ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2020.31.35

Research Article
Antimicrobial Susceptibility and Molecular Characterization of *E. coli* Obtained from Drinking Water in Ecuador

Favian Bayas-Morejón, Riveliño Ramón, Danilo Yánez and Darwin Núñez Torres

Biotechnological Research and Development Center, Research Department, Faculty of Agricultural Sciences, Natural Resources and the Environment, Agricultural Campus, Bolívar State University, Laguacoto II, CP: 020150, Guaranda, Ecuador

Abstract

Background and Objective: Several infectious diseases caused by pathogens have become a major global threat, especially when presenting resistance to multiple medications for clinical use. The objective of the work was to study the antimicrobial susceptibility of *E. coli* strains isolated from drinking water, as well as their characterization by molecular techniques. **Materials and Methods:** In this study 100 samples of drinking water from the city of San José de Chimbo, Ecuador were analyzed, the samples were initially filtered on Petri dishes with nutrient agar for coliforms and incubated under controlled conditions for 24 h after this period, the characteristic colonies *Escherichia coli* were analyzed by Gram stain and biochemical tests. The alleged *Escherichia coli* were confirmed by PCR and finally the isolates obtained were tested for susceptibility to ciprofloxacin and streptomycin. **Results:** After the culture analysis and confirmation by microscopic, biochemical and PCR tests, it was obtained that the prevalence of *Escherichia coli* was 54% with agarose gel bands of 212 bp. The highest number of contaminating samples were found in the sources of direct consumption, unlike the samples obtained directly from the slope that did not appear to be contaminated. After the antibiogram test, all isolates were found to be susceptible to Ciprofloxacin and Streptomycin. **Conclusion:** Although in this study, the isolates were found to be sensitive to the two most used antibiotics in Ecuador, it does not remove the risk of future contamination if the water conduction system is not controlled.

Key words: *E. coli*, molecular characterization, drinking water, susceptibility, contamination, direct consumption

Citation: Favian Bayas-Morejón, Riveliño Ramón, Danilo Yánez and Darwin Núñez Torres, 2020. Antimicrobial susceptibility and molecular characterization of *E. coli* obtained from drinking water in Ecuador. Biotechnology, 19: 31-35.

Corresponding Author: Favian Bayas-Morejón, Biotechnological Research and Development Center, Research Department, Faculty of Agricultural Sciences, Natural Resources and the Environment, Agricultural Campus, Bolívar State University, Laguacoto II, CP: 020150, Guaranda, Ecuador Tel: +5930990316224

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Drinking water, defined as "suitable for human consumption", is free of disease-causing microorganisms. The possible consequences of microbial contamination for health are such that its control must be a primary objective and must never be compromised.

The developing countries continue to struggle with the issue of food security, that is, the amount of food sufficient for consumption by the growing population; there is another dilemma in these countries. It is estimated that more than 200 types of diseases caused by pathogens are foodborne and waterborne causing problems in vulnerable groups of people such as, the elderly, pregnant women and young children.

Therefore, ensuring the safety of food is an important challenge for public health[1], these pathogens are gamma proteobacteria such as; *Escherichia coli*[2]. During recent years, *Escherichia* spp. has been identified as an emerging food borne pathogen world wide and as an indicator for human fecal contamination of water[2,3]. Also, these pathogen associated with diseases such as; bacteraemia, gastroenteritis, abdominal pain, diarrhea, nausea, vomiting and fever[4,5]. Water, mainly due to anthropic activities, is one of the most deteriorated resources and it is increasingly difficult to dispose of it in optimal conditions. In Ecuador, the streams or rivers of the municipalities are the sources of water, which have water pollution, generally of an organic type, with direct effects on flora, fauna and human health[6].

In developing countries, diseases caused by microorganisms como *E. coli* are one of the most important reasons for premature death, especially of children and more commonly associated with clinical conditions[7]. Mortality in newborns with *E. coli* bacteremia is as high[8] as 40%. In particular, *Escherichia* as enteropathogenic *E. coli* (EPEC) orentero hemorrhagic *E. coli* (EHEC) has been classified as a serious hazard to human, besides, these rovar "0157 H7", causes food poisoning due to the production of verotoxin[9]. Committees of experts in sanitation and hygiene of the housing of the World Health Organization have repeatedly highlighted the relationship between major epidemics or endemics and water pollution[10].

When used as a means of eliminating excreta and other organic waste, water becomes a vehicle for the transmission of numerous microorganisms, mainly bacteria of intestinal origin such as; *E. coli.* It is for this reason that sanitary control is carried out based on the presence of this type of bacteria. From the microbiological point of view, the examination of the sanitary quality of the water by means of culture techniques and identification by rapid methods such as PCR.

The present study was designed with an aim to know the occurrence of *Escherichia* spp. in water by culture and molecular methods and to study the sensitivity of the isolates obtained by Ciprofloxacin and Streptomycin.

MATERIALS AND METHODS

Study area: The present study was descriptive, experimental, cross-sectional and carried out during the months of March-October, 2018, where the presence of *E. coli* was determined in 100 samples of waters for human consumption in five areas or locations of the city of Chimbo, province of Bolívar, Ecuador (Table 1).

Technique and process for simple collection: The water samples were collected in plastic containers previously sterilized, two containers were used for each sampling point. The collection was carried out directly from the water supply taps of the mentioned points, using the parameters described in the INEN[11] Norms.

Preparation of the sample and initial culture: About 100 mL of collected drinking water was taken and filtered on a suction ramp using membrane filters (CHM, MPV045047H, Spain), after complete suction, filter paper was carefully removed and placed on nutrient agar plates (OXOID CM0003, England) specific for the growth of enterobacteria. It was allowed to incubate at 37°C for 24 h. Colonies considered suspect were cultured on nutrient agar at the same conditions.

Microscopic confirmation: The colonies with considerable growth were analyzed by Gram stain. The characteristic identification of *E. coli* by staining is a Gram-negative bacillus.

Biochemical confirmation: The colonies suspected by their plate morphology and microscopy were inoculated in 9 mL of bright green bile broth (Difco, 274000) with Durham campaign, to verify if the microorganism has the capacity to ferment lactose. Those tubes containing gas (at least 2/3 of the Durham bell) were seeded on TBX agar (Merck; 1.16122.0500) and incubated at 37°C for 24 h. The suspicious colonies were subcultivated in TBX under the same conditions as in the previous one and to confirm a set of biochemical tests called IMViC (Indol, Methyl Red, Voges-Proskauer and Citrate) was performed. The positive IMViC profile for *E. coli* is that, the Indole test is positive, the Methyl Red test is positive, the Voges-Proskauer test is negative and the citrate test is also negative.

Identification of strains by microscopy: Purified isolates were further confirmed morphologically by Gram staining. The isolates of *Escherichia* spp. (Gram-negative, spiral shaped, motile) were stored in cryovials (Microbank™ Pro Lab Diagnostics, USA) at -80°C, after molecular identification.

Molecular analysis: Five colonies of each strain grown on nutrient agar to *Escherichia* were suspended in 500 µL of buffer TAE 1X and centrifuged at 16,000 g for 10 min at room temperature. DNA extraction total DNA from each characterized *Escherichia* isolate was extracted used Pure Link™ Microbiome DNA Purification Kit (Invitrogen, A29790, USA) which was performed according to the manufacturer's protocol. Almost 5 µL of each DNA simple obtained was used as the DNA template for the PCR assay.

PCR assay: The primers and PCR assay conditions previously described by Lindsey *et al.*[7] were used for specific identification *of Escherichia*. The primers amplify a 212 bp fragment from *E. coli*, the sequence of primers were: EC_F: CCAGGCAAAGAGTTTATGTTGA and EC_R: GCTATTTCCTGCCGATAAGAGA.

PCR to *Escherichia* detection: The PCR reactions were performed in a 50 µL reaction mixture (2X PCR master mix Promega) contained: 2 µL template DNA, 5 µL of 10×PCR buffer, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 0.375 µM of each primer, EC_F and EC_R and 1.25 units of HotStar *Taq*. The thermocycling conditions were as follows: one cycle at 95°C for 10 min; 30 cycles of 92°C for 1 min, 57°C for 1 min and 72°C for 30 sec and one final cycle at 72°C for 5 min and maintenance at 4°C before electrophoresis, the type ATCC 10536 strain was used as a positive control and water as negative control.

Electrophoresis of PCR products: About 5 µL of PCR amplification product of previously mixed with 2 µL of loading buffer Blue/orange 6X, loading Dye (Promega, G190A, USA) was then separated in 1.5% agarose gels prepared with TAE (40 mM Tris-acetate, pH 8.3, 1 mM EDTA) buffer with 2 µL/100 mL SYBR Safe DNA gel stain (Invitrogen, 247110-030, USA) at 100 V for 40 min. A 50 bp molecular weight marker (Invitrogen, 10488-043, USA) was included in each gel. Finally, the bands sizes were visualized with a UV transilluminator.

RESULTS AND DISCUSSION

Microbiological analysis: Cultural isolation of *Escherichia* showed 54 samples out of a total of 100 samples to have the presence of pathogen, with total of 58 isolates (Table 1). The suspect colonies showed typical white to whitish-gray color, Gram-negative after staining.

Identification of *Escherichia* isolates by PCR: In the present study, of the 59 isolates analyzed by PCR with the specific primers (EC_F and EC_R), all turned out to be positive for *E. coli*, in these samples a characteristic band of 212 pb was obtained (Fig. 1).

The highest prevalence was obtained from public areas and education centers, followed by restaurants with values

Table 1: Samples detected with *Escherichia* by culture and PCR

Area of water sampling	Samples	Samples detected by culture (%)	Sample identification by PCR
Restaurants	15	8 (53.33)	8**
Education centers	25	14 (56)	14*
Private homes	18	9 (50)	9*
Public places	32	18 (56.25)	18
Main and secondary slopes	10	5 (50)	5
Total	100	54	54

*More than 1 isolated, **More than 2 isolated

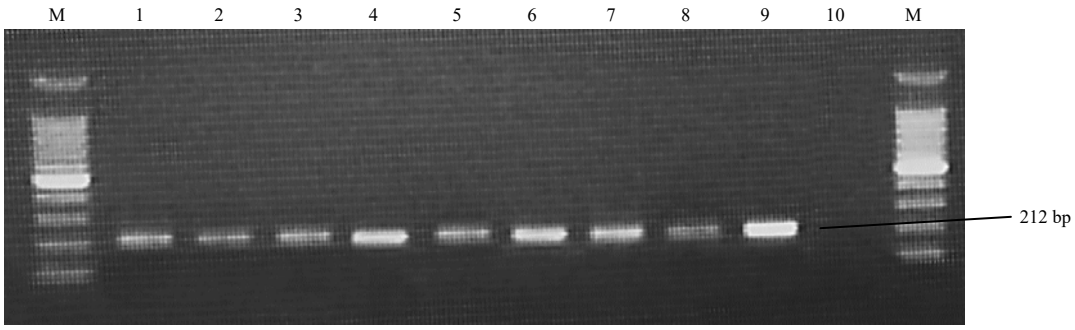

Fig. 1: Conventional PCR detection *Escherichia coli* in chicken, beef and pork meat samples in agarose gel electrophoresis
Lane M: Molecular weight marker, 212-bp, Lane 1-8: *E. coli* in water, Lane 9: Positive control, Lane 10: Negative control

that exceed 53%, both in private homes and in waterways the prevalence was 50% prevalence, in the slopes there should be no contamination, however, the detection of *E. coli* is especially due to the fact that the water conduction costs are directly exposed to the environment to grazing areas of cattle, which possibly leads to contamination of the vital liquid by feces of both cows like wild animals.

The number of isolates obtained in most cases was one isolate per sample, however, there were samples where more than one isolate was obtained (Table 1).

Similar results to this study were obtained in Peru by Tarqui-Mamani *et al.*[12], with *E. coli* prevalence values of 30.8% in the urban sector and 56% in the rural sector. In a study development by Thani *et al.*[13], in Mombasa, the presence values of *E. coli* were higher than in current study, the authors obtained a 60.30% of prevalence and after PCR isolates were characterized as enteroinvasive *E. coli* (EIEC), Similarly, López Cuevas *et al.*[14], obtained values of 98% with an average count of 1.6×104 CFU/100 mL.

Pathogen detection values lower than this study were obtained by Momtaz *et al.*[15], who analyzed drinking water, the results were 26.38 and 2.63% positive for *E. coli* in tap water and bottled respectively, these values were confirmed by PCR. Much lower detection values were obtained by Adzitey *et al.*[16], 10% (38/25) samples, Arriaza *et al.*[17], 14.3% and Al-Nuwaysir *et al.*[18], reported that by PCR they detected the presence of the *hha* and *tuf* genes specific for *E. coli* in 6.41% of the samples. In Ecuador there are no data on research studies for the detection of *E. coli* in drinking water, there are few studies regarding river waters[19]. It should also be noted that according to INEN 1108[20], the level of biological contamination by *E. coli* must be null.

Antimicrobial activity: By diffusion disk plate analysis, all 59 isolates were found to be susceptible to Ciprofloxacin and Streptomycin, which demonstrated that the pathogen can be controlled in case of water consumption infection. In the study developed in Mexico by López-Cuevas *et al.*[14], where it was determined that 46 strains of *E. coli* isolated from water samples in Mexico, 38 were resistant to streptomycin. There is also intermediate resistance for other antibiotics, there are no data on susceptibility studies of *E. coli* to Ciprofloxacin.

CONCLUSION

This paper demonstrates the presence of the *Escherichia coli* microorganism in drinking water of the Chimbo sector, said recognition of the pathogen is given by PCR confirmation analysis, knowing that 54% of the samples are contaminated and pathogens should not be present in drinking water, it can be indicated that vital liquid is not suitable for human consumption.

SIGNIFICANCE STATEMENT

This study discovered the presence of *E. coli* pathogens in water for human consumption, these results contribute to the epidemiology of the pathogen, especially to the existing relationship of cases of chronic gastritis in San José de Chimbo, Ecuador. This study will help researchers and the mayor's office to find alternatives for water purification outside conventional chlorination, for example, ozonation.

ACKNOWLEDGMENTS

This study was supported by the Project: Detección de patógenos mediante m-PCR (Reacción en Cadena de la Polimerasa-múltiple) para determinar la calidad del agua potable del cantón Chimbo (PIDPA 2018-2019), from the Departamento de Investigación y Vinculación, Universidad Estatal de Bolívar, Ecuador. In addition, thanks also to the Ecuador-Spain debt swap project for having provided the equipment used in this study.

REFERENCES

1. Paudyal, N., V. Anihouvi, J. Hounhouigan, M.I. Matsheka and B.S. Monang *et al.*, 2017. Prevalence of foodborne pathogens in food from selected African countries-A meta-analysis. Int. J. Food Microbiol., 249: 35-43.
2. Olivas, E., M. Flores, G.D. di Giovanni, D. Corral and A. Osuna, 2013. Fecal indicators in drinking water at Juarez Valley. Terra Latinoamericana, 31: 135-143.
3. Brown, J.M., S. Proum and M.D. Sobsey, 2008. *Escherichia coli* in household drinking water and diarrheal disease risk: Evidence from Cambodia. Water Sci. Technol., 58: 757-763.
4. OPS., 2012. 12.6 million people die each year due to the unhealthiness of the environment, reveals the WHO report. Organización Panamericana de la Salud, Pan American Health Organization.
5. García-Hernández, A.M., E> Garcia-Vazquez, A. Hernandez-Torres, J. Ruiz, G. Yaguee, J.A. Herrero and J. Gomez, 2011. Bacteraemia due to *Escherichia coli* producing extended-spectrum beta-lactamases (ESBL): Clinical relevance and today's insights. Rev. Espanola Quimioterapia, 24: 57-66.
6. León, R., B. Pernía, R. Siguencia, S. Franco, A. Noboa and X. Cornejo, 2018. Potential of aquatic plants for the removal of total coliforms and *Escherichia coli* in sewage. Enfoque UTE., 9: 131-144.

7. Lindsey, R.L., L. Garcia-Toledo, D. Fasulo, L.M. Gladney and N. Strockbine, 2017. Multiplex polymerase chain reaction for identification of *Escherichia coli*, *Escherichia albertii* and *Escherichia fergusonii*. J. Microbiol. Methods, 140: 1-4.
8. Weston, E.J., T. Pondo, M.M. Lewis, P. Martell-Cleary and C. Morin *et al*., 2011. The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008. Pediatr. Infect. Dis. J., 30: 937-941.
9. Ooka, T., K. Seto, K. Kawano, H. Kobayashi and Y. Etoh *et al*., 2012. Clinical significance of *Escherichia albertii*. Emerg. Infect. Dis., 18: 488-492.
10. De Navia, S.A.L. and S.M.E. Torres, 2012. Bacteriological quality of the water for human consumption in urban and rural areas of the municipality of Guatavita, Cundinamarca, Colombia. Rev. Cubana Higiene Epidemiol., 50: 163-168.
11. INEN., 1998. Water. Water quality, Sampling management and conservation of samples. https://law.resource.org/pub/ec/ibr/ec.nte.2169.1998.pdf.
12. Tarqui-Mamani, C., D. Alvarez-Dongo, G. Gómez-Guizado, R. Valenzuela-Vargas, I. Fernandez-Tinco and P. Espinoza-Oriundo, 2016. Bacteriological quality of water for consumption in three Peruvian areas. Rev. Salud Pública, 18: 904-912.
13. Thani, T.S., S.M.L. Symekher, H. Boga and J. Oundo, 2016. Isolation and characterization of *Escherichia colipathotypes* and factors associated with well and boreholes water contamination in Mombasa County. Pan Afr. Med. J., Vol. 23, No. 1. 10.11604/pamj.2016.23.12.7755
14. López Cuevas, O., J. León Félix, M. Jiménez Edeza and C. Chaidez Quiroz,2009. Detection and antibiotic resistance of *Escherichia coli* and *Salmonella* in water and agricultural soil. Rev. Fitotecnia Mexicana, 32: 119-126.
15. Momtaz, H., F.S. Dehkordi, E. Rahimi and A. Asgarifar, 2013. Detection of *Escherichia coli*, *Salmonella* species and *Vibrio cholerae* in tap water and bottled drinking water in Isfahan, Iran. BMC Public Health, Vol. 13. 10.1186/1471-2458-13-556
16. Adzitey, F., N. Sumaila and C.K.S. Saba, 2015. Isolation of *E. coli* from drinking water sources for humans and farm animals in Nyankpala community of Ghana. Res. J. Microbiol., 10: 126-131.
17. Arriaza, A.E., S.E. Waight, C.E. Contreras, A.B. Ruano, A. López and D. Ortiz, 2015. Bacteriological determination of the quality of drinking water obtained from filters located in the central campus of the University of San Carlos of Guatemala. Rev. Científica, 25: 21-29.
18. Al-Nuwaysir, H., N. Moubayed, M. Al-Othman, A.B. Bacha and I. Abid, 2019. Molecular detection of *Escherichia coli* in Wadi Hanifa, Saudi Arabia. Biomed. Res., 30: 253-258.
19. Campaña, A., E. Gualoto and V. Chiluisa-Utreras, 2017. Physicchemical and microbiological assessment of water quality in Machángara and Monjas rivers from Quito's metropolitan district. Bionatura, 2: 305-310.
20. INEN 1108, 2014. Ecuadorian technical standard, drinking water requirements. Technical Standard Adapted from the WHO Guidelines for the Quality of Drinking Water, 4th Edn., 2011, pp: 10.