Marilao-Meycauayan-Obando River System (MMORS) Harbors Multidrug-Resistant Bacteria Indicating High Risk of Antimicrobial Contamination

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Marilao-Meycauayan-Obando River System (MMORS) is among the dirtiest and most polluted places in the world but it is still utilized for aquaculture, recreation, and water supply. Since it is contaminated with various industrial and commercial wastes, it is likely that effluents with antibiotics are also dumped into the river system. Antibiotic resistance is one of the biggest threats to global health, food security, and development. Nine (9) bacteria isolated from MMORS were screened for antibiotic resistance using eight (8) antibiotics of different classes, *i.e.*, ciprofloxacin (5mcg), erythromycin (15mcg), ampicillin (25mcg), ertapenem (10mcg), ceftriaxone (30mcg), linezolid (30mcg), cephalothin (30mcg), and piperacillin/tazobactam (100/10mcg). MS1 and MS5 had the highest multiple antibiotic resistance (MAR) index at 0.625, followed by MS4, MS6, MS7, and MS8 at 0.375. MS2 and MS9 had MAR index of 0.125. The proportion of isolates with MAR index greater than 0.2 (66.66%) was higher than isolates with MAR index below 0.2 (33.33%), suggesting a high risk of contamination in sampling sites. Sequence analysis revealed that MS1 had 99.87% sequence similarity with *Bacillus pumilus*, MS2 with 99.30% *Bacillus* sp. sequence similarity, MS3 with 93.08% *Brevibacillus* sp. sequence similarity, MS4 with 99.90% *Morganella morganii* sequence similarity, MS5 with 100.00% *Bacillus cereus* sequence similarity, MS6 with 99.78% *Escherichia coli* sequence similarity, MS7 with 100.00% *Bacillus anthracis* sequence similarity, and MS9 with 100.00% *Bacillus* sp. sequence similarity. This study provides initial insight into multidrug-resistant bacteria and its possible risk in safety and public health in MMORS.

KEYWORDS

MMORS, Aquaculture, Antibiotic Susceptibility, Antimicrobial Activity, MAR Index, Antibiotic Resistance, Probiotics

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INRODUCTION

The World Health Organization (WHO) listed antimicrobial resistance (AMR) as currently one of the biggest threats to global health, food security and development (WHO 2018). AMR occurs when microorganisms, primarily bacteria, undergo random mutation in the presence of selective pressure such as antibiotics, resulting to the development of a gene that confers

Table 1: Antibiotics tested and their respective concentrations and standard ZOI

Antibiotic	Classification	Disk Code	Conc. (mcg)	R	I	S
Ciprofloxacin	Fluoroquinolone	CIP	5	≤ 16	17-19	≥ 20
Erythromycin	Macrolide	E	15	≤ 16	17-19	≥ 20
Ampicillin	Penicillin	AMP	25	≤ 11	12-14	≥ 15
Ertapenem	Carbapenem	ETP	10	≤ 15	16-27	≥ 28
Piperacillin/tazobactam	Penicillin	TPZ	100/10	≤ 21	-	≥ 22
Ceftriaxone	Cephalosporin	CRO	30	≤ 23	24-27	≥ 28
Linezolid	Oxazolidinone	LNZ	30	≤ 19	-	≥ 20
Cephalothin	Cephalosporin	KF	30	≤ 26	-	≥ 27

Standard zones of inhibition (ZOI) are based from BSAC standardized disk susceptibility testing method version 8 (Andrews 2009) Conc.: concentration; R: resistant; I: intermediate; S: susceptible

antibiotic resistance (Gebreyes et al. 2017; Koch et al. 2017). Resistance genes may be transferred horizontally through plasmid exchange among bacteria, and vertically through reproduction which give rise to a fully resistant generation (Prestinaci et al. 2015). AMR has been observed worldwide to have increased alarmingly since new resistance mechanisms are discovered which then threatens the effectivity of current treatments to common infectious diseases such as pneumonia, tuberculosis, blood poisoning, gonorrhea, and foodborne diseases (WHO 2018). AMR is primarily influenced by the behavior of the society in prescribing and using antibiotics, where misuse and overuse as well as poor infection prevention and control are manifested (WHO 2018; Michael et al. 2014). Consequently, humans and animals infected with the resistant bacteria become harder to treat than those infected by non-resistant ones.

Anthropogenic environmental sources of resistant bacteria include livestock manure, agricultural run-off, effluent from hospitals, sewage treatment plants, raw meat or other animal products, soil-containing antimicrobial residues, pet feces, and antimicrobial use in aquaculture – all of which can eventually contaminate surface freshwater like rivers, lakes, and lagoons (Davies and Davies 2017; Baquero et al. 2008; Schroeder et al. 2003). Surface freshwaters contaminated with antimicrobial residues become vehicle for antimicrobial gene transmission among bacteria, and aid in spreading resistant bacteria which contribute to the upsurge of AMR (Danner et al. 2019; UNEP 2019). Pollution of surface freshwaters with heavy metals like zinc and cadmium may also contribute to the selection of resistant bacterial strains along with abiotic factors such as pH and nutrients (Chen et al. 2019; Nguyen et al. 2019; Seiler and Berendonk 2012).

In the Philippines, the Marilao-Meycauayan-Obando River System (MMORS) in the province of Bulacan was included in "The Dirty Thirty", a list of the world's 30 most polluted places identified by Pure Earth in 2007 (Inquirer News 2007). MMORS runs along several industries wherein the wastes from leather tanneries, gold and precious metal refineries, legacy lead-smelting factories, wet market, and numerous municipal dumpsites are discharged into the river (Claudio 2015). In spite of this, the river system also supports a thriving aquaculture industry which is one of the main sources of livelihood and food security in the area. Pollution of MMORS poses health risks to the inhabitants as well as to aquaculture consumers since contaminated aquaculture products cultivated in MMORS are consumed largely in Bulacan and in nearby areas in Metro Manila. Hence, this study measured the antibiotic susceptibility and antimicrobial activity of bacteria in MMORS which may provide information on possible health risks and threats.

METHODS AND MATERIALS

Bacterial source and maintenance

Pure cultures of bacteria previously isolated, either from the gut of tilapia (*Oreochromis* sp.) collected from a fishpond located in the Marilao-Meycauayan-Obando River System (MMORS) or from water samples along the river, were obtained from the culture collection of the Microbial Ecology of Terrestrial and Aquatic Systems (METAS) Research Laboratory of the Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City, Philippines. The sampling and first run of experiments (*i.e.* isolation and preliminary screening of isolates) were conducted during the last quarter of 2017 of which 42 isolates were obtained. Purified stocks were stored at –20°C until further use. The second run of experiments (*i.e.* identification of isolates, antagonistic assays, and antibiotic susceptibility testing – results of which were reported in this study) were done during the first quarter of 2019. Only 9 colonies were selected for further tests based on distinct phenotypic characteristics. Bacterial cultures were maintained in Nutrient Agar (NA), grown at 37°C, and stored at 4°C until ready for use. All cultures were revived either in broth or in agar plates.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates were tested against representative antibiotics of different classes and use, namely: ciprofloxacin (5mcg), erythromycin (15mcg), ampicillin (25mcg), ertapenem (10mcg), piperacillin/tazobactam (100/10mcg), ceftriaxone (30mcg), linezolid (30mcg), and cephalothin (30mcg) (HiMedia® Laboratories, India) using the Kirby-Bauer method based on the protocol by Hudzicki (2009). Briefly, antibiotic-impregnated disks were firmly placed on Mueller-Hinton Agar (MHA) plates previously lawned with standardized bacterial suspension from overnight cultures of pure isolates. Plates were incubated at 37°C for 16-18 hours. The diameter of zone of inhibition (ZOI) was measured to the nearest mm after incubation. The disk concentration, classification, and standard ZOI of the tested antibiotics are summarized in table 1, based from BSAC standardized disk susceptibility testing method version 8 (Andrews 2009). Multiple antibiotic resistance (MAR) index was determined by obtaining the ratio of the number of antibiotics to which the isolate displayed resistance, to the number of antibiotics to which the isolate had been evaluated for susceptibility. A MAR index greater than 0.2 indicates a 'high risk' source of contamination (Krumperman 1983).

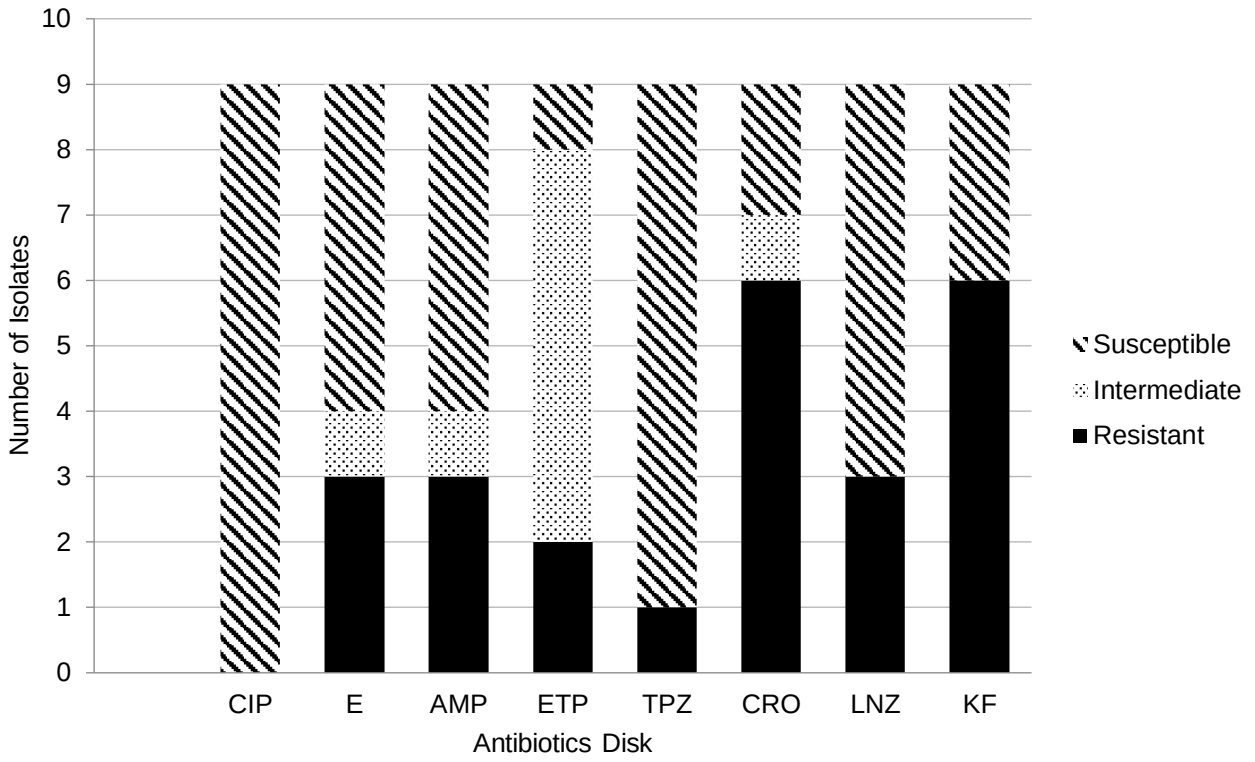

Figure 1: Antibiotic resistance profile of the isolates obtained from MMORS. CIP: ciprofloxacin (5mcg); E: erythromycin (15mcg); AMP: ampicillin (25mcg); ETP: ertapenem (10mcg); TPZ: piperacillin/tazobactam (100/10mcg); CRO: ceftriaxone (30mcg); LNZ: linezolid (30mcg); KF: cephalothin (30mcg)

Antagonistic assay

The potential antagonistic activity of the isolates against the common test pathogens was determined through agar disk and agar well diffusion assays. In the agar disk diffusion assay, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* as competitor test organisms were lawned on NA plates. Filter paper disks with the standardized inhibitor isolate were placed onto the agar surface, and the plates were incubated at 37°C for 16-18 hours. Zone of clearing was measured after incubation. Filter paper disk inoculated with Nutrient Broth (NB) served as negative control. The same set of competitor test organisms were used in agar well diffusion assay. Instead of filter paper disks, wells were punched into the agar and inoculated with standardized inhibitor organisms. Plates were incubated at 37°C for 16-18 hours and were observed for the presence of clearing zones.

Genomic DNA extraction and polymerase chain reaction (PCR)

Bacterial genomic DNA was extracted using Zymo Research Quick-DNA™ Microbe Miniprep Kit according to manufacturer's instructions. The 16S rDNA region of the isolates was amplified in a 30µL reaction mixture consisting 15µL SYBR™ Green PCR master mix, 5µL DNA extract, and 4µL nuclease free water, and 3µL each of 10µM universal forward and reverse bacterial primers (27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-GGYTACCTTGTTACGACTT-3') (Weisburg et al. 1991). PCR was conducted by using a thermal cycler (Thermo Scientific™ Arktik™) with the following amplification conditions: initial denaturation at 95°C for 2 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 2 minutes, and final extension at 72°C for 5 minutes. PCR products mixed with 1µL of 1X Invitrogen BlueJuice™ gel loading buffer were analyzed on agarose gel (0.5g agarose, 50mL 1X TAE) stained with GelRed® nucleic acid gel stain in 1X TAE buffer, and run at 100V for 45 minutes. Vivantis® 100-bp plus ladder was used as molecular weight marker and was visualized under a UV transilluminator (Clinx Science®).

Sequence data and phylogenetic analysis

PCR products were sent to First BASE Laboratories, Malaysia for purification and sequencing. Partial 16S rDNA fragments were matched with the sequences of reference strains in GenBank database through BLAST (Basic Local Alignment Search Tool) (http://www.ncbi.nlm.nih.gov). The nucleotide similarity, e-value, nucleotide coverage, and maximum score were the bases for the putative identification of isolates. Sequence alignment was carried out in BioEdit version 7.0.5.3 (Hall 1999). The Clustal W algorithm (Higgins and Lemey 2009) of the BioEdit Sequence Alignment Editor program was used to create initial alignments with default gap penalties. Saturation of dataset was analyzed using Xia Test (Xia 2009) in DAMBE program. Sequence distance matrices were established in pairwise comparisons through transition model of nucleotide substitution with gamma distribution which was identified as the optimal model using jModelTest v.2.1.10 software (Darriba et al. 2012). Phylogenetic tree was constructed by neighbor-joining method (Saitou and Nei 1987) using the PAUP* version 4.0 software (Swofford 2002). *S. aureus* was chosen as an outgroup.

RESULTS AND DISCUSSION

Antibiotic resistance profile

The antibiotic resistance profile of 9 MMORS isolates are shown in figure 1 and in table 2. Among the list are six common classes of antibiotics (aminoglycosides, macrolides, penicillins, quinolones, sulfonamides, and tetracyclines) that are regularly used in agriculture and aquaculture (Done et al. 2015). All isolates were susceptible to ciprofloxacin of the fluoroquinolone class that can work against a wide range of Gram-negative and

Table 2: Multiple antibiotic resistance (MAR) index of isolates

Disk Code	Isolate								
(in mcg)	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9
CIP (5)	S	S	S	S	S	S	S	S	S
E (15)	I	S	S	R	S	R	S	R	S
AMP (25)	R	S	S	I	R	S	R	S	S
ETP (10)	R	I	S	I	R	I	I	I	I
TPZ (100/10)	S	S	S	S	R	S	S	S	S
CRO (30)	R	R	S	S	R	R	R	I	R
LNZ (30)	R	S	S	R	S	S	S	R	S
KF (30)	R	S	S	R	R	R	R	R	S
MAR Index	0.625	0.125	0	0.375	0.625	0.375	0.375	0.375	0.125

Standard zones of inhibition are based from BSAC standardized disk susceptibility testing method version 8 (Andrews 2009)

CIP: ciprofloxacin; E: erythromycin; AMP: ampicillin; ETP: ertapenem; TPZ: piperacillin/tazobactam; CRO: ceftriaxone; LNZ: linezolid; KF: cephalothin; MAR: multiple antibiotic resistance

Gram-positive microorganisms (Sharma et al. 2017). Isolates MS4, MS6 and MS8 exhibited resistance to erythromycin, a macrolide commonly used in small-scale poultry farms (Kariuki et al. 2013). Vibrios from shrimp larvae and ponds in the Philippines were also found to be erythromycin-resistant (Tendencia and Lavilla-Pitogo 2005). MS1, MS5, and MS7 exhibited resistance to ampicillin of the penicillin class commonly used in hospitals (Wall et al. 2016). MS1 and MS5 exhibited resistance to ertapenem, a restricted carbapenem antibiotic requiring approval from specialists but was found to be non-adherent to prescription guidelines, either due to no de-escalation of the antibiotic or due to an uncompleted course of therapy, according to a study in a Manila private hospital (Mitchell et al. 2019). Hospital sewage and river water in some areas of Metro Manila were found to be contaminated by clinically relevant carbapenemase-producing Enterobacteriaceae strains (Suzuki et al. 2020). MS1, MS2, MS5, MS6, MS7, and MS9 exhibited resistance to ceftriaxone, a cephalosporin extensively used in hospital and community settings (Collignon and McEwen 2019). MS1, MS4, and MS8 exhibited resistance to linezolid, an oxazolidinone used in infections caused by vancomycin-resistant *Enterococcus faecium*, hospital-acquired and community-acquired pneumonia, and other skin infections (Hashemian et al. 2018). MS1, MS4, MS5, MS6, MS7, and MS8 exhibited resistance to cephalothin, a first-generation cephalosporin used in treating uncomplicated skin and soft tissue infections, and as mild surgical prophylaxis (Mehta and Sharma 2016). Only MS5 was resistant to piperacillin/tazobactam of the penicillin class. Unpublished data of healthcare facilities in Manila area also revealed resistance of *P. aeruginosa* to piperacillin/tazobactam and carbapenems (Mitchell et al. 2019). MAR indexing is a rapid and cost-effective method of bacteria source tracking and health risk assessment (Davis and Brown 2016; Sandhu et al. 2016). MAR index for each isolate was calculated by using the formula *a/b*, where *a* is the number of antibiotics to which the isolate exhibited resistance, and *b* is the total number of antibiotics to which the isolate had been evaluated for susceptibility (Krumperman 1983). MS1 and MS5 had the highest MAR index at 0.625, followed by MS4, MS6, MS7, and MS8 with MAR index of 0.375 (table 2). MS2 and MS9 had MAR index of 0.125. Only MS3 was susceptible to all antibiotics with zero MAR index (table 2). Six out of nine isolates (66.66%) have MAR index greater than 0.2. MS1 and MS5 had the highest MAR index at 0.625, followed by MS4, MS6, MS7, and MS8 with MAR index of 0.375. MS2 and MS9 had MAR index of 0.125. The proportion of isolates with a MAR index that exceeded the standard of 0.2 was higher than those with a MAR index below 0.2 (33.33%), suggesting that these isolates originate from sites that are at high risk of contamination (Davis and Brown 2016; Osundiya et al. 2013; Krumperman 1983), thus revealing the threat posed by these potentially pathogenic microorganisms to food safety and public health in MMORS.

Antagonistic activity against common pathogens

Isolates were tested for inhibitory activity against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa,* and *E. faecalis* via agar disk diffusion assay. Out of the 45 pairwise interactions of isolate vs pathogenic bacteria, only MS2 exhibited antagonistic activity against *S. aureus* (data not shown). Isolates were also tested for their antagonistic activity against each other via agar well diffusion assay. Out of the 72 pairwise interactions of isolate vs isolate excluding self-self interaction, none exhibited antagonistic activity against other isolates (data not shown).

Apart from possessing multidrug resistance, the result of the antagonistic activity of isolate vs pathogenic bacteria further demonstrated that most of the isolates obtained from MMORS potentially do not manifest the traits of beneficial microbes such as efficiency in disease management since they are not capable of significantly suppressing the growth of the tested common pathogens. Among all the isolates screened for antibiotic susceptibility and antimicrobial activity, only MS3 remained as a viable candidate that could be further evaluated for probiotic functions. Despite not showing antagonistic activity against common pathogens and against the test isolates, only MS3 conferred sensitivity to all the antibiotics tested. Further analysis of its susceptibility against a wide range of antibiotics as well as its capacity to establish within a host, inhibit fish pathogens as well as provide other benefits that could increase fish survivability and health are necessary (Pandiyan et al. 2013). Only 1 out of the 117 pairwise interactions was classified as competitive but this does not guarantee that the remaining interactions are completely noncompetitive.

Identification of isolates and its implications

All isolates except MS8 were putatively identified through partial 16S rDNA sequencing (table 3) due to null sequence data. Only isolates with the common similarity threshold of 97% 16S rRNA sequence similarity for bacteria (Nguyen et al. 2016) were analyzed for molecular phylogeny. Most of the isolates clustered with *Bacillus* species (fig. 2).

Table 3: Identification of bacterial isolates based on partial 16S rRNA gene sequences

Isolate	Accession Number	BLASTn Result	Percentage Similarity
MS1	MN589823.1	*Bacillus pumilus*	99.87%
MS2	MN704737.1	*Bacillus* sp.	99.30%
MS3	MG214999.1	*Brevibacillus* sp.	93.08%
MS4	MF754135.1	*Morganella morganii*	99.90%
MS5	MN750766.1	*Bacillus cereus*	100.00%
MS6	MN314217.1	*Escherichia coli*	99.78%
MS7	MN733140.1	*Bacillus anthracis*	100.00%
MS9	MN594798.1	*Bacillus* sp.	100.00%

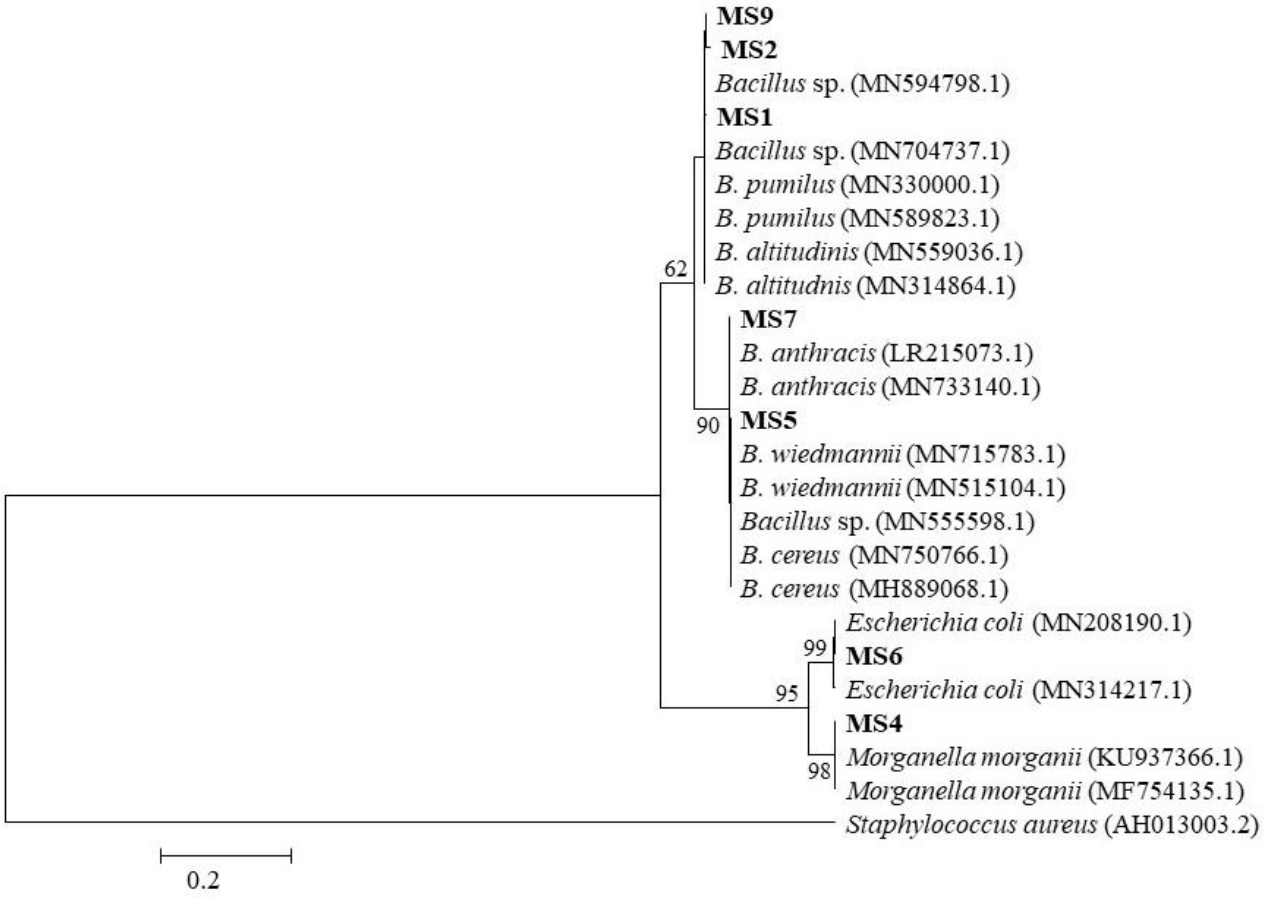

Figure 2: Phylogenetic tree of bacteria in this study. Neighbor-joining tree was constructed based on 807 nucleotides of 16S rRNA gene following the transition model of DNA substitution with Gamma correction. The tree is rooted on *Staphylococcus aureus*. Values on nodes represent bootstrap percentages based on 1000 bootstrap samples. Values less than 50% are not shown. Scale bar represents 20 nucleotide substitutions for every 100 nucleotides.

MS1 with 99.87% sequence similarity with *Bacillus pumilus* and MS5 with 100% sequence similarity with *B. cereus*, both with 0.625 MAR index, were isolated from Meycauayan public market water samples. Although *B. cereus* and *B. pumilus* are found throughout the environment such as soil, and fresh and marine waters (Bintsis 2017; Liu et al. 2013), MS1 and MS5 may be possibly sourced from slaughtered animals such as pig and chicken since Meycauayan is an abattoir. *B. cereus* and *B. pumilus* are used as feed additives in the list of 2018 Registered Products by the Animal Feeds Veterinary Drugs and Biologics Control Division (AFVDBCD 2018) of the Bureau of Animal Industry. Discharged slaughterhouse wastes into the river may eventually lead to fish farms. Widespread application of antibiotics in livestock and poultry acts as a selective pressure for the emergence and persistence of antibiotic resistant bacteria (Gebreyes et al. 2017; Koch et al. 2017).

MS9 from fish gut sample from Obando fishpond matched with *Bacillus sp.* with 100.00% similarity. It had 0.375 MAR index indicating that the fish was obtained from a site at high risk of contamination. Antibiotic resistant bacteria can make their way into the food chain, and the transfer of several resistance factors clearly poses health risks to consumers (Schwartz et al. 2003). MS7, also from a fish gut sample from Obando fishpond, had 100.00% sequence similarity with *B. anthracis*. This *Bacillus* species along with *B. cereus, B. thuringensis*, and *B. pumilus* from effluent water of aquaculture farms were also found to be among the most dominant resistant bacterial genera against tetracycline (Liyanage and Manage 2019), an antibiotic extensively used in aquaculture for treatment of fish diseases (Mortazavi 2014). Ampicillin resistance genes were likewise identified in *B. subtilis* from wastewater treatment plant effluent disposed to a river, indicating that wastewater disposal increases

the reservoir of resistance mechanisms in the environment (Amos et al. 2014). Heavy metal tolerant *Bacillus* species isolated from polluted river waters were also reported as multidrug-resistant (Shammi and Ahmed 2013).

MS2 and MS4 from Obando fishpond water samples harbored *Bacillus* sp. and *Morganella morganii* with 99.30% and 99.90% sequence similarity, respectively. *M. morganii* isolated from shrimp was found to be resistant to azithromycin, an antibiotic prescribed for respiratory infections, skin infections, and inflammatory disease (Khan 2018). *M. morganii* is responsible for shrimp spoilage and neonatal sepsis (Salen and Eppes 1997). MS3, also from Obando fishpond water samples, harbored 93.08% sequence similarity with *Brevibacillus* sp. Studies showed that *Brevibacillus* has potential in wastewater degradation (Hooda et al. 2018; Chebbi et al. 2015), heavy metal tolerance, and as a leachate biological treatment (Er et al. 2018). *Brevibacillus* species isolated from giant freshwater prawn larvae showed antibacterial activity against fish and prawn pathogens, suggesting its potential as probiotic (Mujeeb et al. 2017).

MS6 obtained from Marilao bridge water sample had 99.78% sequence similarity with *E. coli*. Waterborne *E. coli* are a major reservoir of antimicrobial resistance, including but not limited to extended spectrum β-lactamase (ESBL). *E. coli* harboring ESBL from river water and wastewater were reported as pathogenic and exhibited multidrug resistance (Haberecht et al. 2019). In Metro Manila, *E. coli* were prevalent in irrigation waters of some urban farms with multidrug resistance and high resistance to tetracycline (Vital et al. 2018). Similar studies on polluted and non-polluted river and irrigation waters, as well as freshwater and marine food products, reported the presence of multidrug-resistant *E. coli* (Tersagh et al. 2018; Ye et al. 2017; Lyimo et al. 2016; Wambugu et al. 2015). *E. coli* are also commonly found in the intestinal tract of humans and animals, thus may serve as a good indicator of faecal contamination (Cabral 2010) and can be used to track antibiotic resistance in rivers (Wambugu et al. 2015). According to the 2017 Antimicrobial Resistance Surveillance Program (ARSP) Data Summary Report of the Research Institute for Tropical Medicine (RITM), a total of 8,939 *E. coli* isolates were reported, and majority of which were isolated mainly from urine specimens. The highest resistance rates for *E. coli* were for ampicillin at 83% (n=7,792), followed by co-trimoxazole at 61% (n=7,446), ciprofloxacin at 41% (n=7,601), and ceftriaxone at 39% (n=7,802). Resistance to carbapenems were found to be emerging with rates against ertapenem at 3% (n=4,775), imipenem at 5% (n=7,539), and meropenem at 5% (n=8,194). The 8 Metro Manila sentinel sites comprised the 34% of the total 2017 ARSP data.

MMORS is still utilized as industrial water supply, for recreation, and for the propagation and growth of fish and other aquatic resources (DENR 1990) albeit continuous discharge of effluents from industrial, aquaculture and agricultural runoffs, and many other activities of anthropogenic origin. Water is an important vehicle in facilitating the spread of antibiotic residues and resistant genes (Wall et al. 2016), and it alleviates their transfer into the bacterial pathogens and their propagation. With the lack of proper sewage treatment system, the incessant discharge of effluents in reservoirs of multidrug resistant bacteria will continuously contribute to the harmful impacts on human health (Caruso 2016; Mendoza 2006). Increasing cases of multidrug resistance conveys a serious need for 'broad-based, local AMR surveillance, and planning of effective interventions' to reduce AMR, and to prevent further acquisition of resistance genes by these microorganisms (Osundiya et al. 2013).

CONCLUSION

Bacterial isolates from MMORS conferred resistance to some of the antibiotics tested. Despite being highly polluted, Bulacan still contributes to Region III's fish production, hence, the presence of antibiotic resistant and potentially pathogenic microorganisms in water and fish gut samples collected from MMORS poses threat to food safety and public health. Risks include the presence of antibiotic residues in the environment and in aquaculture produce, and eventually the development and spread of AMR.

The high MAR indices revealed that MMORS is at high risk of contamination, indicating the prevalent misuse and abuse of antibiotics in the vicinity of the river system primarily in fish farms, as well as implications in improper treatment of hospital waste. Further investigation on the AMR of MMORS microbial community and the development of strategies and interventions to prevent further transmission of resistance genes are needed.

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