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Swimming pools as emerging environmental reservoirs of antimicrobial-resistant bacteria in two cities of southwestern Colombia: Cali and Jamundí

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ABSTRACT

Antimicrobial-resistant microorganisms are considered emerging environmental contaminants, and chlorination could exacerbate this issue. The main objective of this study was to characterize the antibiotic resistance profile of bacteria isolated from swimming pools in Cali and Jamundí, two cities in the southwest of Colombia. A total of 16 swimming pool water samples were microbiologically analyzed to determine bacterial diversity and assess antibiotic susceptibility to ten broad-spectrum antibiotics using the disk diffusion method. Bacterial isolates showing resistance were identified through 16S rRNA gene sequencing and selective differential growth media. A total of 69% of swimming pool samples did not comply with Colombian microbiological regulations. Among the bacterial isolates, 92% exhibited resistance to at least one antibiotic, with no significant differences between Gram-positive and Gram-negative species. Clinically relevant multidrug-resistant strains were identified, including *Escherichia coli, Pseudomonas otitidis, Staphylococcus warneri, Proteus mirabilis,* and *Bacillus cereus,* some of which showed simultaneous resistance to critical antibiotics such as tetracycline, ceftriaxone, vancomycin, and kanamycin. These results confirm that swimming pools provide a favorable environment for the presence of bacterial resistance. From a One Health perspective, these results emphasize the interconnectedness of human, animal, and environmental health, reinforcing the need for stricter monitoring and control of recreational waters to mitigate the spread of antibiotic resistance.

Keywords: one health, recreational water, waterborne pathogens.

Piscinas como reservatórios ambientais emergentes de bactérias resistentes a antimicrobianos em duas cidades do sudoeste da Colombia: Cali e Jamundí

RESUMO

Os microrganismos resistentes a antimicrobianos são considerados contaminantes ambientais emergentes, e a cloração pode agravar esta questão. O objetivo principal deste

estudo foi caracterizar o perfil de resistência a antibióticos de bactérias isoladas de piscinas em Cali e Jamundí, duas cidades do sudoeste da Colômbia. Um total de 16 amostras de água de piscinas foi analisado microbiologicamente para determinar a diversidade bacteriana e avaliar a suscetibilidade a antibióticos de dez antibióticos de amplo espectro utilizando o método de disco-difusão. Isolados bacterianos que apresentaram resistência foram identificados por meio do sequenciamento do gene 16S rRNA e do uso de meios de cultura seletivos e diferenciais. Um total de 69% das amostras de piscinas não atendera às normas microbiológicas colombianas. Entre os isolados bacterianos, 92% apresentaram resistência a pelo menos um antibiótico, sem diferenças significativas entre espécies Gram-positivas e Gram-negativas. Foram identificadas cepas multirresistentes clinicamente relevantes, incluindo *Escherichia coli, Pseudomonas otitidis, Staphylococcus warneri, Proteus mirabilis* e *Bacillus cereus*, algumas das quais exibiram resistência simultânea a antibióticos críticos como tetraciclina, ceftriaxona, vancomicina e canamicina. Esses resultados confirmam que as piscinas representam um ambiente favorável à presença de resistência bacteriana. Sob a perspectiva da Saúde Única (One Health), esses achados ressaltam a interconexão entre a saúde humana, animal e ambiental, reforçando a necessidade de um monitoramento e controle mais rigorosos das águas recreativas para mitigar a disseminação da resistência aos antibióticos.

Palavras-chave: água recreativa, patógenos transmitidos pela agua, saúde única.

1. INTRODUCTION

Bacterial resistance is a critical threat to global public health, according to the World Health Organization (WHO). It is estimated that more than 700,000 deaths a year are due to infections caused by resistant micro-organisms, exacerbated by practices such as the indiscriminate use of antibiotics, poor management of hospital wastewater and climate change (Bengtsson-Palme *et al.*, 2018). Aquatic environments, both natural and artificial, have been recognized as important reservoirs for the spread of resistant bacteria and antibiotic resistance genes (ARGs) (Marti *et al.*, 2014). Evidence of this includes the isolation of antibiotic-resistant bacteria in water treatment systems after chlorination (Xi *et al.*, 2009; Jia *et al.*, 2015) and in environments where residual chlorine is persistent (Adefisoye *et al.*, 2022), such as swimming pools (Caro-Hernández *et al.*, 2024). Due to inappropriate use of swimming pools, they receive significant loads of commensal, opportunistic and pathogenic microorganisms from bathers, making them a vehicle for the transmission of infectious diseases (Barna and Kádár, 2012). In addition, there is evidence that bather load in swimming pools contributes to the increase in ARGs, and that the absolute abundance of ARGs increases by almost two orders of magnitude when the bather load exceeds 0.1 person/m^2-h (Shuai *et al.*, 2021). Although chlorination is a widely used method of water disinfection (Shannon *et al.*, 2008), in these environments some bacteria can persist for long periods due to their resistance to the disinfectant (Jia *et al.*, 2015; Peters *et al.*, 2018), making this an issue with significant public health implications (Adefisoye *et al.*, 2022, Caro-Hernández *et al.*, 2024). Thanks to co-selection factors, it has been shown that the pressure exerted by chlorine increases the transfer of plasmids carrying resistance genes through natural transformation between bacteria of the same genus and emerging bacteria (Davies, 1994). In addition, inadequate chlorination disinfection could increase the abundance of both intracellular and extracellular antibiotic resistance genes (Liu *et al.*, 2018). This could contribute to pool bacteria not only adapting to a hostile environment, but also becoming a vehicle for bacterial resistance transfer, linking resistant bacteria in the natural environment with human microbiota (Yang *et al.*, 2018; Shuai *et al.*, 2021; Adefisoye *et al.*, 2022). This poses a risk of environmental spread of antibiotic resistance and a potential threat to public health.

Accordingly, this study characterized antibiotic-resistant bacterial isolates from water

samples collected from swimming pools in the cities of Cali and Jamundí, in the southwestern region of Colombia.

2. MATERIAL AND METHODS

2.1. Swimming pools

This study was conducted using water samples from 16 swimming pools, comprised of public recreational pools (n = 2), sports pools (n = 2), and private pools located in residential complexes for adults (n = 10) and children (n = 2). The sampling sites were situated in the southwestern region of Colombia, in the department of Valle del Cauca, specifically in the cities of Cali (geographical coordinates: 3°27′00″ N, 76°32′00″ W) and Jamundí (geographical coordinates: 3°15′39″N, 76°32′22″W). Each sample was collected only once during the indicated months: February, March, June, and September 2019; December 2021; and June 2022.

2.2. Sampling and microbiological procedures

Water samples were collected randomly in a single grab sample using sterile wide-mouth 1000 ml bottles, with sodium thiosulphate added to a final concentration of 100 mg/L. Samples were refrigerated at 4°C and processed within 2 hours. Samples were analyzed using the plate count technique for the enumeration of total heterotrophic bacteria (THB) at 37°C ± 1°C (Plate Count Agar® Cat. No. 1.05463, Merck KGaA, 70152) and membrane filtration using 0.45 µm pore size nitrocellulose filters (individually packed sterile filters, type AC, SC. Sartorius Stedim Biotech) according to standardized methods (APHA *et al.*, 1998). The following parameters were determined: Enteric bacteria per 100 mL at 37 ± 1°C (VRBD Agar®, Merck KGaA 110275) and total coliforms (Chromocult®, Merck KGaA 110426).

From the counts for each of the indicator groups, pure colonies of isolates with different morphology and characteristics were obtained on tryptic soy agar (TSA® Merck KGaA 105458) at 37°C. Gram staining was performed prior to each analysis to confirm their purity. The isolates obtained were characterized based on their growth on differential agars for coliforms (MacConkey® Merck KGaA 100205 and Chromocult® Merck KGaA 110426), *Escherichia coli* (Eosin Methylene Blue EMB, Becton, Dickinson), *Staphylococcus* spp. (Mannitol Salt Becton, Dickinson) and *Pseudomonas* spp. (CHRO-Magar™ Pseudomonas).

Isolates characterized as resistant were subjected to sequencing for molecular identification in a specialized laboratory (CorpoGen, Bogotá). In general, genomic DNA was extracted using a standard silica column–based purification method, involving cell lysis with detergent and proteinase K, removal of proteins and RNA, and elution of high-quality DNA. DNA quantity and purity were assessed spectrophotometrically. Amplification of target regions was performed by PCR, and resulting products were purified and sequenced by the Sanger Method, using 16S rRNA gene primers 337F/880R (V3–V5 region) and 518F/1100R (V4–V6 region). Sequence cleaning and assembly was conducted using BLAST (Basic Local Alignment Search Tool) from NCBI (National Center for Biotechnology Information), comparing it with the reference RNA database RefSeq (Altschul *et al.*, 1997).

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the Kirby–Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). The antibiotic concentrations applied in this study followed the CLSI standards for clinical applications. A single colony from a 16–24 h culture of the isolate of interest was suspended in 5 mL of 0.85% saline, and the turbidity was adjusted to the 0.5 McFarland Standard (approximately 10^8 CFU/mL). The standardized inoculum was then spread onto Müller–Hinton agar plates (Difco Laboratories, USA), which were subsequently incubated at

37 °C for 24 hr. Antibiotic-impregnated disks (BD BBL™ Sensi-Disc™, Becton Dickinson, USA), containing predefined concentrations established by CLSI, were placed on the inoculated agar surface, and susceptibility was determined by measuring the inhibition zone diameters after incubation.

The antimicrobial susceptibility of Gram-negative bacilli was evaluated against eight antibiotics: Sulfamethoxazole/trimethoprim (SXT, 1.25/23.75 µg), ciprofloxacin (CIP, 5 µg), kanamycin (KAN, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CRO, 30 µg), amikacin (AMK, 30 µg), tetracycline (TET, 30 µg) and ceftazidime (CAZ, 30 µg). For Gram-positive bacteria, the following antibiotics were used: Kanamycin (KAN, 30 µg), Ciprofloxacin (CIP, 5 µg), Ceftriaxone (CRO, 30 µg), Rifampin (RIF, 5 µg), Tetracycline (TET, 30 µg) and Vancomycin (VAN, 30 µg). Bacteria were classified as resistant or susceptible based on the inhibition zone diameters around the discs according to CLSI recommendations and antibiotic standardization values using the Kirby-Bauer Technique (Bernal *et al.*, 1984). The following reference strains were used for quality control *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

3. RESULTS AND DISCUSSION

3.1. Microbiological quality

The transmission of waterborne microorganisms to humans is most achieved via the oral route. Nonetheless, exposure to contaminated or inadequately treated recreational water also poses a significant risk, serving as a substantial source for the transmission of infectious diseases. According to the Resolution 1618 of the year 2010 of the Colombian Ministry of Health and Social Protection, the admissible limits for bacterial indicator groups in swimming pool water are as follows: heterotrophs should not exceed 200 CFU/mL, and bacteria such as thermotolerant coliforms *Escherichia coli* and *Pseudomonas aeruginosa* should not be present (Colombia, 2010). The microbiological quality of swimming pool water from the 16 sampled sites, seven pools in Cali and nine in Jamundi, is presented in Table 1. The pools were categorized according to their use: 10 residential adults pools (RA), two residential children pools (RC), two public recreational pools (RT) and two sports adults pools (SA).

Table 1. Number of heterotrophic bacteria and Enterobacteriaceae in the sampled sites.

Code Pool	City	Pool Type	Heterotrophic (CFU/mL)	Coliforms (CFU/100 mL)	Enterobacteriaceae (CFU/100 mL)	Meets Quality Criteria
PUNLVL	Cali	SA	>300	8	13	Not
PURBR	Cali	RA	80	3	5	Not
PURVS	Cali	RT	42	0	0	Yes
PURCRH	Cali	RA	>300	0	0	Not
PURRP	Cali	RA	260	0	0	Not
PDAG	Cali	SA	>300	0	0	Not
PRPC	Cali	RT	44	3	4	Not
PURCAA	Jamundí	RA	20	1	1	Not
PURCAN	Jamundí	RC	>300	2	6	Not
PURCFN	Jamundí	RC	>300	0	1	Not
PURCTA	Jamundí	RA	>300	0	0	Not
PURVH	Jamundí	RA	33	0	0	Yes
PURSP	Jamundí	RA	84	0	0	Yes
PURCAZ	Jamundí	RA	12	0	0	Yes
PURAL	Jamundí	RA	2	0	0	Yes
PURFV	Jamundí	RA	26	1	2	Not

RA: Residential adults, RT: Recreational, SA: Sports adults, RC: Residential children.

The findings of this study revealed that 69% of the pools did not comply with the

microbiological limits established by Resolution 1618 of 2010, issued by the Colombian Ministry of Health and Social Protection. Moreover, 44% exhibited total heterotrophic bacterial counts above the acceptable threshold (<200 CFU/mL). With respect to coliform bacteria, 30% of the pools presented detectable levels, indicating non-compliance with the specification of 0 CFU/100 mL.

The application of selective and differential media, combined with PCR amplification and sequencing of the 16S rRNA gene, allowed the identification of 41 out of 49 bacterial isolates. In certain cases, species-level identification was achieved. These results, in line with previous studies, highlight the clinical, environmental, and public health significance of the bacterial isolates detected in swimming pool waters (Barna and Kádár, 2012; Caro-Hernández *et al.*, 2024).

Among the microorganisms identified, potentially pathogenic bacteria included *Enterobacter cloacae, Escherichia coli, Proteus mirabilis, Pseudomonas otitidis, Klebsiella pneumoniae, Aeromonas hydrophila, Bacillus cereus,* and *Staphylococcus aureus*. Opportunistic pathogens such as *Pseudomonas spp., Providencia spp., Acinetobacter spp., Staphylococcus spp., Enterobacter spp., Staphylococcus warneri, and Kurthia gibsonii* were also detected. Additionally, Gram-negative bacilli such as *Pantoea spp. and Pantoea stewartii, as well as Gram-positive bacilli including Bacillus subtilis, Priestia megaterium, and Exiguobacterium spp.,* were identified. The presence of these microorganisms in recreational waters is alarming, as they are frequently implicated in community and hospital-acquired infections (Leal *et al.*, 2013; Rada *et al.*, 2019., Lupo *et al.*, 2018, Chávez *et al.*, 2018), and their survival in chlorinated environments highlights their resilience and adaptive capacity. Opportunistic organisms such as *S. warneri* recently documented as harboring antibiotic resistance and virulence determinants (Ravaioli *et al.*, 2024), illustrate the potential for commensal species to evolve into clinically significant pathogens under selective environmental pressures. Similarly, *A. hydrophila* is a well-recognized waterborne pathogen capable of affecting both humans and animals, with reports linking it to cases of folliculitis associated with swimming pools (Manresa *et al.*, 2009), as well as broader reviews highlighting its pathogenic potential across aquatic and clinical contexts (Semwal *et al.*, 2023; Abdella *et al.*, 2023).

It is also important to note that the standard is limited in scope, as it does not address the control of antibiotic-resistant bacteria, a significant drawback given the increasing annual prevalence of this problem (O'Neill, 2014). The present study demonstrated that swimming pools contain bacteria capable of persisting under chlorination conditions and exhibit resistance to antibiotics as will be shown later. While five pools satisfied the microbiological quality standard, the presence of antibiotic-resistant bacteria was observed in most of these pools (Tables 2 and 3). These findings are in addition to the results obtained in the ten other pools that did not meet the standard, where resistant and multi-resistant bacteria were isolated. It is notable that among the pools exhibiting heterotrophic counts that exceeded the permissible limit, coliforms were detected in only two pools. However, five Gram-positive bacteria with resistance were identified, with one bacterium, exhibiting multiple resistance.

It is a well-documented fact that swimming pools are a common environment for the transmission of pathogenic and opportunistic bacteria, which can also be resistant to disinfecting agents (Barna and Kádár, 2012; Caro-Hernández *et al.*, 2024). At adequate concentrations, chlorine can kill most bacteria; thus, chlorination is by far the most widely used treatment for water disinfection (Shannon *et al.*, 2008). However, during the process of disinfection, the disinfectant agent itself, along with its byproducts, has been shown to induce oxidative stress (Yuan *et al.*, 2006). This, in turn, has been observed to prompt an adaptive response in the bacteria, enabling them to withstand the adverse conditions imposed by the disinfectant (Adefisoye *et al.*, 2022). The maintenance of lethal chlorine concentration in water

depends on several factors, including pH, temperature, organic load, contact time and the presence of microorganisms. Consequently, swimming pools may contain chlorine concentrations that fall below the required range. This can impede the lethal action of chlorine and augment the ability of bacteria to resist and persist in the water. This has led to the suggestion that chlorination may induce increased resistance in surviving organisms, rendering chlorine-treated swimming pools a potential environment for the spread of antibiotic resistance among bacteria (Shuai *et al.*, 2021). Despite the absence of consensus on the relationship between chlorine and the increase in antibiotic resistance, this is a significant aspect to consider, particularly in the context of regulations pertaining to the microbiological quality of swimming pools. While some authors maintain that chlorine disinfection does not contribute to the selection of resistant strains, others argue that in some bacterial groups it induces the transfer and expression of resistance genes (Shuai *et al.*, 2021; Khan *et al.*, 2016; Siedlecka *et al.* 2021).

Although chlorine and pH levels were not directly measured during the collection of water samples, official pool quality records confirmed compliance with the Colombian regulation (Colombia, 2020), which establishes an acceptable chlorine concentration range of 2.0–4.0 mg/L. This information suggests that disinfection practices were maintained within regulatory standards. Nevertheless, because these parameters were not corroborated at the time of sampling, this constitutes a methodological limitation. The absence of real-time measurements prevents establishing a stronger and more direct relationship between physicochemical water conditions and the persistence of multidrug-resistant bacteria. Direct assessment of chlorine and pH during sampling would have provided more robust evidence to support the microbiological findings.

Despite existing regulatory frameworks, our study demonstrates that swimming pools frequently fail to meet microbiological quality standards and, as reported in other studies (Shuai *et al.*, 2021), can serve as reservoirs for antibiotic-resistant bacteria (ARBs) and could facilitate the horizontal transfer of resistance genes due to selective pressure from disinfectants. Such environments may promote the dissemination of opportunistic and multidrug-resistant bacteria, posing direct public health risks, particularly for vulnerable populations such as children, the elderly, and immunocompromised individuals. From a One Health perspective, the detection of clinically relevant bacteria underscores the interconnectedness of human, animal, and environmental health and highlights the urgent need for enhanced surveillance, regulation, and control of recreational waters (Jin *et al.*, 2020; Serna and Gonzalez-Zorn, 2022).

3.2. Antibiotic-resistant bacteria in swimming pools

Of the 49 bacterial isolates, 45 (92%) exhibited resistance to one or more antibiotics. A total of 27 were identified through 16S rRNA gene sequencing, most of which displayed resistance to two or more antibiotics (n = 27). Both Gram-negative (Table 2) and Gram-positive (Table 3) bacteria were represented in comparable proportions. The most frequently resistant bacteria identified in this study included *Escherichia* spp. (most belonging to *E. coli*, n = 6), *Enterobacter* spp. (n = 6, including one isolate identified as *E. cloacae*), *Pseudomonas* spp. (including two isolates identified as *P. otitidis*), and *Staphylococcus* spp. (most belonging to *S. warneri,* n = 5, and *S. aureus*, n = 2). It is notable to emphasize the presence of *Proteus mirabilis* and *B. cereus* as multi-resistant isolates, in addition to *Exiguobacterium* spp., a Gram-positive bacterium characterized as a psychrophile with multiple resistances.

Although most studies have been conducted in water treatment and distribution systems, there are some studies in swimming pools that support our findings. In 2008, Chrissanthy Papadopoulu *et al.*, published a study evaluating the bacterial resistance of 107 isolates obtained from 462 swimming pool water samples in Greece, between 1997 and 2005. The results demonstrated that 35.5% of the isolates exhibited resistance to antibiotics, including multi-resistant bacteria such as *P. aeruginosa, P. alcaligenes, P. fluorescens, Leuconostoc, Staphylococcus aureus, Staphylococcus warneri, Chryseobacterium indologenes,*

Ochrobactrum anthropi, Aeromonas hydrophila, Enterobacter clocae and *Klebsiella pneumoniae* (Papadopoulou *et al.*, 2008). A 2018 study in Bavaria (Germany), analyzed the surrounding surfaces and water of therapeutic pools in 11 healthcare centers and found 102 antibiotic resistant isolates. Most of the isolates were non-fermenting Gram-negative bacteria such as: *Burkholderia* spp., *Moraxella* spp., *Pseudomonas* spp., *Stenotrophomonas* spp. and *Sphingomonas* spp. and Gram-positive bacteria such as *Staphylococcus epidermidis* and *Bacillus subtilis* (Koech *et al.*, 2018). In the same year, a study carried out in Guangzhou (China) was also published, in which 39 outdoor swimming pools were evaluated, from which were isolated enteric bacteria such as *E. coli*, *Shigella*, *Salmonella* and *P. aeruginosa*, the latter with 27 isolates of varying resistance (Wei *et al.*, 2018).

Our findings show that the sampling point with the highest number of resistant and multiple resistant isolates (three to four antibiotics) was PUNLVL, a pool classified as a sports pool for adults (SA). The location of this point is of particular interest, being situated on a university campus with a health sciences school. Consequently, most of its users are health professionals, as well as medical, nursing and psychology students. These individuals, due to their occupation, spend part of their time in health centers, clinics, and hospitals, or have contact with patients. As has been extensively documented, the routes of dissemination of antibiotic resistance among bacteria are primarily a consequence of person-to-person contact, as evidenced by the research conducted by Livermore and Pearson (2007). However, extraneous to this environment, it has been established that the transfer of ARBs and ARGs transpires between humans, animals, and the environment (Larsson and Flach, 2022), which is the rationale behind the conceptualization of the term “One health”. We hypothesize that the conditions, due to the presence of users in the PUNLVL pool site, could enhance the number of resistant bacteria. According to Shuai *et al.* (2021), a key factor to consider is the microbial load contributed by bathers. To this end, these researchers evaluated the distribution of ARGs in 16 pools, both before and after the closure of the facilities, considering a variable number of users. The study encompassed a comprehensive analysis of the water and skin surfaces of the users. It was found that bather load in swimming pools contributes to the increase of ARGs, and that the absolute abundance of ARGs increases by almost two orders of magnitude when bather load exceeds 0.1 person/m^2-h. Furthermore, the genetic material in question was divided into distinct pools, exhibiting comparable transfer dynamics. Of the ARGs detected in bathers post-swimming, 18 were not detected prior to the activity and remained after showering, suggesting that swimming may trigger the exchange of ARGs and bacteria between skin and water. This finding has also been verified after swimming in the ocean, thus emphasizing the importance of thorough showering before and after aquatic exercise (Nielsen *et al.*, 2021).

3.3. Bacterial phenotype characteristics of antimicrobial resistance

Most bacteria isolated in the present study (92%) showed resistance to at least one antibiotic (Tables 2 and 3). Among the Gram-negative bacteria with resistance profiles, 36% exhibited resistance to at least one of the tested antibiotics, 24% to two, 28% to three and one isolate showed resistance to four antibiotics spanning three different families of antibiotics (details in Table 2). In the case of Gram-positive bacteria, the resistance profiles exhibited significant variation. Among the isolates, 38% exhibited resistance to at least one antibiotic, while another 38% demonstrated resistance to two. Notably, only two isolates (8%) exhibited resistance to three antibiotics, and one strain showed resistance to four (details in Table 3).

Table 2. Resistance profile of Gram-negative bacteria.

Source	Type of pool	Code Pool	Bacteria	Antibiotics								Profile
				KAN	CAZ	CIP	TET	CRO	SXT	GEM	AMK	
Cali	RT	PRPC	*Enterobacteria* spp.	S	S	S	S	S	S	S	S	0
Cali	RT	PRPC	*Pseudomonas*	S	S	S	S	R	R	S	S	2
Cali	RT	PRPC	*Escherichia coli*	S	S	S	S	S	S	R	S	1
Cali	SA	PUNLVL	*Escherichia coli*	S	S	S	R	S	S	S	S	1
Cali	SA	PUNLVL	*Klebsiella pneumoniae*	S	R	S	S	S	S	S	S	1
Cali	SA	PUNLVL	*Enterobacter* spp.	S	S	S	R	S	S	S	S	1
Cali	RT	PRPC	Enterobacter spp.	S	S	S	R	R	S	S	S	2
Cali	SA	PUNLVL	*Escherichia* spp.	S	S	S	R	S	S	S	R	2
Cali	SA	PUNLVL	*Proteus mirabilis*	S	R	S	R	S	S	S	R	3
Cali	SA	PUNLVL	*Enterobacter cloacae*	R	R	S	R	S	S	S	S	3
Cali	RA	PURBR	*Proteus mirabilis*	R	R	S	S	S	S	S	R	3
Cali	RA	PURBR	*Proteus mirabilis*	R	R	S	R	S	S	S	S	3
Cali	SA	PUNLVL	*Escherichia coli*	R	S	S	R	S	R	S	R	4
Jamundí	RC	PURCAN	*Enterobacter* spp.	S	S	S	S	R	S	S	S	1
Jamundí	RC	PURCFN	*Aeromonas hydrophila*	S	S	S	S	S	R	S	S	1
Jamundí	RC	PURCAN	*Enterobacter* spp.	S	S	S	S	S	R	S	S	1
Jamundí	RC	PURCFN	*Pseudomonas* spp.	S	S	S	S	S	S	S	S	0
Jamundí	RA	PURCAA	*Escherichia coli*	S	S	S	R	S	S	S	S	1
Jamundí	RA	PURFV	*Escherichia coli*	S	S	S	R	S	S	S	S	1
Jamundí	RC	PURCAN	*Acinetobacter* spp.	S	S	S	R	R	S	S	S	2
Jamundí	RC	PURCFN	*Pantoea* spp.	R	S	S	S	R	S	S	S	2
Jamundí	RA	PURCTA	*Pseudomonas otitidis*	S	S	S	S	R	R	S	S	2
Jamundí	RA	PURCAA	*Pseudomonas otitidis*	S	S	S	R	R	R	S	S	3
Jamundí	RC	PURCAN	*Providencia* spp.	S	S	S	R	S	R	S	R	3
Jamundí	RA	PURCTA	*Pantoea stewartii*	R	S	S	S	R	R	S	S	3

RA: Residential adults, RT: Recreational, SA: Sports adults, RC: Residential children; KAN: Kanamycin 30 µg, CAZ: Ceftazidine 30 µg, CIP: Ciprofloxacin 5 µg, TET: Tetracycline 30 µg, CRO: Ceftriazone 30 µg, SXT: Sulfamethozazole/Trimethoprim 1,25/23,75 µg, GEN: Gentamicin 10 µg, AMK: Amicacin 30 µg.

Table 3. Resistance profile of Gram-positive bacteria.

Source	Type of pool	Code Pool	Bacteria	KAN	CIP	TET	CRO	VAN	RIF	Perfil
Cali	SA	PDAG	*Staphylococcus* spp.	S	S	S	S	S	S	0
Cali	SA	PUNLVL	Bacilos (SI)	S	S	S	S	S	S	0
Cali	RT	PRPC	*Staphylococcus* spp.	R	S	R	S	S	S	2
Cali	RA	PURBR	*Bacillus subtilis*	S	S	S	S	R	S	1
Cali	RT	PURVS	*Priestia megaterium*	S	S	S	S	S	R	1
Cali	RT	PRPC	*Staphylococcus aureus*	S	S	R	R	R	S	3
Cali	SA	PUNLVL	*Exiguobacterium* spp.	S	S	S	S	R	R	2
Cali	RA	PURBR	*Staphylococcus warneri*	R	S	S	S	R	S	2
Cali	RA	PURCRH	*Staphylococcus* spp.	S	S	S	R	R	S	2
Cali	RA	PURRP	*Staphylococcus warneri*	S	S	S	R	S	R	2
Cali	SA	PDAG	*Staphylococcus warneri*	R	S	S	R	S	S	2
Cali	RT	PURRP	*Staphylococcus warneri*	R	S	R	S	S	S	2
Cali	SA	PUNLVL	*Staphylococcus* spp.	S	S	S	S	R	S	1
Cali	RA	PURCRH	*Staphylococcus warneri*	R	R	S	R	S	S	3
Jamundí	RA	PURCFN	*Kurthia gibsonii*	S	S	S	R	S	S	1
Jamundí	RA	PURVH	Bacilos Gram positivos	S	S	S	S	S	R	1
Jamundí	RA	PURSP	*Staphylococcus aureus*	S	S	S	R	S	S	1
Jamundí	RA	PURCAA	Bacilos en cadena (UI)	S	S	S	S	S	R	1
Jamundí	RA	PURFV	Bacilos largos (UI)	S	S	S	S	S	R	1
Jamundí	RA	PURCAZ	Bacilos (UI)	S	S	R	S	S	S	1
Jamundí	RA	PURCAA	*Staphylococcus* spp.	S	S	R	S	R	S	2
Jamundí	RA	PURCAZ	Bacilos Gram positivos (UI)	S	S	R	R	S	R	3
Jamundí	RA	PURCAZ	Bacilos Gram positivos (UI)	S	S	S	S	R	R	2
Jamundí	RA	PURCTA	*Bacillus cereus*	S	S	R	R	R	R	4

Residential Adults (RA), Recreational (RT), Sports Adults (SA), Kanamycin (KAN, 30 µg), Ciprofloxacin (CIP, 5 µg), Tetracycline (TET, 30 µg) Ceftriaxone (CRO, 30 µg), Vancomycin (VAN, 30 µg) and Rifampicin (RIF, 5 µg), Unidentified (I).

The geographical distribution of the data shows no notable difference between the proportion of resistant bacteria isolated in Cali (52%) and those isolated in Jamundí (48%). Additionally, bacteria resistant to three or four antibiotics were identified in both Cali and Jamundí, the majority being Gram-negative.

Bacterial resistance patterns varied according to pool type. In residential adult pools (RA), 49% of the bacterial isolates were antibiotic-resistant, predominantly Gram-positive (68%). Most RA isolates exhibited resistance to one (41%) or two (14%) antibiotics. Among the Gram-negative bacteria from RA pools, 29% were resistant to three antibiotics, including one *Pseudomonas otitidis* isolate. Residential children's pools (RC) harbored 13% antibiotic-resistant bacteria, primarily Gram-negative. Notably, one *Providencia* spp. isolate displayed resistance to three antibiotics. Sports adult pools (SA) yielded 22% resistant isolates, 70% of which were Gram-negative. Three of these isolates showed resistance to three or more antibiotics, including one *Escherichia coli* strain resistant to four antibiotics. Recreational pools (RT) yielded 16% resistant isolates, most of which were resistant to one or two antibiotics; one *Staphylococcus aureus* isolate exhibited resistance to three antibiotics.

Resistance patterns varied across the antibiotics assessed. The highest resistance rates were observed for tetracycline (41% of isolates) and ceftriaxone (35%). Moderate resistance was detected for vancomycin and rifampicin (38% each among Gram-positive bacteria) (Table 3), as well as for sulfamethoxazole/trimethoprim (32% among Gram-negative bacteria) (Table 2). Lower resistance levels were found for kanamycin (22% overall) and amikacin (20% among Gram-negative bacteria) (Table 2). Ciprofloxacin showed the lowest resistance rate, with only one Gram-positive isolate (*Staphylococcus warneri*) exhibiting resistance (Table 3), and no resistant Gram-negative isolates. Additionally, resistance to gentamicin was observed in a single *E. coli* isolate (Table 2). Resistance to tetracycline and sulfamethoxazole/trimethoprim is well-documented in bacterial populations. A comprehensive meta-analysis of 9,374 articles published between 1990 and 2020 (Zhuan *et al.*, 2021) reported that sulfonamide and tetracycline resistance gene families exhibit the broadest environmental distribution, being widely detected in water bodies, soil, and wastewater treatment plants. This widespread occurrence is attributed to the extensive use of these antibiotics in agriculture. Tetracycline resistance is of particular concern due to several factors. The *tet* genes responsible for tetracycline resistance are often located on mobile genetic elements, such as plasmids, transposons, and integrons, which facilitate horizontal gene transfer among bacteria. Moreover, tetracycline resistance frequently co-occurs with resistance to other classes of antibiotics. Our previous research demonstrated significant associations between tetracycline resistance and simultaneous resistance to ceftriaxone and kanamycin (Caro-Hernández *et al.*, 2022), consistent with the multidrug-resistant profiles observed in this study.

Antibiotic resistance has shown an alarming upward trend in Latin America in recent decades, with concerns regarding cephalosporin and beta-lactam antibiotics. Our findings support this trend, with 35% of tested isolates showing ceftriaxone resistance and 20% of Gram-negative *Enterobacteriaceae* demonstrating ceftazidime resistance. Cephalosporin resistance typically results from extended-spectrum β-lactamases (ESBLs), including CTX-M, SHV and TEM variants, along with AmpC-type β-lactamases, found in *Enterobacteriaceae* (Wilson and Török, 2018). Colombia has reported increasing ESBL frequency in *K. pneumoniae* and *E. coli* since 1990, with most cases occurring in hospital settings (Leal *et al.*, 2013; Rada *et al.*, 2019). Resistance due to ESBLs has become a major concern in clinical settings worldwide (Hawkey, 2015). However, our study revealed a different pattern, most ceftriaxone-resistant bacteria did not belong to *Enterobacteriaceae* but included *Acinetobacter* spp., *P. stewartia*, *Pseudomonas* spp., *P. otitidis* and nine Gram-positive isolates (*S. aureus*, *S. warneri* and *Kurthia gibsonii*). Among 11 isolated *Enterobacteriaceae*, only two *Enterobacter* spp. showed ceftriaxone resistance. This distribution suggests that resistance mechanisms in recreational water

environments may differ from clinical settings and warrants a genotypic characterization to identify the specific genes involved.

Among Gram-positive bacteria, 38% showed resistance to both rifampicin and vancomycin. Vancomycin resistance aligns with established patterns of acquired and intrinsic resistance in Gram-positive bacteria (Stogios and Savchenko, 2020). While rifampicin resistance is well-documented in *Mycobacterium tuberculosis*, resistance to macrolides has emerged as a growing concern across various Gram-positive bacteria (Li *et al.*, 2023).

Exiguobacterium spp. was identified among the rifampicin and vancomycin-resistant isolates. This environmental bacterium, known for its plant growth-promoting properties and potential as psychrophilic bioremediatory (Pandey, 2020), has shown concerning resistant patterns. Yang *et al.* (2014) found that an *Exiguobacterium* strain from marine sediments exhibited multidrug resistance, harboring both resistance genes previously characterized in clinical pathogens and novel resistance genes. Due to their intrinsic resistance and adaptability to extreme conditions, *Exiguobacterium* species have become important models for studying bacterial resistance in diverse environments (Ferheen *et al.*, 2024). Their presence in recreational waters suggests that swimming pools may facilitate horizontal gene transfer between environmental bacteria, commensals, and potential pathogens.

Kanamycin was associated with the highest frequency of multidrug-resistant bacteria (Table 4), despite showing overall resistance in 22% of Gram-negative isolates. Resistance to aminoglycosides such as kanamycin and amikacin typically results from enzymatic inactivation by acetyltransferases, nucleotidyltransferases (adenylyltransferases), and phosphotransferases, though ribosomal alterations and reduced permeability also contribute (Tomalsky, 2000). Mobile genetic elements carrying aminoglycoside resistance genes, such as transposons Tn1331 (Tomalsky, 2000) and Tn5 (Berg, 2017), may show increased transfer rates under high osmotic stress conditions like those found in chlorinated swimming pool water. This enhanced horizontal gene transfer could facilitate the spread of resistance genes in recreational water environments, which could have significant implications for the biological and ecological processes occurring in these environments.

3.4 Multi-resistant bacteria

Multi-resistant bacteria (MRB) are defined as those exhibiting resistance to at least one antibiotic from three or more distinct antimicrobial families. A noteworthy result of this study was that 22% of the resistant isolates exhibited multidrug resistance, defined as resistance to at least three different families of antibiotics (Table 4).

Among the multi-resistant isolates, *Bacillus cereus* from the PURCTA site in Jamundí showed resistance to four antibiotics (TET/CRO/VAN/RIF) from different families: tetracyclines, β-lactams, glycopeptides and rifamycins). Antibiotic resistance in *B. cereus* is not unexpected given its capacity to form endospores and biofilms. These findings align with recent genomic studies. Tong *et al.* (2024) analyzed complete genomes of 191 *B. cereus* samples collected globally from five continents between the 1990s and 2022. Their analysis identified multiple genes associated with biofilm formation and seven ARGs classified into four categories: β-lactam, Fosfomycin, tetracycline, glycopeptide resistance. These results correspond with the resistance patterns observed in our study.

On the other hand, site PUNVL showed the highest number of resistant isolates and multi-resistant bacteria. From this pool, *E. cloacae* and *Proteus mirabilis* were isolated, both showing resistance to kanamycin or amikacin, ceftazidime and tetracycline. Additionally, an *E. coli* strain demonstrated resistance to kanamycin, amikacin, tetracycline and sulfamethoxazole/trimethoprim. These similar resistance profiles may be attributable to their isolation from a common source.

Table 4. Bacteria resistant to 3 or 4 antibiotics.

Antibiotic resistance	Bacteria	Source	Code Pool	Pool type
TET/CRO/VAN/RIF	*Bacillus cereus*	Jamundí	PURCTA	RA
KAN /CAZ/TET	*Enterobacter cloacae*	Cali	PUNLVL	SA
AMK/CAZ/TET	*Proteus mirabilis*	Cali	PUNLVL	SA
AMK/TET/SXT	*Providencia* spp.	Jamundí	PURCAN	RC
KAN/AMK/TET/SXT	*Escherichia coli*	Cali	PUNLVL	SA
TET / CRO / SXT	*Pseudomonas otitidis*	Jamundí	PURCAA	RA
KAN / CRO /SXT	*Pantoea stewartii*	Jamundí	PURCTA	RA
TET / CRO / VAN	*Staphylococcus aureus*	Cali	PRPC	RT
TET / CRO / RIF	Bacilos Gram positivos (SI)	Jamundí	PURCAZ	RA
KAN / CIP / CRO	*Staphylococcus warneri*	Cali	PURCRH	RA

RA: Residential Adults, RC: Residential Children, RT: Recreational, SA: Sports Adults, KAN: Kanamycin 30 µg, AMK: Amicacin 30 µg, CIP: Ciprofloxacin 5 µg, TET: Tetracycline 30 µg, CRO: Ceftriaxone 30 µg, CAZ: Ceftazidime 30 µg, VAN: Vancomycin 30 µg, RIF: Rifampicin 5 µg, SXT: Sulfamethoxazole/Trimethoprim 1,25/23,75 µg.

In this study, four isolates were identified as *Pseudomonas*, including *P. otitidis* isolated from the PURCAA site, which exhibited multi-drug resistance to tetracycline (TET), ceftriaxone (CRO) and sulfamethoxazole-trimethoprim (SXT). *Pseudomonas* species are distinguished by their capacity to withstand a wide spectrum of antimicrobial agents, including broad-spectrum antibiotics. These bacteria have been frequently isolated from highly controlled environments such as swimming pools (Papadopuolou *et al.*, 2008; Koeck *et al*., 2019). *Pseudomonas* species produce biofilms (Thi *et al.*, 2020) and express efflux pumps (Hou *et al.*, 2019), enabling adaptation to oxidative stress conditions and making them excellent candidates for studying bacterial resistance in chlorinated water. Tetracycline resistance in *Pseudomonas* species is associated with overexpression of efflux systems such as MexAB-OprM and MexXY-OprM. Resistance to third-generation cephalosporins is attributed to β-lactamase production, loss of specific porins and activation of efflux pumps (Morita *et al.*, 2014). Additionally, *P. aeruginosa* exhibits high intrinsic resistance to sulfamethoxazole-trimethoprim (Odjadjare *et al.*, 2012).

As expected, *S. aureus* also displayed multiple resistance. The isolate obtained from the PRPC site, a residential children (RC) type pool, showed resistance to tetracycline, ceftriaxone, and vancomycin. Tetracycline resistance in *S. aureus* has been demonstrated to be associated with genes such as *tet*(K), *tet*(M) and *tet*(L), which are known to code for efflux pumps and ribosomal protection proteins (Arabzadeh *et al.,* 2018). Although ceftriaxone, a third-generation cephalosporin, is not the treatment of choice for *S. aureus*, cases of resistance have been documented. For instance, a study conducted in Iran reported the presence of ceftriaxone-resistant *S. aureus* strains characterized by elevated minimum inhibitory concentrations (MICs). The observed resistance is attributed to the production of β-lactamases and the presence of the *mec*A gene (Phe *et al.*, 2015). The emergence of vancomycin-resistant *S. aureus* strains represents a significant public health concern. The development of vancomycin resistance has been linked to the acquisition of the *van*A gene, which results in the modification of the antibiotic's target site (Unni *et al.*, 2021).

4. CONCLUSIONS

The results of this study indicate that public swimming pools serve as a conducive environment for the proliferation and dissemination of antibiotic-resistant bacteria (ARBs),

which poses a significant risk to public health. Despite the existence of specific microbiological regulations, such as Resolutions 1618 of 2010 and 1547 of 2020 in Colombia, it was observed that 69% of the samples analyzed did not comply with the limits established for indicator microorganisms. This highlights the urgent need to strengthen mechanisms of surveillance and control in these recreational spaces.

Alarmingly, 92% of bacterial isolates exhibited resistance to at least one antibiotic, with no significant difference observed between Gram-positive and Gram-negative bacteria. The presence of clinically relevant multi-resistant strains was identified, including *Escherichia coli*, *Pseudomonas otitidis*, *Staphylococcus warneri*, *Proteus mirabilis* and *Bacillus cereus*. It is notable that some of these strains exhibited simultaneous resistance to critical antibiotics such as tetracycline, ceftriaxone, vancomycin and kanamycin.

The prevailing regulations are inadequate as far as they do not encompass the monitoring of ARBs or the presence of ARGs. This omission serves to limit the capacity to respond to this growing problem. In addition, the results suggest that, although chlorine disinfection processes are effective against most bacteria, they may induce oxidative stress in survivors, thereby favoring adaptive mechanisms and facilitating the horizontal transfer of resistance genes, especially under suboptimal chlorination conditions.

Furthermore, it was determined that specific pools, including those situated within university environments with health-related faculties, exhibited a heightened prevalence of multidrug-resistant bacteria. This phenomenon may be attributed to the perpetual circulation of individuals who are potentially exposed to clinical environments. This finding serves to emphasize the necessity of considering the microbial load contributed by users as a relevant factor in the context of water quality control.

Finally, the findings of environmental bacteria such as *Exiguobacterium* spp. with multiple resistance phenotypes highlights the capacity of the aquatic environment to serve as a reservoir and bridge between environmental, commensal and pathogenic bacteria. These results underscore the imperative for the implementation of comprehensive surveillance approaches within the “One Health” concept, encompassing advanced microbiological monitoring, genotypic analysis and risk assessment of resistance transfer in recreational environments.

5. DATA AVAILABILITY STATEMENT

Data availability not informed.

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