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ORIGINAL

# Antimicrobial susceptibility in bacterial isolates from water and agricultural products of the Chanchán river, Chimborazo Province, Ecuador

## Susceptibilidad antimicrobiana en aislados bacterianos de agua y productos agrícolas del río Chanchán, Provincia de Chimborazo, Ecuador

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## ABSTRACT

**Introduction:** river contamination with pathogenic and multidrug-resistant microorganisms is a public health issue due to its impact on health and its potential to transmit infectious diseases. Objective: To determine the presence of multidrug-resistant bacteria in irrigation water samples and agricultural products from the Chachán River.
**Method:** a descriptive, non-experimental study with a quantitative and cross-sectional approach was conducted. Water samples were collected from six different points, and pH and temperature were measured. Additionally, 13 agricultural product samples from the same points were analyzed. The identification and antimicrobial susceptibility testing of bacteria were performed using the VITEK2 system and amplification and sequencing of the 16S rRNA gene.
**Results:** a total of 17 bacterial strains were isolated from the water samples, 16 of which (94 %) belonged to the order Enterobacterales, including Proteus mirabilis, Proteus vulgaris, Morganella morganii, and Citrobacter diversus. From the agricultural products, 24 bacteria were isolated, including species of Klebsiella, Stenotrophomonas maltophilia, and Enterococcus faecalis. Antimicrobial resistance was observed in various strains, notably P. mirabilis, C. diversus, and P. vulgaris with multiple resistances. Resistance was also identified in pathogenic strains such as Yersinia enterocolitica, Plesiomonas shigelloides, and Aeromonas hydrophila.
**Conclusions:** the Chachan River and the agricultural products irrigated with its waters contain multidrug-resistant bacteria, posing a significant risk to public health. It is crucial to implement control and monitoring measures to reduce the spread of these pathogens and ensure food safety.

**Keywords:** Multidrug-Resistant Bacteria; Public Health; Irrigation Water; Agricultural Products; Antimicrobial Resistance.

## RESUMEN

**Introducción:** la contaminación de los ríos con microorganismos patógenos y multirresistentes es un problema de salud pública debido a su impacto sanitario y su capacidad para transmitir enfermedades infecciosas.
**Objetivo:** determinar la presencia de bacterias multirresistentes en muestras de agua de riego y productos agrícolas del río Chachán.
**Método:** se realizó un estudio observacional descriptivo y transversal. Se recolectaron muestras de agua en seis puntos diferentes y se midieron pH y temperatura. También se analizaron 13 muestras de productos agrícolas de los mismos puntos. La identificación y las pruebas de susceptibilidad antimicrobiana de las bacterias se realizaron utilizando el sistema VITEK2 y amplificación y secuenciación del gen 16S rRNA.

**Resultados:** se aislaron 17 cepas bacterianas de las muestras de agua, 16 de las cuales (94 %) pertenecían al orden Enterobacterales, incluyendo Proteus mirabilis, Proteus vulgaris, Morganella morganii y Citrobacter diversus. De los productos agrícolas se aislaron 24 bacterias, entre ellas especies de Klebsiella, Stenotrophomonas maltophilia y Enterococcus faecalis. Se observó resistencia antimicrobiana en diferentes cepas, destacando P. mirabilis, C. diversus y P. vulgaris con resistencias múltiples. También se identificaron resistencias en cepas patógenas como Yersinia enterocolitica, Plesiomonas shigelloides y Aeromonas hydrophila.

**Conclusiones:** el río Chachan y los productos agrícolas irrigados con sus aguas presentan bacterias multirresistentes, lo que representa un riesgo significativo para la salud pública. Es crucial implementar medidas de control y monitoreo para reducir la diseminación de estos patógenos y asegurar la seguridad alimentaria.

**Palabras clave:** Bacterias Multirresistentes; Salud Pública; Agua de Riego; Productos Agrícolas; Resistencia Antimicrobiana.

## INTRODUCTION

Globally, the contamination of water resources driven by human activity impacts food security and the sustainability of the biosphere. In many cases, urban wastewater flows directly into lakes, seas, and rivers, contributing to the degradation of aquatic ecosystems.(1) Rivers, in this context, can act as reservoirs of highly resistant strains, facilitating their spread to animals and humans that depend on the water.(2)

Aquatic ecosystems, especially those exposed to human activities such as rivers, are not only crucial vehicles for the spread of pathogenic microorganisms linked to infectious diseases but also function as reservoirs of bacteria harboring genes resistant to various antibiotics. This facilitates the horizontal transfer of these genes to other bacteria sensitive to the same antibiotics, a phenomenon known as environmental resistomes.(3,4,5,6,7)

The dispersion of bacteria and antimicrobial-resistant genes into the environment from human and animal waste constitutes a global problem with significant consequences for public health.(1) Hospitals are considered one of the most polluting sectors in the world.(8) Wastewater treatment plants, especially those dedicated to hospital waste, are critical points for the spread of antibiotic resistance, posing a threat to health.(9)

Urban areas undergoing rapid expansion are particularly affected by uncontrolled population growth and the lack of progress in infrastructure development. As a result, river pollution emerges as a serious and growing challenge, with antibiotic resistance genes considered critical emerging pollutants. Consequently, aquatic ecosystems are presented as favorable environments for their proliferation.(10,11) The abundance of these genes in the environment has become a major global public health concern due to the widespread use of antibiotics in healthcare systems, agriculture, and livestock.(12,13)

The Chanchán River basin, located in the Andean region of central Ecuador in the Western Cordillera, is formed by the union of the Alausí and Guasuntos rivers, between the Cruzpungo mountains in the province of Chimborazo. The river course heads westward, and its flow contributes to the water system of the Guayas River basin.(14)

The waters of this river have different uses, including the irrigation of agricultural products. Local wastewater is discharged into its waters, which could concentrate pathogenic bacteria that could contaminate them. River pollution is indirectly aggravated by the presence of animals that roam freely in the area, depositing excrement that can reach the river carried by rainwater. In addition, the dumping of garbage along the riverbed turns the river into a landfill, exacerbating environmental pollution.(15) In Ecuador, few studies have been conducted to investigate resistant and multidrug-resistant bacteria in aquatic ecosystems and agricultural products irrigated with water from different watersheds. These investigations seek to analyze and understand the influence and role of the environment in the growing problem of multidrug resistance to antibiotics.(16,17,18,19,20)

It is considered essential to understand the level of contaminating microorganisms and their antimicrobial susceptibility patterns, as they are potential causes of infections in the local population. The primary objective of this study was to investigate the presence of multidrug-resistant bacteria in irrigation water and agricultural products sourced from the Chachán River.

## METHOD

A descriptive study was conducted with a non-experimental field design, quantitative approach, and cross-sectional focus.

### Sampling Sites

Six points were identified for water sampling from the Chachán River, in Chimborazo, Ecuador, at an altitude of 3700 meters above sea level. Altitude was recorded with an altimeter, temperature with a thermometer, and pH with test strips (Fisher Scientific). The strips were immersed in water for two seconds and waited ten seconds to observe the color change.

### *Collection of Water and Agricultural Product Samples*

Two sampling campaigns were conducted at each selected site to collect water samples, each with a volume of 100 mL, collected in duplicate in sterile bottles. These bottles were then transported to the Microbiology Laboratory of the Faculty of Health Sciences of the National University of Chimborazo, located in Riobamba, Ecuador.

Regarding the agricultural products evaluated, thirteen samples were collected in duplicate aseptically in sterile bags. The samples included curly lettuce, head lettuce, coriander (point 1); strawberries and potatoes (point 2); carrots and broad beans (point 3); oca, potato, and mashua (point 4); coriander and radish (point 5); and broad beans (point 6). All samples were hermetically sealed, coded, and kept refrigerated in an ice container during transportation to the Microbiology Laboratory.

### *Isolation and Evaluation of Bacteria in Water and Agricultural Product Samples*

To isolate the bacterial colonies present in water samples from the river, serial dilutions of each sample were performed in sterile physiological solution ($10^{-1}$, $10^{-3}$, $10^{-5}$ y $10^{-7}$). One milliliter of the undiluted sample and each of the dilutions were inoculated using the surface plating technique. Bacterial isolation was carried out using various culture media, such as cystine-lactose-electrolyte-deficient (CLED) agar (Oxoid™), blood agar, MacConkey agar, Salmonella-Shigella (SS) agar (Oxoid™), and thiosulfate citrate bile sucrose (TCBS) agar (Oxoid™). All samples were incubated at 37 °C for 24 to 48 hours.

Twenty-five grams from the center of each vegetable were taken, ground in 225 mL of peptone water, and incubated for 24 hours at 37 °C. Then, 1 mL was inoculated into 9 mL of peptone water and incubated for 24 hours at 37 °C. Subsequently, 10 µL of the culture were plated on MacConkey agar, blood agar, CLED, and TCBS, using the streak plate technique.

The isolated and selected colonies were identified using the VITEK2 system (bioMérieux). Antimicrobial susceptibility tests were performed with VITEK2® (bioMérieux) on Mueller-Hinton agar according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).(21)

### *Molecular Identification of Isolated Bacteria*

Bacterial strains were identified at the species level by analyzing the 16S rRNA gene. Genomic DNA was extracted according to Hesham A et al.(22) PCR was performed with universal primers 27F and 1492R in a final volume of 50 µL. Five microliters of the amplified product were analyzed by agarose gel electrophoresis (1 %) stained with ethidium bromide. Amplicons were purified with the TaKaRa Agarose Gel DNA Purification Kit and sequenced on an ABI 3730 automatic sequencer (Macrogen, Seoul, Korea). The 16S rRNA gene sequences were aligned and compared with GenBank according to Hesham A et al.(22)

### Statistical Data Analysis

The data were processed in descriptive tables, using spreadsheets belonging to the Microsoft Excel 365 operating system.

## RESULTS

Table 1 shows the measurements done at each station, covering altitude, ambient and water temperature, pH, as well as the agricultural products cultivated in the areas near the river. It is observed that at points 4, 5, and 6, characterized by a higher altitude, between 3720 and 3800 meters above sea level, the ambient temperature ranged from 8 to 19°C, the water temperature from 6 to 8°C, and a pH from 7 to 8.

**Table 1.** Altitude, temperature (ambient and water), and pH of the water samples taken from the Chanchán River, and the agricultural products, according to the selected sampling points

| Points | Location | Altitude (m.a.s.l) | Ambient temp. | pH | Agricultural products |
|---|---|---|---|---|---|
| 1 | Rumichaca Alausí Brigde | 2 300 | 20 | 7 | Curly lettuce, cabbage lettuce, and coriander |
| 2 | Illibuchi Brigde | 2 400 | 19 | 8 | Strawberries and potatoes |
| 3 | Tixán Station | 2 860 | 20 | 7 | Carrots and broad beans |
| 4 | Totoras Vía Cocan | 3 760 | 8 | 7 | Oca, potatoes, and mashua |
| 5 | El Cortijo | 3 720 | 10 | 6 | Coriander and radish |
| 6 | Entrance to Palmira | 3 800 | 19 | 8 | Broad beans |

Table 2 shows the susceptibility profiles of the bacteria isolated from different points of the Chachán River. Strain 1 of P. mirabilis showed resistance to 5 of the 12 antibiotics evaluated (CT, TE, CIP, AN, SXT, ATM). Strain 3 of C. diversus was resistant to 3 antibiotics (TE, SXT, AX), as was strain 2 of P. vulgaris (CT, CIP, AX).

**Table 2.** Antibiotic susceptibility profiles of bacteria isolated from water samples at different points of the Chachán River

| **Antibiotics**<br>**Microorganism** | **CN** | **K** | **CT** | **TE** | **CIP** | **AN** | **SXT** | **CRO** | **CAZ** | **IPM** | **ATM** | **AX** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Point 1 Rumichaca Alausi Brigde | | | | | | | | | | | | |
| Proteus mirabilis (cepa 1) | S | S | R | R | R | R | R | S | S | S | S | S |
| Proteus vulgaris (cepa 2) | S | S | R | S | R | S | S | S | S | S | S | R |
| Point 2 Illibuchi Bridge. Main Road | | | | | | | | | | | | |
| Citrobacter diversus (cepa 3) | S | S | S | R | S | S | R | S | S | S | S | R |

In the processed agricultural products from the Chanchán River basin, a variety of bacteria were revealed in the analyzed samples. Curly lettuce exhibited the highest number of isolated bacterial strains (9 strains), followed by cabbage lettuce (4 strains). E. faecalis was the most frequently found bacterium, isolated from various products such as carrots, broad beans, oca, potatoes, mashua, coriander, and radish (table 3).

**Table 3.** Bacterial isolation according to the agricultural product from each sampling point in the Chanchán River basin

| **Sampling Points** | **Product** | **Bacteria** | **Number of strains** |
|---|---|---|---|
| Point 1 | Curly lettuce | *St. maltophilia* | 9 |
| | | *K. pneumoniae* | |
| | | *C. diversus* | |
| | Cabbage lettuce | *C. diversus* | |
| | | *E. aerogenes* | |
| | | *Aeromonas hydrophila* | |
| | | *P. shigelloides* | |
| | Coriander | *K. oxytoca* | |
| | | *Y. enterocolitica* | |
| Punto 2 | Strawberry | *K. oxytoca* | 4 |
| | Potato | *P. aeruginosa* | |
| | | *C. diversus* | |
| | | *E. faecalis* | |
| Punto 3 | Carrot | *E. faecalis* | 3 |
| | | *C. amalonaticus* | |
| | Broad beans | *E. faecalis* | |
| Punto 4 | Ocas | *E. faecalis* | 4 |
| | Potato | *E. faecalis* | |
| | Mashua | *E. cloacae* | |
| | | *E. faecalis* | |
| Punto 5 | Coriander | *E. faecalis* | 3 |
| | Radish | *E. cloacae* | |
| | | *E. faecalis* | |
| Punto 6 | Broad beans | *K. pneumoniae* | 1 |
| Total | | | 24 |

Table 4 presents the susceptibility pattern of the isolated bacteria. Clinically important bacteria, such as Y. enterocolitica, showed resistance to amoxicillin, while P. shigelloides was resistant to kanamycin, tetracycline, and ciprofloxacin. P. aeruginosa and St. maltophilia exhibited resistance to aztreonam, and A. hydrophila to trimethoprim-sulfamethoxazole and amoxicillin. Of the eight strains of E. faecalis, one exhibited resistance to tetracycline and ciprofloxacin, while the others were resistant to ciprofloxacin.

**Table 4.** Antibiotic susceptibility profiles of bacteria isolated from agricultural products collected at different points of the Chachán River

| Sampling Points | Product | Bacteria | GN | K | TE | CIP | AN | SXT | CRO | CAZ | IPM | ATM | AX | CT | VA | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Point 1 | Curly lettuce | *St. maltophilia* | S | S | - | S | - | - | - | S | S | R | - | S | - | - |
| | | *K. pneumoniae* | R | R | S | S | S | S | S | S | S | S | R | - | - | - |
| | | *C. diversus* | R | R | S | R | S | S | S | S | S | S | S | - | - | - |
| | Cabbage Lettuce | *C. diversu* | S | R | S | R | S | S | S | S | S | S | R | - | - | - |
| | Coriander | *E. aerogenes* | S | R | S | S | S | S | S | S | S | S | R | - | - | - |
| | | *Aeromona hydrophila* | S | S | S | S | S | R | S | S | S | S | R | - | - | - |
| | | *P. shigelloides* | S | R | R | R | S | S | S | S | S | S | S | - | - | - |
| | | *K. oxytoca* | S | R | S | R | S | S | S | S | S | S | S | - | - | - |
| | | *Y. enterocolitica* | S | S | S | S | S | S | S | S | S | S | R | - | - | - |
| Point 2 | Strawberry | *K. oxytoca* | S | R | R | S | S | S | S | S | S | S | R | - | - | - |
| | Potato | *P. aeruginosa* | S | S | - | S | - | - | - | S | S | R | - | S | - | - |
| | | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| Point 3 | Carrot | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| | Broad beans | *C. amalonaticus* | S | S | S | S | S | S | S | S | S | S | R | - | - | - |
| | | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| Point 4 | Mashua | *E. cloacae* | S | S | S | S | S | S | R | S | S | S | S | - | - | - |
| | | *E. faecalis* | - | - | R | R | - | - | - | - | - | - | S | - | S | S |
| | Ocas | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| | Potato | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| Point 5 | Coriander | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| | | *E. cloacae* | S | S | S | S | S | S | S | R | S | S | R | - | - | - |
| | Radish | *E. faecalis* | - | - | S | R | - | - | - | - | - | - | S | - | S | S |
| Point 6 | Broad beans | *K. pneumoniae* | S | R | S | S | S | S | S | S | S | S | R | - | - | - |

Legend: Tetracycline (TE), Sulfamethoxazole/Trimethoprim (SXT), Ceftriaxone (CRO), Azithromycin (AZM), Aztreonam (ATM), Imipenem (IPM), Kanamycin (K), Gentamicin (CN), Ceftazidime (CAZ), Amoxicillin (AX), Ciprofloxacin (CIP), Nalidixic Acid (AN), Colistin (CT), Vancomycin (VA), Penicillin (P)

Amplification and sequencing of their 16S rRNA region was performed to identify the selected bacteria. The isolates presented PCR-amplified fragments of approximately 1500 bp (figure 1). The alignment of the 16S rRNA gene sequences of these isolates with sequences obtained through BLAST search revealed an identity of up to 100 %.

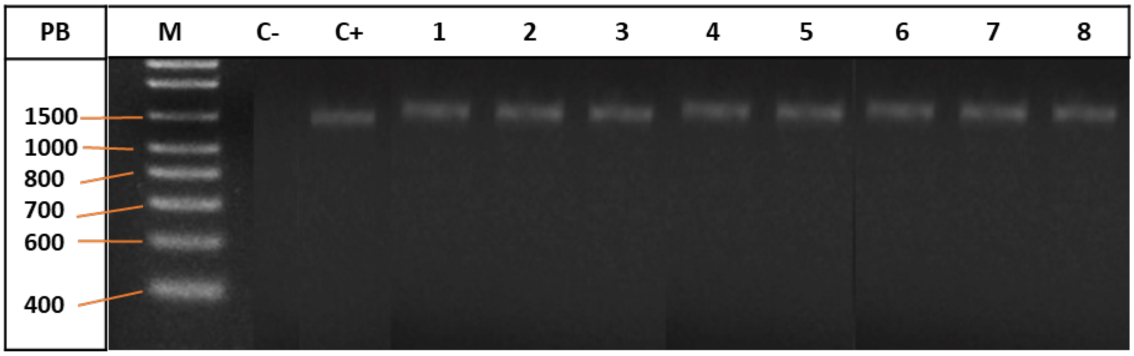

**Figure 1**. Amplified DNA of the 16S rRNA gene for E. faecalis isolates. Lane M: DNA marker; C-: negative control; C+: positive control; Lanes 1 to 8: E. faecalis strains

## DISCUSSION

The Chanchán River basin is located in the Andean region of central Ecuador, in the Western Sierra. It is formed by the confluence of the Alausí and Guasuntos rivers, between the Cruzpungo mountains, in the Chimborazo province.(14) This study analyzed the environmental factors influencing bacterial growth in this region.

Environmental factors, both physical and chemical, are crucial in bacterial growth dynamics. Generally, higher temperatures and a neutral to slightly alkaline pH (7-8), as observed in this study, are conditions that can favor bacterial development and are optimal for many bacterial species. These results align with findings by Shao K et al.(23) who also found that elevated temperatures and a neutral pH promote bacterial growth in similar water bodies.

In this study, 94,0 % of the bacteria isolated from the Chanchán River belonged to Enterobacterales, reinforcing the widespread presence of this group of microorganisms as indicators of fecal contamination in rivers of the region. These results are consistent with previous research conducted on samples from the Chambo, Guano, and Guamote rivers, which also showed contamination by Enterobacterales and E. faecalis.(17,18,19)

The similarities between studies highlight a concerning trend in the microbiological quality of water sources in the region. Agricultural and livestock activities, along with inadequate wastewater disposal, are identified as the main causes of this contamination.(24) The results of this study not only corroborate previous findings but also underscore the urgent need to implement water quality management and monitoring strategies to mitigate microbiological contamination in the rivers of the area.

Regarding the bacteria isolated from agricultural products irrigated with the water of this river, strains of A. hydrophila, P. shigelloides, and Y. enterocolitica were identified. The presence of these species poses a significant risk to public health, as they are pathogenic and can cause gastrointestinal infections, particularly in individuals with compromised immune systems, as well as in young children and the elderly. The presence of these bacteria may be related to fecal discharges from livestock and the use of animal and poultry manure found around the river, as well as waste generated by nearby communities, a situation similar to that observed in other parts of the world.(25)

The main issue addressed in this study was the presence of multidrug resistance in bacteria isolated from Chanchán River water, as well as in agricultural products irrigated with its water. In recent years, the rise of antibiotic-resistant and multidrug-resistant bacteria in aquatic environments has highlighted the need for a comprehensive approach to control and epidemiological surveillance. This involves considering the environment as a crucial component of public health, which has led to the "One Health" perspective.(26)

Antibiotic-resistant bacteria enter aquatic habitats through human and animal sources, and in this way, they can transfer their resistance genes to microorganisms present in those water sources.(27) It is known that this is how resistance has spread globally, representing a multidimensional threat. Antibiotics and other substances, such as heavy metals, have comparable origins and travel routes; therefore, they will eventually coexist in aquatic habitats. Furthermore, despite advancements, existing treatment technologies are not designed to eliminate them, and there are no published health regulations providing guidance on how to manage them.(28)

The finding in this study of strains of A. hydrophila, P. shigelloides, and Y. enterocolitica resistant to different classes of antibiotics, such as beta-lactams, sulfamethoxazole/trimethoprim, aminoglycosides, tetracyclines, and quinolones, represents a significant risk to public health. These bacteria can cause difficult-to-treat infections, especially in vulnerable populations. Additionally, their presence in agricultural products affects food safety and exposes the population to health risks.

Similarly, other studies have reported the presence of Aeromonas strains in agricultural products irrigated with water from the Guano River, with 100 % resistance to amoxicillin and a reduced sensitivity of 66,66 % to imipenem.(17) Likewise, in the irrigation waters of the Chibunga River, P. shigelloides strains resistant to ceftazidime, aztreonam, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole have been reported.(20)

It is important to note that most of the E. faecalis strains isolated from agricultural products showed resistance to quinolones. This finding aligns with previous research documenting the presence of these strains in aquatic environments, resistant to this family of antibiotics.(30) The relevance of this bacterium in water bodies is highlighted by several critical factors: it acts as an indicator of contamination, has the capacity to transfer resistance genes, represents public health risks, affects biodiversity, and is crucial in environmental monitoring and management programs.

The bacteria isolated in this study were identified through 16S ribosomal RNA gene sequencing, an important tool for determining phylogenetic relationships between bacteria.(31)

## CONCLUSIONS

This research confirms the presence of multidrug-resistant bacteria in the Chanchán River and in agricultural products irrigated with these waters, representing a significant risk to public health and food safety.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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