

## A Review of Reported Bacterial Diseases and Antibiotic Use in Tilapia Culture in the Philippines

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### ABSTRACT

Aquaculture has become important to meet the demand for animal food both in local and international markets due to the increasing world population. Tilapias are one of the significant cultured species worldwide, in which the Philippines is one of the leading tilapia-producing countries. Tilapias are the second most preferred fish in the Philippines, constituting about 12% of its total aquaculture production in 2018. Cultivation of tilapias is a practice nationwide, mostly performed in fish ponds and cages in various environments. Despite being an almost hardy fish, the investigation of tilapias for bacterial infections also allowed us to follow the changing bacterial world. In this study, we have reviewed articles that previously reported bacterial diseases and the use of antibiotics in tilapia culture in the Philippines. Streptococcosis, Motile *Aeromonas* Septicemia, and *Pseudomonas* infection caused by *Streptococcus agalactiae* and *S. iniae*, *Aeromonas hydrophila*, and *Pseudomonas fluorescens* and *P. aeruginosa*, respectively, were the identified fish diseases. Chloramphenicol, ampicillin, tetracycline, and erythromycin were among the most commonly used antibiotics in tilapia culture.

### INTRODUCTION

Tilapias are a prime aquaculture commodity marketed globally in which the Philippines is among the top tilapia-producing countries (Miao & Wang, 2020). Tilapias are introduced fish in the Philippines, which is now considered as the second most preferred cultured fish after milkfish (*Chanos chanos*). The first tilapia species introduced to the country in 1950 was Mozambique tilapia (*Oreochromis mossambicus*) which was originated from Thailand. In 1972, Nile tilapia (*O. niloticus*) and other species (Wami tilapia *O. hornorum*, blue tilapia *O. aureus*, and blackchin tilapia *Sarotherodon melanotheron*, and Redbelly tilapia *Coptodon zillii*)

were introduced (Guerrero III, 2019). Pond culture (freshwater and brackish water), cage culture (dam reservoirs, lakes, and seawater), monosex male tilapia culture, saltwater culture, rice-fish (tilapia) culture, and aquaponics are among the most preferred culturing techniques (Romana-Eguia et al., 2020). In 2018, tilapia production from the aquaculture sector was nearly 277,000 metric tons, constituting around 12% of the total Philippine aquaculture production (PSA, 2019).

There are several main factors limiting the sustainability of aquaculture such as habitat conversion, poor management of aquaculture systems, feed availability and management, degradation of water quality, occurrence of harmful algal

blooms, infectious diseases, lack of capital and government support, etc. (Öztürk & Altinok, 2014; Guerrero III, 2019; Boyd et al., 2020; BFAR, 2021; Tahiluddin & Terzi, 2021). One of the main risks to the sustainability of fish aquaculture globally is the proper control and maintenance of infectious diseases (Rodger, 2016; Terzi, 2018). Despite being somewhat hardy fish (Romana-Eguia et al., 2020), tilapias are still susceptible to diseases such as bacterial, parasitic, fungal, and viral diseases (Amal & Zamri-Saad, 2011; El-Sayed, 2019; Romana-Eguia et al., 2020), even with having antibacterial properties (Sajorne & Mabuhay-Omar, 2020). Triggering stressful factors are poor water quality in the farm environment, increasing intensification, and insufficiency of proper health maintenance (Romana-Eguia et al., 2020).

Bacterial abundance and composition in intestine and gills of healthy tilapia (*O. niloticus*) in brackish water fish ponds in the Philippines have been determined by Pakingking et al. (2015). They revealed that under optimum conditions, the total heterotrophic aerobic bacterial counts were ranged from  $10^4$  to  $10^7$  CFU g<sup>-1</sup> with 31 species dominated by *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Plesiomonas shigelloides*, *Bacillus* spp., *Vibrio cholerae*, and *Staphylococcus* spp. While in diseased tilapias, the commonly isolated bacteria were *Streptococcus agalactiae*, *A. hydrophila/caviae*, *A. sobria*, *P. shigelloides*, and *V. cholerae* (Limbauan, 2018).

Bacterial fish diseases in aquaculture are not only a problem in the Philippines but also a severe hurdle in other Southeast Asian countries and worldwide (Boran et al., 2013; Rodger, 2016; Kayansamruaj et al., 2020; Terzi et al., 2021). In the past 20 years, the production of tilapia in the Philippines has decreased, and numerous contributing factors have been pointed out (Guerrero III, 2019). One of the confirmed contributing factors to the reduction in Philippine tilapia production is bacterial diseases (Legario et al., 2020). Baleta et al. (2019) assessed the tilapia cage culture practices in relation to the fish mortality incidence at Magat Reservoir, Philippines. They revealed that, as perceived by the farmers, one of the causes of fish mortalities is diseases alongside other primary triggers like temperature fluctuation, water quality, pollution, predation by birds, and parasite infestation generally occurred during the hot season.

The important bacterial diseases reported globally which has an adverse effect on tilapia aquaculture are Streptococcosis, Motile *Aeromonas* Septicemia, Vibriosis, Staphylococcosis, *Pseudomonas*, Francisellosis, Columnaris disease, Edwardsiellosis, Mycobacteriosis, and others. Pathogens in tilapia were identified as *S. iniae*, *S. agalactiae*, *Edwardsiella tarda*, *Pseudomonas* spp., *Francisella noatunensis*, *Nocardia seriola*, *Aeromonas* spp., and *Flavobacterium* spp.

(Rodger, 2016; El-Sayed, 2019; Romana-Eguia et al., 2020; Kayansamruaj et al., 2020).

In the Philippines, the most commonly reported bacterial diseases in tilapia culture were Streptococcosis, Motile *Aeromonas* Septicemia, and *Pseudomonas* infections (Duremdez & Lio-Po, 1988; Yambot, 1998; Limbauan, 2018; Reyes, 2018; Reyes et al., 2019; Legario et al., 2020). This paper reviewed these reported bacterial diseases of tilapia in the Philippines, including antibiotic use and associated antibiotic-resistant bacteria in tilapia culture. Literatures related to this paper published from 1988 to 2021 were considered by searching in Google and Google Scholar databases using keywords such as antibiotics, antibiotic-resistant bacteria, disease, *Oreochromis*, Philippines, and tilapia.

### Streptococcosis

Streptococcosis is a worldwide bacterial infection hurdle in numerous cultured and wild fish species in various culturing environments (Rodger, 2016). *S. iniae* and *S. agalactiae* are the identified etiological agents of the disease. Streptococcosis-infected tilapia display gross signs like pop-eye, appetite loss, displacement of the spine, hemorrhages (eye, fin bases, and opercula), and corneal opacity (Amal & Zamri-Saad, 2011).

In the Philippines, Legario et al. (2020) reported that grow-out farms (ponds and cages) and hatcheries of tilapia in different parts of the country (Pampanga, Laguna Lake, Taal Lake, Nueva Ejica, Calauan, Laguna, Batad Iloilo, Silay Negros Occidental, and Panabo Davao del Norte), were infected with Streptococcosis. Noted clinical signs of the disease were hemorrhages, exophthalmia, lethargy, eye opacity, ascites, and erratic swimming. Two species were identified as causative agents: *S. agalactiae* and *S. iniae*. The former was found to be a ubiquitous and geographically predominant aetiological agent, and the latter seemed to be restricted to a particular area. A similar study showed that the *S. agalactiae* count in the tilapia farm, Pampanga, were ranged from  $10^4$  to  $10^5$  CFU g<sup>-1</sup> (Reyes et al., 2019). Besides, about 20% of the 181 bacterial isolates from diseased tilapias in Taal Lake were identified as *S. agalactiae*, which was confirmed as a pathogen through a pathogenicity test (Limbauan, 2018). Streptococcosis in tilapias caused by these pathogens have been reported in neighboring Southeast Asian countries (Anshary et al., 2014; Jantrakajorn et al., 2014; Barkham et al., 2019; Kayansamruaj et al., 2019; Syuhada et al., 2020 in Legario et al., 2020) and worldwide as well (Perera et al., 1994; Li et al., 2014; Liu et al., 2016; Su et al., 2019).

## Motile Aeromonas Septicemia

Motile *Aeromonas* Septicemia is a bacterial disease mainly caused by *A. hydrophila*. Other causative agents of this infection are *A. veronii*, *A. sobria*, and *A. caviae*. Pathogens of this disease occur globally among various hosts in brackish water and freshwater, periodically in seawater (Öztürk & Altinok, 2014). Clinical signs of the disease are ulcerations, hemorrhages, abscesses, anemia, and ascitic fluid (Stratev & Odeyemi, 2017).

*Aeromonas* spp. are usually present in the gills and intestines of healthy tilapia, as investigated by Pakingking et al. (2020) in the Philippines' fishponds. The counts of presumptive *Aeromonas* in these tilapia's parts ranged from  $10^2$  to  $10^5$  CFU g<sup>-1</sup>. It was identified that the most predominant species were *A. hydrophila*, followed by *A. sobria* and *A. salmonicida*, constituting 94%, 4%, and 2% of all presumptive counts, respectively, which suggest under stressful environments, could initiate bacterial disease. Likewise, Limbuan (2018) isolated 181 bacterial isolates from disease-infected tilapia in a cage farm in Taal Lake, Luzon, and revealed that about 42% of the isolates were identified as *A. hydrophila/caviae*.

Since *A. hydrophila* is naturally occurring in tilapias, it is not surprising that the outbreak of a disease caused by this pathogen has been observed since the early 1990s. For instance, Yambot (1998) investigated the causative agent of disease outbreaks of Nile tilapia (*O. niloticus*) in several aquaculture farms in Luzon, Philippines, from 1994-1996. The observed disease signs were exophthalmia, dislodged eyeball, eye opacity, ulcerations, mouth sore, body discoloration, skin lesions, fin rot, and sluggishness. In cage farming of tilapia, high death rates were remarkably noted during the cold and rainy seasons when the temperature was low. Through isolation and examination of bacterial isolates from the various tissues of diseased tilapias, the researcher identified the causative agent of the disease as *A. hydrophila* and confirmed through experimental infection that this agent caused septicemia in Nile tilapia. In addition, *A. hydrophila* has been isolated from the diseased tilapias in the grow-out farms in Minalin, Pampanga, Philippines. Most of the sampled tilapias exhibited clinical signs like eye opacity, skin/fin rot, lesion, and abnormal body coloration. The attack rate varied from 31.75% in male tilapias to 62.5% in female tilapias. The source of water and total dependency on feeding were the significant risk factors pointed out for the *A. hydrophila* incidence in a grow-out phase of tilapia in the study area (Reyes, 2018).

## *Pseudomonas* Infection

The first report of *P. fluorescens* infection in the country took place among the fry of Nile tilapia cultured in a hatchery. This disease development intensified under stressful conditions such as overcrowding and poor handling. This causative agent is a Gram-negative and rod bacteria that appeared greenish to yellowish on Pseudosel Agar medium. This bacterium can survive and flourish only in freshwater and brackish rear environments under an optimum temperature of 25-30°C. In both healthy and infected tilapia *O. mossambicus*, this species has been commonly reported to arise mainly in the tilapia's internal organs and tissues (Duremdez & Lio-Po, 1988). Recently, an outbreak of *Pseudomonas* infection in the tilapia grow-out farms in Minalin, Pampanga, Philippines, has been investigated by Reyes (2019). The noted clinical signs of this infection were skin/fin rot, lesion, eye opacity and/or abnormal body coloration. The identified causative agent of this infection was *P. aeruginosa* with a computed attack rate of 54% in the entire municipality.

## Antibiotics Treatment and Antibiotic-Resistant Bacteria in Tilapia

In aquaculture, antibiotics have been considered the most utilized chemicals for more than half-century worldwide. Antibiotics are a group of organic or chemical compounds that kill or inhibit the growth of pathogens and are also used as a growth promoter and disease treatment or prevention (Lulijwa et al., 2020). The global use of antibiotics in aquaculture was reviewed by Lulijwa et al. (2020), and they found out that 11 countries utilized 67 antibiotic compounds from 2008 to 2018. They reported that most of the applied antibiotics in aquaculture were florfenicol, sulphadiazine, and oxytetracycline. Out of 50 papers reviewed, 24% were applying antibiotic treatments in tilapia culture. In the culture of tilapia, the utilization of antibiotics for disease surveillance or development of water quality is limited (Boyd, 2004) and needs extra attention.

In the Philippines, there are few reports on the usage of antibiotics in aquaculture. In *Penaeus monodon* (giant tiger prawn) hatcheries in the country, antibiotics used for disease control were erythromycin, tetracycline, chloramphenicol, rifampicin, and nitrofuran with a concentration ranging from 0.1 to 4 ppm on applied every 1, 2, and 3 days (Primavera, 1993; Cruz-Lacierda et al., 2000). Yambot (1998) tested the sensitivity of *A. hydrophila* isolates isolated from diseased tilapias and revealed that all isolates were susceptible to oxolinic acid (2 µg) and chloramphenicol (30 µg). Besides, 68% and 78% of the isolates were sensitive to streptomycin (10 µg) and oxytetracycline (30 µg), respectively. Also,



Limbauan (2018) reported 181 bacterial isolates, predominated by *A. hydrophila/caviae*, *S. agalactiae*, *P. shigelloides*, *A. sobria*, and *V. cholerae* were all exhibited susceptibility to chloramphenicol and ceftriaxone. Furthermore, Reyes et al. (2019) tested various antibiotics on *S. agalactiae* – a bacterium isolated from the soil of tilapia farm in Pampanga, Philippines. Three groups of antibiotic concentrations were tested in antibiogram test a) 10 µg for ampicillin, gentamicin, and penicillin, b) 20 µg for nalidixic acid and amoxicillin and, c) 30 µg for tetracycline, vancomycin, and chloramphenicol. Their findings revealed that four antibiotics (e.g., chloramphenicol, gentamicin, nalidixic acid, and tetracycline) efficiently inhibited the growth of *S. agalactiae* isolates. Thus, these antibiotics were recommended as a treatment for *S. agalactiae* infections in tilapia. Furthermore, they found out that other antibiotics, such as amoxicillin, ampicillin, penicillin, and vancomycin, resulted in intermediate susceptibility.

Antibiotics in tilapia culture are also applied in other nearby Asian countries, such as Thailand. The most commonly used antibiotics were reported as enrofloxacin, oxytetracycline, amoxicillin, and sulfadiazine, potentiated with trimethoprim (Rico et al., 2014). However, antibiotic utilization in aquaculture may give rise to the development of antibiotic resistance bacteria (Capkin et al., 2015; Corum et al., 2020; Terzi et al., 2020). For example, Niu et al. (2019) reported that isolates of *E. tarda* – pathogen causing Edwardsiellosis, obtained from hybrid red tilapias cultured in a cage in Ping River, Thailand, were resistant to colistin sulfate (10 µg), oxolinic acid (2 µg), oxytetracycline (30 µg), sulphamethoxazole/ trimethoprim (25 µg), ampicillin (10 µg), and ceftazidime (30 µg). Also, hatchery-reared tilapia in Indiana, USA, was found to have 30 bacterial isolates resistant to ampicillin isolated from the guts (Karki et al., 2013).

In the Philippines, studies on antibiotic-resistant bacteria in tilapia are inadequate. Yambot (1998) reported that 25 *A. hydrophila* isolates, a causative agent of Motile Aeromonas Septicemia, which were isolated from diseased Nile tilapia in Luzon, Philippines, were resistant to ampicillin (10 µg), and 64% of the isolates were also resistant to erythromycin (15 µg). Langaoen et al. (2018) determined antibiotic-resistant bacteria in tilapia *O. niloticus* obtained from Lingayen fish farm in Pangasinan, Philippines. A swab test was employed to isolate bioluminescent vibrios from different fish parts such as gills, intestines, stomach, and eyes. In their results, four strains of bioluminescent vibrios were found to be resistant to ampicillin (10 µg). Moreover, two of these vibrio strains were found to be multiple-drug resistant. Furthermore, 50 out of 181 bacterial isolates obtained from diseased tilapia in the Taal Lake dominated mainly by *A.*

*hydrophila/caviae*, *P. shigelloides*, *A. sobria*, *S. agalactiae*, and *V. cholerae*, were resistant to cefoxitin (Limbauan, 2018).

## CONCLUSION

In conclusion, based on the available articles reviewed, tilapias in the Philippines are still prone to bacterial infections despite being hardy fish. Streptococcosis, Motile *Aeromonas* Septicemia, and *Pseudomonas* infection caused by *S. agalactiae* and *S. iniae*, *A. hydrophila*, and *P. fluorescens* and *P. aeruginosa*, respectively, were the reported bacterial diseases in the country. Chloramphenicol, ampicillin, tetracycline, and erythromycin were among the most commonly used antibiotics in tilapia culture. Antibiotic-resistant bacteria in tilapia cultivation have also been reported. Hence, the use of antibiotics in tilapia culture gives rise to the development of antibiotic-resistant bacteria. Further investigations may be conducted in other parts of the country, especially those with fish mortalities incidence. Isolation of antibiotic-resistant bacteria may be done in tilapia farms or hatcheries utilizing antibiotics as disease treatments. Also, antibiotic resistance genes, plasmids, and transferable resistance genes of the bacteria isolated from tilapia and their surrounding environments should be given special attention to determine horizontal gene transfer among pathogenic and non-pathogenic bacteria.

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## Compliance With Ethical Standards

### Authors' Contribution

ABT and ET conceptualized the study, ABT wrote the first draft of the manuscript, and then ET edited the manuscript. Both authors have read and approved the final version of the manuscript.

### Conflict of Interest

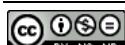
The authors declare that there is no conflict of interest.

### Ethical Approval

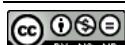
For this type of study, formal consent is not required.

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