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RESEARCH ARTICLE

Isolation, identification and antibiogram profiling of bacteria isolated from water, seafood, and macroplastic samples from Baseco Beach, Manila Bay

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ABSTRACT

Background and Objective: Manila Bay plays an important role both in economics and ecology because it serves as the major economic center of the Philippines and as it harbors different habitats and biodiversity. Unfortunately, it is threatened by various pollutions including the unregulated discharge of wastewater from industrial, agricultural, and household sectors and improper disposal of trash such as macroplastics among others. All these contributes to the current state of Manila Bay. This study identified bacteria isolated from water, seafood and floating macroplastic samples from Baseco Beach, Manila Bay and determined their antibiogram profiles.

Methodology: Bacterial isolates were obtained from water, seafoods and macroplastic samples from Baseco Beach, Manila Bay using conventional culture techniques. Identification of the isolates was done using Vitek-2 Automated System and antibiogram profiling was done using Kirby-Bauer Disk Diffusion Susceptibility Test.

Results and Conclusions: A total of 30 bacterial isolates were obtained from different samples from water, seafood and macroplastic samples from Baseco Beach, Manila Bay. These isolates were identified and found to belong to 13 different bacterial species with *Bacillus* spp. comprising 33.33% of the isolates (10 out of 30), and *Vibrio alginolyticus* comprising 23.33% of the isolates (7 out of 30) and the other species comprise the remaining 43.34% (*Pseudomonas* spp., *Vibrio fluvialis*, *Klebsiella pneumoniae*, *Shewanella alga*, *Sphingomonas paucimobilis*, *Staphylococcus haemolyticus*, *Chryseobacterium indologenes*, *Myroides* sp. and *Aeromonas salmonicida*). Of these, six out of 30 isolates (20%) showed susceptibility to all six representative antibiotics used (Cefazolin 30µg, Gentamicin 10 µg, Chloramphenicol 30 µg, ampicillin 10 µg, Cefuroxime 30 µg, Ceftazidime 30 µg) while 7 isolates (23.33%) were resistant to only one class of antibiotic. Moreover, 17 out of 30 isolates (56.66%) were resistant to two or more classes of antibiotic while only one isolate (3.33%) was found to be resistant to gentamicin. All 30 isolates (100%) were susceptible to chloramphenicol. Interestingly, three antibiotic resistant (AMR) bacteria were isolated from macroplastics namely *Pseudomonas oleovorans* (S2), *Vibrio alginolyticus* (S5), and *Pseudomonas alcaligenes* (S29) which were all resistant to ampicillin and cefazolin. This is the first study in the Philippines to isolate AMR bacteria from macroplastics from Manila Bay. The presence of AMR bacteria in macroplastics shows that these materials can be a reservoir for its dynamics and distribution. Lastly, with the emergence of antimicrobial resistant bacteria, the elucidation of the antibiogram profile of bacteria is necessary to determine its implication sand threats to public health. This study served as a baseline study of presence of AMR bacteria in macroplastic samples from Manila Bay.

Keywords: *Antibiogram profiling, Baseco Beach, Manila Bay, Macroplastics, Vitek-2 Automated System, Kirby-Bauer Disk Diffusion Susceptibility Test*

Introduction

Antibiotic resistance has been referred to as "the silent tsunami facing modern medicine" [1]. It is now one of the leading causes of diseases and deaths worldwide [2], and thus continues to be a serious public health concern. Several studies have shown that the use of antibiotics at a high concentration and frequency yields higher rate of resistance to humans and animals [3]. Antibiogram profiles from other studies show that several bacterial species have emerged as major clinical problems due to its developing resistance to antibiotics such as carbapenems, cephalosporins, and other beta-lactam antibiotics [4,5]. Mechanisms of antibiotic resistance come from the expression of chromosomal genes or may be acquired through plasmids via horizontal gene transfer [2,5].

Aquatic environments provide diverse ecological habitats and important environmental resources for organisms. As a result, major food sources of Manila come from communities that thrive along its coasts [6]. However, with the diminishing environmental quality of the waters, a significant decline in the populations developed over the last 30 years [7]. Moreover, samples from Manila Bay such as milk fish, tilapia, mussel, and shrimp were observed to exceed food safety standards for *E. coli* in seafood [8].

Manila Bay is an estuary with a semi-closed landscape that accommodates effluents from 17,000 km watersheds with 26 catchment areas [9]. The bay continues to be the country's major center for commercial and industrial activities [10] and serves as one of the main avenues for trade and commerce, potentially attracting many tourists and businesses worldwide [11]. However, the increased human activity within the area, the massive effluent discharges, and macroplastic pollution caused its deteriorating water quality [12]. This has led to the destruction of many marine habitats and loss of biodiversity within the waters [13,14]. Because of this, assessment of the ecological status of Manila Bay must be given importance. Recently, the government initiated the rehabilitation of Manila Bay and the water quality has continued to improve (*i.e.* reduced coliform count) [15]. This improvement of water quality is attributed to the rehabilitation program and the closure of several establishments that directly dump their wastes to the body of water.

In the Philippines, only a few studies focused on the ecological and microbial status of Manila Bay. These studies are limited to the isolation of bacteria from water samples which are observed to have developed resistance against sulfamethoxazole [16]; pollution study in Manila Bay [17]; the effects of the water quality of Manila Bay on allometric parameters and histological biomakers of selected organs of *Perna viridis* [18]. Several studies have shown that seafood and macroplastics are possible reservoirs of both gram-positive and gram-negative bacteria and more significantly, antimicrobial resistant bacteria [19,20]. High possibility of horizontal gene transfer can occur in large bodies of water like Manila Bay because of wastewater discharge from different industries which are significant environmental reservoirs of antimicrobial resistant bacteria [21,22]. While different studies have been conducted globally on isolating antimicrobial resistant bacteria from different substrates, there has been limited studies available about antimicrobial resistant bacteria from the various environments such as soil, sediments, and river catchments like Manila Bay [23].

The objectives of this study are to determine the antibiogram profiles of bacteria isolated from water, seafood, and macroplastic samples from Baseco Beach, Manila Bay using complete antibacterial susceptibility tests according to the standards of the Clinical Laboratory Standards Institutes (CLSI) and to identify the putative identity of isolates using VITEK-2 Automated System. This study may give additional information on the presence of antibiotic resistant bacteria in water and seafood samples from Baseco Beach, Manila Bay. To the knowledge of the authors, this is the first study that provides baseline information about antibiotic resistant bacteria from macroplastics of Manila Bay. This study may also serve as preliminary assessment of the possible risk and threats to public health in this area using the pathogen-host-environment interplay.

Methodology

Sample Collection

Procurement of samples was done at the Baseco Beach, Manila Bay, Baseco Compound Manila with global positioning system (GPS) location (14°35'24.4"N 120°57'11.9"E). Five sampling points with at least 10-meter apart were selected. For each sampling point, samples were collected on three different sample collection dates. For water samples, five 1-liter samples were collected for every sampling point on three different days (n=75). The water samples were obtained using sterile 1-liter polypropylene bottles. The sampling bottles were directly dipped in the surface of the water and at least 2.5 cm was left out for ample air space in the bottle and to facilitate mixing by vigorous shaking prior to filtration. The water samples were labelled properly and transported on ice to ensure preservation of samples. For macroplastic samples,

three samples such as one-time use shopping bags and food wrappers were collected for every sampling point on three different days (n=45). Macroplastic samples were placed in Ziploc bags with 20 mL seawater and labelled properly. Macroplastic samples were transported on ice and brought to the laboratory. For seafood samples, 35 Squids, 4 Tilapia, and 60 Mussels were purchased per sample collection day (squid: n=105; tilapia: n=12; mussel: n=180). Samples were contained in separate Ziploc bags to avoid cross contamination then placed in a cool ice box for transport to the laboratory.

Isolation, Purification, and Identification of Bacteria

For water samples, three volumes of 500 mL were filtered through 0.20 µm pore-size filter (Nylaflo, 0.20um, 47mm Gelman 66602) using a commercially available water pump (Nalgene Rapid-Flow Bottle and Vacuum Pump). The filter membrane was aseptically transferred to Brain Heart Infusion (Hi-Media) plates with 2% sodium chloride to mimic the seawater environment [24]. Plates were incubated at 37 ºC for 24 hours. Ten grams of seafood sample was processed in a sterile blender with 90 mL 0.85% saline solution. Serial dilution was done and the last three dilutions (10^{-5}, 10^{-6}, 10^{-7}) were plated in BHI agar plates with 2% NaCl using spread plate method. Inverted plates were incubated at 37°C for 24 hours. Swab inoculation method was employed for macroplastic samples. Sterile cotton swab was moistened using 0.85% saline solution then swabbed onto the surface of the plastic samples. The cotton swab was then inoculated onto brain heart infusion broth and incubated at 37°C for 24 hours [25]. Gram-staining was done to confirm the morphology of the bacteria from BHI medium. The bacterial isolates were grown in BHI slants with 2% NaCl and incubated at 37°C for 24 hours prior to storage at -20°C. Cultures were sent to the Microbiology Section, Department of Laboratories of the Philippine General Hospital for identification using VITEK-2 Automated System. Generated results from this system is up to species level.

Antibiogram Profiling

The Kirby-Bauer Disk Diffusion Assay was employed for the antimicrobial susceptibility of the isolates. The breakpoints were based on the standards set by the Clinical and Laboratory Standards Institute (CLSI). The antibiotics used were cefalozin 30 µg, gentamicin 10 µg, chloramphenicol 30 µg, ampicillin 10 µg, cefuroxime 30 µg and ceftazidime 30 µg. Twenty-four hour old cultures were evenly streaked on Mueller-Hinton agar plates using sterile cotton swab before inoculation of antibiotic disks. After incubation, the inhibition zone diameters were measured. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. A representative sample was also subjected to VITEK 2 Automated System for accuracy. Results were certified by a clinical pathologist from the Microbiology Section of PGH.

Results

Identification of Bacteria Isolated from Baseco Beach, Manila Bay

Table 1 shows the identity of isolates based from the results obtained from the VITEK-2 Automated System. Eleven isolates were gram-positive while 19 isolates were gram-negative (seven isolates from water, one isolate from squid, one isolate from fish, five isolates from mussel, and five isolates from macroplastics). A total of ten genera were isolated, eight were gram-negative and two were gram-positive.

Antibiogram Profiling of Bacteria Isolated from Baseco Beach

The antibiogram of Gram-negative bacteria isolated from water sample is presented in Table 2. Only one isolate was susceptible to all the four classes of antibiotic while one isolate was resistant to only one antibiotic. Six isolates were resistant to two or more classes of antibiotic. For the cephalosporin group, six were resistant to cefazolin, four were resistant to cefuroxime and one isolate was resistant to third-generation cephalosporin (ceftazidime). Only one isolate showed resistance against gentamicin and all isolates were susceptible to chloramphenicol.

Table 3 shows the antibiogram profiles of bacterial isolates from seafood samples. Four isolates were susceptible to all antibiotic used in this study. Also, three isolates were found to be resistant to one class of antibiotic. These were two *Shewanella alga* isolates and one *Bacillus* sp. isolate. Meanwhile, six isolates were resistant to two or more classes of antibiotic. Nine isolates were resistant to first-generation cephalosporin (cefazolin), three isolates showed intermediate susceptibility to second-generation cephalosporin (cefuroxime), and three isolates showed resistance to third-generation cephalosporin.

Table 4 shows the antibiogram profiles of gram-negative isolates from macroplastic samples. Two isolates were susceptible to all antibiotics and three isolates were resistant to at least two classes of antibiotics-ampicillin and first-generation cephalosporin (cefazolin).

Table 1. *Identification of bacterial isolates from water, seafood, and macroplastic samples based from then VITEK-2 Automated System.*

Sample	Gram-negative		Gram-positive	
	Identity	**Number of isolates**	**Identity**	**Number of isolates**
Water	*Pseudomonas stutzeri (S1)*	1	*Bacillus sp. (S23)*	1
	Vibrio fluvialis (S4)	1		
	Vibrio alginolyticus (S6,S17,S20,S22)	4		
	Klebsiella pneumoniae (S7)	1		
Squid	*Shewanella alga (S8)*	1	*Bacillus sp.(S18,S26)*	2
Fish	*Sphingomonas paucimobilis (S24)*	1	*Bacillus sp. (S13,S14,S25)*	3
Mussel	*Shewanella alga (S9)*	1	*Staphylococcus haemolyticus (S10)*	1
	Chryseobacterium indologenes (S11)	1	*Bacillus sp. (S15,S16,S27,S28)*	4
	Myroides sp. (S12)	1		
	Vibrio alginolyticus (S19,S21)	2		
Macroplastic	*Pseudomonas oleovorans (S2)*	1		
	Pseudomonas stutzeri (S3)	1		
	Vibrio alginolyticus (S5)	1		
	Pseudomonas alcaligenes (S29)	1		
	Aeromonas salmonicida (S30)	1		
TOTAL		**19**		**11**

Table 2. *Antibiogram profiles of bacterial isolates from water sample from Baseco Beach, Manila Bay using Kirby-Bauer Disk Diffusion Susceptibility Test.*

Isolate	Susceptibility Category					
	Ampicillin	Cefazolin	Cefuroxime	Ceftazidime	Gentamicin	Chloramphenicol
Pseudomonas stutzeri (S1)	S	S	S	S	S	S
Vibrio fluvialis	R	R	S	S	R	S
Vibrio alginolyticus (S6)	R	R	R	S	S	S
Klebsiella pneumoniae ss. pneumoniae	R	S	S	S	S	S
Vibrio alginolyticus (S17)	R	R	I	S	S	S
Vibrio alginolyticus (S20)	R	R	R	S	S	S
Vibrio alginolyticus (S22)	R	R	R	I	S	S
Bacillus sp. (S23)	R	R	R	R	S	S

Legend: S - susceptible; I - intermediate; R - resistant

Table 3. *Antibiogram profiles of bacteria isolated from seafood sample from Baseco Beach, Manila Bay using Kirby-Bauer Disk Diffusion Susceptibility Test.*

Type	Isolate	Susceptibility Category					
		Ampicillin	Cefazolin	Cefuroxime	Ceftazidime	Gentamicin	Chloramphenicol
Squid	*Shewanella alga (S8)*	S	R	S	S	S	S
	Bacillus sp. (S18)	R	R	R	R	S	S
	Bacillus sp. (S26)	S	S	R	R	S	S
Fish	*Sphingomonas paucimobilis*	I	I	R	R	S	S
	Bacillus sp. (S13)	R	R	R	R	S	S
	Bacillus sp. (S14)	R	R	R	R	S	S
	Bacillus sp. (S25)	S	S	S	S	S	S
	Shewanella alga (S9)	S	R	S	S	S	S
	Chryseobacterium indologenes	R	R	I	S	S	S
Mussel	*Myroides sp.*	R	R	I	S	S	S
	Vibrio alginolyticus (S19)	R	R	I	S	S	S
	Vibrio alginolyticus (S19)	R	R	I	S	S	S
	Staphylococcus haemolyticus	S	S	S	S	S	S
	Bacillus sp. (S15)	S	S	R	R	S	S
	Bacillus sp. (S16)	S	S	R	R	S	S
	Bacillus sp. (S28)	S	S	R	R	S	S
	Bacillus sp. (S30)	S	S	S	S	S	S

Legend: S - susceptible; I - intermediate; R - resistant

Table 4. *Antibiogram profiles of Gram-negative bacteria isolated from macroplastic sample from Baseco Beach, Manila Bay using Kirby-Bauer Disk Diffusion Susceptibility Test.*

Isolates	Susceptibility Category					
	Ampicillin	Cefazolin	Cefuroxime	Ceftazidime	Gentamicin	Chloramphenicol
Pseudomonas oleovorans	R	R	S	S	S	S
Pseudomonas stutzeri(S3)	S	S	S	S	S	S
Vibrio alginolyticus (S5)	R	R	S	S	S	S
Pseudomonas alcaligenes	R	R	S	S	S	S
Aeromonas salmonicida	S	S	S	S	S	S

Legend: S - susceptible; I - intermediate; R - resistant

Discussion

Gram-negative Bacteria from Water

The gram-negative bacteria isolated from water of Manila Bay are *Pseudomonas stutzeri*, *Vibrio fluvialis*, *Vibrio alginolyticus*, and *Klebsiella pneumoniae*. *Pseudomonas* and *Vibrio* are ubiquitous in marine environment [26]. *P. stutzeri* has been isolated from human as an opportunistic pathogen and another study shows that immunocompromised individuals can be easily infected with *P. stutzeri* [27,28]. It has a high degree of physiological and genetic adaptability and can be present in many different natural environments [29]. One study reported that *P. stutzeri* has type IV pili that is essential to genetic transformation and can be utilized for horizontal gene transfer [30]. Two species of *Vibrio* were isolated - *V. fluvialis* and *V. alginolyticus*. Numerous studies have shown the prevalence of *Vibrio* species in surface water across the world and its prevalence is influenced by season and location [31]. It is important to note that based on previous studies, more than a dozen of species of *Vibrio* are pathogenic and can cause diseases to humans [32,33]. On the other hand, *Vibrio fluvialis*, another isolate from this study, is known to be a pathogen commonly found in coastal environments and was recently correlated with the increase in numbers of diarrheal outbreaks and sporadic extraintestinal cases [34]. Another isolate, the *V. alginolyticus* was formerly known as biotype 2 of *V. parahaemolyticus* and was previously reported to be considered as a rare factor for human infection [35]. It usually causes superficial wound, ear (otitis media, otitis externa), and conjunctival infections [36]. On the contrary, one study reported that an immunocompromised patient developed necrotizing fasciitis due to *V. alginolyticus* after an injury from a coral reef while bathing in the Caribbean Sea [36,37]. With this, this isolate may be of potential threat to people bathing in the marine waters near the sample collection site in Baseco, Manila especially that the area is known to cater households and a number of industrial establishments in the vicinity.

Klebsiella pneumoniae was also isolated from water samples. This organism can be found in various environment such as soil, water, or vegetation. Aquatic environments that receive wastewater from different industries can be reservoir of *Klebsiella* [38]. Some studies shown that a significant proportion of hospital-acquired urinary tract infection (UTI), pneumonia, septicemias, and infections in soft tissues is accounted to *Klebsiella pneumoniae* [38]. As to their antibiogram profiles, this study found that the *P. stutzeri* isolate was susceptible to all the antibiotic tested while seven of eight gram-negative isolates from water (87.5%) were resistant to at least one of the six antibiotics. Gram-negative bacteria can show resistance to different antibiotics because of its membrane having lipopolysaccharides that prevents antibiotics from penetrating the cell and thus prevents cell death. Intrinsic antibiotic resistances of gram-negative bacteria can be attributed to the peptidoglycan cell wall inhibiting the function of certain antibiotics. *V. fluvialis* showed resistance to ampicillin, cefazolin, and gentamicin while all four *V. alginolyticus* showed resistance to ampicillin and to the first two generations of cephalosporin tested. Meanwhile, the isolate *Klebsiella pneumoniae* was only resistant to ampicillin. Ampicillin-resistant gram-negative bacteria have emerged due to the transformation of plasmid under the stress of ampicillin and new protein (cpxP) can be produced by gram-negative bacteria even in the absence of ampicillin [39]. The mode of action of beta-lactam antibiotics on sensitive organisms can be considered to be a two-step process: In the first step, the drug binds to primary receptors called membrane-bound penicillin-binding proteins (PBPs). These proteins perform vital roles in cell cycle-related, morphogenetic formation of cell wall peptidoglycan. Inactivation of PBPs by bound antibiotic has immediate arresting actions on their function. The second stage comprises the physiological effects caused by this receptor-ligand interaction. PBPs are involved in the late stages of peptidoglycan synthesis in the cell wall. Because peptidoglycan maintains the integrity of the cell wall, which resides in a hypotonic environment, its disruption causes lysis and cell death [40]. Meanwhile, aminoglycosides like gentamicin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically, it binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids is inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes [41].

Gram-negative Bacteria from Seafood

In the seafood samples, *Shewanella alga* was isolated from squid and mussel. This organism has been isolated from various environments, including freshwater and sea [42]. Generally, it is considered as an opportunistic pathogen in humans if an open wound is exposed in contaminated marine environment [43]. Furthermore, this bacterial species tends to be associated with

ingestion of raw seafood [44,45]. On the other hand, *Sphingomonas paucimobilis* was the only gram-negative bacteria isolated from fish (Tilapia). This is consistent with a study that also isolated *S. paucimobilis* from fish samples from Sardinia, Italy [46]. This species is also one of the most frequently isolated bacteria from fishes that manifest clinical signs of disease [47]. However, there are reports that immunocompromised patients are more susceptible to infections caused by *S. paucimobilis* [48]. Meanwhile, four gram-negative bacteria were isolated from mussel. These are *S. alga*, *Chryseobacterium indologenes*, *Myroides* sp., and *V. alginolyticus*. These organisms were previously isolated from seawater, fishes and marine invertebrates including mussel. *Chryseobacterium indologenes* has been reported to cause pneumonia, sepsis and abdominal infections [49]. It also infects newborns and immunocompromised individuals across all age groups [50,51]. To the authors' knowledge, this is the first study in the Philippines to isolate *Myroides* sp. from mussel. This is possible because of the filter feeding mode of nutrition of bivalve samples. These bivalves can process large volumes of water that might contain *Myroides* sp. and other microorganisms [52]. However, infections by *Myroides* sp. are rare and have only been reported to infect immunocompromised individuals [53].

In this study, *Shewanella alga* was resistant only to cefazolin. This can be attributed to the OXA-48-type carbapenem-hydrolyzing β-lactamase genes, which were increasingly reported in enterobacterial species and have originated from *Shewanella* [54]. Cefazolin binds to and inactivates penicillin-binding proteins (PBP) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This results in the weakening of the bacterial cell wall and causes cell lysis [55]. A previous study showed that *S. alga* is multidrug-resistant to ampicillin and cefazolin [56]. Also in this study, the isolated *S. paucimobilis* from fish shows resistance to cefuroxime and ceftazidime. Cefuroxime, like the penicillins, is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefuroxime interferes with an autolysin inhibitor [57]. On the other hand, ceftazidime is a beta-lactam, third-generation cephalosporin antibiotic with bactericidal activity. It binds to and inactivates penicillin-binding proteins (PBP) located on the inner membrane of the bacterial cell wall. PBPs participate in the terminal stages of assembling the bacterial cell wall, and in reshaping the cell wall during cell division. Inactivation of PBPs interferes with the cross-linkage of peptidoglycan chains necessary for bacterial cell wall strength and rigidity. This results in the weakening of the bacterial cell wall and causes cell lysis [58]. On the contrary, previous report suggested that third generation cephalosporins or aminoglycosides were best choice of treatment of *S. paucimobilis* infections [59]. No antibiotic resistance mechanisms have yet been elucidated to explain the resistance of this bacteria to cefuroxime and ceftazidime. Isolates from this study which are from mussels are resistant to at least one of the antibiotics. The mechanism of the resistance of these bacteria can either be inherent or acquired from other bacteria through horizontal gene transfer [26].

Gram-negative Bacteria from Macroplastic

A unique habitat can be provided by the physical properties of plastic, thus, can be capable of supporting the growth and proliferation of different microbial communities [60]. Its buoyancy and abundance can contribute to the possibility of the survival and long-distance dissemination of microorganisms thriving in its surface. Thus, plastics can be a potential vector for the spread of these organisms [61]. Reports have shown that an ecologically diverse group of gram-negative bacteria known as Gammaproteobacteria contain several potentially pathogenic strains of *Salmonella* spp. and *Vibrio* spp. that might be harmful to human health [62]. Based on previous studies, certain strains of *Vibrio* spp. have been recognized to readily colonize plastics, therefore, pathogenic species of *Vibrio* can colonize plastic as well [60]. Interestingly, *V. alginolyticus* was isolated in this study. Other gram-negative species that were isolated were *Aeromonas salmonicida* and three *Pseudomonas* species (*P. oleovorans*, *P. stutzeri*, and *P. alcaligenes*). In a previous study employing a laboratory-based microcosm setup containing sterile artificial seawater and inoculated with low-density polyethylene (LDPE) microplastics, colonization of plastics by morphologically distinct prokaryotic cells, predominantly bacteria, occurred over time, thus, plastics can be indeed reservoir of these microorganisms [63]. For their reactions to antibiotics, both *A. salmonicida* and *P. stutzeri* were susceptible to all six antibiotics while *P. oleovorans*, *P. alcaligenes*, and *V. alginolyticus* were all resistant to ampicillin and cefazolin. This might be explained by a previous study that has shown that *Pseudomonas* are known to have chromosomal AmpC that may be responsible for the beta-lactam (ampicillin and cefazolin) resistance [64]. Also, species of *Pseudomonas* are known to acquire

multiple intrinsic and acquired resistance genes from other families of gram-negative bacteria [65]. In this study, the isolated *V. alginolyticus* showed 100% resistance to ampicillin and cefazolin. Based from published papers, the *blapl* gene is responsible for the resistance of *V. alginolyticus* against ampicillin and cefazolin [66].

Gram-positive Bacteria from Water and Seafood

In this study, the dominant gram-positive bacteria that were isolated from water and seafood are *Staphylococcus haemolyticus* and *Bacillus* sp. Based from previous reports, *Staphylococcus haemolyticus* is one of the most common causes of staphylococcal infections and is considered as an important hospital pathogen that has methicillin resistance genes [67,68]. On the other hand, *Bacillus* spp. are known to be ubiquitous in nature but can also be found in higher concentration in soil, water, and food samples [69]. This is true for the results of this study as tabulated in Table 1 where *Bacillus* sp. is apparently present in all the samples collected except from macroplastics. Unfortunately, because of some limitations, the VITEK-2 Automated System was not able to identify the bacterial isolates down to its species level. A study that evaluated the identification of *Bacillus* spp. using VITEK-2 cards found that the automated software itself was not sufficient to identify species within the genus *Bacillus* because they are indistinguishable by most phenotypic and molecular methods and even softwares are having trouble with the identity of these species thus it is recommended that the manufacturer of these cards should have a way to address this particular issue [70].

Based from the results of this study, a total of six out of 30 isolates (20%) show susceptibility to all six representative antibiotics used and three antibiotic resistant (AMR) bacteria were isolated from macroplastics. The data generated from this study may be used to assess the risk and threat of the isolated AMR bacteria. The adaptability, dispersal and survival efficiency, and reproductive fitness of a pathogen like the isolated AMR bacteria are some of the factors of disease development. Microorganisms that have multi-drug resistance have emerged not only in the hospital environment but are now often identified in community settings, suggesting that reservoirs of antibiotic-resistant bacteria are present outside the hospital. The bacterial response to the antibiotic "attack" is the prime example of bacterial adaptation and the pinnacle of evolution. From an evolutionary perspective, bacteria use two major genetic strategies to adapt to the antibiotic "attack". One is through mutations in gene(s) often associated with the mechanism of action of the compound, and another acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer (HGT). Acquisition of foreign DNA material through HGT is one of the most important drivers of bacterial evolution and it is frequently responsible for the development of antimicrobial resistance [71].

Aside from those mentioned above, the conditions of the environment can permit the growth, proliferation, and dissemination of pathogens to individuals can contribute to the development of an outbreak. Lastly, the overall health conditions and susceptibility of the host and its community can also become factors of disease development. The interplay of the pathogen, host, and environment can be used to pose the threats and risks to the community near the Baseco Beach, Manila Bay. No data is available about medical cases of infections from Baseco Beach due to bathing and other recreational activities done by people in Manila Bay or ingestion of contaminated undercooked seafood. The Department of Health (DOH) warned the public that swimming or bathing in polluted bodies of water like Manila Bay can lead to skin, eye, and gastrointestinal infections including diarrhea and cholera.

Conclusion

Thirteen (13) species of bacteria were isolated from the different samples from Baseco Beach, Manila Bay. Six out of 30 isolates (33.33%) were susceptible to all antibiotics used, seven out of 30 isolates (22.33%) were resistant to one class of antibiotic while 17 out of 30 isolates (56.66%) were multidrug-resistant or resistant to two or more classes of antibiotic. Antibiogram profiles of the bacterial isolates showed that 17 out of 30 isolates (56.66%) are resistant to ampicillin, 18 out of 30 isolates (60%) are resistant to cefazolin, 13 out of 30 (43%) are resistant to cefuroxime, nine out of 30 isolates (30%) are resistant to ceftazidime and only one out of 30 isolates (3.33%) is resistant to gentamicin. Multidrug resistance can also be seen from the antibiogram profiles where 17 out of 30 isolates (56.66%) are resistant to two (2) or more classes of antibiotic. To the author's knowledge, this is the first study in the Philippines that has isolated antimicrobial resistant (AMR) bacteria from macroplastics in Manila Bay. The presence of these antimicrobial resistant (AMR) bacteria shows that Manila Bay is indeed a reservoir of these potentially harmful bacteria. It is recommended that a survey of antibiotic resistance genes present in the isolates must be done to have a molecular support to the results from this study.

Metagenomics can be employed for future studies to determine the different antimicrobial resistance genes that may be present in the bay.

Acknowledgments

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