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Microbiological contamination and antimicrobial resistance in seawater samples from three beaches in Trujillo, Peru

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Abstract*– Beaches worldwide are popular tourist destinations that attract both local and international tourists and the increase in population density in the summer season generates an increase in domestic and industrial waste dumped into the seas and oceans, the present work of The objective of the research is to determine the antibacterial sensitivity of bacteria isolated in seawater; For which the three most popular beaches of La Libertad were sampled during October and November of 2023. The results obtained showed that the beaches of Salaverry have good microbiological quality, while the microbiological quality of the beaches of Las Delicias and Huanchaco is poor; E coli being the bacteria that is isolated most frequently and presents resistance to Ampicillin (AMP) and Ciprofloxaxin (CIP): this research concludes that due to the dumping of wastewater in coastal areas, microbiological contamination of the beaches is produced, which is a danger. latent for bathers who come during the summer.

Keywords*-- Wastewater, Microbiological contamination of beaches, Bacterial resistance, Bathers.

I. INTRODUCTION

Beaches worldwide are popular tourist destinations that attract both local and international tourists [1,2] and especially during high season such as the summer season where tourists tend to come more frequently in search of relaxation and fun [3, 4]. After the COVID-19 pandemic, beaches have become a very popular coastal and marine tourist destination [5,6], where people carry out activities such as swimming, surfing, sunbathing, as well as tasting local cuisine, ecotourism with sightings of marine flora and fauna [1,7].

Tourist activities in coastal areas cause the beaches to receive a large number of visitors, with a positive impact on the local economy [8], but often negative for the environment because the natural spaces and the health of the people are threatened. people [9]. The increase in population density in coastal areas turns them into densely populated areas, generating an increase in domestic and industrial waste that usually reaches the seas and oceans through direct or poorly controlled discharges into rivers [3,10].

Over time, the seas and oceans have been used as dumping grounds for waste generated by the domestic and industrial activity of the populations that are located near the coast, with devastating consequences for the marine environment and that is increasing over time. global level [11]. It is mentioned that 80 to 90% of the wastewater generated is discharged without adequate treatment into natural water bodies, which ultimately flow into marine ecosystems, this practice being more frequent in developing countries [12]. Among the waste dumped in rivers, which reach the sea directly, directly affecting marine life and coastal ecosystems, are domestic and industrial waste, untreated or inadequately treated wastewater, plastics and chemicals [13,14].

The presence of different types of contaminants in seawater affects its quality and the biodiversity of marine life, also affecting the health of people who have direct contact with these contaminated waters [15,16]. Microbiological contamination of waters coastal areas has generated concern at a global level because its contamination with industrial and domestic wastewater causes serious problems for people's health and alters marine ecosystems in areas close to said discharges due to the high number of pathogenic and opportunistic microorganisms found. in wastewater [17,18].

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Swimming at beaches where the water or sand is contaminated can cause illness, with children, the elderly, and people with weak immune systems being the most likely to contract illnesses [10,14,18]. The World Health Organization (WHO) and the United States Environmental Protection Agency (EPA) have indicated that various substances can be found in beach water that can cause serious health problems for people, mainly to bathers, where gastrointestinal and skin diseases are the most frequent diseases that occur when contacting or swallowing contaminated seawater [6,18,19]. Gastroenteritis is the most common disease that is acquired when we swim in water contaminated by sewage. This disease appears in various forms and can present one or more of the following symptoms: nausea, vomiting, stomach pain, diarrhea, headache or fever; while contact of contaminated water with skin, eyes or exposed wounds can cause a mild infection that can later be severe if it does not receive adequate treatment [20,21].

Peru is a country that is home to a great diversity of beaches, from the quiet and peaceful ones to the most lively and crowded, which makes them suitable for all types of audiences [3,22]. The beaches of Trujillo are located on the northern coast of Peru, with Salaverry, Las Delicias and Huanchaco beaches being the most popular and visited by families, groups of friends and foreign visitors [23], due to their impressive landscapes, warm climate and to the rich cultural and gastronomic offering that offers visitors a place to relax, practice water sports, swim, enjoy the sun and explore the history and beauty of the region [5,24].

The Regional Health Management (GERESA), as well as the Regional Health Directorates (DIGESA) are the bodies in charge of preventing, controlling and evaluating the health quality of the beaches on the Peruvian coast, which are governed under Health Directive No. 038. /MINSA-DIGESA [25]; With the information that is gathered weekly, at the beginning of summer 2024, it was reported that 77% of the beaches in Peru are considered unhealthy for not meeting quality standards, such as correct cleaning and availability of functional hygienic services; This information shows that of the 312 beaches evaluated, in 78 a concentration of bacteria higher than the levels recommended for the safety of bathers was found [26].

In La Libertad from 2018 to the present, the DIGESA portal in its healthy beaches section has reported the presence of thermo-tolerant coliforms on the beaches of Salaverry, Las Delicias and Huanchaco; reporting peaks of up to 110, 560 and 1600 thermotolerant coliforms in 100 mL of seawater, respectively [27]. In different studies where the presence of thermotolerant coliforms in seawater and marine species has been reported, bacteria from this group have also been isolated and identified in order to study the degree of sensitivity to antibiotics [28,29,30]; The results obtained in these studies show cultures of enteropathogenic E coli (EPEC) resistant to penicillins and sulfonamides [31] and strains of *Proteus vulgaris, Enterobacter sp.* and quinolone-resistant *Klebsiella spp.* [30]. Antimicrobial resistance represents a major threat to patients and healthcare systems because the treatment and pharmacological therapy of bacterial infections is limited because many microorganisms are already developing or acquiring resistance to antibiotics [32,33].

Based on the aforementioned and with respect to the health problems caused by the presence of thermotolerant coliforms and *E. coli* in seawater, this research work seeks to quantify these microorganisms in seawater, isolate and identify the bacteria. present in seawater, as well as their sensitivity and resistance to antimicrobials in order to expand our knowledge and alert the authorities about the exposure to the danger that people who visit the three most popular beaches in Trujillo, La Libertad are exposed to.

II. Material and Methods

A. Study area

The study was carried out on three beaches located in the central area of Trujillo, including Salaverry, Las Delicias and Huanchaco (Fig. 1) because these beaches are popular tourist destinations that attract both local and international tourists, especially during the season. summer where a greater presence of tourists is observed on the beaches; Furthermore, the study area is influenced by the discharge of the Moche River and other small rivers where poorly treated wastewater is discharged, which comes from the wastewater treatment plants (WWTP) of Covicorti and Cortijo, as well as domestic wastewater. that are discarded along the riverbeds that reach the beaches, causing a significant impact on the local ecosystem and the organisms that inhabit it [34].

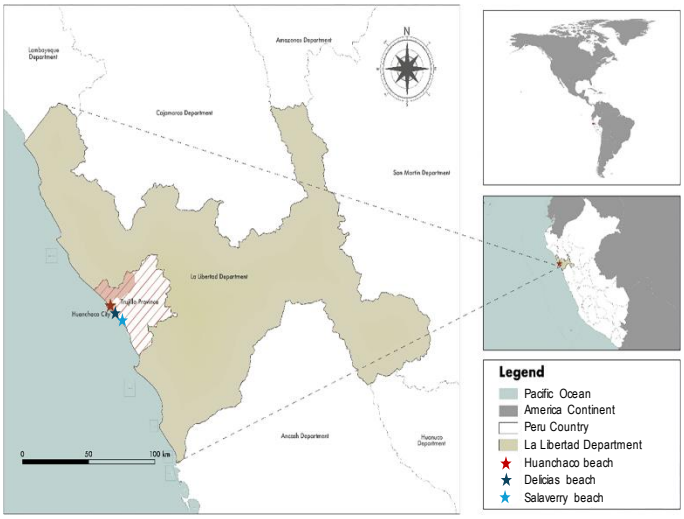

Figure 1. Geographic location of the beaches of Salaverry, Las Delicias and Huanchaco, La Libertad, Peru (Source: The figure is similar but not identical to the original image shown by Regalado-Pezúa et al. (2023)).

B. Sample collection

Four samples were carried out during the period from October 2 to November 20, 2023, prior to the summer season of 2024, where a total of 12 samples were taken, 4 samples for each beach. The collection of seawater samples was carried out in accordance with the provisions of the Technical Guide "Procedure for Sampling Seawater in Bathing and Recreational Beaches" RM No. 553-2010/MINSA issued by the General Directorate of Health. Environmental [35]. This standard mentions that the sterile bottle must be submerged 30 cm below the surface of the water and the sample will be taken in countercurrent.

The samples were collected in areas where there was a greater influx of bathers (bathing area) and that were close to the shore, proceeding to submerge a sterile flask of 500 ml capacity, in points where the depth of the water was approximately 1 meter. where the sampling bottle was introduced approximately 30 cm below the surface of the water and in countercurrent to fill it up to 2/3 parts, then the bottle was closed, identified and stored in an isothermal cooler at 4°C for transfer to the laboratory of the Science and Technology Research Institute of the Cesar Vallejo University - Trujillo Campus where the microbiological studies were carried out.

C. Determination of Thermotolerant Coliforms and E coli

Analyzes were performed weekly using the multiple tube fermentation technique established in the Standard Methods for the Examination of Water and Wastewater [36]. This procedure was developed by inoculating the seawater sample in a series of 5 tubes in decreasing dilutions of 10 ml double concentration, 1 ml and 0.1 ml of single concentration of Lauryl Sulfate Tryptose (LST) broth for a presumptive phase where the tubes inoculated were incubated at 37°C for 24 to 48 hours; Then these presumptively positive tubes were subjected to the confirmatory test for Thermotolerant Coliforms and *E. coli*, using EC broth where an aliquot of the positive pre-tubes from the presumptive phase was inoculated and then incubated in a water bath at 44.5°C for 24 hours. To confirm the presence of thermotolerant coliforms, the presence of turbidity and gas production must be observed in the tube (bubbles inside the Durham tube that is inverted in the EC broth). The calculation of the results is established based on the NMP tables and is expressed in NMP/100ml. While confirmation of the presence of *E. coli* was carried out by the Indole test and by the characteristic growth on Mac Conkey agar and EMB agar.

D. Isolation and Identification of Bacteria present in seawater.

The procedure consisted of sowing, using the streaking technique, an aliquot of seawater on the surface of Petri dishes with Mac Conkey agar and EMB agar; then the plates were incubated at 35°C for 24 to 48 hours. The isolated colonies were replicated in tubes with nutrient agar where the purity of the culture was evaluated before proceeding to identify them on the Vitek 2 equipment present in the Microbiology laboratory of the Institute for Research in Sciences and Technologies of the Cesar Vallejo University - Trujillo Campus. The Vitek2 microbiological identification system is an automated bacterial identification system where a bacterial suspension is inoculated on identification cards that have 62 biochemical tests, which makes it possible to identify the bacterial species to be identified with up to 99% specificity [37].

E. Antibiotic sensitivity testing

In each isolated culture, sensitivity to 11 antimicrobials was evaluated, such as: Ampicillin 10ug (AMP), Amoxicillin - Ac. Clavulanic 20/10ug (AUG), Cefuroxime 30ug (CXM), Ceftriaxone 30ug (CRO), Imipenem 10ug (IMI), Meropenem 10ug (MRP), Ertapenem 10ug (ETP), Gentamicin 10ug (CN), Amikacin 30ug (AK), Ciprofloxaxin 5ug (CIP) and Trimethoprim-sulfamethoxasole 1.25/23.75ug (SXT).

To determine antimicrobial susceptibility, the disk diffusion method was used. The procedure consisted of mass seeding of a known concentration of the bacteria on the surface of the Mueller-Hinton agar, with the help of a sterile swab, achieving a uniform dispersion, and then placing the disks of known antibiotics. The inoculated plates were incubated at 35°C for 24 to 48 hours. After this period, the growth zones and halos of bacterial growth inhibition were observed, which will be measured in millimeters and compared with the reference tables to determine 3 categories. (Susceptible, Intermediate or Resistant) [38,39].

III. Results

In Table I shows the results of Thermotolerant Coliforms and *E. coli* present in seawater samples from three beaches in La Libertad, between October and November 2023; where it is observed that over time the Salaverry beach has less microbiological load compared to the beaches of Las Delicias and Huanchaco, and is the only one suitable in reference to the current national regulations that establish that the maximum limit is 200 and <1.8 NMP /100 mL of thermotolerant Coliforms and E coli, respectively, as established in SUPREME DECREE No. 004-2017-MINAM where Environmental Quality Standards (ECA) for Water are Approved [40].

TABLE I
THERMOTOLERANT COLIFORMS AND *E. COLI* PRESENT IN SEAWATER SAMPLES FROM THREE BEACHES IN LA LIBERTAD, BETWEEN OCTOBER AND NOVEMBER 2023

Date	Beach	Thermotolerant Coliforms (NMP /100 mL)	*E. coli* (NMP /100 mL)	*Thermotolerant Coliforms (NMP /100 mL)	**Microbiological qualification of the beach
10-Oct-23	Salaverry	2	<1.8	2	GOOD
	Las Delicias	140	140	130	BAD
	Huanchaco	540	540	920	BAD
24-Oct-23	Salaverry	2	<1.8	2	GOOD
	Las Delicias	140	140	130	BAD
	Huanchaco	350	350	220	BAD
07-Nov-23	Salaverry	0	0	0	GOOD
	Las Delicias	170	170	240	BAD
	Huanchaco	920	540	540	BAD
21-Nov-23	Salaverry	0	0	0	GOOD
	Las Delicias	1600	1600	920	BAD
	Huanchaco	33	33	23	BAD

(*) Values reported by DIGESA on its healthy beaches web portal.

(**) Qualification based on *E. coli* count obtained in comparison with the maximum value established in SUPREME DECREE N° 004-2017-MINAM

The presence of thermotolerant coliforms and *E. coli* in seawater is indicative of fecal contamination and/or wastewater that was not properly treated. The high values of microbiological contaminants are mainly related to the discharges of wastewater that were not properly treated that reach the beaches directly [41,42]. The discharge of untreated wastewater continues to be a common practice, especially in developing countries, because they do not have the necessary infrastructure, technical and institutional capacities and financing [11,14,43]; The presence of these microorganisms is worrying due to which can cause gastrointestinal infections in bathers due to contact with contaminated water when people bathe on the shores of beaches or when they do some recreational water sport [18,19,23]. During the period that the monitoring was carried out, it was evident that Las Delicias and Huanchaco beaches are categorized as not microbiologically suitable because they exceed the maximum limit of thermotolerant coliforms and E coli, according to those established by the Environmental Quality Standards (ECA) for Water. [40].

In Table II, shows the bacterial species isolated and identified in seawater samples from three beaches in La Libertad, between October and November 2023; where it is observed that of the total of 22 isolated cultures, *E. coli* is the most prevalent bacteria and was identified in 8 cultures from seawater.

TABLE II
BACTERIAL SPECIES ISOLATED ON THREE BEACHES IN LA LIBERTAD, BETWEEN OCTOBER AND NOVEMBER 2023.

Beach	N° of Isolated Cultures	Identified microorganism (amount) - (species)
Salaverry	02	01 - *Enterobacter cloacae*
Las Delicias	09	04 - *Escherichia coli,* 03 - *Enterococcus faecalis,* 02 - *Enterobacter aerogenes*
Huanchaco	11	04 - *Escherichia coli,* 03 - *Enterococcus faecalis,* 02 - *Enterobacter aerogenes,* 02 - *Klebsiella pneumoniae*

In Latin America and the Caribbean, only about 60% of the population is connected to a sewage system and only 30% to 40% of the region's wastewater collected is treated efficiently [44]. It has been reported that, in the province of Trujillo, the wastewater treatment plants do not have the capacity to treat the volume of wastewater generated by the population of Trujillo, it has also been shown that along the Moche River bed and other rivers discharge untreated wastewater which reaches the beaches directly [45,46]. The presence of *E. coli* on the beaches of Libertad due to contamination with wastewater is not a current problem, and the presence of Thermotolerant Coliforms and *E. coli* was reported on the beach of Huanchaco in 2015 [47] and in 2017 [48]. This problem of microbiological contaminants has not only been reported in Trujillo; Various investigations have reported thermotolerant coliforms and *E. coli* on the beaches of the Costa Verde [49], Chorrillos and Ancón [31], in Moquegua [50], Huacho [51], Pucusana [52] and Arequipa [53]; The presence of *Klebsiella pneumoniae* is being reported for the first time in seawater off the Peruvian coast because most studies focus on determining the microbiological quality of seawater through the count and presence of Thermotolerant Coliforms and *E. coli*; and they do not direct the search to determine the presence of *Klebsiella pneumoniae* in seawater and marine species as is done in other countries,

where it is vitally important to demonstrate the presence of this microorganism in seawater, marine sediments, bivalve mollusks and fish, since It is known that this microorganism is capable of acquiring resistance to antibiotics [28].

In Table III, shows the bacterial sensitivity and resistance to 11 antibiotics that were evaluated in all the microorganisms isolated in seawater from three beaches in La Libertad, between October and November 2023.

TABLE III
BACTERIAL SENSITIVITY OF THE BACTERIAL SPECIES ISOLATED ON THREE BEACHES IN LA LIBERTAD, BETWEEN OCTOBER AND NOVEMBER 2023.

Bacterial species	Antimicrobial sensitivity		
	Sensitive	Intermediate	Resistant
Escherichia coli	AUG,CXM, IMI, MRP, ETP, AK, CIP y SXT.	CRO, CN	AMP, CIP
Enterococcus faecalis	AMP, AUG, CXM, CRO, IMI, MRP, ETP, CN, AK, CIP y SXT.		
Enterobacter aerogenes	AMP, AUG, CXM, CRO, IMI, MRP, ETP, CN, AK, CIP y SXT.		
Klebsiella pneumoniae	AMP, AUG, CXM, CRO, IMI, MRP, ETP, CN, AK, CIP y SXT.		
Enterobacter cloacae	AMP, AUG, CXM, CRO, IMI, MRP, ETP, CN, AK, CIP y SXT.		

Ampicillin 10ug (AMP), Amoxicillin - Ac. Clavulanic 20/10ug (AUG), Cefuroxime 30ug (CXM), Ceftriaxone 30ug (CRO), Imipenem 10ug (IMI), Meropenem 10ug (MRP), Ertapenem 10ug (ETP), Gentamicin 10ug (CN), Amikacin 30ug (AK), Ciprofloxaxin 5ug (CIP) and Trimethoprim-sulfamethoxasole 1.25/23.75ug (SXT).

Worldwide, a progressive increase in diseases caused by resistant and multi-resistant bacteria has been observed; which is resulting in ineffectiveness in the treatment of bacterial infections with antimicrobials [54].

In South America, the presence of enterobacteria resistant to beta-lactams has been demonstrated in hospital wastewater that was not adequately treated, making these effluents a potential risk for people when they contaminate surface waters and coastal waters [55]. In the present study it was found that *E. coli* presents resistance to Ampicillin (AMP) and Ciprofloxacin (CPX), these results coincide with the studies carried out on the beaches of the Costa Verde [49] and on the beaches of Ancon and Chorrillos [31], while the strains of *Enterococcus faecalis, Enterobacter aerogenes, Klebsiella pneumoniae* and *Enterobacter cloacae* are sensitive to the 11 antibiotics evaluated, and these drugs can be used in antibiotic therapy for lesions acquired on the beaches as recommended in other studies carried out. in North America where gram-negative bacteria isolates were susceptible to cefepime, lomefloxacin and levofloxacin [29].

It is known that *E. coli* is the microorganism that has the greatest tolerance for developing in seawater [56,57], this is because small genes that affect its sensitivity to seawater can mutate, thus allowing this microorganism to adapt to marine environments. The mutation of the rpoS gene is the one that stands out the most when a transfer of this bacteria to a marine environment occurs [58].

The abundance and diversity of antibiotic resistance in oceans and seas is affected by gene transfer between bacteria and the distance between coastal areas and locations where untreated or poorly treated wastewater is disposed of [59]. Antibiotic resistance genes can be transferred horizontally by multiple mechanisms including conjugation, transformation and transduction [60, 61]. In various studies it has been reported that antibiotic resistance genes can be transferred horizontally through the facilitation of the class 1 integron-integrase gene (intl1) in urban beaches through the process of plasmid conjugation between bacteria [59]; and this occurs more frequently in areas where seawater and wastewater mix, where genes for resistance to chloramphenicol (floR, cmlA) and sulfonamides (sul1) have been found [62, 63], while in areas coastal areas of Montevideo, Uruguay, the presence of resistance genes to β-lactams was found [64]

V. CONCLUSION

Only one of the three busiest beaches in Trujillo has good microbiological quality, putting the health of the people who come to bathe on these beaches at risk.

E. coli is the bacteria most frequently found in seawater samples due to its ability to tolerate and grow in seawater. This bacteria is the one most reported in other research and is also the cause of gastrointestinal infections in people who consume contaminated water.

Despite not finding bacterial species that present a high degree of resistance to antibiotics, the exposure of people to the beach waters continues to be considered a latent danger for bathers who would come in the next summer season.

Despite not finding bacterial species that present a high degree of resistance to antibiotics, the exposure of people to these contaminated waters continues to be considered a latent danger for bathers who would come in the next season, for this reason it is important to continue monitoring and research that helps to understand the risks and biological dangers to which people are exposed.

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