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Motile aeromonads recovered from tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines: assessment of antibiotic susceptibility and multidrug resistance to selected antibiotics

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Abstract

A total of 177 motile *Aeromonas* spp., comprised of *A. hydrophila* (n=162) and *A. sobria* (n=15), isolated from the water, sediment, gills and intestines of tilapia collected from the grow-out culture earthen ponds in the Philippines were investigated for their antimicrobial susceptibility to 10 antibiotics commonly used in fish aquaculture. All motile *Aeromonas* spp. tested generally exhibited a very high percentage of resistance to amoxicillin (*A. hydrophila*: 95%; *A. sobria*: 100%), erythromycin (99%;100%), neomycin (83%;100%), and oxytetracycline (92%;100%), respectively. On the contrary, they showed substantial susceptibility to chloramphenicol, nitrofurantoin, doxycycline, enrofloxacin and norfloxacin. Results of the antimicrobial susceptibility testing demonstrated that 94.4% and 100% of *A. hydrophila* and *A. sobria* isolates tested were resistant to antibiotics belonging to at least 3 or more categories and therefore considered as multiple drug resistant strains. The multiple antibiotic resistance (MAR) indexing of *A. hydrophila* and *A. sobria* strains showed that they originated from high-risk sources.

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Introduction

Tilapia (*Oreochromis* spp.) is among the major aquaculture species intensively farmed worldwide. In the Philippines, tilapia has by far emerged as a major commercially important fish species ranking second to milkfish with regard to the country's economic and social development (Romana-Eguia et al., 2004, 2022). The Nile tilapia *O. niloticus* is the most widely cultured species in freshwater and brackishwater ponds, lakes, and reservoirs owing to its capability to be cultured even under marginal conditions (Romana-Eguia et al., 2004, 2022). However, a semi-intensive and intensive culture of tilapias in earthen ponds, typified by high stocking density, can inescapably instigate the diminution of the quality of the rearing environment thereby rendering tilapias become more susceptible to diseases. An amalgamation of various abiotic factors including environmental degradation exemplified by abnormal water physicochemical parameters such as low dissolved oxygen and high ammonia levels can consequently inflict remarkable stress that most often than not result in fish morbidity and eventual mortality. Concomitantly, environmental degradation plausibly facilitates the multiplication of opportunistic bacterial pathogens which in reality are members of the natural bacterial microbiota ubiquitously residing in tilapia and their rearing environment (Pakingking et al., 2015, 2020). Hence, under harsh environmental conditions typified by the build-up of excessive organic matter in the rearing water due to excessive accumulation of uneaten feeds, logarithmic proliferations of these opportunistic bacteria in the water and concomitantly in tilapia's gills and intestines may inadvertently result to the occurrence of disease epizootics (Al-Harbi and Uddin, 2005; Pakingking et al., 2015, 2020). Several bacterial species have been implicated in diseases of different fish species including tilapia, however, *Aeromonas* spp., known pathogenic bacteria harmful to fish and humans, have by far been extensively reported (Austin and Austin, 2012; Janda and Abbott, 2010).

Aeromonas spp. are common aquatic microorganisms widely distributed in freshwater, brackish water, river water, groundwater, spring water, irrigation water, and sewage-contaminated water among others, clearly showing that aquatic environment represents an important reservoir for aeromonads (Al-Harbi and Uddin, 2005; Austin and Austin, 2012; Janda and Abbott, 2010; Pakingking et al., 2015, 2020). The extensive distribution of aeromonads in diverse aquatic ecosystems underlines their capacity to adapt to environments with different trophic levels (Janda and Abbott, 2010). Motile pathogenic *Aeromonas* spp. such as *A. hydrophila, A. caviae, A. sobria, A. jandei*, and *A. veronii*, have been implicated in bacterial diseases with resultant mortality reaching up to 80 to 100% in cultured tilapia (Dong et al., 2017; El Latif et al., 2019; Li and Cai, 2011; Raj et al., 2019; Roy et al., 2019). Infection with *Aeromonas* spp. is typically characterized by hemorrhagic ulcers on the skin and fin erosion (AlYahya et al., 2018; Janda and Abbott, 2010, Ly et al., 2009, Xia et al., 2022). Because *Aeromonas* spp. can ubiquitously inhabit marine and freshwater environments, under unfavorable rearing conditions, they can inevitably emerge as opportunistic pathogens that can devastatingly impact the tilapia farming industry (Austin and Austin, 2012; Janda and Abbott, 2010; Santos et al., 2011).

In the Philippines, mortalities of cultured tilapias due to bacterial infections, i.e. with *A. hydrophila* frequently isolated from the kidney or exophthalmic eye of infected fish, have been reported (Yambot, 1998). To mitigate losses, farmers have resorted to using antibiotics and other chemical agents as prophylactic and therapeutic agents for various bacterial diseases of tilapia. However, over the years, the indiscriminate use of antibacterial agents has not only caused death and morphological deformities in fish but consequently led to the development of resistant bacterial strains thereby posing serious public health hazards (Janda and Abbott, 2010). For example, the incorporation of sub-therapeutic doses of antibiotics into feeds to treat aeromonad infection of tilapia reared in ponds and floating net cages in lakes and rivers has become a serious problem in developing countries. In the Philippines, although regulation on the use of these antimicrobials and chemicals in aquaculture has been recently worked out by the Bureau of Fisheries and Aquatic Resources

(BFAR) (Regidor, 2020), the application of antibiotics in animal husbandry and aquaculture is still widely practiced in the country. This should be seriously taken into account considering the fact that the widespread application of antibiotics is the most critical factor responsible for amplifying the level of resistance in a given reservoir (Wegener and Frimodt-Moller, 2000). In fact, multiple antibiotic resistance (MAR) among *Aeromonas* spp., including *A. hydrophila* and *A. sobria* which are regarded as pathogens of tilapia, has been reported from many parts of the world (Pettibone et al., 1996; Son et al., 1997; Ko et al., 1998; Vivekanandhan et al., 2002; Hatha et al., 2005). Additionally, the widespread and indiscriminate use of antibiotics to prevent and treat bacterial infections in tilapia has consequently resulted in a global increase in antibiotic resistance among fish pathogenic bacteria (Eid et al., 2022; Pauzi et al., 2020).

Based on the surveys conducted by the competent authority of the Philippines, the Bureau of Fisheries and Aquatic Resources (BFAR), amoxicillin and oxyteracycline were the antibiotics frequently used by farmers to treat bacterial infections of tilapia (Regidor, 2020). It should be also noted that based on the survey conducted by the BFAR and as per personal communication with Dr. S. Somga (Head, National Fisheries Laboratory Division, BFAR), fish farmers observed that the efficacy of amoxicillin and oxytetracyline incorporated in feed as a treatment against bacterial infections, particularly when the occurrence of mortality is encountered, has apparently reduced, plausibly due to the emergence of drug-resistant bacteria. Moreover, aside from amoxicillin and oxytetracycline, the indiscriminate use of other antibiotics in tilapia aquaculture over the past several years may have likewise led to the emergence of multiple drug-resistant bacteria. However, to date pertinent data that could be used as a reference to demonstrate the occurrence of multiple antibiotic-resistant *Aeromonas* spp. in pond-produced tilapia in the Philippines are unavailable. Because dominance of *A. hydrophila* and *A. sobria* in the water and sediment, and gills and intestines of apparently healthy *O. niloticus* grown in brackishwater earthen ponds has been comprehensively documented (Pakingking et al., 2020), under these circumstances, we investigated in the current study the prevalence of antibiotic resistance among *A. hydrophila* and *A. sobria* strains isolated from the water, sediment, gills and intestines of tilapia collected from grow-out culture earthen ponds (Pakingking et al., 2020) to antibiotics commonly used in aquaculture (Regidor, 2020; Somga et al., 2012) and identified the high-risk source. Resolute results generated from this study would plausibly serve as springboard aimed at developing strategies toward the responsible use of antibiotics for effective disease management of cultured tilapia species.

Materials and Methods

Bacterial isolates

A total of 177 motile *Aeromonas* spp., i.e. comprised of *A. hydrophila* (n=162) and *A. sobria* (n=15), previously isolated from the water, sediment, gills and intestines of tilapia periodically collected from grow-out culture earthen ponds located in Barangay (village) Lantad and Barangay Balaring, Silay City, province of Negros Occidental, Philippines (**Figure 1**) (Pakingking et al., 2020), were subjected to antibiotic sensitivity test (**Tables 1 and 2**). These isolates were taxonomically identified at the species level using the criteria specified in the Bergey's Manual of Systematic Bacteriology (Holt, 1994) and API 20 E or API 20NE (bioMeriuex, France) methods (Pakingking. et al., 2020). Pure cultures of these isolates were stocked in tryptic soy broth (TSB; Merck) supplemented with 15% glycerol at −80ºC.

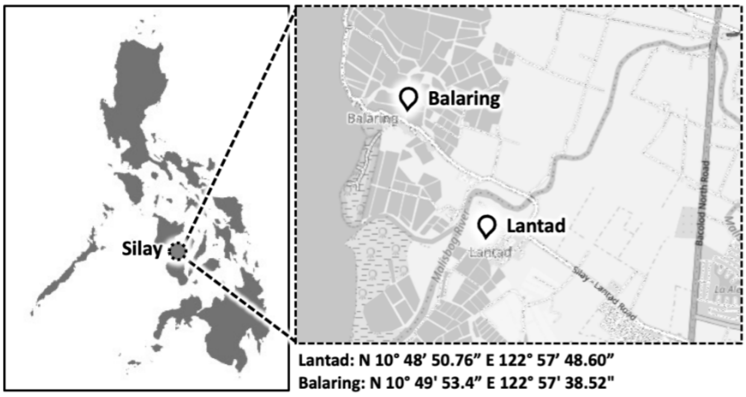

Figure 1 Map of Silay city, province of Negros Occidental, showing the locations of the sampling sites in Barangay Balaring and Barangay Lantad.

Antibacterial susceptibility testing and determination of multiple antibiotic resistance (MAR) index

The susceptibility of *A. hydrophila* and *A sobria* isolates was determined on Mueller-Hinton agar (MHA; Merck, Germany) employing the disc diffusion technique (Bauer et al., 1966) as described by Gołaś et al. (2022). The antibacterial susceptibility tests involved 10 antimicrobial drugs belonging to the most popular classes of antibiotics including beta-lactams, phenicols, macrolides, aminoglycosides, nitrofurans, fluoroquinolones, tetracyclines, and sulfonamides. Commercially available antibiotic sensitivity discs (Mastdiscs™, Mast Group Ltd., UK) used in the assay included amoxicillin (25 µg), chloramphenicol (30 µg), doxycycline (30 µg), erythromycin (15 µg), enrofloxacin (5 µg), neomycin (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), oxytetracycline (30 µg), and trimethoprim-sulphamethoxazole (25 µg). 24h cultures of the bacterial isolates grown on tryptic soy agar plates (TSA; Merck, Germany) were suspended in 0.85% sterile normal saline solution (NSS), and the turbidity of the bacterial suspension was adjusted to match 0.5 McFarland standard. The bacterial inocula were then streaked on MHA plates using a sterile cotton swab. After 30 min, the discs were placed on the agar surface using a disc dispenser, i.e. sufficiently separated so as to prevent the overlapping of the inhibition zones. The plates were then incubated at 30ºC for 18-24 h (Gołaś et al., 2022). The antibiotic susceptibility of the tested bacterial isolates was evaluated based on the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2012). To verify the antibacterial effect of the antibiotics tested, *A. hydrophila* BIOTECH 10090 was obtained from the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna 4031, Philippines, was used as a control microorganism.

The multiple antibiotic resistance (MAR) index was computed according to the method described by Krumperman (1983). To determine the MAR index of a single isolate, the number of antibiotics to which the isolate was resistant was divided by the number of antibiotics to which the isolate was exposed. MAR index value higher than 0.2 is considered to have originated from high-risk sources of contamination such as humans, commercial poultry farms, swine, and dairy cattle wherein antibiotics are frequently used. MAR index value of ≤0.2 is regarded as bacterial strains whose origin comes from animals that are seldom or have not been exposed to antibiotics (Krumperman, 1983).

Results

Antimicrobial susceptibility of A. hydrophila and A. sobria isolates

As shown in **Table 1**, *A. hydrophila* isolates obtained from various sources generally exhibited a very high percentage of resistance to four commonly used antibiotics in fish aquaculture including neomycin (83%), oxytetracycline (92%), amoxicillin (98%) and erythromycin (99%). Surprisingly, while 35% of the *A. hydrophila* isolates were found susceptible to trimethoprim sulfamethoxazole, 72% of these isolates obtained from the pond water exhibited resistance. Notably, these *A. hydrophila* isolates examined were highly susceptible to chloramphenicol, nitrofurantoin, doxycycline, and fluroquinolones including norfloxacin and enrofloxacin (**Table 1**). In the case of *A. sobria* isolates, a similar trend was likewise observed, i.e. these aforesaid isolates generally exhibited a very high level of antibiotic resistance to amoxicillin (100%), erythromycin (100%), neomycin (100%), and oxyteracycline (100%) (**Table 2**). Although 53% of the *A. sobria* strains examined were resistant to trimethoprim sulfamethoxazole, it is quite alarming to note that 80 and 100% of these strains isolated from sediment and intestines of tilapia were resistant to this antibiotic, respectively. Additionally, *A. sobria* isolates tested were likewise highly susceptible to chloramphenicol, nitrofurantoin, doxycycline, norfloxacin and enrofloxacin (**Table 2**).

Table 1 Prevalence of antibiotic resistance among *Aeromonas hydrophila* strains isolated from the water, sediment, gills and intestines of tilapia.

Antibiotic Class	Antibiotic	Percent (%) resistant strains				
		Water (n=43)	Sediment (n=38)	Gills (n=42)	Intestines (n=39)	Mean (n=162)
Beta-lactams	Amoxicillin	100	100	95	95	98
Phenicols	Chloramphenicol	0	5	0	0	1
Macrolides	Erythromycin	100	97	100	100	99
Aminoglycosides	Neomycin	100	76	64	90	83
Nitrofurans	Nitrofurantoin	2	8	0	0	3
Fluoroquinolones	Norfloxacin	0	0	0	0	0
	Enrofloxacin	12	34	10	18	18
Tetracyclines	Oxytetracycline	100	95	83	90	92
	Doxycycline	0	5	0	3	2
Sulfonamides	Trimethoprim sulfamethoxazole	72	18	19	26	35

Table 2 Prevalence of antibiotic resistance among *Aeromonas sobria* strains isolated from the water, sediment, gills and intestines of tilapia.

Antibiotic Class	Antibiotic	Percent (%) resistant strains				
		Water (n=6)	Sediment (n=6)	Gills (n=1)	Intestines (n=2)	Mean (n=15)
Beta-Lactams	Amoxicillin	100	100	100	100	100
Phenicols	Chloramphenicol	0	0	0	0	0
Macrolides	Erythromycin	100	100	100	100	100
Aminoglycosides	Neomycin	100	100	100	100	100
Nitrofurans	Nitrofurantoin	0	0	0	0	0
Fluoroquinolones	Norfloxacin	0	0	0	0	0
	Enrofloxacin	0	0	0	0	0
Tetracyclines	Oxytetracycline	100	100	100	100	100
	Doxycycline	0	0	0	0	0
Sulfonamides	Trimethoprim sulfamethoxazole	33	80	0	100	53

MAR index of A. hydrophila and A. sobria isolates

Results of the antimicrobial susceptibility testing demonstrated that 153 out of 162 (94.4%) *A. hydrophila* isolates tested were resistant to antibiotics belonging to at least 3 or more categories (**Table 3**) and therefore considered as multiple drug resistant strains. As shown in **Table 3**, 32 (74.4%) of the *A. hydrophila* strains obtained from the pond water exhibited remarkable resistance to at least 5 antibiotics. Overall, about 35.8% of the *A. hydrophila* strains obtained from the pond water, sediment, gills and intestines of tilapia were resistant to 4 antibiotics followed by 35.2%, 14.8% and 7.4% of 5, 3, and 6 antibiotics, respectively. Interestingly, 0.6 % of the strains were resistant to 7 and 8 antibiotics, respectively (**Table 3**). In the case of *A. sobria* strains, 15 (100%) of the isolates tested were remarkably resistant to antibiotics belonging to at least 3 categories as shown in **Table 4**. In particular, 53.3% of the *A. sobria* strains examined were resistant to 4 antibiotics followed by 40% and 6.7% of 5 and 3 antibiotics, respectively.

Table 3 Multiple antibiotic resistance (MAR) index of *Aeromonas hydrophila* strains isolated from the water, sediment, gills and intestines of tilapia.

MAR Index	Source				Total (n=162)	Percentage (%) occurrence
	Water (n=43)	Sediment (n=38)	Gills (n=42)	Intestines (n=39)		
0.1	0	0	0	0	0	0
0.2	0	2	5	2	9	5.6
0.3	0	6	13	5	24	14.8
0.4	10	13	16	19	58	35.8
0.5	32	10	4	11	57	35.2
0.6	1	5	4	2	12	7.4
0.7	0	1	0	0	1	0.6
0.8	0	1	0	0	1	0.6
0.9	0	0	0	0	0	0

Table 4 Multiple antibiotic resistance (MAR) index of *Aeromonas sobria* strains isolated from the water, sediment, gills and intestines of tilapia.

MAR Index	Source				Total (n=15)	Percentage (%) occurrence
	Water (n=6)	Sediment (n=6)	Gills (n=1)	Intestines (n=2)		
0.1	0	0	0	0	0	0
0.2	0	0	0	0	0	0
0.3	0	0	1	0	1	6.7
0.4	4	2	0	2	9	53.3
0.5	2	4	0	0	5	40.0
0.6	0	0	0	0	0	0
0.7	0	0	0	0	0	0
0.8	0	0	0	0	0	0
0.9	0	0	0	0	0	0

Discussion

As shown in **Tables 1** and **2**, the *A. hydrophila* and *A. sobria* strains that we examined, particularly those isolated from the pond water, demonstrated a high level of resistance to amoxicillin, a beta-lactam antibiotic, and this finding concurs with the previous reports of many researchers, for example, from diseased cultured carp (Guz and Kozińska, 2004) and rearing water of carp ponds in Poland (Zdanowicz et al., 2020), from farmed mud loach (*Misgurnus mizolepis*) in Korea (Yu et al., 2015), and from marine species of shrimps cultured at inland low salinity ponds in Thailand (Yano et al., 2015). As beta-lactam antibiotics like amoxicillin have low toxicity, this class of antibiotics is by far the most extensively used antibiotics, i.e. approximately 50% of global antibiotic consumption, to treat a broad range of bacterial infections in cultured fish. As pointed out by Saavedra et al. (2004), the genus *Aeromonas* is considered naturally resistant to beta-lactam antibiotics. This is because of chemically unstable beta-lactam ring in the structure of beta-lactam antibiotics wherein they are readily susceptible to bacterial hydrolysis by chromosomal beta-lactamases produced by *Aeromonas* spp., thus, easily eliminated (Goñi-Urriza et al., 2000). Furthermore, the resistance of *Aeromonas* spp. to beta-lactam antibiotics can also be attributed to their ability to synthesize three extracellular enzymes, i.e. beta-lactamase, acylase and penicillinase (Janda and Abott, 2010). These enzymes can hydrolyze the amide bond of the beta-lactam ring of beta-lactam antibiotics and likewise limit the permeability of cytoplasmic membranes to these antibiotics (Guz and Kozińska, 2004; Saavedra et al., 2004). It should be also noted that the number of *Aeromonas* strains producing an extended spectrum of beta-lactamases capable of hydrolyzing beta-lactam antibiotics has conspicuously increased in recent years and therefore, resistance to beta-lactams may plausibly become a serious global problem. The high level of antibiotic resistance against amoxicillin noted for both *A. hydrophila* and *A. sobria* strains may also indicate that the beta-lactamase gene might be widely present in the gene pool of microbes in the studied aquatic environment (Lin et al., 2004).

A high level of resistance to erythromycin, a macrolide antibiotic, was also observed for both *A. hydrophila* and *A. sobria* strains tested in the present study. This finding is comparable to the data obtained by Son et al. (1997) who reported that 43% of the 21 *Aeromonas* strains recovered from *Tilapia mossambica* were resistant to erythromycin. Additionally, 64-100% of the motile *Aeromonas* strains that we examined were resistant to neomycin, coherent to the recent report of Fauzi et al. (2021) in *A. hydrophila* and *A. sobria* strains isolated from various fish samples such as *Oreochromis* spp., *Clarias gariepinus*, and *Pangasius hypophthalmus*, collected from Kelantan and Terengganu, Malaysia.

Enrofloxacin has been commonly used against motile aeromonad septicemia caused by *A. hydrophila* in Thai aquaculture together with oxyteracycline (Baoprasertkul et al. 2012). This could be attributed to the fact that enrofloxacin in general possesses an excellent antibacterial activity against virulent strains of *A. hydrophila* as previously documented by Ren et al. (2014). In the Philippines, the use of enrofloxacin in aquaculture has also been reported by Somga et al. (2012), although the specific target pathogen has not been identified. However, oxyteracycline has by far gained popularity among fish farmers as the preferred antibacterial agent against *Aeromonas* infection in cultured fish, which is usually administered as medicated feed (Julinta et al., 2017). The high sensitivity result obtained for enrofloxacin against *A. hydrophila* and *A. sobria* strains in the current study could be perhaps attributed to the fact that this antibiotic has not been frequently used compared to oxyteracycline. On the contrary, the very high percentage of oxytetracycline-resistant *A. hydrophila* and *A. sobria* strains noted in this study, without doubting, have developed resistance overtime due to the indiscriminate use of oxyteracycline in the facility where these *Aeromonas* strains were isolated. It is worth noting that norfloxacin and doxycycline are also commonly employed by tilapia farmers in the Philippines (Somga et al. 2012), however, pertinent data on in vitro and in vivo studies about their efficacy against *Aeromonas* infection in tilapia are currently unavailable (Regidor, 2020).

The chloramphenicol resistant strains were significantly low (1%) among *A. hydrophila* strains isolated from the pond water. None of these strains isolated from sediment, gills, and intestines of tilapia was found resistant to chloramphenicol. Ansary et al. (1992) reported similar findings in Malaysian and American strains of *A. hydrophila* isolated from fish. However, resistance to chloramphenicol has also been detected among *A. hydrophila* strains recovered from *T. mossambica* (Son et al. 1997). It should be noted that the use of chloramphenicol in fish and shrimp aquaculture has long been banned worldwide (Bondad-Reantaso et al., 2012). It should be also taken into account that although the *Aeromonas* strains examined in this study were all sensitive to nitrofurantoin, its application as a prophylactic or therapeutic agent against bacterial infections in cultured fish species including tilapia, is no longer feasible because BFAR, the competent authority, has banned the use of this drug in food-producing animals including aquaculture (Regidor, 2020).

As large quantities of pathogenic *A. hydrophila* and *A. sobria* strains coming from the feces of tilapia are released into the aquatic environments, their accumulation in the aquatic environments can consequently contaminate fish harvested from these areas. With the presence of these bacteria in tilapia's rearing environment, exchanges of plasmids between bacteria can result in a higher frequency of multiple antibiotic-resistant strains (Hatha et al., 2005). It is therefore imperative that the use of antibiotics including amoxicillin, erythromycin, neomycin, oxytetracycline, and trimethoprim-sulfamethoxazole, which are frequently used in hatcheries and grow out as prophylactic agents and medicated feed, respectively (Somga et al. 2012), be regulated by the competent authority as their continued applications in tilapia culture could promote greater dissemination of virulent and drug-resistant strains of bacterial pathogens in the natural environment thereby posing some impending threats to human health. This recommendation is evidently supported by our current data highlighting the role of tilapia, pond water, and sediment as reservoirs of multi-drug-resistant bacteria that may pose some risks to humans as food-borne infection or following direct contact. The competent authority (BFAR) has recently implemented its residue program to detect any illegal use of banned antibiotics, i.e. chloramphenicol and nitrofurans, and comply with the maximum residue limits for the regulated antibiotics used in food-producing animals including aquaculture (Regidor, 2020).

The present study illustrated multiple antibiotic resistance (MAR) among motile *Aeromonas* strains recovered from tilapia and their rearing environment. In fact, majority of the *A. hydrophila* and *A. sobria* strains tested were resistant to 3 up to 8 antibiotics examined, indicating that these aforesaid motile *Aeromonas* strains were capable of detoxifying those antibacterial organic compounds investigated in this study (Orozova et al., 2008). Moreover, current data clearly indicate that *A. hydrophila* and *A. sobria* strains ubiquitously inhabiting the ponds where they were isolated originated from high-risk sources of contamination where antibiotics have been often used (Orozova et al., 2008). It should be also noted that most classes of antibiotics that are used in medicine and veterinary have been inadvertently introduced into water basins (Ko et al., 2003; Lin et al., 2004). The occurrence of antibiotic resistance genes against different groups of antibiotics in the genus *Aeromonas* derived from aquaculture is widely known (Piotrowska and Popowska, 2014). Undoubtedly, the adaptive responses of bacterial communities to several antibiotics have implications for the health of fish being reared in the studied aquaculture environments (Orozova et al., 2008) and may plausibly reflect the history of antibiotic application in these aquaculture facilities (Bondad-Reantaso et al., 2012). Multiple antibiotic resistance in *Aeromonas* spp. inhabiting different water basins have been documented globally by several authors (Deng et al., 2016; Matyar et al., 2010; Yano et al., 2015). The rapid increase in the number of resistant and multi-resistant *Aeromonas* spp. is attributed to the ability of these organisms to transfer antibiotic resistance by mobile genetic agents (plasmids, transposons, IS elements, gene cassettes, class 1 integrons) among bacterial populations by cell to cell contact (Dar et al., 2016; Patil et al., 2016; Piotrowska et al., 2017). Majority of these mobile elements may harbor multiple antimicrobial resistance determinants that may propel the propagation of antibiotic resistance in aquaculture environments (Patil et al., 2016). Thus, the development of multiple antibiotic resistance by different *Aeromonas* spp. including *A. hydrophila* and *A sobria* isolated from tilapia and their culture environments in recent years has emerged as a major problem in many parts of the world.

In summary, our current study constitutes the first comprehensive report elucidating the high occurrence of multiple antibiotic resistant *A. hydrophila* and *A. sobria* strains inhabiting tilapias' gills and intestines, and their culture environment to antibiotics commonly used in fish aquaculture. Our data further demonstrate that these multiple antibiotic resistant bacteria originated from high-risk sources of contamination. Thus, tilapia growers should be admonished to discontinue the indiscriminate and irresponsible use of antibiotics in tilapia aquaculture in the Philippines as a pragmatic approach to control if not abrogate the acceleration of emerging fish and human pathogenic *Aeromonas* spp. possessing resistance to multiple antibiotics prescribed for veterinary and clinical use. This recommendation should be aggressively implemented in tilapia hatcheries and grow-out ponds because the release of multiple antibiotic resistant bacteria from the feces of fish into their rearing environments will undoubtedly pave the way for the vaster spreading of virulent bacterial pathogens in the natural aquatic environments and thus potentially into the human food chain. Additionally, the occurrence of these multi-drug resistant *Aeromonas* spp. in tilapia's culture environments urgently requires prompt and thorough assessment with regard to their potential role in fish infections in which antibiotic therapy would be required. Further studies are essential for better understanding of antibiotic resistance of *Aeromonas* spp. in fish aquaculture in general particularly in areas where uncontrolled and extensive use of antibiotics may cause the frequent occurrence of multiple antibiotic resistance. The emergence of multiple antibiotic resistant bacteria including the opportunistic fish pathogens such as *Aeromonas* spp. would undoubtedly become an important problem in the future not only in fish health but importantly, in public health as a consequence of the possible zoonotic transmission of these antibiotic resistance to humans through consumption of bacterially contaminated fish.

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