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Preliminary Microbiological Coastal Water Quality Determination along the Department of Atlántico (Colombia): Relationships with Beach Characteristics

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Abstract: Beach water quality is an important factor concerning public health and tourism linked to the "Sun, Sea and Sand" market and is usually assessed in international regulations by the quantification of *Escherichia coli* and enterococci counts. Despite *Salmonella* spp. detection not being included in international normative, the presence/absence of this bacteria is also an indicator of seawater quality. The objective of this study was to determine microbiological quality of beach water at 14 beaches along the Department of Atlántico (Colombia) and its relationship with beach characteristics as beach typology (i.e., urban, village, rural and remote areas), presence of beach facilities (e.g., bars, restaurants, etc.) and streams outflowing into the coastline. Sampling program aimed to analyse *E. coli* and *Salmonella* spp., by culture-based and real time PCR methods, respectively. Microbiological outcomes were compared with beach characteristics, and a cluster analysis was performed. *E. coli* and *Salmonella* spp. were detected in 70% and 20% of samples, respectively. Highest *E. coli* counts were observed at beaches classified as urban and at Sabanilla, a rural beach with presence of numerous beach restaurants/bars. *Salmonella* spp. presence was associated with streams that lack wastewater treatment systems. Cluster analysis clearly evidenced the relationship between *E. coli* and *Salmonella* spp. and beach characteristics, allowing to obtain indications to implement management programs. According to data obtained, monitoring programs have to be especially carried out in urban areas and at places with beach facilities. This could enhance microbiological water quality and consequently, beachgoers safety and touristic beach attractiveness to international visitors.

Keywords: seawater quality; *Salmonella* spp.; *E. coli*; PCR real time; beach classification

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1. Introduction

According to the World Tourism Organization, "travels and tourism" was one of the fastest growing and most dynamic economic sector in the world in 2019 [1]. International tourist arrivals have increased from 25 million in 1950 to 1.5 billion in 2019 and are estimated to reach 1.8 billion by 2030 [1]. In 2019, tourism generated 10.3% of global Gross Domestic Product, supporting 330 million jobs. Colombia recorded the arrival of c. 4.5 million travellers in 2019, which represented an increase of 2.7% with respect to 2018 [2].

Leisure tourism represented the most common sector (56%) among the travel purposes [1] and, within this, the "Sun, Sea and Sand (3S)" is the most attractive market [3]. Concerning the preferences of beachgoers, thousands of questionnaires have been carried out in different countries to find out that, despite travelling distance being a relevant selection condition, five aspects (the "Big Five"), i.e., safety, facilities, water quality, absence of litter, and scenery, are of the greatest importance to coastal visitors [3,4]. Beach users have an idyllic concept of the beach since they prefer sunny white sand beaches bordered by dark blue waters and luxuriant vegetation on land [5,6] as above. Therefore, according to the desires of beachgoers, tourism planners must be conscious of the 3S potentialities in their countries and have to enhance the attractiveness of their beaches in different ways if present natural conditions show low attractiveness or environmental characteristics [7,8].

In Colombia, tourism activity has a great potential in the Caribbean for its exuberant natural beauty and richness [9]; however, beaches close to Barranquilla, which from an administrative point of view belong to the Department of Atlántico, have not adequate environmental conditions to offer appropriate quality touristic services because the presence of abundant beach litter, vegetation debris and poor water quality [10–14]. Unfortunately, poor water quality conditions are common in the Caribbean and Latin America and in most cases are linked to the discharge of untreated or inadequately treated wastewaters, which contain a large load of microorganisms of faecal origin [15], many of which are human pathogens causing several diseases such as gastroenteritis, diarrhoea, typhoid fever, cholera, dysentery, etc. [16]. The detection and quantification of pathogens microorganisms is labour-intensive and not easy to perform because pathogens appear intermittently and at low concentrations in natural waters [17]. For this reason, water quality is evaluated by the use of several indicators of faecal contamination (FIB), being the most common the bacteria *Escherichia coli* and enterococci according to different environmental standards as the ones established by the European Union Council [18] (2006/7/EC) and the United States Environmental Protection Agency [19] (USEPA, 2012 RWQC). In Colombia, the quality of recreational waters (surface water, groundwater, seawater and estuarine water) is regulated by the decree No. 1594 of 1984 [20] (and modifications [21], i.e., decree No. 3930 of 2010), which establishes only the determination of total and faecal coliforms by the technique of most probable number (MPN). The most used methods to quantify FIB in seawater are based on bacteria cultivation mainly due to their easy implementation, low cost and the existence of robust relationships between results obtained with such methods and data on swimming-illness [22]; however, despite the wide use of FIB for seawater quality evaluation, these present numerous limitations because the short survival time of microorganisms in a water body, their possibility of multiply after their release in the water column, inability to identify the source of faecal contamination and low levels of correlation with the presence of pathogens, among others [17,20]. Furthermore, time required to obtain analysis results is a limiting factor because beaches remain open to bathers during the analytical processing period (from 18 to 96 h) and the contamination event has often disappeared meanwhile the analysis are still being carried out, that is, before the results are obtained [22]. For this reason, nowadays, the use of molecular techniques based on nucleic acid detection in water samples is greatly increasing: it gives a higher sensibility, specificity and reduces time analysis and therefore is possible to have results available for decision makers in a short time [23–25]. Such techniques allow the detection and quantification of pathogens as *Salmonella* spp., *Legionella* spp., *Vibrio cholerae*, and *entomopathogens* such as *E. coli*, among others. *Salmonella* spp. is a Gram-negative rod with more than 2500 serotypes classified according to the flagellar antigen H and somatic antigen O. It is considered a world-wide distributed pathogen causing gastroenteritis, typhoid and paratyphoid fever [26]. There are few studies related to the transmission of *Salmonella* spp. through seawater; however, recently its presence has been demonstrated by culture methods in coastal areas of India and Italy [27,28].

The main objective of this paper was the evaluation of seawater microbiological quality in the beaches of the Department of Atlántico, Colombia, through simultaneous detection by real time PCR of *Salmonella* spp. as pathogen microorganism and *E. coli* quantification by culture-based methods as faecal indicators and to determine relationships with beach characteristics based on natural and anthropogenic dimensions taking into account factors such as beach typology [4], and presence of facilities and streams/artificial channels. Further, such information was complemented with data presented by other investigations in the area in order to give a preliminary but global view on microbiological water quality characteristics along the whole coastal length of the department of Atlántico. The results presented here are not comparable with the limits imposed by the Colombian Standard (decrees No. 1594 of 1984 and No. 3930 of 2010) but can be compared with the standards presented in international literature/directives and/or required by international Beach Awards (e.g., the Blue Flag) and can be used to actualize the Colombian standard of microbiological quality of seawater. Therefore, this paper constitutes a preliminary study that can serve as a baseline for future research in an area with relevant environmental and degradation problems essentially linked to human pressure. The experimental design used here helps to identify microbiological health hazards in an area with no historical monitoring data, which is heavily used for bathing purposes. Information obtained, which relates water quality with beach typology and other aspects related to human interventions and impacts, is especially useful to local coastal managers and administrators to determine coastal sites that need main attention, e.g., a detailed monitoring program, and sound management actions, e.g., the regulation of human interventions, in order to enhance safety conditions for beachgoers and beach tourist attractiveness. The information obtained and the methodology used can be easily applied at other coastal areas in South America or other continents with similar settings and hence be useful to optimise monitoring programs that are time consuming and often too expensive and complicate for local administrations in developing countries.

2. Materials and Methods

2.1. Study Area and Seawater Sampling

Study area, which is about 60 km in length, is located in the Caribbean coast of Colombia, in the department of Atlántico, and includes 15 beaches between Sabanilla and Punta Astillero (Figure 1) [29]. The area is a tropical environment with two rain periods in April–May and October–November and two dry periods in November–April and July–September. Maximum precipitations are circa 2500 mm/year and mean temperature values are ca. 27 °C. Tidal range is mixed semi-diurnal, with maximum amplitudes of 60 cm [30], and Trade winds (*Alisios*) are frequent during December–March. Waves approach the coast from the third and fourth quadrants giving rise to a predominant SW directed longshore transport [31].

In situ sampling was carried out on 18 and 24 of May 2015, and thirty seawater samples were gathered in natural and, especially, tourist beaches (Table 1). In larger tourist areas, to have a better representation of water quality along the whole beach length, more than one sampling point was established with an average distance apart of ca. 150 m. Despite the relatively low number of samples, this survey constitutes the first attempt and approximation to cover the whole coastline of the department taking into account that 14 out of the 15 beaches of the department were surveyed and that many coastal sectors, ca. 9 km in total, are constituted by cliffed areas, which are totally inaccessible from land. Sampling was performed during early morning under low sunshine conditions and no bathers in the beach. At each location, 2 L of seawater were taken in the nearshore at a distance from the shoreline corresponding to approximately one-meter depth, using sterile Whirl-pack sampling bags. Sampling bags were introduced into the water at 30 cm depth to avoid water layer affected by ultraviolet radiation. Water samples were identified and immediately transported under refrigeration at 4 °C to the laboratory.

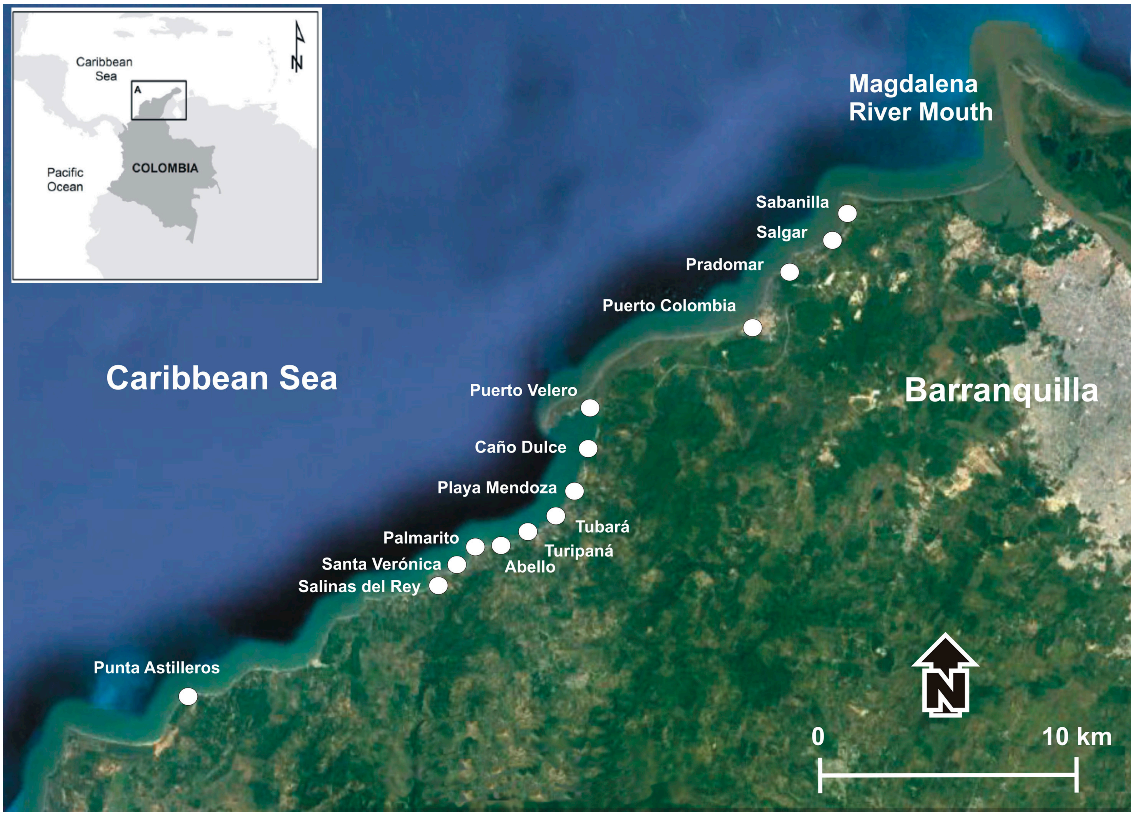

Figure 1. Beaches sampled along the department of Atlántico, Colombia.

Table 1. Average values of microbiological parameters and beach characteristics. *E. coli* counts are expressed in cfu/100 mL. Error is expressed as one standard deviation.

Beach	**Sample Number**	***E. coli***	***Salmonella* spp.**	**Stream/Channel**	**Beach Facilities**	**Beach Typology**
Sabanilla	1, 2, 3	248 ± 111	Absence	Absence	Presence	Rural
Salgar	4, 5, 6, 7	84 ± 48	Presence	Presence	Presence	Urban
Pradomar	8, 9, 10	51 ± 42	Absence	Presence	Presence	Urban
Puerto Colombia	11, 12	135 ± 8	Presence	Absence	Presence	Urban
Puerto Velero	13, 14, 15	21 ± 34	Absence	Absence	Presence	Rural
Caño Dulce	16, 17, 18	5 ± 4	Absence	Absence	Presence	Rural
Playa Mendoza	19, 20	0 ± 0	Absence	Absence	Presence	Village
Tubará	21	2 *	Absence	Presence	Absence	Rural
Turipana	22, 23	32 ± 43	Absence	Absence	Presence	Remote
Abello	24	0 *	Absence	Presence	Absence	Rural
Palmarito	25	7 *	Presence	Presence	Presence	Rural
Santa Veronica	26, 27, 28	1 ± 1	Presence	Absence	Presence	Village
Salinas del Rey	29	3 *	Absence	Absence	Absence	Remote
Astilleros	30	0 *	Absence	Absence	Absence	Remote

* Only one sample was taken.

2.2. Strains and Culture Media

Bacterial strains of *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076 were used to assess culture media. Culture media used for microbiological analyses were OXOID and MERCK brand, and their performance was evaluated according to the International Organization for Standardization (ISO) 11133-2/2011.

2.3. *Salmonella* spp. Detection Using Real Time PCR Method

DNA extraction was performed by filtering 1 L of seawater sample and the membrane filter was added to 250 mL of buffered peptone water (Merck, Darmstadt, Germany) and, incubated at 37 °C for 24 h. After incubation, 1.5 mL of culture media was used for DNA extraction. In a following step, bacterial biomass was suspended in 600 µL of TE buffer 1X (10 mM Tris-HCl Scharlab, Barcelona, Spain; 1 mM EDTA Duksan, Phnom Penh, Cambodia and pH 8.0). Afterwards, cellular lysis was performed with Lysozyme A (10 mg/mL pH 8.0) (VWR Solon, Ohio USA) and SDS 0.5% v/v (Scharlab, Barcelona, Spain). The mixture was incubated for 90 min at 37 °C to eliminate residual peptides and lipids and, next it was added 80 µL of ammonium acetate 3 M (Merck, Darmstadt, Germany) and 100 µL of NaCl 5M (Scharlab, Barcelona, Spain) and incubated during 10 min at 65 °C. Resulting suspension was treated with double volume of mixture Chloroform: Isoamyl alcohol (24:1) (Merck, Darmstadt, Germany) and centrifuged for 5 min at 12,000 rpm. DNA was precipitated with cold Isopropanol (Scharlab, Barcelona, Spain) and then washed with ethanol 70% (Merck, Darmstadt, Germany). After ethanol evaporation, it was hydrated with 20 µL of nuclease free water. DNA purity and concentration were determined at λ260 and λ280 in a Biospectrophotometer Eppendorf® (Hamburg, Germany).

Real time PCR amplification for *Salmonella* spp. detection was performed with the commercial kit All pathogenic *Salmonella* species genesig® Advanced Kit (PrimerDesign TM Ltd., Camberley, UK), which uses a Taqman® probe read in FAM channel. Reaction mixture was prepared according to manufacturer instructions, using DNA samples at 10–50 ng/µL concentration. Amplification protocol was carried out in a Real Time system CFX96TM Bio-Rad Laboratories, Hercules California and a thermocycler C-1000TM. It consisted of an initial step at 95 °C for 2 min, followed by 50 cycles of 95 °C for 10 s and 60 °C for 1 min. On all samples it was used an internal extraction control added during the step of cell lysis in the DNA extraction process, which was amplified together with DNA sample using another TaqMan® probe detected on VIC channel. The validation parameters of the method were a 100% of sensitivity, 87% of specificity, 94% predictive positive value, 100% predictive negative value, 0% of false negatives rate, 13% of false positives rate, 96% of efficiency and a detection limit of 1 cfu/L.

2.4. E. coli Quantification

E. coli analyses were performed according to ISO 16649-1:2013 (recently replaced by ISO 16649-1:2018) for *Escherichia coli* determination method, filtering 100 mL of sample and transferring the membrane filter to a petri dish with Trypton-Galle-X-glucuronid (TBX) Agar (Merck, Darmstadt, Germany) and incubated at 44.5 °C for 24 h. After incubation time, blue colonies β-glucuronidase positives were counted as true *E. coli*.

2.5. Sites Characteristics Determination

Information useful to beach characterization was collected during sampling, i.e., the presence of streams/artificial channels outflowing in the area, the presence of beach facilities (bars, restaurants, kiosks, etc.) and beach typology, the latter was determined according to the Bathing Area Registration and Evaluation (BARE) classification system [4]. This method classifies beaches on an anthropogenic dimension as remote, rural, village, urban and resort, taking into account criteria such as environmental conditions, accessibility, habitation/accommodation level and community services [4]. The categories identified in this paper are described below:

Remote areas are largely defined by difficulty of access (usually by boat or on foot—a walk of 300 m or more). They are not reached by public transport and have very limited temporary summer housing. Very limited restaurants and second homes may be found in the holiday season, occupied by a few people who may live there permanently [4].

Rural areas are located outside the urban/village environment. They are not readily reached by public transport and have virtually no facilities. Housing in rural areas is limited and is of a temporary (holiday months) or permanent nature but without community focal centres. They are valued by beachgoers for their quietness and natural qualities [4].

Village areas are situated outside the main urban environment but reached by public transport and associated with a small, but permanent, population with an organized community service. The village environment would also include 'tourist villages', mainly frequented during holidays months [4].

Urban areas serve large populations with well-established public services. In the proximity of most important urban areas are usually found commercial activities such as fishing/boating harbours and marinas. Urban beaches are located within or adjacent to the urban area [4].

2.6. Data Analysis

Beach characterization data and microbiological results were analysed with R software version 3.6.3 and R studio version 1.1.456. Measures of central tendency and dispersion were calculated for *E. coli* counts. Point-biserial correlation tests and Kruskal–Wallis rank sum test were utilized to evaluate relationships among *Escherichia coli* counts, *Salmonella* spp., presence of streams/channels, presence of beach facilities and beach typology. For the comparison of *E. coli* counts in categorical variables with more than two levels, was utilized a pairwise Wilcoxon test with Benjamini & Hochberg correction. Chi-square test was used to assess relationships between categorical variables.

Cluster analysis was performed to group beaches according to their microbiological and locative characteristics using the R packages pvclust version 2.2-0 and Cluster version 2.1.0. Hierarchical clustering was done using Ward method and creating a Euclidean distance matrix. The matrix created was organized on a Euclidean space and visualized using Principal components analysis (PCA) method. All statistical analyses utilized an alpha value of 0.05.

3. Results

Microbiological parameters were analysed and related to beach typology and characteristics such as the presence/absence of beach facilities and streams/channels outflowing on the shore. A cluster analysis was also performed to relate all previous aspects.

3.1. Beach Characteristics

Analysed beaches, as far as their typology, were classified as urban (21%), rural (43%), village (14%) and remote (22%), (Table 1). Urban beaches are located in the northern part of the Department of Atlántico near the city of Barranquilla and present the best access roads and always show facilities (restaurants, bars, kiosks, toilets, etc.). Streams were observed to discharge dark colour and unpleasant smell waters directly onto the beach in most of beaches belonging to this typology (Table 1). Even if no samples were taken in correspondence of streams, it was observed as they are continuous flows apparently linked to natural inland freshwater sources that record illegal pouring of wastewaters. Rural beaches, with the exception of Sabanilla, are located in the central-south part of the area and therefore relatively far away from large urban centres. In most of them, there are no facilities, and streams discharging wastewaters are often observed (Table 1). Village and remote beaches are located in the southern part of the department, with the exception of Santa Veronica. These beaches have no facilities and are very far away from urban centres, this aspect greatly limits the arrival of tourists. No streams were observed on such beaches.

3.2. Microbiological Parameters

Real time PCR method allowed to detect the presence of *Salmonella* spp. in 20% (6 out of 30) of samples (Tables 1 and 2; Figure 2A). Results showed that 3 out of 4 samples collected at Salgar were positive for *Salmonella* spp., i.e., at that place were collected 50% of all samples positive to *Salmonella* spp. Other beaches that recorded the presence of *Salmonella* spp. were Puerto Colombia, Palmarito and Santa Veronica (Table 1, Figure 3A).

Table 2. *Salmonella* spp. and *E. coli* counts according to the four different clusters in which beaches were grouped.

Cluster Number	Beach	Beach (%)	Number of Beaches *Salmonella* spp. Positive (%)	Number of Beaches per *E. coli Counts* (%)				
				<1 cfu/ 100 mL	1–10 cfu/ 100 mL	10–100 cfu/ 100 mL	100–200 cfu/ 100 mL	200–300 cfu/ 100 mL
1	Pradomar Puerto Velero Turipana	21	0	-	-	21.43	-	-
2	Abello Astillero Caño Dulce Palmarito Playa Mendoza Salinas del Rey Santa Veronica Tubará	57	14	21.42	35.71	-	-	-
3	Pto. Colombia Salgar	14	14	-	-	7.14	7.14	-
4	Sabanilla	7	0	-	-	-	-	7.14
Total		100	28	100				

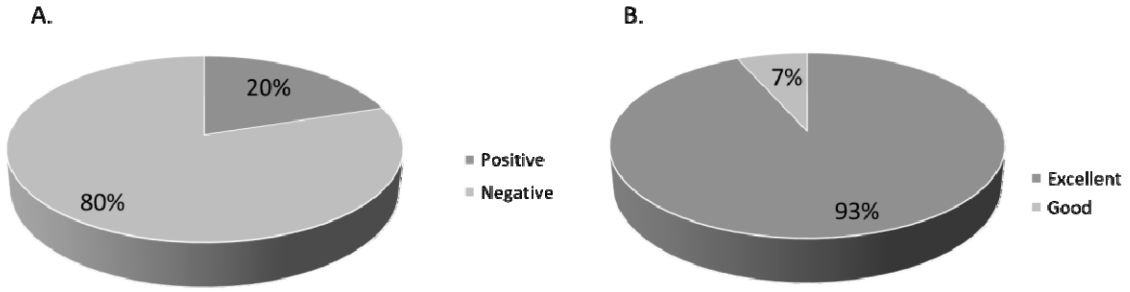

Figure 2. Pie chart of percentage of samples positive and negative for *Salmonella* spp. detection (**A**), percentage of samples complying Directive 2006/7/EC regarding *E. coli* concentration in seawater (**B**).

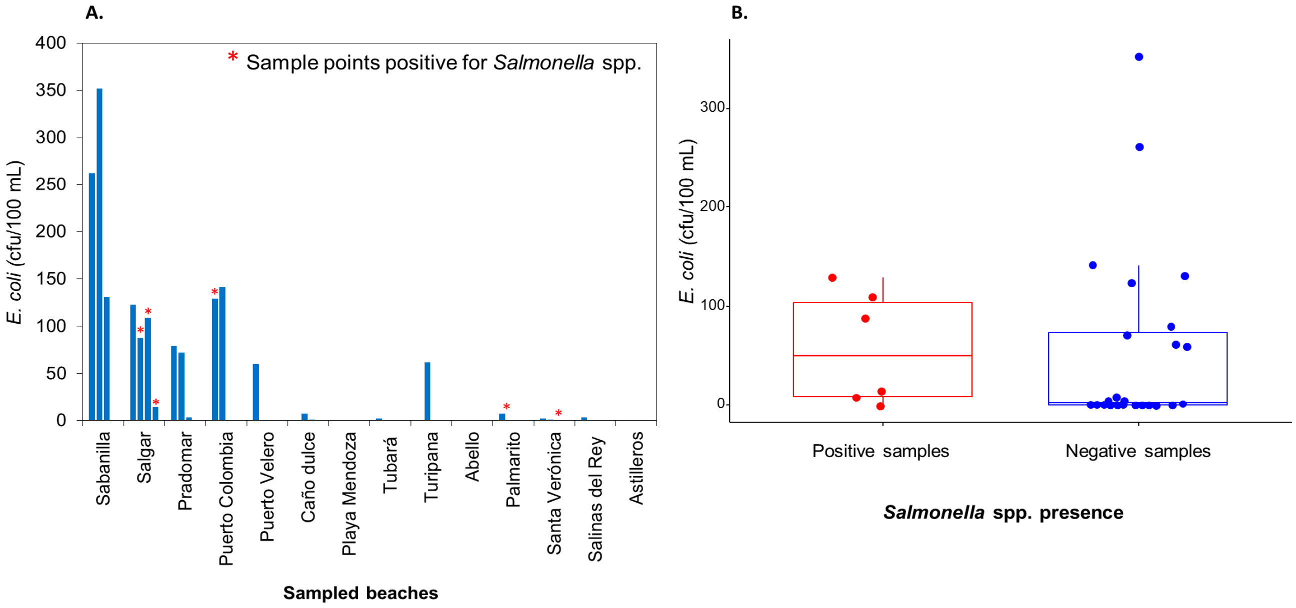

Figure 3. (**A**) Barplot representing *E. coli* count per each sampling point at beaches investigates. (**B**) Boxplot representing *E. coli* counts sorted according to their co-occurrence with *Salmonella* spp.

Regarding *E. coli*, 70% (21 out of 30) of samples presented this faecal indicator (Table 1). Specifically, 23.3% (7 out of 30) had *E. coli* concentrations above 100 cfu/100 mL and 6.7% (2 out of 30) presented values above 200 cfu/100 mL. Beaches located in the northern part of the coast, from Sabanilla to Puerto Velero (Figure 1), presented the highest counts with mean values between 21 and 248 cfu/100 mL. Beaches located in the southern part, from Caño Dulce to Punta Astillero (Figure 1), presented the lowest *E. coli* counts with values between 0 and 7 cfu/100 mL. Only the Turipana beach presented an average count of 32 cfu/100 mL. According to the Directive 2006/7/EC, all samples, except for Sabanilla, presented excellent water quality, with maximum *E. coli* values of 141 cfu/100 mL (Figures 2B and 3A).

Regarding the co-occurrence of *Salmonella* spp. and *E. coli* in samples, Kruskal–Wallis rank test showed neither any dependency nor any association between these two microorganisms (*p*-value 0.3052; Figure 3B) meanwhile a point-biserial analysis demonstrated a week correlation coefficient between them (R^2 = 0.0173). Only at Salgar beach (Sample no. 6, Table 1) and Puerto Colombia (Sample no. 13, Table 1) *E. coli* counts above 100 cfu/100 mL and the presence of *Salmonella* spp. were detected meanwhile samples gathered at Sabanilla, which showed *E. coli* counts above 200 cfu/100 mL, were not positive for *Salmonella* spp. Samples collected at Santa Veronica (Sample no. 28), which did not present *E, coli,* and at Palmarito, which presented only 7 cfu/100 mL of *E. coli*, were positive for *Salmonella* spp. (Table 1, Figure 3A).

3.3. Water Quality Versus Beach Characteristics

Cluster analysis allowed to group beaches investigated into 4 clusters according to their microbiological parameters and their typology and presence of beach facilities and streams (Table 2, Figure 4). Regarding microbiological parameters, beaches belonging to Cluster 1 presented *E. coli* counts between 21 and 51 cfu/100 mL, but the presence of *Salmonella* spp. was not detected. Concerning beaches characteristics, all beaches belonging to Cluster 1 were characterized by the presence of beach facilities, included urban, rural and remote areas, and streams were observed at 2 out of 3 sites (Table 2, Figure 4A,B).

Beaches belonging to Cluster 2 presented *E. coli* counts between <1 and 7 cfu/100 mL, and two of them were positive for *Salmonella* spp., and regarding beach typology, this cluster included rural, remote and village typologies. The 50% of beaches had facilities and only 3 out of 8 presented streams (Table 2, Figure 4A,B). Cluster 3 comprised Puerto Colombia and Salgar beaches and showed *E. coli* counts between 84 and 135 cfu/mL, and additionally, at four sampling points (3 in Salgar and 1 in Puerto Colombia), *Salmonella* spp. was detected. It included urban beaches with beach facilities and only a beach presented a stream (Table 2, Figure 4A,B).

Last, Cluster 4, including only a rural beach with facilities (i.e., Sabanilla), presented the highest average values of *E. coli* with 248 cfu/100 mL; nonetheless, no evidence of *Salmonella* spp. was found (Table 2, Figure 4A,B). No streams/channels were observed at this location (Table 1).

Furthermore, relationships between microbiological parameters distribution and beach characteristics indicated that *E. coli* counts presented a certain relationship with beach typology according to Kruskal–Wallis rank test (*p*-value 0.0125). Urban beaches had significant higher *E. coli* counts respect to village (*p*-value 0.0096), rural (*p*-value 0.0083) and remote beaches (*p*-value 0.0406) (Figure 4C). Noticeably, Sabanilla beach had significant higher *E. coli* values than urban ones (*p*-value 0.045). Likewise, *E. coli* counts are correlated to the presence of beach facilities (e.g., restaurants, kiosks, etc.) according to the Point-biserial test (R2 = 0.4249), having beaches with beach facilities on average values of *E. coli* of 78.19 ± 92.6 cfu/100 mL, meanwhile the ones with no facilities showed an average value of 0.6 ± 1.1 cfu/100 mL (Figure 4D). Similarly, Kruskal–Wallis rank test demonstrated significant dependency between these two variables (*p*-value 0.0009). On the contrary, no association was found between *E. coli* counts and presence of streams/artificial channels (*p*-value 0.2954). Chi-squared test confirmed previous observations indicating an association of *Salmonella* spp. with streams (*p*-value 0.049). Nevertheless, it was not observed any relation between the presence of *Salmonella* spp. and beach typology (p-value 0.1459) and the presences of beach facilities (p-value 0.7651).

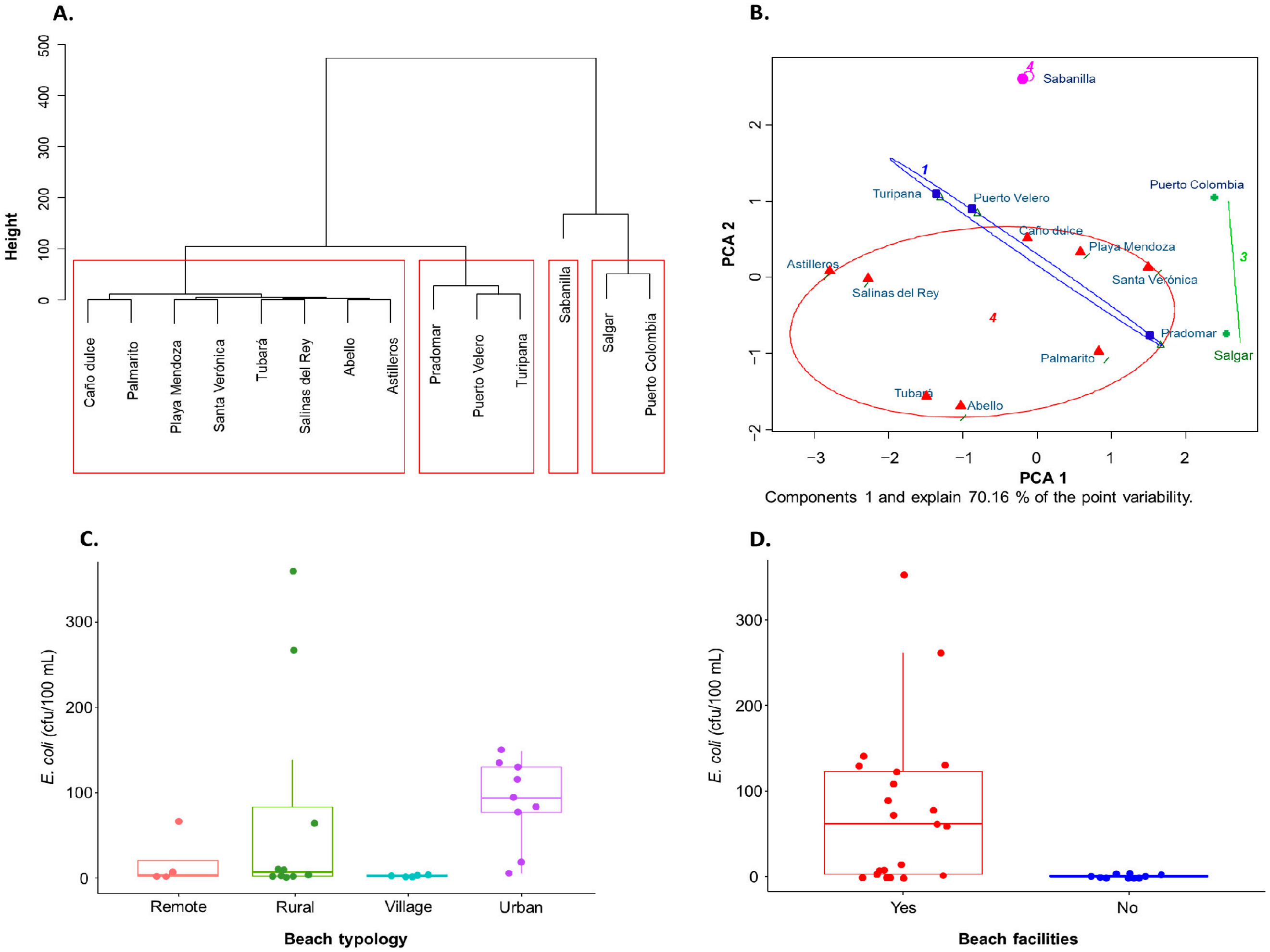

Figure 4. Clusters analysis grouping beaches according to microbiological parameters and beach characteristics. (**A**) Dendrogram of hierarchical clustering using Ward method. (**B**) Principal Components Analysis (PCA) calculated based on a Euclidian dissimilarity distance matrix. (**C**) Boxplot of *E. coli* counts according to beach typology. (**D**) Boxplot of *E. coli* counts according to presence or absence of beach facilities.

4. Discussion

4.1. E. coli and Salmonella spp. Distribution

Beaches are coastal ecosystems that are subject to great human influence, mainly related to the development of tourist activity [32]. For recreational purposes, five criteria have been identified by beachgoers when choosing a site: safety, facilities, water quality, absence of litter and landscape [4,33]. As for water quality, this parameter refers to both aesthetic, e.g., water turbidity, presence of algae, etc., and non-visible aspects, i.e., the microbial load [33]. The area investigated, i.e., the Department of Atlántico, is located in the Colombian Caribbean region and has a coastline length of approximately 64.5 km (4% of the Colombian Caribbean). Different studies have assessed seawater quality at specific places of such coastal sector, using only the detection of indicator bacteria of faecal contamination (e.g., Faecal Coliform, *E. coli*, *Enterococcus* and *Clostridium*) [12–14], but none took into account beach typology or used molecular techniques for the detection of pathogens (e.g., *Salmonella* spp. presence/absence).

Concerning *E. coli*, this microorganism is one of the most used microbiological parameters to evaluate water quality [23,34,35], and results obtained in the present study regarding this indicator are locally comparable to Torres et al. [36], who reported faecal coliform counts with a maximum value of 18 cfu/100 mL and 33 cfu/100 mL for Puerto Velero and Caño Dulce beaches, respectively. INVEMAR [14] reported that Puerto Colombia, Pradomar, Salgar, Caño Dulce, Puerto Velero and Santa Veronica beaches presented thermotolerant coliforms values between 78–920 MPN/100 mL and 2–1400 MNP/100 mL in 2018 and 2019, respectively. Sánchez et al. [12], which only covered the northern part of the area investigated in this paper, established the highest values of *E. coli* $\geq$ 500 cfu/100 mL on beaches located in urban areas (e.g., Salgar, Pradomar, Puerto Colombia and Northern zone of Puerto Velero), while beaches located in rural areas (Sabanilla and the southern zone of Puerto Velero) presented the lowest counts with values between 16 and 54 cfu/100 mL. Such authors associated high *E. coli* counts with the presence of polluted streams outflowing directly on the beach (Table 3). Sánchez et al. [12] reported low *E. coli* counts at Sabanilla beach that, in this study, was the beach with the highest counts (262–352 cfu/100 mL). Such differences may be related to *E. coli* survival time in seawater, i.e., the lapse time between the contamination event and the survey [37], that is, the intermittent nature of this kind of contamination (i.e., the contamination source is not constant) as observed by Torres-Bejarano et al. [13] in Puerto Velero, or the different location of the sampled points (Table 3). Additionally, Gram-negative bacteria lose quickly their ability to form colonies in seawater, although they can still be detected at high levels by viable counting methods [38], and *E. coli* in seawater by effect of solar radiation can enter into a reversible state of "viable but not cultivable": this can strongly influence count values [39]. The differences in the cfu numbers obtained for *E. coli* on this beach by this study and Sanchez et al. [12] may be associated with the sampling season, i.e., the survey in this study was carried out during the rainy season meanwhile Sanchez et al. [12] carried out the sampling during the dry season. From one side, increased runoff during rainy season favours the deposition of faecal bacteria on beaches. From the other side, high temperatures recorded during the dry season can generate VNC that cannot be detected by in vitro culture.

Despite *E. coli* presenting a great variability in this study, the level of contamination observed along the Department of Atlántico is relatively relevant if compared with values observed along the beaches of the Iberian Peninsula by Garrido et al. [40] where the highest *E. coli* count was only 17–22 cfu/100 mL and 89% of the beaches investigated presented counts between 0 and 8 cfu/100 mL and in studies carried out in north America, in Miami beaches, where the microbial load did not exceed the United States base guideline for faecal coliforms [41]. Data concerning counts of indicators microorganisms in Table 3, especially of *E. coli*, presented a similar trend in all the studies carried out in the study area: High counts are evident in urban beaches and in those with beach facilities. Therefore, is especially mandatory the development of permanent microbiological quality monitoring programs in this type of beaches to guarantee beachgoers' safety.

Concerning the detection of *Salmonella* spp., which represents itself a potential public health risk due to its pathogenicity causing multiple infections like stomach flu, bacteraemia, endocarditis, osteomyelitis, among others [42,43], was detected by means of the DNA method, this being so far the only study that detected a pathogen like *Salmonella* spp. on the Colombian coast with molecular techniques as real time PCR. DNA detection techniques have emerged as an alternative for the detection of pathogens in seawater, one limitation being the lack of differentiation between viable and non-viable cells [44]. Thus, an aspect that is considered critical in the use of molecular techniques in the water analysis is the persistence of DNA in aquatic systems [45], which is influenced by conditions such as DNA characteristics (e.g., conformation, size) and environmental factors both biotic (e.g., microbial communities, extracellular enzymes) and abiotic (e.g., temperature, pH, salinity) [46]. Furthermore, it has been established that nutrient limitation (e.g., phosphate) also plays a role in DNA weighting in marine waters [47]. Regarding this, Collins et al. [48] estimated that environment DNA in seawater can be detected for about

48 h, degrading 1.6 times faster in the terrestrially influenced inshore environment than the ocean-influenced offshore one. Bae and Wuertz [49] studied the persistence of Bacteroids in seawater, establishing that DNA could be detected for 177h. Due to this long persistence of DNA, the authors proposed that DNA detection, by PCR technique, can provide data on the origin and fate of faecal contamination in seawater, which allows the establishment of management mechanisms to ensure the quality of these waters.

Table 3. Seawater microbiological parameters recorded by different authors along the department of Atlántico.

Beach	**Indicator Microorganisms and Pathogens**						
	E. coli **(cfu/100 mL)**	***Enterococcus*** **(cfu/100 mL)**	**Fecal Coliform (MPN/100 mL)** (1)	**Fecal Coliform (cfu/100 mL)**	***Clostridium*** **(cfu/100 mL)**	***Salmonella*** **(Presence/Absence)**	**Reference**
Puerto Mocho	16 ± 6	7 ± 2	-	-	<20	-	[12]
Sabanilla	54 ± 12	≈50	-	-	<20	-	[12]
	248 ± 111	-	-	-		Absence	This study
Salgar	≈52 ± 8	≈50	-	-	<20	-	[12]
	-	-	170 and 79	-	-	-	[14]
	84 ± 48	-	-	-	-	Presence	This study
Pradomar	≈500	440 ± 16	-	-	<20	-	[12]
	-	-	78 and 490	-	-	-	[14]
	51 ± 42	-	-	-	-	Absence	This study
Puerto Colombia	>500	≈ 50	-	-	≈ 40	-	[12]
	-	-	170 and 1400	-	-	-	[14]
	135 ± 8	-	-	-	-	Presence	This study
Puerto Velero	>500	125 ± 20		-	≈ 120	-	[12]
	-	-	-	18	-	-	[36]
	-	-	-	67	-	-	[13]
	-	-	79 (2)	-	-	-	[14]
	21 ± 34	-	-	-	-	Absence	This study
Caño Dulce	-	-	-	33	-	-	[36]
			920 and 2				[14]
	5 ± 4					Absence	This study
Playa Mendoza	0 ± 0					Absence	This study
Tubará	2					Absence	This study
Turipana	32 ± 43					Absence	This study
Abello	0					Absence	This study
Palmarito	7					Presence	This Study
Santa Veronica			790 and 22				[14]
	1 ± 1					Presence	This study
Salinas del Rey	3					Absence	This study
Astilleros	0					Absence	This study

(1) Measurements performed in 2018 and 2019; (2) measurement performed in 2018.

Further, in this study, high sensitivity of real time PCR method allowed the detection of *Salmonella* spp. Percentages of *Salmonella* spp. (20%) detected in this study agree with the anthropogenic activity of the sampling area. In this regard, *Salmonella* spp. was detected in 6 sampling points (5, 6, 7, 11, 25 and 28) of which 67% were located in urban beaches. The most likely source of *Salmonella* spp. on these beaches is the discharge of wastewater (from polluted streams) without prior treatment, which contains a high variety of pathogenic microorganisms (e.g., *Salmonella, Shigella, Vibrio*, among other) that can cause disease outbreak [50]. Sanchez et al. [12] detected high concentrations of *E. coli* and *Enterococcus* in these streams, which flow directly into the beaches of the studied area. The average *E. coli* and *Enterococcus* counts reported by Sanchez et al. [12] were 4.1 × 10^5 cfu/100 mL and 4.3 × 10^2 cfu/100 mL, respectively, which demonstrated the elevated pollution of those streams with faecal matter that could also contain *Salmonella* spp. In addition, wastewater treatment plants are mostly designed to effectively eliminate faecal bacteria but are not able to eliminate pathogenic microorganisms [51]; therefore, wastewaters that are deposited directly on the beach can be a source of *Salmonella*, as was detected in this study.

Similar observations and trends were also observed in other countries, e.g., Yamara et al. [52] reported that 15% of analysed beaches had *Salmonella* spp. in sand, and *E. coli* was detected in 68% of California beaches. Similarly, Steele et al. [53] reported in California the presence of *Salmonella* spp. in up to 25% of samples, 6–86 gene copies per 100 mL using digital PCR. Moreover, Massinai et al. [54] analysed *Salmonella* spp. by culture-based methods in six different bathing beaches in Makassar City (Indonesia) during the rainy season. *Salmonella* spp. was detected in the 6 beaches with counts between 648 and 1081 cfu/100 mL, attributing such high counts to the presence of runoff waters and contaminated wastes, with highest count of *Salmonella* spp. observed in a densely populated area because of the absence of proper wastewater treatment. High counts were also observed in the Port Blair Bays, South Andaman (India) in the order of up to 10^{5} cfu/100 mL, and the occurrence of *Salmonella* spp. was significantly higher in water column samples than in marine sediments [27]. Percentages lower than those of this study have been determined in the province of Ferrara, Emilia Romagna region (Italy), where 2.8% of 137 seawater samples were positive for the presence of *S. enterica* subsp. *enterica* [28].

The lack of correlation between *E. coli* and *Salmonella* spp. recorded in this paper demonstrates that the absence of *E. coli* detected by culture-based methods does not indicate the absence of *Salmonella* spp. measured by real time PCR, as it was observed at Santa Verónica beach, where *E. coli* was not detected but *Salmonella* spp. was; these inconsistencies may be due to the differences in the sensitivity of such analytical methodologies, being the PCR much more sensitive than the culture methods. This facilitates the use of PCR especially in the diagnosis of pathogens and not of contamination indicators since quantitative data at low concentrations are difficult to obtain with a qPCR that, furthermore, is an expensive method [55]. Although the European bathing water quality criteria does not establish the risk associated with microorganisms as *Salmonella* spp. and *Candida albicans* [56], some studies, such as Bolton et al. [57], assessed the presence of *Salmonella* spp. in beach sand in North West and South West of England. Those beaches fulfilled the European Quality Standard (76/160/EEC), but 8 out of 10 beaches were positive for *Salmonella* spp.

As indicated by previous authors, beach sand can act as a refuge for microorganisms and act as a mechanism for replanting seawater with enteric pathogens [57–59]. However, more sensible methods, such as microfluidic quantitative PCR (MFQPCR), are able to correlate quantities of potential pathogens and the faecal indicator genetic markers in beach water and wastewater samples, but such correlation was not observed in sand and sediment samples [60]. Nonetheless, Yamahra et al. [52] established that faecal indicators and pathogens were slightly correlated between each other in sand. Baudard et al. [61] also demonstrated a strong correlation between Enterobacteriaceae counts and standard faecal indicators in seawaters using fluorescent in situ hybridization and solid phase cytometry. Consequently, the combination of culture-based method to determine bacterial indicators and PCR to target waterborne pathogens can provide a wider overview for a complete assessment of beach water quality.

4.2. Microbiological Load and Beach Characteristic

Several studies concerning beach characterization along the department of Atlántico (Caribbean Sea of Colombia) took into account their anthropogenic dimension, i.e., Gallardo et al. [29] carried out an evaluation of beach touristic potential, establishing that, in general, beaches have good accessibility, being Puerto Colombia and Salgar the ones with the best touristic infrastructures and facilities, and those aspects were confirmed in this study that grouped aforementioned beaches in the same cluster. They are both urban beaches close to Barranquilla, so they agglomerate a great number of tourists. Gallardo et al. [29] also evaluated services as potable water, energy and sanitation, indicating that Palmarito and Puerto Colombia beaches got a low score, while Santa Verónica, Sabanilla, Puerto Velero and Caño Dulce presented good conditions regarding services, lower water contamination, minor visual and acoustic pollution and great extensions of green zones represented by mangrove forests, dunes and creeping vegetation, being these beaches grouped principally in this paper in clusters 1 and 2; they reflected a low impact of anthropogenic activity and associated good microbiological quality.

Data recorded in this paper regarding *E. coli* and *Salmonella* spp. highlighted as elevated counts of *E. coli* and presence of *Salmonella* spp. were observed in densely populated areas, with the presence of streams that discharge wastewater directly onto the beach; a clear example is shown in Puerto Colombia Municipality because of the presence of streams that carry untreated, or not adequately treated, wastewaters [12]. This is probably the reason for high counts of *E. coli* [29], in what is considered one of the most critical health problems on tourist beaches in Latin America and the Caribbean [15,54].

The link between water quality and population density, indicated in this study by beach typology, explains the high *E. coli* counts reported by Sánchez et al. [12]. Regarding the *E. coli* values recorded in this paper, they can be related to the population increment from 2015 to 2019, especially in coastal municipalities located in the northern part, and the associated increase of discharged wastewaters directly into the beach that is also manifested by the detection of *Salmonella* spp. in different beaches, which is confirmed by the association between this pathogen and the presence of polluted streams [50,51]. The lack of appropriate systems for wastewater treatment observed in the central and southern parts of the coast, e.g., at Tubará, Piojó and Juan de Acosta and the discharge directly into the sea of wastewaters coming from treatment plants (e.g., Puerto Colombia) could be the main sources of those microorganisms in the Department of Atlántico [62,63].

The presence of faecal indicators as *E. coli* and *Salmonella* ssp., as shown in studies carried out in Europe [40] and at other places [64], is related to the large number of seasonal tourists, the presence of animals and the lack of adequate wastewater management policies. Such inadequate management, which gives rise to relevant health problems for bathers, highlights such beaches have not adequate conditions to provide good services to international tourists mainly due to their poor environmental aspects [36,65,66]. Efforts have to be carried out to improve their quality, i.e., water quality and also reduce the presence of solid wastes that would enhance their landscape value [67]. As observed by Rangel at al. [67], Caño Dulce, Puerto Velero and Sabanilla beaches are classified in Class IV of the Coastal Scenic Evaluation System method, i.e., as areas with low landscape values because they have been highly damaged by anthropogenic activities. Palmarito, Puerto Colombia, Salgar and Santa Verónica beaches are classified in Class V, i.e., as urban areas that are not very attractive with intensive development associated with a very high use and with low landscape values. In the case of Puerto Colombia and other urban beaches, the score obtained was due to the presence of beach litter and evidence of sewage discharge. Last, the best rated beaches were those located in rural areas that present low human impacts.

5. Conclusions

In this paper, the microbial contamination was evaluated, by counting *E. coli* and detecting *Salmonella* spp., of almost all coastal length of the department of Atlántico (Caribbean Sea of Colombia), and it was related to beach characteristics, based on anthropogenic and natural dimensions, as presence of streams, beach facilities and beach typology. As a result, it was possible to group the beaches investigated into 4 clusters. This information is of great interest for local coastal managers and administrators to design sound monitoring and management actions at each place according to beach typology and human activities developed there.

It was found that *E. coli* counts were correlated to beach typology and the presence of facilities and ranged from 0 to 241 cfu/100 mL giving to the sites analysed a good quality according to the Directive 2006/7/EC. It has to be highlighted that beaches with high *E. coli* counts were those close to urban areas and beaches with facilities; hence, special attention has to be devoted in the future to those areas to maintain and enhance their water quality and design sound monitoring controls. Likewise, it was possible to determine the presence of *Salmonella* spp. by real time PCR in 20% of the samples analysed, essentially in urban beaches and in presence of streams outflowing on the beach, this being the first work that reports the presence of this microorganism in the department of Atlántico, therefore constituting a baseline research for this pathogen. All previous results evidenced that it is necessary to implement of plans and policies that promote the upgrading of beach water quality and a more sustainable tourism. Since monitoring and water analysis are expensive and time consuming, urban areas and sites with beach facilities have to be especially examined respect to remote areas that show low or null microbiological load. Concerning beach facilities, especially bars and restaurants emplaced onto the beach, usually have no toilettes or restrooms, and those that have such services are not usually connected to the sewage system, as a result wastewater end up always directly into the sea. Special attention has to be also devoted to characterising discharges of streams, which seem to be a source of contamination because of the illegal pouring of wastewaters, and appropriate wastewater treatment has to be implemented and enhanced by local administrations.

Last but not least, it is mandatory to actualize and/or develop a new national regulation in Colombia for seawater microbiological characterization based on modern methods/techniques and hence enhance it to fit to international standards and allow to obtain more reliable and quick results, an aspect often of great relevance for local decision makers.

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