Bacteriological assessment of the recreational water of Bacuit Bay, El Nido, Palawan, Philippines

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ABSTRACT

In early 2018, the recreational water of Bacuit Bay in El Nido, Palawan, Philippines was declared by the Department of Environment and Natural Resources (DENR) to be contaminated with a coliform count of 1,139 MPN 100 ml^{-1} sample water. Although water quality assessment was done in the area, no study was conducted on the associated clinically important bacterial pathogens. This study was conducted to assess the microbial contamination and presence of some pathogens, and to compare microbial contamination between 2018 and 2019 in Bacuit Bay. Surface water samples were collected from 11 stations in Bacuit Bay in June 2018 and June 2019. The multiple tube fermentation technique was done to assess the coliform contamination. Selective and differential microbial culture media were used to isolate some clinically important pathogens. Results showed that in 2018, all stations were positive with coliform where nine out of 11 stations exceeded the acceptable level of coliform count for recreational waters. Also, there were eight clinically important putative bacterial pathogens isolated, the *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella* sp., *Salmonella* sp., *Vibrio parahaemolyticus* and *Vibrio cholerae*. In 2019, the coliform count and the associated bacterial pathogens decreased in number with only two out of 11 stations exceeded the acceptable level and two of eight bacterial pathogens were detected. These could be attributed to the activities that were conducted by the local government unit of El Nido to mitigate the pollution in the coastal environment.

Keywords: beach, coliform, bacterial pathogens, water quality

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INTRODUCTION

Recreation is one of the most important components in life of people inhabiting coastal areas (Saba and Tekpor 2015). Recreational waters are coastal waters used for recreational activities such as bathing, sailing, boating, surfing and diving (MFE 2002). The use of marine waters for recreation has been increasing in many countries. It is estimated that foreign and local tourists together spend around two billion days annually at coastal recreational resorts (Shuval 2003). According to Fogarty et al. (2003), recreational waters are susceptible to a variety of sources of microbiological pollution. However, little information exists when it comes to the quality of water for recreation (van Heerden et al. 2005).

In coastal environments, the contamination of waters raises a major concern due to public health issues (Griffin et al. 2001). Among the leading sources of chemical and biological contamination of recreational waters are the uncontrolled releases of human waste in water, which is one of the problematic consequences of increasing population (Jackson et al. 2001; Howarth et al. 2002; Dwight et al. 2004). The release of untreated sewage is the number one contributor of waste into the oceans and is a major source of nutrient input, environmental damage and eutrophication (Shahidul-Islam and Tanaka 2004). Sewage release can also increase mortality of marine organisms (Hernández et al. 1998) when infected with pathogens (Bossart et al. 1990). The quality of recreational waters is not only critical to environmental health, but also to human health (Fleming et al. 2006).

The presence of fecal indicator bacteria (e.g. *Escherichia coli, Klebsiella* sp. and *Enterobacter* sp.) in recreational waters measures the quality of water and indicates the presence of some clinically important bacterial pathogen (Korajkic et al. 2018). Recreational exposures to pathogens in the water environment may result in disease (Pond and WHO 2005). Although recreational waters do not serve as potable water to many individuals across the globe, its quality must meet that of drinking water since many swimmers accidentally drink it during swimming and there is high risk of microbial contamination from the environment which poses serious health threat to humans (Saba and Tekpor 2015).

El Nido, Palawan is known to be one of the prime tourist destinations in the Philippines because of its diverse ecosystem and beautiful, white sand beaches (WWF-Philippines 2005). It also hosts unique bird species and is frequented by large marine wildlife like the sea or marine turtles, sea cow or dugong, dolphins, sharks and rays (Maguya 2018). Tourism industry of El Nido increased significantly in the number of tourist arrivals starting in 2010 with a number of 37,383 and further increased to 64,896 in 2013 (Aguila et al. 2015) and reaching ~200,000 in 2016 (Maguya 2018). Bacuit Bay is the area

in El Nido where business establishments are located nearshore and also serves as the entrance and exit area for tourists who do island hopping. However, in February 2018, the microbial contamination of El Nido coastal water in Bacuit Bay was reported in. According to the Department of Environment and Natural Resources (DENR) in MIMAROPA region, water tests in Bacuit Bay showed that the coliform count has reached 1,139 most probable number (MPN) 100 ml^{-1} water samples (Bajo 2018). This count is a lot higher compared to the level of coliform count acceptable for swimming which is 100 MPN 100 ml^{-1} (DAO 2016) and 200 colony forming units (cfu) 100 ml^{-1} water sample (US EPA 1976).

Though there were studies conducted in El Nido on the assessment of water quality (DENR 2019; Maguya 2019), none of it assessed the presence of clinically important pathogenic bacteria and looked into the effects of rehabilitation projects. Thus, this study was conducted to assess the microbial contamination and presence of some pathogens, and to compare microbial contamination between 2018 and 2019 in the recreational water of Bacuit Bay, El Nido, Palawan.

METHODS

Locale of the Study

This study was conducted in Bacuit Bay, El Nido, Palawan, Philippines (Figure 1). The study site is located near residential and business areas (11.181744°N, 119.3913803°W). There are many tourist activities going on in the area because El Nido is a major tourist destination of the Philippines. There are also business establishments like hotels and restaurants built on the 3-meter coastal easement zone. Some establishments were found to have discharged wastewater that greatly exceeded the allowable DENR effluent standards which is a violation of Republic Act 9275 or the Philippine Clean Water Act of 2004. The wastewater of these hotels drains to Bacuit Bay through the Barangays Masagana, Buena Suerte, Maligaya and Corong-corong outfalls, which were likewise recorded with soaring fecal coliform count (Maguya 2019). There were 11 stations identified in Bacuit Bay for surface water sampling (Figure 1).

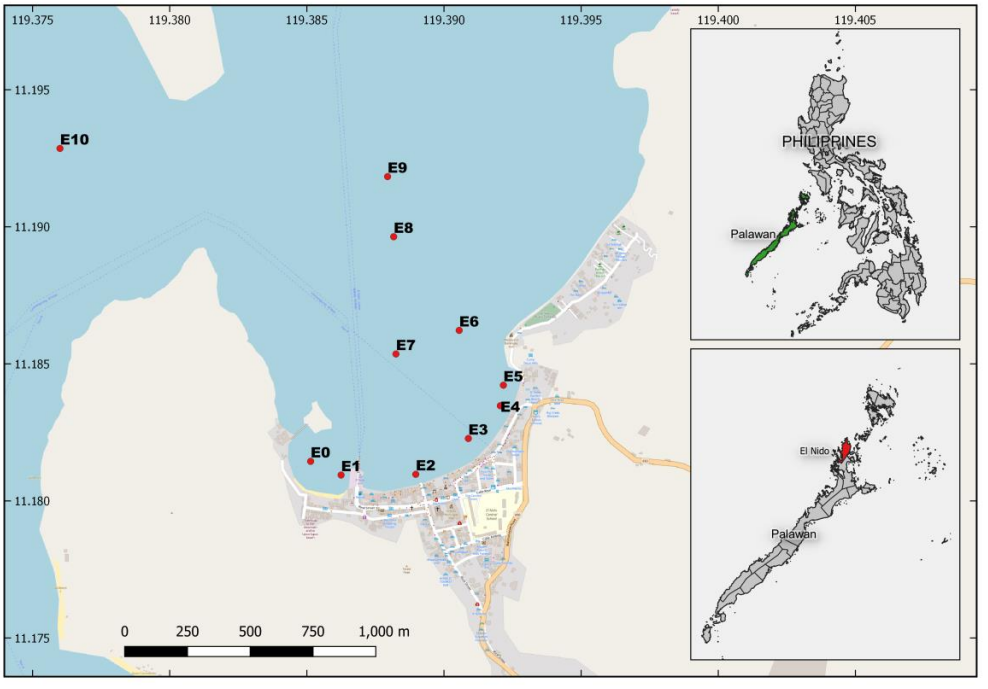

Figure 1. Map of the Bacuit Bay, El Nido, Palawan, Philippines showing the 11 sampling stations; E0 (11.18144444°N, 119.3851389°W), E1 (11.18094444°N, 119.38625°W), E2 (11.18097222°N, 119.3889722°W), E3 (11.18227778°N, 119.3908889°W), E4 (11.18347222°N, 119.3920556°W), E5 (11.18422222°N, 119.3921667°W), E6 (11.18622222°N, 119.3905556°W), E7 (11.18536111°N, 119.38825°W), E8 (11.18963889°N, 119.3881667°W), E9 (11.19183333°N, 119.3879444°W), E10 (11.19286111°N, 119.376°W).

Collection of Water Samples

Water samples were collected using sterile polyethylene plastic bottle in surface waters approximately up to 10 cm depth. Three samples were collected from each station and homogenized in a sterile container. Collection was done within the distance ranges from 100 m to >1,000 m away from the seashore. Seawater was collected using sterile polyethylene plastic bottle. The samples were stored in 100 ml sterile vials at 6°C and were then transported to the Western Philippines University-Puerto Princesa Campus, Microbiology Laboratory for the analysis within six hours. The first sampling was conducted on 20 June 2018, four months after the news on water contamination broke out and

on 13 June 2019, approximately one year after the first sampling and the start of rehabilitation project of the recreational area. This is to find out if the rehabilitation project conducted by the Local Government Unit was successful.

Coliform Detection

Coliform load in the samples was determined using the conventional three-tube MPN (most probable number) method (Rompre et al. 2002). Ten milliliter of water sample was added in test tube containing 10 ml of double strength lactose broth (DSLB). One milliliter and 0.1 ml of each water samples were added separately in test tube containing 10 ml of single strength lactose broth (SSLB). The total sets were incubated at 35°C for 24 hours and examined for the presence of growth accompanied by gas production. Those cultures positive for gas formation were inoculated into Eosin Methylene Blue (EMB) agar and were incubated at 35°C for 24 hours. After incubation, EMB Agar plates were examined for differential colony-forming units. *Escherichia coli* colonies grow with a metallic sheen and a nucleated center, *Aerobacter aerogenes* colonies have a brown center, and nonlactose-fermenting Gram-negative bacteria appear pink. A loopful of sample from positive EMB agar were inoculated in DSLB tubes and incubated for 24 hours at 35°C. Quantification was determined using the standard MPN table and coliform was reported as MPN 100 ml^{-1} water sample.

Isolation of Putative Pathogenic Bacteria

Isolation of putative pathogenic bacteria was done using selective and differential culture media following the manufacturers standards. One milliliter of each water sample from 11 stations were poured in thiosulfate citrate bile sucrose salt agar (TCBS) for the isolation of *Vibrio* species, xylose lysine deoxycholate agar (XLD) for *Salmonella-Shigella* species and McConkey agar for *E. coli*. All plates were incubated at 35°C for 24 hrs. After incubation, the plates were examined for the growth of putative pathogenic bacteria. The colonies of bacteria were identified based on the description of Zimbro et al. (2009). Isolates were chosen based on different characteristics of the colony forming units. These were transferred into sterile agar slants for pure culture, incubated for 24-48 hours and Gram stained to check for purity.

RESULTS

Coliform

The data showed that all water samples collected from all sampling stations in Bacuit Bay in June 2018 (first sampling) were positive for total coliform, with counts ranging from 93 to 1,100 MPN 100 ml^{-1} (Table 1). According to the DENR water standard, the stations are categorized in a range from Class SB (recreational water class I for bathing, swimming, skin diving, etc.) to class SC (recreational water class II for fishing and boating, etc.)

Out of 11 stations tested in 2018, nine stations exceeded the recommended fecal coliform bacteria, which is 100 MPN 100 ml^{-1} (DENR 2016) 200 CFU (colony-forming units) 100 ml^{-1} (US EPA 1976). These are stations E0, E1, E2, E3, E4, E5, E6, E9 and E10 (Table 1). Results also showed that contamination was higher in sampling sites that are close to the coast and other islands like E0, E1, E3 and E10. The category wise distribution of coliform count showed that six stations (E0, E1, E3, E5, E9 and E10) were unfit even for bathing and swimming having a water grade of poor.

Table 1. Coliform count and category wise distribution (categories: I-drinking, II-bathing and swimming, III-bathing and swimming, IV-Unfit) of eleven stations in El Nido Bay in June 2018 and June 2019 (Pandey and Sharma 1999; Saleem et al. 2013).

Sampling stations	June 2018		June 2019	
	MPN Index per 100 ml	Category (grade)	MPN Index per 100 ml	Category (grade)
E0	1,100	IV (poor)	21	II (good)
E1	1,100	IV (poor)	240	III (fair)
E2	240	III (fair)	7	II (good)
E3	1,100	IV (poor)	7	II (good)
E4	240	III (fair)	240	III (fair)
E5	460	IV (poor)	23	II (good)
E6	240	III (fair)	4	II (good)
E7	150	III (fair)	9	II (good)
E8	93	III (fair)	0	I (excellent)
E9	460	IV (poor)	0	I (excellent)
E10	1,100	IV (poor)	0	I (excellent)

On the other hand, coliform count in June 2019 (second sampling) decreased in number as compared to 2018 (Figure 2). Table 2 shows that out of 11 stations, only two stations (E1 and E4) exceeded the recommended fecal coliform, and all other stations were acceptable for bathing and swimming with water grade ranging from fair to excellent.

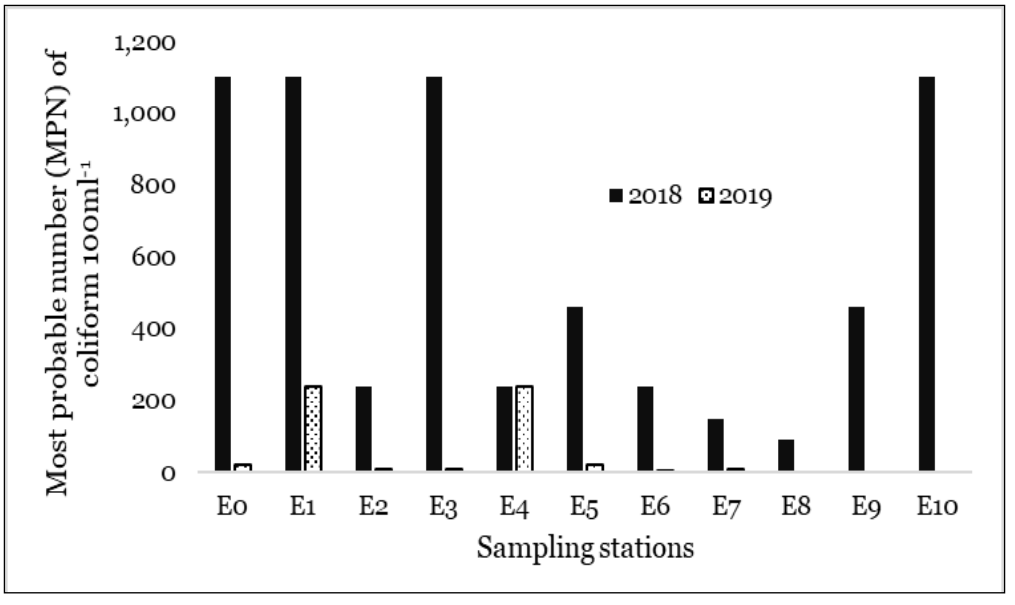

Figure 2. Comparison of coliform count 100 ml^{-1} water sample at 11 stations in June 2018 and June 2019.

Clinically Important Putative Pathogenic Bacteria

There were eight different putative pathogenic bacteria isolated from Bacuit Bay, El Nido, Palawan during the first sampling in June 2018. These are the *E. coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella* sp.*, Salmonella* sp., *Vibrio parahaemolyticus* and *V. cholera* (Table 2 and Figure 3). On the other hand, only two bacterial species, *E. coli* and *Salmonella* sp., were detected and isolated in the second sampling in June 2019. Results also showed that sampling sites with high coliform count also have high number of pathogenic bacteria (Table 2).

Table 2. Clinically important putative pathogenic bacteria from 11 stations (presence is indicated by √) in Bacuit Bay, El Nido, Palawan in June 2018 and June 2019.

Year	Putative Pathogenic bacteria	Sampling Stations										
		E0	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
2018	*Escherichia coli*	√	√	√	√	√	√	√	√	√	√	√
	Proteus mirabilis	√	√									
	Klebsiella pneumoniae	√	√	√	√	√						
	Pseudomonas aeruginosa	√	√	√		√						√
	Salmonella sp.	√	√	√	√	√	√	√	√		√	√
	Shigella sp.	√	√	√	√	√	√					√
	Vibrio cholerae	√	√	√	√	√						√
	Vibrio parahaemolyticus	√	√	√	√	√	√					√
2019	*Escherichia coli*	√	√			√						
	Salmonella sp.		√									

Figure 3. Isolated clinically important putative (further characterization is needed) bacterial pathogens from Bacuit Bay, El Nido, Palawan growing on selective culture media.

DISCUSSION

In this study, the multiple fermentation (MTF) technique revealed that the water samples collected from 11 stations of Bacuit Bay, El Nido in June 2018 sampling were all contaminated with coliform specifically, *E. coli*. Nine out of eleven stations exceeded the acceptable level of coliform count in recreational waters (US EPA 1976). The water grade ranged from fair to poor where most areas were unfit even for swimming and bathing. Among the coliform bacteria, *E. coli* is the most abundant and can be found in the gastrointestinal tracts of humans, birds and animals, but rarely found in water or soil that has not been subjected to fecal pollution. The use of contaminated or polluted marine water for recreational purposes poses a number of health risks which depend on factors such as the nature of the hazard, the characteristic of the water body and the immune status of the user (Pond and WHO 2005). According to Dwight et al. (2004), one of the main reasons of the degradation of the recreational waters is the increasing number of population and uncontrolled release of human waste. The release of human, animal and industrial sewage is the number one contributor of waste into the oceans and is a major source of nutrient input, leading to environmental damage and eutrophication (Shahidul-Islam and Tanaka 2004). Sewage release can also increase mortality of marine organisms (Herńandez et al. 1998) and infect these animals with pathogens (Bossart et al. 1990). On the other hand, in June 2019 sampling, results showed that coliform count decreased in number and the water grade ranged from fair to excellent. Recreational waters can now be used for bathing and swimming.

The quality of recreational waters is not only critical to environmental health but is also closely tied to human health (Fleming et al. 2006). In this study, there were eight clinically important pathogenic bacteria isolated from El Nido Bay, Palawan. These are the *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella* sp.*, Salmonella* sp., *Vibrio parahaemolyticus* and *V. cholerae*. Presence of these bacteria in recreational water is alarming because these are waterborne pathogens that can cause illness with severe outcomes even in an average population (WHO 2003). Although not always severe, infection by these pathogens can result in hospitalization, surgery and death (Pond and WHO 2005).

Escherichia coli is the common cause of non-bloody diarrhea, which can progress to bloody diarrhea and haemolytic uraemic syndrome (McLellan et al. 2001). Most strains of *E. coli* are not regarded as pathogens, but they can be opportunistic that can cause infections in immune-compromised hosts. Presence of *E. coli* indicates presence of other pathogens like *Klebsiella, Vibrio* and other clinically important bacterial pathogens (WHO 2001). After *E. coli*, *P. mirabilis* is one of the most frequent etiological agents associated with urinary tract infections (UTIs). The most widely occurring species of

Klebsiella are *K. pneumoniae* and *K. oxytoca*. They are found in nature associated with vegetable matter and surface waters, including surface waters used for recreational purposes. *Klebsiella* strains have low pathogenicity for human but can cause serious human infections only when the resistance of the host is impaired. However, some literatures state that *Klebsiella* is a serious pathogen (Brown and Seidler 1973; Knittel 1975; Seidler et al. 1975; Bagley and Seidler 1977; Caplenas et al. 1981) and should not be taken for granted. It has been suggested that this organism causes a significant number of cases of community pneumonia which have a high mortality rate (Caplenas et al. 1981) and that it is an important cause of urinary tract infections and various other infections in the community (Knittel 1975; Bagley and Seidler 1977). *Pseudomonas aeruginosa* is an opportunistic pathogen capable of infecting immunocompromised individual and causing severe pulmonary disease (Alhazmi 2015). *Salmonella* and *Shigella* species are normally found in water polluted with human excrement (Saha et al. 2009). Most common illness associated with these bacteria is diarrhea. *Vibrio parahaemolyticus* is recognized throughout the world as the leading causal agent of human gastroenteritis resulting from ingestion of contaminated water or consumption of raw seafood (Iida et al. 2006). On the other hand, *V. cholerae* causes cholera with symptoms of acute watery diarrhea. It has been a common disease reported since the 19th century in the United States (Sack et al. 2004) and remains an internationally widespread though neglected disease (Ryan 2011).

Monitoring the number of indicator microorganisms such as fecal coliform and *E. coli* is a common approach to quantifying pathogenic microorganisms present in the surface waters (Pandey et al. 2014). However, there is still much debate concerning the ability of the indicator microorganisms to represent the potential pathogens present in sampled water. One noteworthy result of this study is the relationship of the coliform load and the number of pathogenic bacterial species found in the recreational waters of El Nido. It was shown that as the coliform load increases, the number of pathogenic bacteria also increases.

From June 2018 to June 2019, both coliform load and the number of clinically pathogenic bacteria in Bacuit Bay decreased. These can be due to the rehabilitation activities that were conducted by the local government unit (LGU) of El Nido. To address the contamination reported in 2018 and the pressure from the national government, the local government of El Nido planned and executed actions on addressing the sewage problem in the area. Business establishments directing their sewerage pipes towards the sea were closed temporarily. The Department of Environment and Natural Resources (DENR) issued cease-and-desist orders (CDO) for establishments polluting Bacuit Bay, especially those found to have discharged wastewater that greatly exceeded the allowable DENR effluent standards, in violation of Republic Act

9275 or the Philippine Clean Water Act of 2004 (Maguya 2019). The DENR also created a task force El Nido for establishments violating the 3-meter easement zone, as provided for urban areas under the Water Code of the Philippines (Maguya 2018). Tourist establishments with 30 or more rooms are required to build their own dislodging plants before the sewage is piped into the sewage treatment plant. Those who would not comply would face a Php 500,000 fines (Cimatu 2019).

The LGU also passed the no-plastic ordinance that requires all those going to the islands to surrender their single use plastic bottles, plastic bags and other disposable plastic materials before boarding their boats; tourists are to take portable jugs and reusable water containers only (Cimatu 2019). Restaurants, bars and other tourist establishments ceased selling bottled water and other drinks using polyethylene terephthalate (PET) and other plastics under the supervision of the Department of Health. In addition to that, the Protected Area Management Board (PAMB) of El Nido-Taytay Protected Area passed a resolution that limits tourist entry and activities in three of the most visited places in El Nido. In the Big Lagoon, one of the major attractions in the area, only 60 guests are allowed at any one time or a maximum of 720 guests per day. In the Small Lagoon, a maximum of 30 guests are allowed at any one time or a total of 360 persons per day. For the Secret Beach, only 12 visitors are allowed at any one time or a total of 144 a day. Limits on the number of conveyances have also been set – maximum of five boats in the anchorage area and 30 kayaks inside the Big Lagoon, 15 kayaks inside the Small Lagoon, and two boats in the anchorage area of Secret Beach (DENR 2019).

The rehabilitation conducted in the study area to address the water contamination remarkably decreased the coliform load as well as the associated pathogens within a year. The mitigation of coastal water contamination, which includes proper wastewater disposal and municipal wastewater treatment, is an effective measure to improve the water quality in El Nido. It is recommended that a regular monitoring of the presence of pathogenic bacteria must be conducted not just in El Nido but in other tourist attractions in the Province of Palawan, such as Coron, Ulugan Bay, Port Barton, and Puerto Princesa Bay. The results of this study can be used as basis for the formulation of management strategies for coastal tourist sites in Palawan and the Philippines to ensure safety, healthy and aesthetically pleasing coastal environment.

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