Original Article

Detection Of E. Coli By Culture From Human Water Consumption Of Guaranda – Province Of Bolivar (Ecuador)

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Abstract

Background: Escherichia coli is a Gram negative opportunistic microorganism, which is directly related to gastroenteritis in depressed immune persons, this pathogen has been found in several biological niches including water; the objective of this work was to detect E. coli by culture techniques.

Methods: fifty samples of water for human consumption in the city of Guaranda, Bolivar (Ecuador) were collected. Cultures were performed using selective media and after incubation under controlled conditions, they were confirmed through biochemical and microscopic tests by Gram stain.

Results: After the analysis, 18 samples were found to be contaminated with E. coli, which is equivalent to 36% prevalence in the analyzed simples.

Conclusion: This contamination level is probably due to the lack of adequate treatments in the water, also the transfer of the fluid and the possible contact with cattle feces in the water springs.

Keywords: Detection, E. coli, culture, water for human consumption

INTRODUCTION

The quality of water for people's consumption is a concern that remains constant both in the population and in the Ecuadorian government. If it is true, there is a progressive growth of the population, both in the urban area and in the rural area, but also, the increase in the levels of microbiological contamination of the water increases the risk of diseases in the population. Waterborne diseases remain the critical risk factor trigger for drinking water quality (**Deepa et al., 2011; Rodríguez Miranda et al., 2016).**

The WHO (World Health Organization) Guidelines for Drinking Water Quality state that microbiological quality control of drinking water is largely based on the examination of indicator bacteria such as total coliforms, Escherichia coli, Salmonella spp. and Pseudomonas aeruginosa. Coliforms regroup some bacterial species of the Enterobacteriaceae family, bacillary, Gram negative, aerobic or facultative anaerobic, oxidase negative, non-sporulating, with acid production at 37ºC at 24-48 hours (**Olivas Enríquez et al., 2013; Ríos-Tobón et al., 2017; González-Mendoza et al., 2017**)

Escherichia coli is a generally pathogenic bacterium that causes abdominal pain, nausea, diarrhea, vomiting and fever; on the other hand, Klebsiella is related to respiratory diseases; Citrobacter produces alterations at the colon and intestinal level (**Ameer et al., 2021; Ashurst et al., 2022; Ramos-Vivas et al., 2020**).

In developing countries, diseases caused by microorganisms are one of the most important reasons for premature death, especially of children. Normally, these microbes reach the water in the feces and other organic remains produced by the infected people (**Larrea Murrell et al., 2013; Salas-Salvadó et al., 2020; Poaquiza-Caiza et al., 2022**).

The main causes of mortality are linked to the environment, the report shows, after the analysis of more than 100 categories of diseases and injuries, where the majority of deaths linked to the environment, and among which stand out diarrheal diseases with 846 000 deaths annually reports (**OPS, 2012**).

Diarrhea and gastroenteritis are fourth in the list of most frequent hospitalizations in Ecuador. These stomach discomforts affect almost the entire population, regardless of age or sex, with 30,078 cases in 2016, after Apencicitis, cholelithiasis and pneumonia (**INEC, 2016**).

Among the different diseases related to the consumption of non-potable water are: diarrheal diseases, cholera, typhoid fever, salmonellosis, hepatitis A, among others (**INEC, 2007; Amicizia et al., 2019; Bayas-Chacha et al., 2022**).

Considering the previously described, the objective of this work was to detect E. coli by means of culture techniques from samples of water for human consumption of Guaranda, Province of Bolivar (Ecuador).

METHODS

The present study was descriptive, experimental, cross-sectional, carried out during the months of December to April 2022, where the presence of E. coli was determined in water for human consumption in certain sectors of the canton Guaranda canton, (Ecuador). , where the type strain ATCC 10536 was used as a positive control.

Population and sample

The population was represented by the urban sectors of the city, 10 sampling points were randomly considered for water collection, In this sense, 5 samples were selected for each point in a given period of 10 monthly samples. Table 1 shows the analyzed sectors.

Table 1. Distribution of the sectors of sampling

Sector	**Code**
Mantilla	A
La Humberdina	B
Centros educativos	C
Universidad	D
Alpachaca	E
Guanujo centro	F
Veintimilla	G
Primero de Mayo	H
Bellavista	I
Las colinas	J
Total	50

Technique and process for sample 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 Norms (INEN, 1998).

The samples collected in a time not exceeding 4 hours were taken to the Microbiology area of the General Laboratory of the State University of Bolivar.

Preparation of the Sample and Initial Culture

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 hours. 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.

The 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 hours. 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.

Then, in a cryovial (Cryobank, Cryo / M, USA), each of the isolates were refrigerated at 2-4ºC for further analysis

RESULTS AND DISCUSSION

Through the analysis of plate culture, microscopy and biochemical tests, it was determined that 18 samples of water for human consumption in the city of Guaranda were found to be contaminated with E. coli, this means that 36% of the samples were in contact with feces, or with some other agent before finally reaching consumers (Table 2).

Table 2. Isolates of E. coli obtained

Sectors	Positive (%)	Total
A	2 (40)	5
B	3 (60)	5
C	3 (60)	5
D	1 (20)	5
E	0 (0)	5
F	3 (60)	5
G	2 (40)	5
H	1 (20)	5
I	0 (0)	5
J	3 (60)	5
Total	**18 (36)**	**50**

Our result was quite similar to that obtained in Peru by **Tarqui-Mamani et al.** (**2016**), where they analyzed the human consumption of water in the regions of Cajamarca, Huancavelica and Huánuco, the authors detected a prevalence level of E. coli of 30.8% in the urban sector and 56% in the rural sector.

It should also be noted that according to **INEN 1108. (2014)** the level of biological contamination by E. coli must be null.

In another study carried out in Mexico (Nezahualcóyotl), the authors conclude that drinking water does not meet quality standards in view of the fact that both total coliforms and E. coli show values that exceed the regulations (± 63 CFU according to NMP) (**Cázares and Alcantara, 2014**).

In a study carried out in Guatemala by **Arriaza et al. (2015),** the authors determined after cultivation, that the water for human consumption of an educational center was contaminated with E. coli in 14.3%. On the other hand, in a work conducted by **Olivas et al. (2013)** in 84 samples of drinking water in the valley of Juares Mexico, determined the absence of E. coli.

In our study, of the 18 positive samples, sectors B, C, F and J showed a higher prevalence (60%), followed by sectors A and G, with a prevalence greater than 40%, while in sectors D and H there was a prevalence of 20%, only in samples E and I the presence of microorganisms was not identical.

In Ecuador, there is no record that similar work has been carried out, however, there is a work to detect pollutants in river waters, such as the Machángara River in Quito, where the results of general microbiology obtained by the authors were: average concentration of total Coliforms for agricultural and livestock use and was 337.7 times higher than the norm, presenting a value of 337777 NMP / 100 mL (**Campaña et al., 2017**).

Based on the criteria mentioned, the microbiological indicators of water contamination have generally been bacteria of the intestinal saprophyte flora, among which are Bacteroides fragilis, mesophilic bacteria, total coliforms, and fecal [thermotolerant], Escherichia coli and fecal streptococci. Some of these, of animal origin [generally from livestock farms], represent a high zoonotic potential, (**Kim et al., 2014**).

In conclusion, it can be said that after the analysis and biochemical tests, the levels of contamination by E. coli are high and are due to the fact that the water is in contact with faeces that may be of animal origin, in addition to the purification mechanisms used in the city of Guaranda there are deficits.

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