

Isolation and Characterization of Pathogenic Bacteria in Sewage Wastewaters in Tuguegarao City Cagayan

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Abstract—Residential wastewater is considered as an ideal environment for the survival of broad arrays of pathogenic bacteria. The study aimed to characterize the bacteria isolated from residential sewage wastewater collected from four flood-prone barangays in Tuguegarao City. Morphological and biochemical tests were used to identify the bacterial isolates using Vitek 2 Compact Machine. The antibiotic susceptibility of identified bacteria in the study area was also investigated using determination of Minimum Inhibition Concentration. The findings revealed the presence of gram negative, non-lactose fermenting bacterial genera characterized as *Aeromonas hydrophila*/ *Aeromonas caviae* with a probability of 99% in all of the isolates, *Aeromonas sobria* with a probability of 98% and *Klebsiella pneumoniae* spp. *pneumoniae* with a probability of 99%. Moreover, the isolate *Klebsiella pneumoniae* spp. *pneumoniae* showed resistance to Ampicillin and Ticarcillin. This indicates that the sewage water is conducive to supporting the growth and proliferation of these gram negative bacteria. These pathogens also have the potential to cause diseases in humans especially when the sewage water is mixed with flood water which is an important health concern in flood-prone areas.

Keywords—sewage wastewater, residential sewage, antibiotic resistant pathogens, morphological characterization.

I. INTRODUCTION

Wastewaters are essentially the water supply of the community after it has been fouled by a variety of uses. It is also defined as a combination of the liquid or water carrying wastes removed from residences, institutions, commercial and industrial establishments, together with such groundwater, surface water and storm water as may be present (Topare, Attar, & Manfe, 2011). Wastewater, which is primarily domestic

sewage, possibly contains a proportion of non-domestic sources, such as industries (Moo-Young, 2019). The different types of substrates from the different sources of domestic wastewater were combined and hence it may act as a best medium for the proliferation of numerous microorganisms, which will be detrimental to human health if left uncontrolled.

The sewage wastewater environment is an ideal medium for both pathogenic and nonpathogenic microorganisms (Abdel-Raouf et al., 2012). Domestic wastewater is considered a favorable environment for the survival of broad arrays of microorganisms (Essa & El-Gayar, 2018). Sewage water commonly includes the bacteria belonging to coliforms, clostridia, enterococcus, lactobacilli, *Micrococci*, *Proteus*, *Pseudomonas*, *Streptococcus* and *Staphylococcus*. These bacteria may produce certain toxins which can be responsible for causing different types of illness (Mahesh, Basha & Kavitha, 2017; Moges et al., 2014). Regarding the pathogenic microorganisms, coliform bacteria are a chief portion of the human intestinal flora that is discharged into the sewage wastewater. They include various pathogens that cause an assortment of diseases such as cholera and typhoid (Essa & El-Gayar, 2018). Some bacteria or viruses found in wastewaters were also found to be resistant to antibiotics and can survive harsh environments and remain infectious even for a long period of time even if the sewage will be treated (Ventola, 2015). Recent epidemics of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, multidrug-resistant respiratory pathogens, and extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* are a few examples of the worldwide proliferation of antimicrobial resistance (Linares et al., 2010; Calderon et al., 2016). The wastewater microbiome brings together bacteria of environmental, human

and animal origins, many harboring antibiotic resistance genes (ARGs) (Manaia et al., 2018).

In the last five years, the Philippines experienced a series of waterborne disease outbreaks which could have been minimized if improvement in access to adequate treatment and disposal of human excreta and sewage had been given full attention (WHO, 2016). Some bacteria and diseases carried by sewage and wastewater are *E. coli*, shigellosis, typhoid fever, salmonella, and cholera. Other diseases caused by bacteria in wastewater include wound infections (*Pseudomonas aeruginosa*), respiratory infections (*L. pneumophila*, *Mycobacterium avium*) and leptospirosis (*Leptospira*) (Chahal et al., 2016). Disposal facilities are rare and domestic wastewater is discharged without treatment (Claudio, 2015). Consequently, the major sources of wastewater pollution in the Philippines comes from inadequately treated domestic wastewater or sewage (48%) and the rest compose of agricultural wastewater (37%) and industrial wastewater (15%) (Greenpeace Philippines, 2015). Poor wastewater management may also lead to contaminated flood water. In extreme weather conditions excessive rain water can also cause our drains and sewage systems to overflow into flood water, which can then contain sewage sludge and waste. Cagayan Province is among the provinces that are vulnerable to severe flooding especially during seasons of heavy rainfall; and Tuguegarao City is among the municipalities commonly affected by such flooding, yet there is no locally drafted policy for sewage management (Dela Cruz, 2016; TCENRO, 2017). The flood water may contain sewage, and may pose potential health risks to citizens exposed to pathogens in these waters, increasing the risk of waterborne diseases due to increased frequency of intense extreme weather events (Veldhuis et al., 2010; NIH, 2017).

The lack of adequate facilities for the proper treatment, collection and disposal of sewage wastewater in the city may lead to serious health risk to the people of Tuguegarao. The vulnerability of the city gives rise to the testing of sewage wastewater for the presence of pathogens and finding all possible controls in eradicating it, lessening threats to the community's safety.

Given that this bacteria remains viable even after a certain duration in the sewerage and that Tuguegarao City lacks a robust sanitary infrastructure to fully capture all generated sewage, the growing concern regarding the risks and danger that the sewage wastewater imposes to people have pricked interest of the researchers to dig deeper with the main goal of isolating and characterizing pathogenic bacteria present in sewage wastewater and to determine the antibiotic sensitivity of the isolated bacteria in the flood-prone barangays in Tuguegarao City. This study therefore aimed to isolate and identify the characteristics of pathogenic bacteria present in sewage waste water found in flood-prone areas in Tuguegarao City. Physical characteristics (color, turbidity and pH) of the wastewater samples were identified as well morphology, biochemical characteristics and antibiotic sensitivity of the isolated bacteria were determined.

II. METHODS

This study is an applied descriptive quantitative design. The researchers collected sewage wastewater samples in Tuguegarao City to identify and determine the characteristics of the bacteria present. The study was conducted in four barangays of Tuguegarao City namely, Annafunan East, Linao West, Linao Norte and Bagay. The DPWH Tuguegarao Flood Hazard Map shows that these four barangays are flood-prone areas of the city.

A. Collection and Handling of water samples

The water samples were collected aseptically from sewage water from the different identified barangays. The researchers followed the procedure based on Donegal County Council Laboratory on Standard Operating Procedure of Wastewater Sampling Procedures (2013).

The sample bottles/containers were clearly labelled and identified. The time and date were recorded together with all relevant details of location and sampling conditions that may be present at time of sampling. The sample bottles were securely sealed following sampling and stored securely for safe transport to the laboratory in cooler boxes where necessary. The samples were analyzed within 24 hours of sample collection, as a general rule; however, there may be specific requirements for particular tests.

The samples were sent to the Laboratory of Department of Agriculture, wherein the isolation and characterization of bacteria were performed.

B. Isolation of bacteria from residential sewage water sample

Isolation of the microorganisms was done by using the Vitek 2 Compact machine. This method used pure isolates of the organisms to be tested and taken from Trypticase Soy Agar with 5% sheep blood (BAP), chocolate agar, Maconkey, CPSE, and Columbia Sheep Blood Agar (CBA). EMB plates are not acceptable. The plates were incubated at 35 °C - 37° C for 18-24 hours. After successful growth of microorganisms, the pure colonies of bacteria were used for the suspension preparation for the ID Cards.

C. Characterization of bacterial culture

Samples were observed under microscope and characterized based on morphology and cell features.

a) *Morphological characteristics:* Morphological characteristics such as shape, size and color were studied through microscopic observation. The shape of the colony was studied by observing their margin and elevation and the colonies may fall into either round, rod or cocci shape. The colour of the bacteria was identified by observing the colony under microscope (Mahesh et al., 2017).

b) *Biochemical characteristics:* The biochemical tests were performed to identify the unknown cultures. Biochemical test was performed using the Vitek 2 Compact Machine. The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Test kits available include ID-GN (gram negative bacillus

identification), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility) and AST-GP (gram positive susceptibility). Suspension for the biochemical tests were prepared by transferring 3ml of Saline into a tube. A colony was selected and suspended in the saline tube. After thorough mixing, the optimal density with the DensiCHECK was checked. The ID card and tube were then placed into the cassette. All the cards and tubes with suspension were set in a cassette. From the PC workstation, Cassette Worksheet and record Lab ID and barcode for each card were printed. The cassette was loaded into the instrument and the Fill door was closed. Then the Start Fill button was pressed. Audible and visual indicators signal the completion of the fill process, then the cassette was transferred into the loading station and the Load door was closed. The cassette from Loading station was removed when the Indicator was seen flashing and Remove status was clicked.

c) *Antibiotic Susceptibility Assay:* The antibiotic susceptibility assay was performed using the Vitek 2 Compact Machine to determine the antibiotic susceptibility of the isolates to common antibiotics: Amikacin, Amoxicillin, Ampicillin, Cefalotin, Cefoxitin, Cefotaxime, Ceftazidime, Ciproflaxacin, Ertapenem, Gentamicin, Imipinem, Nalidixic acid, Nitrofurantoin, Ofloxacin, Piperacillin, Ticarcilli, Tobramycin, Trimethoprim.

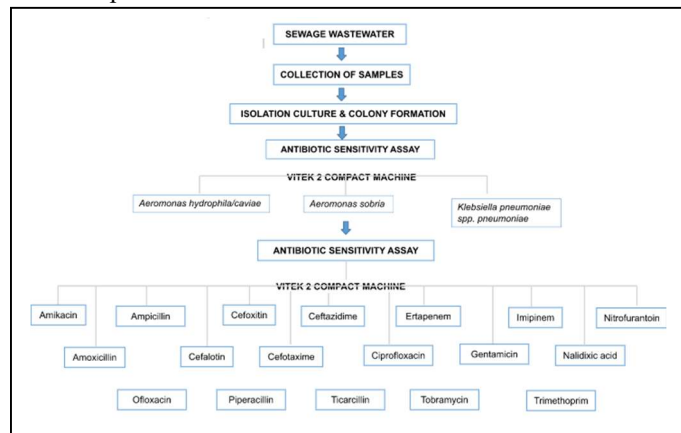


Fig. 1. Methodological framework of the study

D. Waste Disposal Management

The disposal of samples was accomplished by the Department of Agriculture laboratory personnel with the requisite protective equipment and with proper handling.

E. Data Analysis

The data from the tests performed on the waste water samples from the different barangays were presented in tabular format. Mean Minimum Inhibitory Concentration (MIC) was used to determine the susceptibility of the identified bacteria to common antibiotics. The Minimum Inhibitory Concentration is the lowest concentration (in µg/mL) of an antibiotic that inhibits the growth of a given strain of bacteria. MIC is reported as either sensitive, intermediate or resistant. These interpretive standards have been established by the Clinical and Laboratory Standards Institute (CLSI). The table below presents the corresponding values for interpreting MIC.

TABLE I. QUALITATIVE INTERPRETATION FOR MINIMUM INHIBITORY CONCENTRATIONS

MIC Value (in µg/mL)	MIC Value (in µg/mL)	MIC Value (in µg/mL)
≤ 0.25 - ≤ 20	Sensitive	The organism is inhibited by the serum concentration of the drug that is achieved using the usual dosage
0.5 - 64	Intermediate	The organisms are inhibited only by the maximum recommended dosage
≥ 8 - ≥ 128	Resistant	The organisms are resistant to the usually achievable serum drug levels.

F. Ethical Considerations

The researchers asked for permission from the Dean of the School of Health and Allied Sciences, University Ethics Board, Vice President for Academics and President of University. All protocols that were used in conducting this study were subjected for approval by the ethics committee of University of Saint Louis (USL).

The researchers also asked for permission from the Barangay Captains of Barangay Annafunan, Bagay and Linao to ensure that the study does not cause any harm, and have a beneficial outcome.

The researchers also requested assistance from the Environmental Management Bureau – Department of Environmental and Natural Resources, San Gabriel, Tuguegarao City for the validation of wastewater samples.

Lastly, the researchers ensure to protect and store all the data collected for it is regarded as confidential.

III. RESULTS

TABLE II. PHYSICAL CHARACTERISTICS OF WASTEWATER SAMPLES COLLECTED

Barangay/ Sample	Color	Turbidity	pH
1	No color	Low turbidity	8.17
2	Black	High turbidity	7.76
3	Light Tan	Medium turbidity	8.72
4	Grey-Brown	High turbidity	7.79

Table 1 presents the physical characteristics of the different samples. Sample 1 exhibits no color and low turbidity which indicates that the water is clearer and contains fewer particles; sample 2 exhibits black color and high turbidity that indicates a form of pollution; sample 3 exhibits light tan color and medium turbidity indicating the presence of small particles in the water; and sample 4 displays a grey-brown color and high turbidity which is linked to the presence of a support medium for the transport of pathogenic microorganisms and a rise in disinfection demand. From Samples 1 to 4, all of them show high levels of alkalinity due to the presence of particulates, type of dissolved inorganic and organic compounds, the amount of suspended organic and bicarbonate in the water.

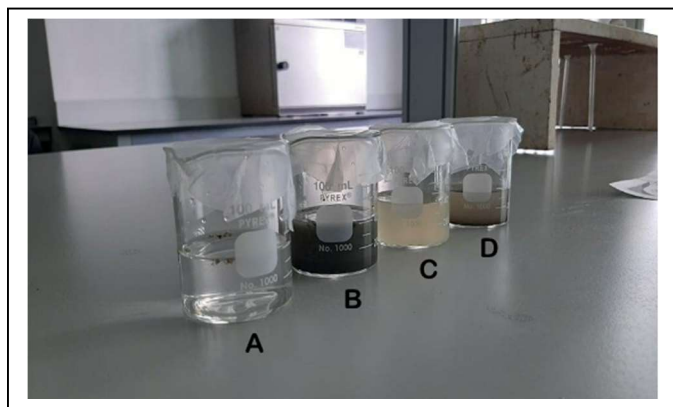


Fig. 2. Physical characteristics of sewage wastewater samples.

The first figure above showing a clear in color is the sample obtained from Barangay 01 (specimen A); a black color shows the sample obtained from Barangay 02 (specimen B); a light tan color shows the sample obtained from Barangay 03 (specimen C) and a grey-brown color shows the sample obtained from Barangay 04 (specimen D). Moreover, the figure presents differing characteristics of the wastewater samples from the different barangays which may indicate differing contaminants and levels of contamination or pollution.

TABLE III. MORPHOLOGICAL CHARACTERISTICS OF PATHOGENIC BACTERIA IDENTIFIED IN WASTEWATER SAMPLES

Barangay/ Sample	Colony No.	Characteristics		Bacteria Identified
		Color	Shape	
1	1	White to yellowish	Mucoid	<i>Aeromonas hydrophila/caviae</i>
	2	White	Mucoid	<i>Aeromonas hydrophila/caviae</i>
2	1	Gray	Flat, mucoid	<i>Aeromonas sobria</i>
	2	White to yellowish	Mucoid	<i>Aeromonas hydrophila/caviae</i>
3	1	White to yellowish	Mucoid	<i>Aeromonas hydrophila/caviae</i>
4	1	White	Mucoid	<i>Klebsiella pneumoniae spp. pneumoniae</i>

The bacteria isolated in the sewage wastewater sample from the colony 1 of the Barangay 01, colony 2 of Barangay 02 and Barangay 03 exhibits white to yellowish color and mucoid in shape which is consistent with the bacteria species of *Aeromonas hydrophila/caviae* (with a 99% probability). The colony 2 in Barangay 01 exhibits white color and mucoid in shape with the bacteria specie of *Aeromonas hydrophila/caviae* (with 99% probability). Colony 1 of Barangay 02 exhibits gray and flat mucoid in shape which is consistent with the bacteria *Aeromonas sobria* (with 98% probability). The bacterial colony formed in the Barangay 04 exhibits white color with mucoid in shape which is consistent with the bacteria *Klebsiella pneumoniae spp. pneumoniae* (with 99% probability).

TABLE IV. BIOCHEMICAL CHARACTERISTICS OF PATHOGENIC BACTERIA IDENTIFIED IN WASTEWATER SAMPLES

Barangay/ Sample	Colony No.	Characteristics		Bacteria Identified
		Gram Staining	Lactose Fermentation	
1	1	Gram negative	Non-lactose fermenter in MAC	<i>Aeromonas hydrophila/caviae</i>
	2	Gram negative	Non-lactose fermenter in MAC	<i>Aeromonas hydrophila/caviae</i>
2	1	Gram negative	Non-lactose fermenter in MAC	<i>Aeromonas sobria</i>
	2	Gram negative	Non-lactose fermenter in MAC	<i>Aeromonas hydrophila/caviae</i>
3	1	Gram negative	Non-lactose fermenter in MAC	<i>Aeromonas hydrophila/caviae</i>
4	1	Gram negative	Lactose fermenter in MAC	<i>Klebsiella pneumoniae spp. pneumoniae</i>

The bacterial colony isolated in the sewage wastewater sample from Barangay 01, Barangay 02 and Barangay 03 showed cells exhibiting gram negative stain with non-lactose fermenter in MAC which is consistent with the bacteria species of *Aeromonas hydrophila/caviae* (with 99% probability) and *Aeromonas sobria* (with 98% probability). Barangay 04 showed cells displaying gram negative stain with lactose fermenter in MAC which is consistent with the bacteria *Klebsiella pneumoniae spp. pneumoniae* (with 99% probability).

TABLE V. ANTIBIOTIC SENSITIVITY OF *AEROMONAS HYDROPHILA/CAVIAE* IDENTIFIED IN WASTEWATER SAMPLES

Antibiotics Tested	Mean Minimum Inhibitory Concentration (µg/mL)	Qualitative Interpretations
Amikacin	≤ 2	Sensitive
Amoxicillin	4	Sensitive
Cefoxitin	≤ 4	Sensitive
Ceftazidime	≤ 1	Sensitive
Ciprofloxacin	≤ 0.25	Sensitive
Gentamicin	≤ 1	Sensitive
Imipenem	≤ 0.25	Sensitive
Piperacillin/Tazobactam	≤ 4	Sensitive
Trimethoprim/Sulfamethoxazole	≤ 20	Sensitive

The table above presents the antibiotic sensitivity testing of *Aeromonas hydrophila/ caviae* which was isolated in three of the four barangays. It can be gleaned from the table that the bacterial colony isolated is sensitive to all common antibiotics used for the bacteria.

TABLE VI. ANTIBIOTIC SENSITIVITY OF *AEROMONAS SOBRIA* IDENTIFIED IN WASTEWATER SAMPLES

Antibiotics Tested	Mean Minimum Inhibitory Concentration ($\mu\text{g/mL}$)	Qualitative Interpretations
Amikacin	≤ 2	Sensitive
Amoxicillin	≤ 2	Sensitive
Cefoxitin	4	Sensitive
Ceftazidime	≤ 4	Sensitive
Ciprofloxacin	≤ 1	Sensitive
Gentamicin	≤ 0.25	Sensitive
Imipenem	≤ 1	Sensitive
Piperacillin/ Tazobactam	1	Sensitive
Trimethoprim/ Sulfamethoxazole	≤ 4	Sensitive

The table above presents the antibiotic sensitivity testing of *Aeromonas sobria* which was isolated in one of the four barangays. It can be gleaned from the table that the bacterial colony isolated is sensitive to all common antibiotics used for the bacteria.

TABLE VII. ANTIBIOTIC SENSITIVITY OF *KLEBSIELLA PNEUMONIAE* IDENTIFIED IN WASTEWATER SAMPLES

Antibiotics Tested	Mean Minimum Inhibitory Concentration ($\mu\text{g/mL}$)	Qualitative Interpretations
Amikacin	≤ 2	Sensitive
Amoxicillin/ Clavulanic Acid	≤ 2	Sensitive
Ampicillin	≥ 32	Resistant
Cefalotin	≤ 2	Sensitive
Cefoxitin	≤ 0.4	Sensitive
Cefotaxime	≤ 1	Sensitive
Ceftazidime	≤ 1	Sensitive
Ciprofloxacin	≤ 0.25	Sensitive
Ertapenem	≤ 0.5	Sensitive
Gentamicin	≤ 1	Sensitive
Imipenem	≤ 0.25	Sensitive
Nalidixic acid	≤ 2	Sensitive
Nitrofurantoin	64	Intermediate
Ofloxacin	≤ 0.25	Sensitive
Piperacillin/ Tazobactam	≤ 4	Sensitive
Ticarcillin	≥ 128	Resistant
Tobramycin	≤ 1	Sensitive
Trimethoprim/ Sulfamethoxazole	≤ 20	Sensitive

The table above presents the antibiotic sensitivity testing of *Klebsiella pneumoniae* which was isolated in one of the four

barangays. It can be gleaned from the table that the bacterial colony isolated is sensitive to most of the common antibiotics used for the bacteria. However, it was also found to be resistant to Ampicillin and Ticarcillin indicating the potential to cause serious clinical and public health issues and cause treatment failure to current antibiotic therapy. Moreover, it is reported to have intermediate susceptibility to Nitrofurantoin, which indicates that it requires a higher dose of the antibiotic to prevent its growth.

IV. DISCUSSION

The 4 samples from the 4 different flood prone residential areas of Tuguegarao City possess different physical characteristics. Light brownish grey color is seen in fresh sewage but old sewage is seen as dark grey and black due to its anaerobic activities (Jamal, 2017). The pH of the samples is considered to be basic, considering that most microorganisms survive well in wastewater environments within a pH range of 6.5 to 8.5 (EBS, 2021). This alkaline environment therefore favors the growth and proliferation of many microorganisms. However, some microorganisms can tolerate extreme pH's and will thrive in acidic or basic environments. Risk factors favoring *Aeromonas* abundance in water include high turbidity for the reason that turbidity can provide food and shelter for pathogens, it has been associated with increased densities of bacteria (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012; Figueras & Ashbolt, 2019). If not removed, the causes of high turbidity can promote regrowth of pathogens in the water. A lightly clear color and low turbidity was obtained from Barangay 01; this may be from a fresh sewage wastewater from the households of Barangay 01. A black in color and high turbidity wastewater sample was obtained from Barangay 02. This sample was collected in a wet, damp, and warm atmosphere near a cornfield, which affects the color, turbidity, and microorganisms present in the sample. Barangay 03 showed a sample exhibiting light tan color with medium turbidity. The sample was taken from wastewater which is mixed with flood water from the previous storm. It is light tan in color and had a medium turbidity due to floodwater and a mixture of household sewages. A gray color is seen in the sample obtained from Barangay 04. The houses in this barangay are situated very close to each other. The sewage's gray color and high turbidity may be due to variation in water use among households. Some of these households have an open sewage making it more exposed and vulnerable to various forms of dirt.

The study isolated and characterized the bacterial populations of the 4 samples. A total of 6 isolates were characterized along with their antibiotic sensitivity. During the investigation, the findings revealed the presence of bacterial genera characterized as *Aeromonas hydrophila*/*Aeromonas caviae* with a probability of 99% in all of the isolates, *Aeromonas sobria* with a probability of 98% and *Klebsiella pneumoniae* with a probability of 99%. Some of these bacteria were also found in a study of stabilization ponds of the wastewater treatment system which showed *Aeromonas* species isolated *A. caviae* (25.09%), *A. hydrophila* (2.12%) and *A. veronii sobria* (3.53%) (Martone-Rocha et al., 2010). *Aeromonas sobria* is also seen in domestic wastewaters (El-Gayar & Essa, 2018).

Aeromonas spp. were detected in the majority of isolates in this study. These bacteria live in a variety of aquatic environments, which comprises a collection of gram negative, facultative anaerobic, rod-shaped, oxidase positive bacteria under the family *Aeromonadaceae* (Janda & Abbott, 2010; Khajanchi et al., 2010). Although the sampling points were geographically distant, the detection of *A. hydrophila*, *A. caviae* and *A. sobria* from the six isolates, suggests that these strains exist widely in residential wastewaters. Of the many species of aeromonads capable of causing infections in humans, these three strains are among the species mainly responsible for causing disease in humans (Miyagi et al., 2016). *Aeromonas* are known to cause septicemia in aquatic organisms, gastroenteritis and extraintestinal diseases such as septicemia, skin, eye, wound, respiratory tract infections in humans, opportunistic systemic disease in immunocompromised patients, diarrheal disease and wound infections both in healthy and diseased states (Bhowmick & Bhattacharjee, 2018; Sekizuka et al., 2019). Many infections are related to water exposure (traumatic accidents, near-drowning, natural disasters etc.), leech therapy (due to their symbiotic relationship with these bacteria) or consumption of contaminated water or food. *Aeromonas* have a high prevalence in sewage (approximately 100 percent positive samples), and their high prevalence in sewage and wastewater at comparable similar counts to fecal coliforms suggests that they can multiply in this environment (Salvat & Ashbolt, 2019). Considering that fecal matter is a major component of domestic sewage and the source of the majority of human pathogens in wastewater (Symonds & Breitbart, 2015), enteric pathogens enter wastewater from human and animal fecal wastes or from fecal-contaminated water from other household uses such as bathing or laundry. Disease-causing organisms (pathogens) from humans can also enter a community's wastewater from patients at hospitals, or from anyone who is sick or a carrier of disease (Symonds & Breitbart, 2015), as well as industrial waste from food production, particularly from animal processing and slaughter house (Chahal et al., 2016). Water and contaminated food are considered the main sources of *Aeromonas* transmission (Janda & Abbott, 2010; Figueras & Beaz-Hidalgo, 2015). *A. hydrophila* and *A. caviae* were the species most commonly isolated from clinical and environmental samples. In addition, significant concentrations of *Aeromonas* have been isolated in flood waters especially following natural disasters when sewage wastewaters combine with flood waters (Miyagi et al., 2016; Salvat & Ashbolt, 2019). This results in outbreaks of *Aeromonas* infections in people who have survived floods where exposure to this bacterial species was from contact with or ingestion of flood water contaminated with sewage wastewater (Miyagi et al., 2016; Salvat & Ashbolt, 2019). *Aeromonas* plays an important role in infections with people who have survived natural disasters, for instance it was the most isolated microbe (22.6%, 145 isolates) of all isolated bacteria recovered from the infected wounds of tsunami survivors in southern Thailand and something similar occurred in 2005, after hurricane Katrina in New Orleans (Figueras & Ashbolt, 2019). Hence, the area where samples were collected are of high risk of contaminating *Aeromonas* infection when flood water combines with their sewage wastewaters. Waterborne outbreaks have also been described involving *Aeromonas* (Ventura et al., 2015) and are commonly observed during rainy seasons (Bhowmick &

Bhattacharjee, 2018). Dermal exposure of water contaminated with *Aeromonas* has also been found to result in wound or burn infections (Azzopardi et al. 2011). Contact with ambient untreated water to extinguish flames was recognized as the main risk factor associated with the infection (Azzopardi et al., 2011).

Another pathogenic bacterium isolated from the wastewater samples of the current study is *Klebsiella pneumoniae*. They are frequently found in water, sewage, soil, and plant surfaces (Martin & Bachman, 2018). *Klebsiella pneumoniae* is the most medically important species of the genus and remains among the world's most common nosocomial pathogens, where they cause pneumonia and urinary tract infections in catheterized patients (Ashurst & Dawson, 2021). *Klebsiella pneumoniae* is a commensal and saprophyte *Enterobacteriaceae* ubiquitously present in the fecal flora of humans and animals and in the environment; it is responsible for nosocomial and community infections (urinary and respiratory tract infections, blood, and wound infections (Ginkel, 2017). It is also a major cause of neonatal sepsis, ranking in the top three causative agents in most settings (Okomo et al., 2019). The lack of clean water and adequate sanitation contributes to the resurgence of infectious diseases. *Klebsiella* bacteria are normally found in the human intestines (where they do not cause disease). They are also found in human stool (feces) (CDC, 2010). The findings of our study supports the result of the study of Koberg et al. (2016) and Padhi et al., (2013) (ESBL)-producing *Klebsiella pneumoniae* strain 182 and *Klebsiella pneumoniae* CF-S9 strain was isolated from raw domestic sewage. This implies that there is a possibility where the septic tanks of the households where the *K. pneumoniae* were isolated are directly connected in the sewage system of the Barangay.

The isolated *Aeromonas* species in the current study showed sensitivity to different antibiotics. This is not consistent with studies that assessed the same from wastewater treatment plants which showed prevalence of *Aeromonas* strains resistant to amoxicillin (Popovic et al., 2015; Varela et al., 2014) and ciprofloxacin (Varela et al., 2014). Studies have demonstrated that aeromonads play an important role in the dissemination of antimicrobial resistance (Figueira et al., 2011; Osińska et al., 2016). Our results did not support these observations. All of the studied isolates were sensitive to antibiotics. The difference in the results is attributed to the source of the sample. The *Aeromonas* that presented resistance to antibiotics were isolated from wastewater treatment plants and from hospital effluent. In contrast to our study, the *Aeromonas* from our sample presented sensitivity to common antibiotics for the reason that our samples are taken from residential sewage wastewaters. Because hospitals use more antibiotics and use them more frequently than the community, the development of antibiotic-resistant bacteria and the discharge of antimicrobial residues and resistant bacteria in these effluents are expected to have a significant impact on the spread of antibiotic-resistant bacteria in the environment (Varela et al., 2014).

Wastewater environment is considered a significant reservoir of antibiotic-resistant bacteria. It was found out in the present study that the bacterial strain of *Klebsiella spp.* is resistant to ampicillin and ticarcillin. Same finding is reported in the study of Sidjabat et al., (2011), Balkhair (2016), and Sakkas (2019) confirming the resistant activity of *Klebsiella*

spp. to the same antibiotics. This species has become resistant to antibiotics and can transmit this resistance to other species of bacteria due to a constitutively expressed chromosomal A β -lactamase (Gundogan, 2014; Pendleton, Gorman & Gilmore, 2013). The World Health Organization recognizes extended-spectrum β -lactam (ESBL)-producing and carbapenem-resistant *K. pneumoniae* (CRKp) as a critical public health threat (WHO, 2017). Among the organisms capable of extended-spectrum β -lactamase (ESBL) production, *K. pneumoniae* is an important pathogen (Gundogan, 2014).

In the present study, bacterial isolates from various genera showed both sensitivity and resistance to the majority of antibiotics tested. The isolates that show sensitivity to different antibiotics present no threat to the public; however, the antibiotic resistance of *Klebsiella* species to different antibiotics is becoming a serious public health concern especially when these bacteria are evident in residential wastewaters as revealed in this study. Considering that clinical settings are essential reservoirs of antibiotic resistance (Varela, Nunes & Manaia, 2016), it can be argued that the discharge of hospital sewage into environmental wastewaters increases the risk of dissemination of antibiotic resistance. The ineffectiveness of hospital sewage treatment, or the lack thereof, can also contribute to the spread of KPC-2-producing and other multidrug-resistant bacteria from the hospital to the environment (Poluri et al., 2021). In developed countries, the vast majority of hospitals lack sewage treatment plants, exacerbating the problem and may have important public health implications. Earlier researchers also reported that the uncontrolled use of antibiotics and self-medication is responsible for building a selection pressure in favor of organisms possessing genes that code for antibiotic resistance.

V. CONCLUSION

The present study confirmed the presence of various bacterial genera characterized as *Aeromonas hydrophila*/*Aeromonas caviae*, *Aeromonas sobria* and *Klebsiella pneumoniae*. This indicates that the sewage water is conducive to supporting the growth and proliferation of these gram negative bacteria. These pathogens have the potential to cause diseases in humans especially when the sewage water is mixed with flood water. This is also very likely as these pathogens were isolated in sewage waste waters of flood prone barangays in the city. One of the isolated pathogens also exhibited antimicrobial resistance which is another cause of concern for public health. This further implies the need to improve or strengthen policies and measures for proper sewage wastewater management and treatment in these areas.

VI. RECOMMENDATIONS

Based on the findings and conclusion presented, the researchers recommend conducting a similar study to cover other flood-prone barangays in the city and to conduct the collection and testing of wastewater samples during the rainy season. The determination of chemical parameters of the wastewater should also be tested, in order to determine if there could be a possible relationship with the kind of bacteria growing in the area. The researchers also recommend to inspect if there are any *E. coli* bacteria present in the wastewater.

The results of the study further imply the need to treat sewage wastewater and impose strict quality control measures to be put in place to ensure the effective treatment of the sewage wastewater. This would decrease the load of microorganisms and other contaminants allowed to be disposed of in the environment. Such measures will improve the overall quality of sewage wastewaters, preventing outbreaks and spreading water-borne diseases. Antibiotic resistance surveillance can be used as a tool to control the problem of antibiotic resistance and to educate the public on the consequences of the misuse of antibiotics and also regulate the usage of drugs in both human and veterinary medicine. The study will help the City officials and the city engineers to formulate guidelines and produce innovative wastewater treatment technologies for the proper collection, disposal and treatment of sewage wastewaters.

Further studies should be conducted to assess the level of antibiotics in water and the potential risks associated with untreated sewage wastewaters. It is also very significant that findings from studies such as this one should be disseminated to the health sector and barangay officials of the affected communities which would help them come up with intervention programs for the improvement of wastewater management in the different households.

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