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ORIGINAL

ESBL superbacteria in fresh waters of Tungurahua: risks and regulatory gaps

Superbacterias BLEE en aguas dulces de Tungurahua: riesgos y vacíos normativos

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ABSTRACT

Introduction: currently, antimicrobial resistance and more specifically extended-spectrum beta-lactamase (ESBL)-producing bacteria are a growing global threat. The fresh waters of Tungurahua-Ecuador are very important because of the diversity of uses they provide and could also be a source and dissemination route of ESBL with a potential risk to public health and the environment.
Method: a descriptive, qualitative-quantitative observational study was carried out in the province of Tungurahua. Thirty points (5 rivers, 25 pools) were analyzed by non-probabilistic sampling. Physicochemical variables (pH, chlorine, temperature) were measured in situ and in the laboratory. Bacteria were isolated and phenotypically characterized. A qualitative regulatory analysis was performed to detect regulatory gaps in antimicrobial resistance and ESBL.
Results: ESBL phenotype was detected in 28 of the 30 sites (93,3 %): 5 rivers and 23 of the 25 pools. In rivers, E. coli ESBL (72,7 %) and KEC (Klebsiella, Enterobacter, Citrobacter) (27,3 %) were the most frequent. In swimming pools, Acinetobacter ESBL (60,6 %), KEC (15,2 %), Pseudomonas (15,2 %) and E. coli (9,1 %) were the most frequent. Regulatory analysis revealed that TULSMA lacked specific AMR/ESBL parameters, mandatory periodic monitoring and alert thresholds.
Conclusions: the evidence revealed severe contamination by ESBL, linked to wastewater and agricultural discharges, which increases the health and environmental risk. There is an urgent need to improve water treatment, discharge controls and monitoring. The TULSMA lacks parameters for AMR, ESBL, ARGs and “One Health” approach; it requires PCR and intersectoral surveillance and active training.

Keywords: Fresh Water; Drug Resistance, Microbial; Extended-Spectrum Beta-Lactamases (ESBL); One Health; Environmental Legislation - TULSMA; Public Health.

RESUMEN

Introducción: en la actualidad, la resistencia antimicrobiana y más específicamente las bacterias productoras de betalactamasas de espectro extendido (BLEE) constituyen una creciente amenaza mundial. Las aguas dulces de Tungurahua-Ecuador son muy importantes por la diversidad de usos que prestan y podrían ser también una fuente y vía de diseminación de las BLEE con un potencial riesgo para la salud pública y el medioambiente.
Método: se llevó a cabo un estudio observacional descriptivo, cualitativo-cuantitativo en la provincia de Tungurahua. Se muestrearon 30 puntos (5 ríos, 25 piscinas) mediante un muestreo no probabilístico. Se midieron in situ y en el laboratorio las variables fisicoquímicas (pH, cloro, temperatura). Se aislaron y caracterizaron fenotípicamente las bacterias. Se realizó un análisis cualitativo normativo para detectar

vacíos regulatorios sobre resistencia antimicrobiana y BLEE.
Resultados: se detectó fenotipo BLEE en 28 de los 30 sitios (93,3 %): 5 ríos y 23 de las 25 piscinas. En ríos, E. coli BLEE (72,7 %) y KEC (Klebsiella, Enterobacter, Citrobacter) (27,3 %) las más frecuentes. En piscinas, Acinetobacter BLEE (60,6 %), KEC (15,2 %), Pseudomonas (15,2 %) y E. coli (9,1 %) las más frecuentes. El análisis normativo reveló que el TULSMA carecía de parámetros específicos de RAM/BLEE, monitoreo periódico obligatorio y umbrales de alerta.
Conclusiones: la evidencia reveló severa contaminación por BLEE, vinculada a descargas residuales y agropecuaria, esta eleva el riesgo sanitario y ambiental. Es urgente mejorar el tratamiento de aguas, controles de descarga y monitore. El TULSMA carece de parámetros para RAM, BLEE, ARGs y enfoque "Una Salud"; requiere PCR y vigilancia intersectorial y capacitación activa.

Palabras clave: Agua dulce; Farmacorresistencia Microbiana; Betalactamasas de Espectro Extendido (BLEE); One Health; Legislación Ambiental - TULSMA; Riesgo para la Salud Pública.

INTRODUCTION

The province of Tungurahua, located in the central region of Ecuador, has a varied and rich hydrography, allowing for agricultural and industrial development and human consumption. One of its rivers (Ambato) is one of the primary water sources in the province, as it is used extensively to irrigate crops in nearby towns. This use makes it a fundamental water resource in the area.[1,2]

Antimicrobial resistance (AMR) poses a threat to public health and negatively affects the efficacy of antibiotic treatments;[3,4] the emerging environmental spread of extended-spectrum beta-lactamase (ESBL)-producing bacteria is a significant problem.[3,5] ESBL-producing bacteria are resistant to critical antibacterial molecules such as third-generation cephalosporins, and their spread leads to treatment failure in humans and animals infected with these multidrug-resistant bacteria.[6]

Aquatic environments are the target of anthropogenic impacts due to pollutants.[5,7] The discovery of ESBL-producing enterobacteria in surface waters around the world is a fact, with surface waters being a reservoir and a means of transport for ESBL-producing enterobacteria to humans and/or ecosystems.[3,5] The release of pollutants leads to an increase in AMR.[3] Colistin resistance has been detected in sewage, industrial, and river water,[8] highlighting the urgent need for control and intervention.[8] In this context, anthropogenic action, i.e., all human activities that impact the environment, has an impact on environmental AMR.[9]

In this context, anthropogenic action, i.e., all human activities that impact the environment, impacts environmental AMR—BLEEs in freshwater risk exposure through contact, ingestion, and the food chain.[7,10] The qualitative assessment of the risk of BLEE prevalence considers pathogenicity and water use.[11]

Rural areas without quality water are vulnerable.[7] Faecal organic contamination (measured by fecal/total coliforms) is a phenomenon that will predominate and be present in water resources where wastewater and agricultural use originate due to poor water management and lack of sewerage,[12] as well as its involvement in the transport of antimicrobial-resistant bacteria.[13]

Freshwater ecosystem conditions act as reservoirs for AMR genes, which can promote the phenomenon of horizontal transfer.[3,5] Untreated sewage and industrial effluents can facilitate the spread of resistant bacteria.[3] The expansion of agriculture and deforestation of water bodies could reduce water quality[14,15] and promote the persistence/dissemination of resistant bacteria,[7,16] while riparian areas favor the reduction of pollution.[16]

It is essential to compare the findings of this study with Ecuadorian regulations: the Consolidated Text of Secondary Environmental Legislation (TULSMA),[17] a law that regulates the quality of water for human consumption. Regulation No. DIR-ARCA-RG-012-2022 stipulates the requirements to be met in the event of a risk to water quality, requiring communication and the development of contingency plans.[18] TULSMA is also responsible for regulating solid waste production.[17] The National Environmental Authority exercises oversight. Quality criteria exist, although there may not be specific criteria for BLEE in freshwater;[7] the standard also determines discharge limits.[17]

The study of environmental RAM 'hot spots' (wastewater treatment plants, wastewater overflows, and sewage and industrial effluents, as well as rivers under anthropogenic influence) is key to understanding the phenomenon of dissemination.[19,20,21]

The objectives of this study are to evaluate the presence, characteristics, and distribution of Extended Spectrum Beta-Lactamase (ESBL)- producing bacteria in freshwater sources in the province of Tungurahua, Ecuador; analyze the health and environmental risk associated with these superbugs; and identify gaps in Ecuadorian environmental regulations (TULSMA) to propose recommendations to strengthen their monitoring, prevention, and control.

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METHOD

A qualitative-quantitative descriptive observational study was conducted between July and December 2023. Thirty non-probabilistic sampling points were established for convenience, including five rivers and 25 recreational pools, prioritizing locations related to public health. Each sampling point was then georeferenced and coded. Using sterile 250 mL bottles, a spot sample of surface water was collected at each site. In the case of swimming pools, sodium thiosulphate was added as a mediator to neutralize chlorine. An identifier was dispensed in swimming pool water samples after informed consent was obtained. Once collected, the samples were refrigerated (< 4 °C) and transported to the laboratory within < 6 hours. The water and air temperatures were recorded in situ; the pH and free residual chlorine were measured in the laboratory. For total aerobes, Petrifilm® was used for the initial bacterial count, which was supplemented with the Most Probable Number (MPN) method in serial dilutions for rivers. Representative colonies were reseeded on MacConkey agar to select Gram-negative bacilli.

Phenotypic detection of ESBL was performed in a selective chromogenic medium (e.g., CHROMagar™ ESBL), incubated at 37 °C for 24 hours. Colonies with characteristic coloration were considered presumptive ESBL+ and classified at the genus/group level using basic biochemical tests. LEE confirmation and resistance profile were determined by microdilution (Sensititre™ GNX2F). Bacterial suspensions were adjusted to 0,5 McFarland and inoculated with different antimicrobials. After incubation for 18 to 24 hours, the minimum inhibitory concentration (MIC) was determined, and the results were interpreted as sensitive, intermediate, or resistant, confirming the BLEE phenotype with antimicrobial susceptibility testing, according to CLSI (Clinical and Laboratory Standards Institute) standards and guidelines for clinical laboratories.

For confirmed BLEE+ isolates, their presumptive identification was recorded. The frequencies of BLEE+ taxonomic groups and the prevalence of positive sites (%) were calculated. The presence and prevalence of BLEE+ isolates were analyzed according to the type of water source.

The qualitative component of this research adopted a normative analysis approach, seeking regulatory gaps in Ecuadorian environmental legislation, specifically in the TULSMA, regarding antimicrobial resistance in surface water.

To this end, a methodological strategy was used, consisting of five phases: I) a critical review of the current TULSMA was carried out to detect omissions in terms of bacterial resistance parameters; II) a comparative regulatory analysis was carried out concerning international reference frameworks; III) a qualitative assessment of health risks was carried out and compared with water quality standards; IV) the environmental risk was interpreted and regulatory gaps were identified; and V) technical and operational recommendations were developed to strengthen the TULSMA.

RESULTS

Thirty sites (five rivers and twenty-five recreational pools) selected by non-probabilistic sampling were analyzed to determine the prevalence of sites with the BLEE phenotype:

- River samples: M1-M7 (Río Verde, Río Ulba, Río Cutuchi, Río Pachanlica, Río Ambato).
- Pool samples: M8-M32 (Pool A-Y).

Seven river water samples and 25 recreational pool samples were analyzed in Tungurahua. The mean pH of the river and pool water was similar (rivers: 6,86; pools: 6,68), with medians of 6,8 and 6,7, respectively. The pH range was higher in pools (6,1-7,7) than in rivers (6,5-7,2).

Residual chlorine levels were substantially higher in pools (mean: 0,111 mg/L; median: 0,096 mg/L) than in rivers (mean: 0,016 mg/L; median: 0,01 mg/L), with higher ranges (0,02-0,3 mg/L in pools versus 0-0,04 mg/L in waterways). The water temperature was considerably higher in swimming pools (mean 28,15 °C, median 28 °C) compared to the river (mean 16,06 °C, median 16,4 °C).

The average ambient temperature was practically the same (approximately 27,4-27,5 °C), but the pools had a higher median (30,1 °C compared to 27,5 °C) and a greater temperature range (12,8-36,2 °C in the pools compared to 22-32,6 °C in the rivers). Similarly, the average, median, and temperature range of the ambient humidity were higher in the pool environment (average 55,72 %, median 56,7 %, and range 24,5-88,7 %) than in the rivers (average 48,86 %, median 44 %, and range 36-67 %).

The results showed the presence of bacteria with an ESBL phenotype in the five rivers studied and in twenty-three of the twenty-five swimming pools; no ESBLs were isolated in swimming pool R (M23) and swimming pool S (M24). Twenty-eight (93,3 %) of the thirty sites had multidrug-resistant bacteria producing extended-spectrum β-lactamases (ESBLs).

The following table shows the frequency of isolates of extended-spectrum β-lactamase-producing bacteria (ESBLs) and samples without growth, broken down by rivers and pools in Tungurahua.

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Table 1. Total BLEEs+ isolates characterised by water source

Category	F. Rivers	F. Swimming pools
	0	20
BLEE Acinetobacter	8	3
BLEE E. coli	3	5
BLEE KEC	0	5
BLEE Pseudomonas	0	4
No growth (-)	11	37

In this analysis, the results recorded for river water (n = 11) showed that 100 % of cases were resistant isolates, predominantly ESBL E. coli (72,7 %) and KEC (27,3 %). The results for swimming pool water (n = 37) show that 89,2 % of the samples were positive, with Acinetobacter ESBL isolates predominating (60,6 %), together with KEC (15,2 %), Pseudomonas (15,2 %) and E. coli (9,1 %). Overall, 41,7 % of all samples analyzed contained resistant Acinetobacter, 22,9 % E. coli, 16,7 % KEC, 10,4 % Pseudomonas, and 8,3 % without bacterial isolation.

Based on the quantitative results, in accordance with the methodology, we generated a normative qualitative analysis to identify gaps in the TULSMA, compare its parameters with international standards, and assess environmental and health risks. The TULSMA is an environmental management framework in Ecuador that covers water quality regulation, environmental pollution control, hazardous and special waste management, and dangerous chemical management. It establishes water quality according to its physical, chemical, and biological characteristics and proposes the assessment and control of water quality through sampling, bacterial modeling, coliform counts, etc., but does not describe parameters for AMR, BLEEs, or ARGs.(17)

Studies in Ecuador(1,7,22,23,24) show continuous contamination with AMR linked to wastewater discharges, high levels of E. coli BLEE, and the prevalence of blaCTX-M-15.(25) These results, combined with evidence from other sources,(19,24,26,27) demonstrate the high prevalence of antibiotic-resistant and multi-resistant (MDR) bacteria in freshwater bodies in Ecuador.

Research in the United States, Brazil, Malaysia, and other countries has monitored ARGs (blaTEM, blaCTX-M, blaPSE, blaCMY-2, blaOXA-48, MCR, blaNDM, blaKPC) using PCR (polymerase chain reaction)(26,27,28,29) and ARI indices to quantify multiple resistance;(13) and the prevalence of BLEE E. coli and specific genes in wastewater,(7,8,23,24,30) under the WHO One Health Tricycle protocol.(31) These practices establish technical standards beyond fecal coliforms(7) and highlight the need to incorporate ARGs and AMR as regulatory indicators.

Recent studies confirm that aquatic environments contain multidrug-resistant bacteria and MRA genes with 'serious implications for public health'(3,13,24,32) and potential negative impacts on aquatic diversity and agricultural systems that use these waters.(5,15) Domestic drinking water can harbor multidrug-resistant Enterobacterales (including E. coli BLEE) because water quality is measured solely with fecal indicators,(7,8,10,14,27,29,33,34) putting both aquatic fauna and flora and irrigated crops at risk and, without specific monitoring,(15,19) water considered 'safe' by current regulations can silently spread AMR.

Qualitative analysis revealed that the TULSMA lacks specific indicators of antimicrobial resistance, does not include BLEE phenotypic parameters despite their high prevalence in Tungurahua and the technical feasibility based on the regulation of faecal coliforms;(17) does not define mandatory periodic monitoring of AMR in recreational water bodies, although studies demonstrate the need for early detection in rivers and swimming pools;(17,35) it does not provide warning thresholds that trigger investigation protocols without immediate sanctions,(17) even though these levels are recommended by the WHO(35) and are used as baseline values;(17) it omits the 'One Health' approach, which recognises the interdependence of environmental, human and animal health in the spread of AMR(36,37) and the obligation of inter-institutional coordination;(17,36) and does not include the detection of ARGs (blaTEM, blaCTX-M, mcr, blaNDM, blaKPC) by PCR(35,36) or the use of multiple resistance indices,(35) both of which are standards in international frameworks.

Similarly, Ecuador's 2019–2023 National Plan for the Prevention and Control of Antimicrobial Resistance does not include surveillance of resistant organisms in freshwater bodies or environmental parameters for monitoring.(38) Most national plans at the international level do not have this either and only maintain traditional controls such as coliforms or BOD (Biochemical Oxygen Demand) and do not include resistant organisms or resistance genes. (39) This absence contradicts the 'One Health' approach, which assumes the spread of resistant microorganisms in soil and water bodies is a global health risk.

DISCUSSION

The data obtained in Tungurahua show a high presence of ERBE enterobacteria found in rivers and swimming pools (93,3 %),(24) consistent with reports on resistant E. coli and Acinetobacter in environments influenced by effluents.(19,23,24) In rivers, E. coli (72,7 %) and KEC (27,3 %) predominated, and in swimming pools, Acinetobacter (60,6 %), KEC (15,2 %), Pseudomonas (15,2 %), and E. coli (9,1 %), highlighting contamination dynamics linked to waste discharges and agricultural practices. Physicochemical differences, such as similar pH (6,86 vs. 6,68), higher residual chlorine in swimming pools, and higher water temperatures (>27 °C) in swimming pools, favor the survival of superbugs despite chlorine levels, with 23 out of 25 swimming pools testing positive, suggesting chlorine-tolerant strains.

The identification of BLEE shows the presence of fecal coliform bacteria, signs of serious contamination,[7] and exceeds the internationally permitted limits of 25 CFU/mL.[26] The WHO prioritizes the elimination of E. coli in drinking water, and its presence requires immediate investigation.[35] The discovery of ESBL in water bodies shows widespread antibiotic resistance, which complicates the treatment of infections.[24] Untreated wastewater contains bacteria exposed to antibiotics used in hospitals, agriculture, and communities.[24] In addition, livestock activities spread them into ecosystems.[7,24,40]

Studies conducted in the Chimbo River show resistance to multiple beta-lactams and nitrofurantoin;[24] This also justifies using molecular analyses (ARGs blaTEM, blaSHV).[41] Studies conducted in the Mocache sector suggest that vegetation alone does not significantly reduce pollution,[7] highlighting the need for more comprehensive actions than mere measures, such as the risk of passive action.

Water bodies near cities are continuously contaminated with resistant bacteria, which is exacerbated by poor wastewater treatment.[5,24] Wastewater treatment plants (WWTPs) receive and release drug-resistant bacteria (ARBs), antimicrobial resistance genes (ARGs), and antibiotics, even after disinfection by chlorine or ultraviolet light,[5] making them hotspots for the spread of AMR in the aquatic environment.[5,19] Rainwater running off agricultural fields and feces from domestic and wild animals contribute to fecal coliforms and other contaminating microorganisms.[7,42]

The detection of high levels of E. coli BLEE in rivers and lagoons is frequent and alarming due to its remarkable ability to adapt.[24] Resistance to several β-lactam antibiotics, such as cefotaxime, piperacillin-tazobactam, cefoxitin, ertapenem, amikacin, nitrofurantoin and azithromycin, and the MDR profile[24,28] increase the health risk. This is because these strains generally live in our bodies and can pass on resistance genes to disease-causing bacteria in water.[43]

A qualitative TULSMA (Unified Text of Secondary Environmental Legislation) analysis reveals a gap with international standards. Although it regulates water quality and discharges[17] and includes physical, chemical, and microbiological parameters (coliforms10), it lacks indicators of antimicrobial resistance (e.g., BLEE). In contrast, countries like the United States, Brazil, and others use PCR to detect antibiotic resistance genes (ARGs) and RAM indices to measure resistance levels.[13] The high prevalence of blaCTX-M-15 in Ecuador's waters[10,23] demonstrates the importance of monitoring specific genes, as this gene is one of the most common causes of antibiotic resistance worldwide.[23,44]

Even when contamination can be detected, TULSMA only focuses on fecal markers, ignoring the risk of resistance. WHO recommendations for drinking water prioritize E. coli as an indicator,[35] but detecting resistant strains in Tungurahua represents an additional threat.

The absence of mandatory periodic monitoring of antimicrobial resistance in recreational waters is notable despite its high prevalence in rivers and swimming pools. Studies highlight the importance of systematically evaluating physical, chemical, and microbiological parameters.[7] The TULSMA requires sampling and monitoring[17] but does not establish clear parameters for AMR, which allows this contamination to go undetected. It does not establish alert thresholds for resistance that would trigger rigorous studies. In the global context, the generation of baseline values and the estimation of parametric limits is essential;[7,35] the WHO opts for a tiered approach, considering acute pathogens first[35] but in which AMR is already considered a high-priority risk.[36,37] Excessive levels of E. coli require rapid responses,[35] so resistant strains would require stricter protocols.

In this context, the clinical and agricultural approach of Ecuador's 2019-2023 National Plan for the Prevention and Control of Antimicrobial Resistance highlights its inadequacy for the microbial quality of freshwater bodies by neglecting the specific surveillance mechanisms that should be applied for the detection and control of multidrug-resistant bacteria in this environment.[38] It is, therefore, necessary to reform the TULSMA to implement mandatory surveillance of resistance in surface waters and create early warning indicators in line with the recommendations of competent international bodies.[5,39] The updated regulations would not only bring environmental management into line with international requirements. Still, they would also effectively allow the ecological dimension of AMR to be incorporated into public policies, counteracting the health and environmental risks arising from the spread of superbugs.

Another critical gap is the lack of a 'One Health' approach, which integrates human, animal, and environmental health into Ecuadorian regulations.[36] The spread of resistance genes through agricultural effluents/runoff highlights the importance of inter-institutional collaboration,[36,37] as already promoted in global plans.[36]

Finally, the TULSMA does not provide for detecting resistance genes (ARGs) by PCR or MAR (Multiple Antibiotic Resistance Index), which are international standards.[7,13] The WHO-FAO-OIE is developing guidelines for environmental surveillance of antimicrobials;[37] their incorporation into national regulations would improve the characterization of the risk of AMR spread at the ecosystem and community levels.

This methodological and regulatory reinforcement is essential to coordinate AMR surveillance in water bodies with the global 'One Health' approach, recognizing the interconnection between human, animal, plant, and environmental health.(36,37) The WHO Global Action Plan on Antimicrobial Resistance(37) and the Joint Action Plan on 'One Health'(36) prioritize surveillance and research to strengthen knowledge on AMR.(36,37,45) These plans explicitly mention and call for integrating the environment into the One Health approach(36) and for integrated surveillance of AMR in human health, animal health, agriculture, and the environment.(36,37,45,46)

CONCLUSIONS

The results of the work carried out in Tungurahua, together with previous evidence obtained in Ecuador and the applicable regulations, reveal severe microbiological contamination with ESBL bacteria: Acinetobacter in swimming pools and E. coli in rivers. This contamination is associated with waste discharge and agricultural activities, with a consequent increase in potential health and environmental risks. These results highlight the urgent need to improve wastewater treatment, increase discharge controls, and implement an active ecological monitoring system to halt the spread of antimicrobial resistance.

The literature and international practice also show the absence of TULSMA methods for regulating these parameters: specific parameters for AMR, BLEEs, and ARGs, lack of controls for periodic monitoring, absence of alert thresholds, and omission of the 'One Health' approach. To fill this regulatory gap, it is necessary to establish methodologies that include the detection of ARGs by PCR and resistance levels, incorporate an intersectoral approach, and carry out active surveillance. Future research should seek to systematically quantify ARBs and ARGs, characterize resistance genes at the molecular level, and validate the management measures introduced.

Therefore, TULSMA recommends: 1. Including quality standards with indicators of resistant bacteria and ARG load; 2. Periodic monitoring of AMR in water bodies and effluents; 3.- waste and wastewater management with an emphasis on AMR; 4.- control of chemicals (antimicrobials) and promotion of good practices; 5.- promotion of research and technological development; and 6.- strengthening of intersectoral coordination and training.

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