters its characteristics. This correlates with findings in the Tomebamba and Tarqui rivers in the city of Cuenca, where pollution is of human origin and the main sources are non-intercepted domestic wastewater discharges (Pauta et al. 2020).

In terms of stagnant water, 4 pools in the province of Tungurahua do not meet the TULSMA standards, showing CFU ranges from 254.7 to 14,053, indicating bacterial contamination, improper cleaning and maintenance, and an excess of bathers for the capacity of the aquatic

environment. Arrieta and Bonifaz (2019) in the pools of Baños, Ecuador, found a microbial load of 6550 CFU/ml in the busiest pools of the area. In that study, it was observed that the main causes of CFU formation were the increase in the number of visitors, poor cleaning and disinfection of the tanks, and inadequate control of the pool piping (Arrieta and Bonifaz 2019). These results are consistent with the findings presented, indicating that the situation has not changed.

The analysis of samples collected from pools has revealed the presence of extended-spectrum beta-lactamase-producing bacteria, such as E. coli, Pseudomonas, Klebsiella, and Acinetobacter, with resistance to most antibiotics. These findings highlight an alarming scenario of microbial resistance in rivers and pools in Tungurahua, emphasizing the importance of addressing this emerging phenomenon in discussions on water quality for recreational use. Analyzing this issue, Ruiz and Enriquez (2021) note that the antibiotic resistance of these strains poses a significant risk to human health. Exposure to these resistant bacteria compromises the effectiveness of conventional medical treatments, generating negative consequences for public health (Ruiz et al. 2021). According to Giono et al. (2020), the high antibiotic resistance of microorganisms in freshwater is due to contamination by residual antibiotics released through various sources, such as agricultural runoff, sewage discharges, and leachates from nearby farms (Giono et al. 2020). In various countries, guidelines have been established to monitor antibiotic-resistant strains, however, these considerations have not been implemented in Ecuadorian regulations (Martínez et al. 2020).

The strains isolated in rivers were E. coli and Klebsiella producing extended-spectrum beta-lactamases, resistant to antibiotics and susceptible to specific pharmacological groups. In the 4 rivers, the sensitivity of the isolated pathogens to antibiotics differed, with carbapenems (meropenem, doripenem, ertapenem, imipenem), ciprofloxacin, and gentamicin being the most effective antibiotics against these strains. Zhong et al. (2021) isolated 9 strains of E. coli producing ESBLs with different genomes and different origins of replication, including a high-risk strain belonging to the ST131 lineage (a multi-resistant pandemic clone) (Zhong et al. 2021). This reveals the presence of various antibiotic resistance genes for ESBLs such as: fluoroquinolones, aminoglycosides, phenicols, sulfonamides, and indicates that the aquatic environment serves as a reservoir for these resistance determinants.
The sensitivity of ESBLs is directly related to the inhibitory concentration, making a higher minimum inhibitory concentration of the antibiotic necessary: Trimethoprim/sulfamethoxazole, ticarcillin/clavulanic acid, and piperacillin/tazobactam, ranging from 8.0 µg/ml to 128.0 µg/ml to achieve treatment efficacy. This finding differs from the report by Giebułtowicz et al. (2018) on the Vistula River, Poland, which showed greater resistance to macrolides, lincosamides, and streptogramins (Giebułtowicz et al. 2018).

The strains isolated in the pools were E. coli, Klebsiella, Acinetobacter, and Pseudomonas producing extended-spectrum beta-lactamases. In the isolated strains, antibiotic sensitivity varied from pool to pool, but in general, there was sensitivity to levofloxacin, ciprofloxacin, and gentamicin, with resistance to trimethoprim/sulfamethoxazole, piperacillin/tazobactam, and aztreonam. The results are similar to those obtained by Koeck et al. (2018), who analyzed pools in Bavaria - Germany, finding gram-negative bacilli and Pseudomonas spp. However, the sensitivity was different as these bacteria were sensitive to piperacillin, ceftazidime, cefepime, and

trimethoprim-sulfamethoxazole, and resistant to ciprofloxacin, levofloxacin, imipenem, ertapenem, and fluoroquinolones (Koeck et al. 2018).

The National Institute of Statistics and Census of Ecuador (INEC:2016), in assessing progress on the Sustainable Development Goals, determined that: "15.4% of the urban population and 31.8% of the rural Ecuadorian population consume water contaminated with fecal coliforms" (Cedeño 2020).

The results presented express an unfortunate reality of moving and stagnant water in the province, with alarming contamination by superbugs, turning these environments into a threat to human health and the ecosystem.

Conclusion

The analysis of river and pool samples conducted in this study found a relationship between the concentration of dissolved chlorine, water temperature, and the concentration of colony-forming units, which in turn affects the prevalence of antibiotic-resistant pathogens (superbugs). It is necessary to take measures to reduce the CFU concentration in both rivers and pools, and it is recommended to add to the TULSMA regulations a maximum permissible amount of antibiotic-resistant multiresistant colonies.

The research reveals a significant impact of pollution on the Ambato, Pachanlica, and Cutuchi rivers, highlighting the presence of bacteria resistant to multiple antibiotics. This pollution poses not only environmental risks but also potential threats to human health and the ecosystem. Additionally, variations in water quality in different pools highlight the need for effective measures to ensure microbiological safety in aquatic environments intended for recreational use.

Finally, the research underscores aspects that pose challenges to current regulations, which mainly focus on conventional physicochemical parameters and do not address the presence of antibiotic-resistant bacteria. The lack of consideration of these microorganisms in Ecuadorian regulations calls for an urgent need to review and strengthen water quality standards, incorporating microbiological indicators that do not threaten health. This comprehensive approach is crucial for addressing emerging risks related to antibiotic resistance and ensuring the protection of both aquatic environments and public health.

The failure to comply with current regulations has caused irreparable damage to water quality and the biodiversity of rivers, making it necessary for public administrations and the population to commit to maintaining healthy rivers to ensure the health of future generations.

References

Arrieta, K., & Bonifaz, A. (2019). Estudio físico-químico y microbiológico de las aguas termales en las piscinas " El Salado " en el Cantón Baños de Agua Santa , Provincia de Tungurahua. *Universidad Estatal Amazónica*. https://repositorio.uea.edu.ec/handle/123456789/485

Astocondor, L. (2018). Betalactamasas: la evolución del problema. *Revista Peruana de Investigación En Salud*, *2*(2), 42–49.

Carrasquero Ferrer, S. J., Mendoza Guillén, A., Pino Rodríguez, J. F., Jativa, H., & Díaz Montiel, A. R. (2024). Análisis de la operatividad y mantenimiento de piscinas de la ciudad de

Maracaibo, Venezuela. *Revista Universidad de Guayaquil*, *138*(1), 62–72. https://doi.org/10.53591/rug.v138i1.2377

Cedeño, H. (2020). Análisis de los parámetros de calidad del agua del efluente del río muerto para su posible reutilización del Cantón Manta, Ecuador. *Polo Del Conocimiento: Revista Científico - Profesional, ISSN-e 2550-682X, Vol. 5, Nº. 2, 2020, Págs. 579-604*, *5*(2), 579–604. https://doi.org/10.23857/pc.v5i2.1299

Conejero S, J. C. (2020). UNA APROXIMACIÓN A LA INVESTIGACION CUALITATIVA. *Neumología Pediátrica*, *15*(1), 242–244. https://doi.org/10.51451/np.v15i1.57

Flores, R., Albornoz, C., & Cáceres, J. (2019). Enterobacterias productoras de b-lactamasas de espectro-extendido y plásmido-ampc en aguas de riego, zona maica, cochabamba. *Revista Científica Ciencia Médica*, *22*(2), 15–21.

Freixa, A. (1998). *Exposición a cloro en piscinas cubiertas*. Ministerio de Trabajo y Asuntos Sociales de España. https://www.insst.es/documents/94886/326827/ntp_341.pdf/7af0d487-75d8-4fd4-a4e9-19147328a6f5

Giebułtowicz, J., Tyski, S., Wolinowska, R., Grzybowska, W., Zaręba, T., Drobniewska, A., Wroczyński, P., & Nałęcz-Jawecki, G. (2018). Occurrence of antimicrobial agents, drug-resistant bacteria, and genes in the sewage-impacted Vistula River (Poland). *Environmental Science and Pollution Research International*, *25*(6), 5788–5807. https://doi.org/10.1007/s11356-017-0861-x

Giono, S., Santos, J., Morfín, M., Torres, F., & Curiel, A. (2020). Resistencia antimicrobiana. Importancia y esfuerzos por contenerla. *Gaceta Médica de México, ISSN 0016-3813, Vol. 156, Nº. 2, 2020, Págs. 172-180*, *156*(2), 172–180. https://doi.org/10.24875/GMM.20005624

Juliño, M., Ocaña, F., & Concha, J. (2021). 5.-Contaminación ambiental y su influencia en la salud. *ReNaCientE - Revista Nacional Científica Estudiantil - UPEL-IPB*, *2*(1), 75–90. https://doi.org/10.46498/renacipb.v2i1.1566

Koeck, D. E., Huber, S., Hanifi, N., Köster, M., Schierling, M. B., & Höller, C. (2018). Occurrence of Antibiotic-Resistant Bacteria in Therapy Pools and Surrounding Surfaces. *International Journal of Environmental Research and Public Health*, *15*(12). https://doi.org/10.3390/ijerph15122666

Lenart-Boroń, A. M., Kulik, K., & Jelonkiewicz, E. (2020). Antimicrobial resistance and ESBL genes in E. coli isolated in proximity to a sewage treatment plant. *Journal of Environmental Science and Health, Part A*, *55*(14), 1571–1580. https://doi.org/10.1080/10934529.2020.1826774

Martínez, A., Garza, U., Sampedro, M. L., González, J., Nava, G., & Toribio, J. (2020). PATOTIPOS Y RESISTENCIA A ANTIBIÓTICOS DE Escherichia coli EN AGUA RESIDUAL. *Revista Internacional de Contaminación Ambiental*. https://doi.org/10.20937/RICA.53711

Melzer, M., & Petersen, I. (2007). Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. *Journal of Infection*, *55*(3), 254–259. https://doi.org/10.1016/j.jinf.2007.04.007

Ministerio del Ambiente, A. y T. E. (2017). *Texto unificado de legislación secundaria de medio ambiente*. Lexis Finder. www.lexis.com.ec

Morales, E., Reyes, L., Barrantes, K., & Chacón, L. (2022). Evaluación temporal y espacial en la calidad microbiológica del agua superficial: caso en un sistema de abastecimiento de agua para consumo humano en Costa Rica. *Revista de Ciencias Ambientales*, *56*(1), 120–137. https://doi.org/http://dx.doi.org/10.15359/rca.56/1.6

Pascual Araoz, D. J., Flores Ocampo, A. B., Quiroga Perez, A. C., Almendras Terrazas, B., & Crespo Osinaga, K. R. (2020). Presencia de Pseudomonas aeruginosas, cepas BLEE y resistencia en Salas del Hospital Solomon Klein, Cochabamba. *Revista Cientifica Ciencia Medica*, *23*(2), 129–135. https://doi.org/10.51581/rccm.v23i2.191

Pauta-Calle, G., Vázquez, G., Abril, A., Torres, C., Loja-Sari, M., & Palta-Vera, A. (2020). Indicadores bacteriológicos de contaminación fecal en los ríos de Cuenca, Ecuador. *MASKANA*, *11*(2), 46–57. https://doi.org/10.18537/mskn.11.02.05

Pérez, E. (2016). Control de calidad en aguas para consumo humano en la región occidental de Costa Rica. *Revista Tecnología En Marcha Scielo*, *29*(3), 3–14. https://doi.org/10.18845/TM.V29I3.2884

Ruiz, D. R. F., Enríquez, M. Q., & Pérez, O. L. C. (2021). Los antibióticos y su impacto en la sociedad. *MediSur*, *19*(3), 477–491. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1727-897X2021000300477&lng=es&nrm=iso&tlng=es

Salazar, L. (2018). Betalactamasas: la evolución del problema. *Revista Peruana de Investigación En Salud*, *2*(2), 42–49.

Santos, P Muñoz, L Cruz, C Navarrete, J Pinilla, G. (2021). Péptidos antimicrobianos LL-37 y sus derivados frente a microrganismos de importancia clínica: Una alternativa a la resistencia microbiana. *Edgar Serna M.*, *197*. https://doi.org/http://doi.org/10.5281/zenodo.5139646

Schmiege, D., Zacharias, N., Sib, E., Falkenberg, T., Moebus, S., Evers, M., & Kistemann, T. (2021). Prevalence of multidrug-resistant and extended-spectrum beta-lactamase-producing Escherichia coli in urban community wastewater. *Science of The Total Environment*, *785*, 147269. https://doi.org/10.1016/j.scitotenv.2021.147269

Vidal, C., Gutiérrez, A., & Robayo, L. (2019). Membrana externa de Helicobacter pylori y su papel en la adhesión al epitelio gástrico. *Universitas Médica*, *56*(1), 44–62. https://revistas.javeriana.edu.co/index.php/vnimedica/article/download/16344/13126

Vink, J., Edgeworth, J., & Bailey, S. L. (2020). Acquisition of MDR-GNB in hospital settings: a systematic review and meta-analysis focusing on ESBL-E. *Journal of Hospital Infection*, *106*(3), 419–428. https://doi.org/10.1016/j.jhin.2020.09.006

Zhong, Y., Guo, S., Seow, K. L. G., Ming, G. O. H., & Schlundt, J. (2021). Caracterización de aislados de Escherichia coli productores de beta-lactamasas de espectro extendido del lago Jurong, Singapur, con secuenciación del genoma completo. *International Journal of Environmental Research and Public Health*, *18*(3), 1–12. https://doi.org/10.3390/IJERPH18030937