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Escherichia coli as a fecal contamination indicator for public health risk prevention at the Canoa recreational beach area

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ABSTRACT

The research was carried out in the Canoa beach resort, its objective was to evaluate the microbiological characteristics of water and sand in the bathing area. Sampling points were selected every 500 m in the strip between the high and low tide lines, in areas of calm waves. The water and sand samples were collected at storage temperatures between 1 and 4 °C, and processed in less than 8 hours, according to Standard Methods regulations. Microbiological analyses were performed for *Escherichia coli* in seawater using the petrifilm technique and in sand by agitation and sedimentation. The results were evaluated according to OMS quality standards and Ecuadorian regulations, with a limit of 200 CFU/100 ml for water. The Anderson-Darling test was applied to verify normality and the Pearson correlation coefficient to evaluate the relationship between water and sand. On holidays, *E. coli* concentrations in water exceeded 250 CFU/100 ml, especially in December and January, due to the greater influx of tourists. On normal days, concentrations were less than 50 CFU/100 ml. In sand, concentrations were also higher on holidays, reaching 131 UFC/g in dry sand and more than 230 CFU/100 ml in wet sand. A moderate negative correlation was found between *E. coli* in water and dry sand (-0.622), and a strong positive correlation with wet sand (0.812), suggesting that moisture favors its persistence.

Keywords: bathing area, seawater, wet sand, dry sand, *Escherichia coli*.

INTRODUCTION

From the twentieth century to the present, sun and beach tourism on the coasts has represented a fundamental pillar for the economic development of these areas, playing a key role in the growth of countless tourist destinations (Prario et al., 2024). However, the massive attendance of visitors, driven solely by production and without planning, has generated a large number of negative impacts, such as overexploitation of resources, coastal erosion, and microbiological contamination, which has become a health problem and a risk to public health (Lucero et al., 2019).

The microbiological contamination of seawater is linked to several sources, among them the most significant is the direct discharge of wastewater that has not received treatment (Soto et al., 2022), this is due to the fact that in coastal areas there is no adequate sewage system, causing human and animal waste to be dumped into saltwater bodies (Zambrano et al., 2022), this problem is compounded by the dragging of pollutants from rivers and streams in urban and rural areas that contribute to the presence of bacteria such as *Escherichia coli* in seawater (Plúas et al., 2020).

Escherichia coli *contamination* does not only affect water, but its impact is also reflected in the sands of beaches where it has been discovered that these microorganisms can remain as a reservoir, increasing the probability of exposure to humans (Moreno et al., 2022), through several studies carried out in different resorts around the world. alarming concentrations of fecal coliforms have been detected in both sand and water, making it a biological risk for tourists who frequent beaches due to exposure to these pollutants (Him et al., 2022).

Due to this problem, the World Health Organization (2006) has established microbiological parameters for human contact with seawater, selecting as a basis two indicator bacteria, *Escherichia coli* (500–250 CFU/100 ml) and *Enterococcus faecalis* (200–100 CFU/100 ml), because these indicators are the ideal ones to evaluate the health status of beaches.

It has been proven that bacterial colonies are more prevalent in sand in contrast to water, however, little interest has been given to research that evaluates microbiological contamination in water and in the emerged strip of sand (Párraga et al., 2022), this situation is particular for public health, because direct exposure to or accidental ingestion of seawater contaminated by *Escherichia coli* during recreational activities such as swimming and water sports can cause gastrointestinal, respiratory, and dermal infections (Pauta et al., 2020).

This research originated due to the need to evaluate the microbiological contamination of water and sand, for this purpose the contamination by fecal coliforms in the resort of Canoa was evaluated to guarantee public health by ensuring a safe environment for visitors and demonstrating its compliance with the parameters established for human contact. To this end, the following idea was raised, the concentration of fecal coliforms in bathing areas varies according to the influx of tourists.

MATERIALS AND METHODS

Study area

This research was carried out in the Canoa beach resort in the Province of Manabí (S 0° 27' 42.44", W 80° 27' 12.88). It is characterized by a warm climate and an average annual temperature of 25 °C and average rainfall of 163.5 mm, due to its tourist area it is characterized as a very productive sector, dedicated to fishing and crafts (Figure 1).

Establishment of the research area in the resort

For the determination of the research area, the beach areas where recreational activities with primary contact are carried out and that have a large influx of bathers were taken as a criterion, for them it is stipulated that on beaches whose extensions are greater than 500 m, at least one sampling point is defined for every 500 m. covering the entire area used by bathers (Secretaría de Salud de México [SSM], 2019).

Delimitation of the strip by tide line and description of the sampling points

The sampling strip was delimited by the high tide line, which marks the upper limit reached by

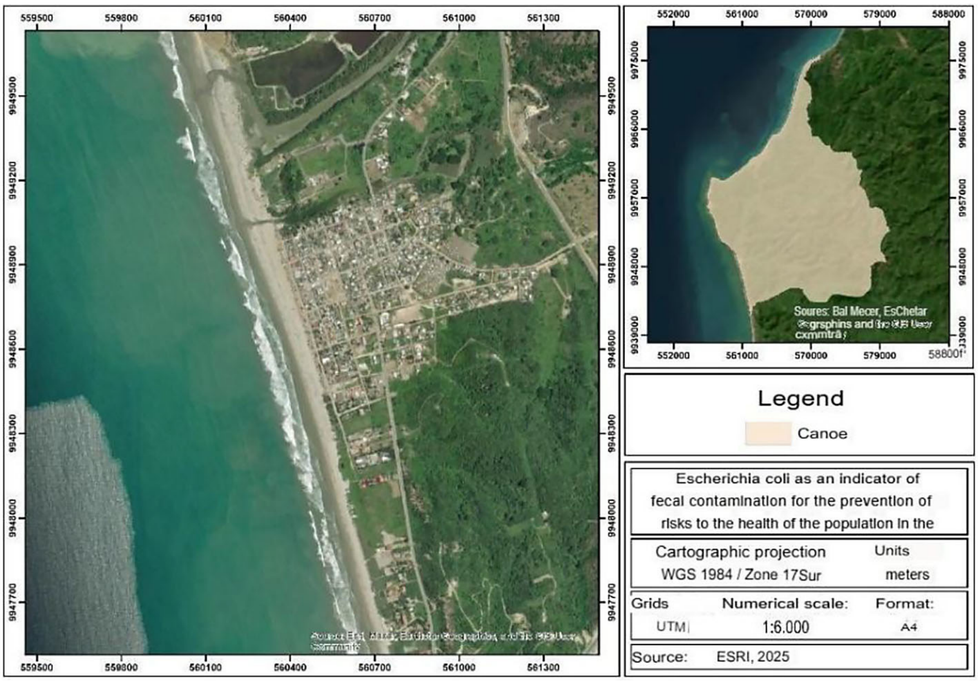

Figure 1. Location map of Canoa resort, San Vicente

the highest tide recorded during the study period, and the low tide line, which defines the furthest point reached by the water when receding, within this band 4 points were selected at random and 3 points located at different distances towards the sea (5 m, 10 m and 20 m) (SSM, 2019), the tidal coefficient was taken into consideration, which is a measure that reflects the amplitude of the tidal cycle, and is defined by the difference between the levels of high tide and low tide, this coefficient affects the spatial delimitation and representativeness of the study area, so the samplings were scheduled during spring tides when the coefficient was between 60 and 80 (Quintana, 2022).

Sample rate

In accordance with the Operational Manual for Primary Contact Water Surveillance on beaches and freshwater bodies, the sampling frequency was carried out monthly for 3 months; it was also established that for water two monthly sampling frequencies are defined, which consists of taking a sample of seawater on the first days of each month, during the months that do not include the pre-vacation periods and during the two weeks prior to each holiday period for the faithful departed, Christmas and New Year (SSM, 2019).

Seawater and sand sampling

In areas of calm waves, samples should be taken in areas where the depth of the water reaches approximately one meter (or at the height of the verifier's waist). The sample should be taken against the current of the incoming flow and approximately 30 centimeters below the surface of the water. Seawater samples were collected following the methodology described in the 23rd edition of the Standard Methods for the Examination of Water and Wastewater; then, they were placed on ice and transferred to the laboratory in dark conditions at a temperature between 1 and 4 °C for subsequent analysis, the samples were processed in less than 8 h (APHA-AWWA-WEF, 2017).

Dry sand was collected in non-flooded areas, near the high tide line, approximately 25 m inland. The wet sand samples were collected in an intermediate zone between dry sand and seawater, for which 100 g of sand per point were taken. The samples were placed on ice and transported in dark conditions, at a temperature between 1–4 °C and processed in less than 8 h (Pinto et al., 2020).

Microbiological analysis

In the case of seawater, the analysis of *E. coli* was performed using the petrifilm technique and following the instructions described in the 23rd edition of the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2017). The sand analysis was carried out as follows: 10 g of sand was stirred in 90 ml of sterile distilled water for 2 min. Then, the mixture was sedimented for 1 min. The CFU of *Escherichia coli* were calculated using the supernatant of the mixture using the petrifilm technique (González and Pinto, 2018).

Evaluation of seawater quality using quality criteria

For the analysis of the microbiological quality due to the contamination of *E. coli* in water for recreational use, the regulations proposed by the World Health Organization and Book VI, Annex 1: Environmental Quality and Water Resource Effluent Discharge Standard, Table 7 of the Unified Text of Secondary Environmental Legislation (TULSMA) were considered. in which a series of indicator values associated with the increase in the frequency of different types of diseases are defined, in this regard, the United States Environmental Protection Agency has established the criteria shown in Table 1, to classify recreational areas considering specific samples.

Correlation between the different matrices (sand and seawater)

The concentrations of microorganisms (CFU) obtained in the determinations made with the sands were standardized to CFU/100 g to allow an evaluation of the relationship between the sand samples and the seawater samples. For the above, a specific density for water of 1 g/mL was assumed. The concentrations of microorganisms were transformed to a scale of log10, then the data obtained in the determinations of *E. coli* and the matrices were analyzed for normality. Subsequently, by means of the Anderson-Darling statistical test, the Spearman correlation coefficients of fecal coliform concentrations were determined, all tests were performed using a 95% confidence level ($\alpha = 0.05$) (Badilla and Mora, 2019).

Table 1. Maximum limits of *E. coli* in seawater according to various regulations

Regulations	Limit
Ecuadorian Regulations TULSMA Annex 1 Table 7	200 CFU 100 ml^{-1}
World Health Organization	200 CFU 100 ml^{-1}
U.S. Environmental Protection Agency	235 CFU 100 ml^{-1}

RESULTS

Sampling points

The satellite image illustrates the spatial arrangement of four sampling locations (P1, P2, P3, and P4) situated along the coastal zone or designated bathing area at Canoa Beach, in the north-central region of Manabí Province (Figure 2).

Table 2 describes the sampling points located in the Canoa Resort, specifying their geographical coordinates, ambient temperature, and characteristics of the coastline as a function of the tidal coefficient. Data that allow contextualizing the environmental conditions of the study area and the distribution of *Escherichia coli*.

Bacteriological concentration of *E. coli*

Below, the concentrations of *E. coli* in the three matrices evaluated are detailed: seawater, dry sand and wet sand, Figure 1 presents the concentrations of *E. coli* in seawater, the results offer a detailed view of its behavior, on holidays an increase in *E. coli* concentrations is observed in December and January, with values that exceed 270 CFU 100 ml^{-1} at points 3 and 4, as for days without holidays, the concentration of *E. coli* is remarkably low compared to the former, at most points and months, the values do not exceed 70 CFU 100 ml^{-1} (Figure 3).

In the month of November, on holidays, concentrations were between 100–150 CFU 100 ml^{-1}, while on days without holidays these concentrations are lower, with values that do not exceed 60 CFU 100 ml^{-1}, in December during the Christmas holiday, P4 presented values higher than 250 CFU 100 ml^{-1} that are outside the established limit, while on normal days the values are less than 50 CFU 100 ml^{-1}, reflecting a reduction of more than 80%, in January the greatest difference is observed, on New Year's holidays, the concentrations in P4 reach 300 CFU 100 ml^{-1}, while on days without holidays the values are practically zero, it was evidenced that in point 3 in the months of December and January on holidays, the concentrations of *E. coli* exceed the maximum limits indicated in Table 1, while on the other sampling days they found values below 150 CFU 100 ml^{-1} that did not exceed this limit.

Figure 4 details the results of the evaluation of *E. coli* in the dry sand, the analysis of the data during the holidays indicates an increase

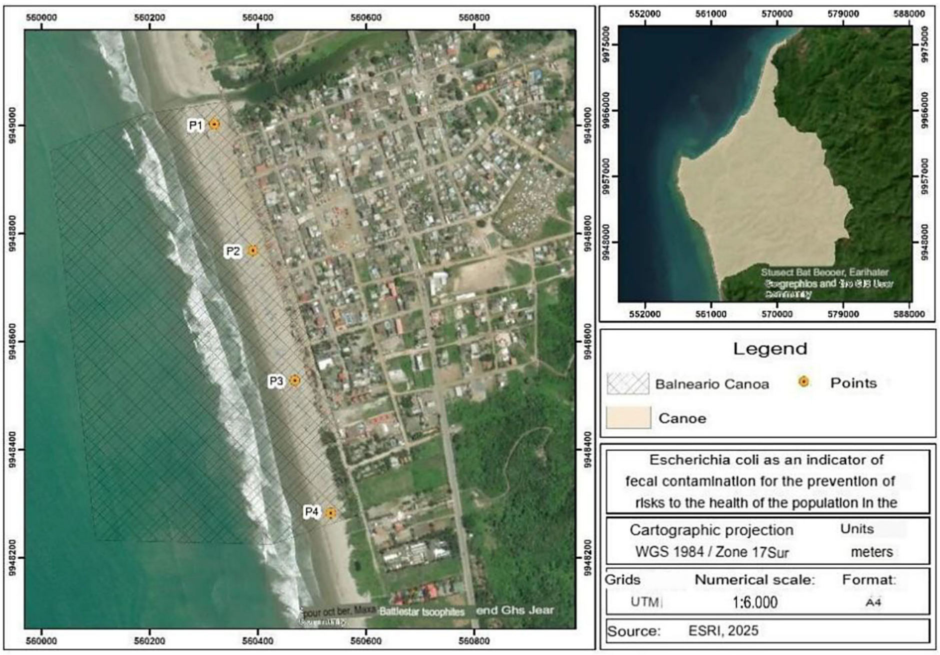

Figure 2. Establishment of the bathing area and monitoring points in the Canoa resort

Table 2. Description of the conditions of the monitoring points

Description of the points	Photography
P1.– Location 560319.28 m E and 9949002.31 m S. Ambient temperature 29 °C and a tidal coefficient of 57–78, reaching a coastline that ranged between 56-78 meters. Reference point of the location of the river mouth.	
P2.– Location 560390.98 m E and 9948768.84 m S. Ambient temperature 28 °C and a tidal coefficient of 57–78, reaching a coastline that ranged between 44-62 meters. Reference point of the central beach location.	
P3.– Location 560468.79 m E and 9948282.48 m S. Ambient temperature 28 °C and a tidal coefficient of 57–78, reaching a coastline that ranged between 50–68 meters. Reference point for fishermen's landing area.	
P4.– Location 560534.35 m E and 9949002.31 m S. Ambient temperature 29 °C and a tidal coefficient of 57–78, reaching a coastline that ranged between 35–52 meters. Reference point area with the greatest tourist influx.	

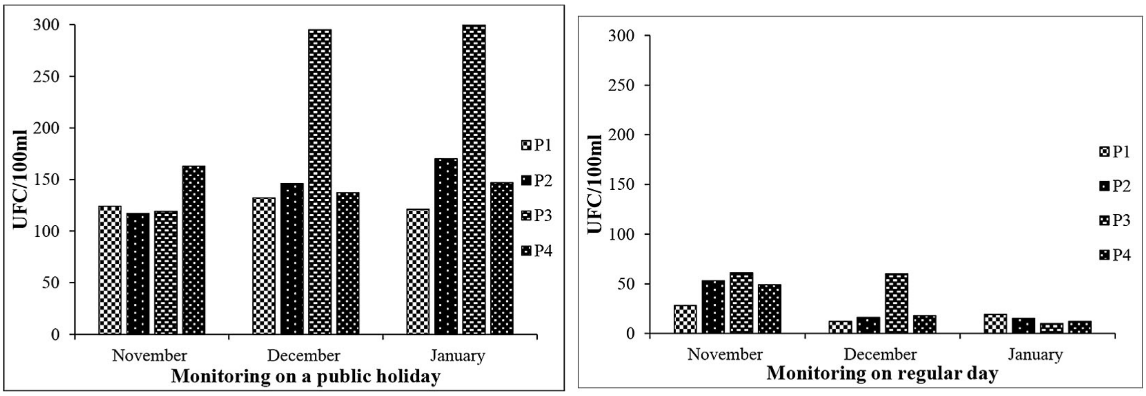

Figure 3. *E. coli* concentrations in seawater during and after the holiday

in concentrations as the months progress, at all sampling points the values increase significantly, in P1, concentrations went from 56 CFU 100 g^{-1} in November to 100 CFU 100 g^{-1} in January, this trend is repeated in the other points, with P4 registering the highest concentrations in all months, as for the days without a holiday, an opposite trend is observed, the concentrations are considerably lower compared to the holidays.

In November, concentrations vary between 56 and 99 CFU 100 g^{-1} at all sampling points, while, on non-holiday days, the values remained between 12 and 16 CFU 100 g^{-1}, reflecting a difference in concentrations, in December the values increased between 72 and 131 CFU 100 g^{-1}, while, on days without holidays, concentrations remained between 14 and 38 CFU 100 g^{-1}, finally in January concentrations reached their highest peaks on holidays, with values between 100 and 131 CFU 100 g^{-1}, while, on days without holidays, values continue to be lower, reaching a maximum of 53 CFU 100 g^{-1} in P2, dry sand being the matrix that presented the lowest concentrations of *E. coli*.

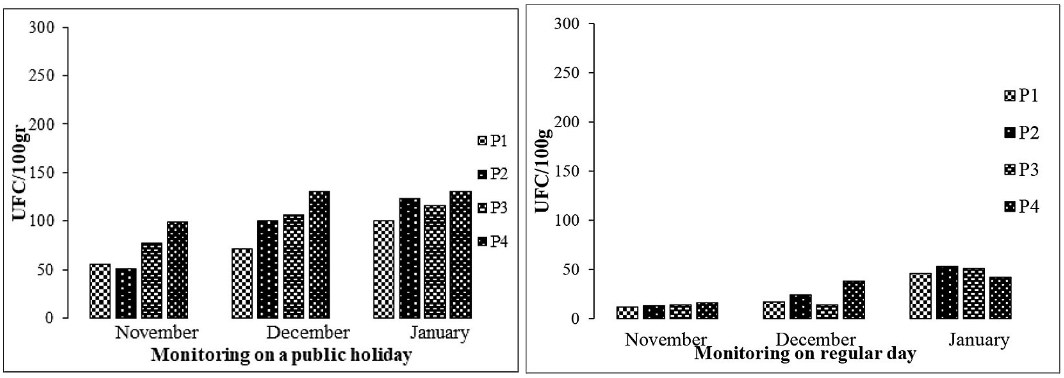

Figure 4. *E. coli* concentrations in wet sand during and after the holiday

On the other hand, Figure 5 shows the concentrations of *E. coli* in the wet sand, finding that during the holidays an increase in concentrations is observed, registering the highest values in January, where some measurements exceed 230 CFU 100 g^{-1} (P2 and P4), on the other hand, in the analysis on days without holidays, values are lower at all sampling points, concentrations remain mostly below 50 CFU 100 g^{-1}, with values closer to zero, indicating that during periods without high tourist influx faecal contamination is lower.

On the November holiday, for All Souls' Day, E. *coli* concentrations were between 100 and 150 CFU 100 g^{-1}, while on days without holidays these values are lower, not reaching 50 CFU 100 g^{-1}. In December, concentrations above 250 CFU 100 g^{-1} are observed in P4, while on days without holidays, concentrations remain below 50 CFU 100 g^{-1}. In January, concentrations reach values above 230 CFU 100 g^{-1} at points P2 and P4, while on other days, concentrations are below 20 CFU 100 g^{-1}.

Anderson-darling normality tests

The analysis of the data from the three samples: seawater, dry and wet sand, each with a sample size of 24 (Gl = 24), shows that Significance (Sig.) or p-value of Seawater: 0.475, Dry Sand: 0.317 and Wet Sand: 0.475, are greater than 0.05, suggesting that the three variables follow a normal distribution within the 95% confidence level (Table 3).

Pearson correlation

Once the normality assumptions were completed, Pearson's correlation coefficient analyses were performed to determine the relationship between the levels of E. *coli* contamination in water, dry sand, and wet sand. the results of these analyses are presented in Figure 6.

Graph 6 of dispersion, together with Pearson's correlation coefficient (-0.622 and p = 0.000), reveals a moderate negative relationship between the concentrations of *E coli* in seawater vs. dry sand, suggesting that as the influx of tourists on the beach increases, the concentrations of *E coli* increase, in terms of the properties of the sand if it is dry, the concentration decreases since in conditions of lower humidity, the bacterium is unable to maintain itself, possibly due to exposure to solar radiation and desiccation, on the other hand

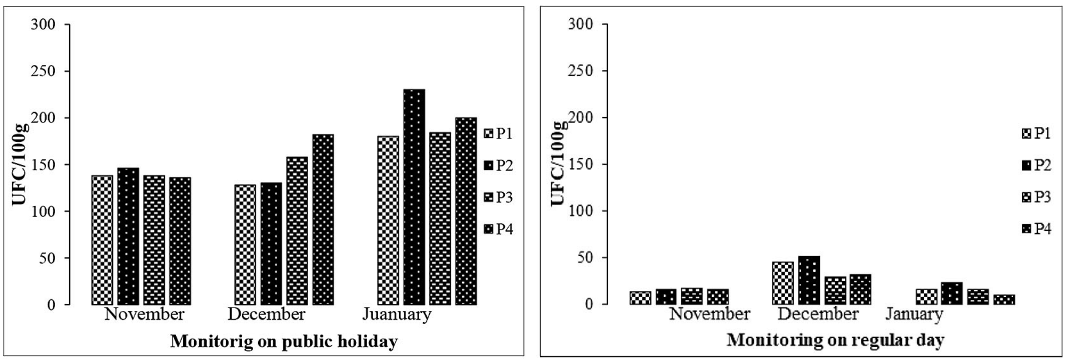

Figure 5. *E. coli* concentrations in dry sand during and after the holiday

Table 3. Anderson-Darling's normality test

Variable	Gl	Minimal	Maximum	Stocking	Gis.
Seawater	24	1.000	2.477	1.783	0.475
Dry Sand	24	1.079	2.117	1.748	0.317
Wet Sand	24	1.000	2.362	1.767	0.475

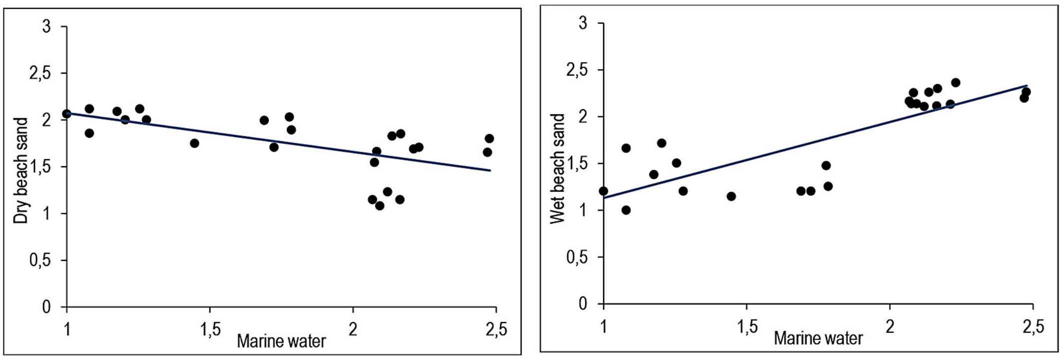

Figure 6. Pearson correlation test for the different matrices

a strong positive correlation is shown between (0.812 and p = 0.000) the concentrations of *E. coli* in seawater vs. wet sand, suggesting that as the concentrations of *E coli* increase In seawater, the amount in wet sand also increases due to moisture retention that favors its persistence, increasing the risk of contamination in coastal environments.

DISCUSSION

During the execution of the study entitled bacteriological distribution in seawater carried out in Cullera, between July and April the concentrations of *E. coli* varied significantly in relation to the variations of the area, with station 1 in Cabo Cullera registering the highest levels, reaching 1600 CFU 100 ml^{-1} in July and 406 CFU 100 ml^{-1} in August, while in September, most of the stations had values below 100 CFU 100 ml^{-1}, evidencing spatial and temporal variations in fecal contamination, this bacteriological distribution in the coastal zone was related to the hydrodynamics of the area, given the surface circulation of coastal waters (Cupul et al., 2020).

The study carried out by Pauta et al. (2020) bacteriological indicators of fecal contamination in Cuenca demonstrated the adaptability demonstrated by *E. coli*, since these bacteria were able to survive for long periods of time in a water for recreational use after the Easter holiday period, a gradual decrease in concentrations was observed, however, there was the presence of this bacterium until 16 days of monitoring, highlighting the adaptability to different conditions and environments, which makes it a health risk. However, Pico and Mendoza (2020) mention that the oceans have a natural mechanism to self-purify, this process is conditioned by several factors such as currents, solar radiation and temperatures, allowing these bodies of water to decompose more than 70% of organic pollutants in 72 hours, in the same way a reduction in the concentrations of pathogenic pollutants such as *E. coli* was evidenced and coliforms of more than 90% in 48 hours in regions with tropical climates

A study carried out in the Ayangue resort, Santa Elena province, showed that the concentrations of *E. coli* in the different points sampled during the days with the greatest influx of tourists, did not show statistically significant differences with a value of p = 0.3160; however, variations were found in the concentrations, where point one presented the highest concentration (144 CFU ml^{-1}). followed by seven (123 CFU ml^{-1}) and two (104 UFC ml^{-1}), and finally points six (86 CFU ml^{-1}), eight (74 CFU/ml), four (55 CFU ml^{-1}), three (42 CFU ml^{-1}), ten (41 CFU ml^{-1}) and five (37 CFUml^{-1}) (Velasco, 2023).

Velasco (2023) evaluated the concentration of *E. coli* between high tide and low tide conditions, here it was evidenced that there are no statistically significant differences between the concentrations obtained (p = 0.0707), on the one hand the total average of the sampling during low tide was 44.3 CFU ml^{-1}, while in high sand 171.08 CFU ml^{-1}, results that do not exceed the maximum permissible limit of 250 CFU ml^{-1}. On the other hand, Plúas et al. (2020) reported in the Chulluype Estuary concentrations of *E. coli* at low and high tide ranging from 300 CFU/ml to 2200 CFU/ml, however they attributed this increase to other variables, the correlation was positive in the parameters of pH (0.47), salinity (0.5) and conductivity (0.48) suggesting that as these parameters and the tide increase, so does the concentration of *E. coli*.

In Playa Blanca, Punta Leona, the highest pollution by *E. coli* it was detected in dry sand, this because more microorganisms accumulate here, while in wet sand, being exposed to tidal action and the presence of protozoa, there tends to be lower bacterial concentrations (Whitman et al., 2024), while in Jacó Beach, Costa Rica, the highest concentration was found in wet sand, influenced by variations in humidity caused by factors such as location, particle size, and water table, which influences the time of sampling and therefore concentrations (Byappanahalli et al., 2022), in addition this beach showed greater pollution in seawater influenced by the discharge of the Copey rivers, Naranjal and Madrigal in times of high tourist load that exceeded the limits allowed by the United States Environmental Protection Agency (235 CFU 100 ml^{-1}) (Badilla and Mora, 2019).

CONCLUSIONS

The characterization of the sampling points allowed the identification of factors such as ambient temperature, tidal coefficient, and coastline, which range between 35 and 78 meters, this coefficient has an influence on their concentration and dilution, depending on the dynamics of the waves and waves of the area under study.

The data show that, during holidays, the concentration of *Escherichia coli* increases, especially in December and January, contamination levels exceed 200 CFU/100 ml, which is the limit established by Ecuadorian regulations, the WHO, and the EPA for recreational water, which represents a risk to public health. On normal days, concentrations are much lower, complying with legislation.

Normality tests indicate that all three variables (seawater, dry and wet sand) follow a normal distribution. Pearson's correlation shows a strong positive relationship between the concentration of *Escherichia coli* in seawater and wet sand (r = 0.812), while the negative correlation between seawater and dry sand (r = -0.622). This indicates that wet sand may be a reservoir of *E. coli*, increasing the risk of contamination in coastal areas.

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