# Drug Resistance of Bacteria in Cage Cultured Tilapia from Tapi River, Nakhon Si Thammarat Province

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**Abstract**

The objective of this study was to investigate antibiotic resistance of bacteria isolated from Nile tilapia (*Oreochromis niloticus*) obtained from 10 farms located in Chawang District, and Thungyai District, Nakhon Si Thammarat province. The samples were collected during April and June 2019. *Streptococcus agalactiae* and *Aeromonas hydrophila* were found in the samples and confirmed by PCR technique. Disk diffusion assay was used to test bacteria response to 8 antibiotics, namely, Amoxicillin-clavulanate 30 µg, Cephalexin 30 µg, Gentamicin 10 µg, Enrofloxacin 5 µg, Ciprofloxacin 5 µg, Sulfamethoxazole 25 µg, Oxytetracycline 30 µg, and Tetracycline 30 µg. It was found that *Aeromonas hydrophila* could resist 30% of Amoxicillin-clavulanate, Oxytetracycline, Tetracycline, 20% of Enrofloxacin, 10% of Gentamicin, and 0% of Ciprofloxacin and Slufamethozazole. While *Streptococcus agalactiae* could resist 75% of Cephalexin, 50% of Tetracycline, 25% of Amoxicillin-clavulanate, Oxytetracycline, Gentamicin, and 0% of Enrofloxacin, Ciprofloxacin, and Sulfamethoxazole. It was suggested that antibiotic resistance partially arose from the contaminants from household sewage, wastewater from hospital or fish farms operated at the beginning of the river. This might cause genomic development in bacteria in the river to resist the antibiotics. It was recommended that biosecurity, specie selection, development of disease-resistant aquatic animals, and use of microorganism and immune-stimulants should be employed to lessen the use of antibiotics.

**Key words:** Tilapia, Drug resistance, Bacteria

**Introduction**

Nile tilapia culture is one of potential industries in Thailand and there is generally a large number of fish in a cage. Without an effective farm management, tension in fish could be increased and leads to bacterial infection (Shoemaker, Parson & Adamo, 2006). This can cause serious negative effects on the farm, especially bacterial related diseases such as Streptococosis caused by *Streptococcus* spp. or other diseases caused by *Flavobacterium columnare,* and *Aeromonas hydrophila.* These bacteria are significantly harmful to Nile tilapia industry (Chitmanat, 2013).

Antibiotics have been used by fish farmers as a preventive treatment and a cure for bacterial diseases during culture process. Moreover, an overdose of antibiotics has been found repeatedly which may lead to antibiotic resistance in pathogenic bacteria. Unfortunately, misusing of antibiotics will not only increase drug resistance in bacterial disease carriers, but also in other microorganism found in humans and animals (Chitmanat et al., 2011). Chelossi et al. (2003) reported that there was a change in bacteria population in a neighboring area of fish farms, and the number of antibiotic resistant bacteria had increased.

Bacterial development, to resist antibiotics, is a very complex process which can be stimulated by several factors, including environmental contamination (Alanis, 2005). A development of antibiotic resistant bacteria happened when bacteria received several antibiotics, and then developed themselves into various new bacteria species (Pathak and Gopal, 2005). Because of that, this study aimed to investigate antibiotic resistance in bacteria found in cage culture Nile tilapia in Tapi river in Nakhon Si Thammarat province. The result could be used as a guideline for farmers to appropriately control and prevent disease infection in Nile tilapia.

**Materials and methods**

**1. Data and Samples Collection**

A survey questionnaire was conducted on 10 (out of 20) cage culture Nile tilapia farms to examine farming practice, medicine application, and occurrence of diseases in the culture. Random sampling was employed to collect samples from 10 cage culture farms in Nakacha and Thung-yai subdistricts from Nakhon Si Thammarat province, between April and June 2019. Observation on behavior and skin appearance caused by pathogenic bacteria and gross lesion was employed to identify fish with signs of illness, and 10 of them would be randomly retrieved as samples.

The symptoms of sick fish included consuming less or none diet; moving very inactively or sluggishly; imbalanced swimming; discolored skin; bulging eyes; mouth, gill, and body

hemorrhage; fin rot; pale gills or gill rot; swollen, pale, or pus on livers; swollen or enlarged intestine; enlarged spleen; and blood spots or small pus on kidney.

In preliminary screening, observation on behavior, gross lesion, evidence of sickness on skin, and kidney and brain infection were used as criteria before moving affected fish to a laboratory of Research Center and Aquatic Animal Clinic of Veterinary Faculty.

Appearance of affected fish was recorded to be used in an analysis of infection in accordance with 2 methods proposed by National bureau of agricultural commodity and food standards; microbiology test and molecular test.

**2. Examining of Bacteria Infection in the Samples**

Bacteria from sick fish organs or tissue was cultured on Tryptic soy agar (TSA) medium, Nutrient agar NA medium, and Shieh medium. Bacteria were identified through morphological test and biochemical property test by collecting 1 gram of fish skin, gill, kidney, and brain from each sample. The 4 ml of 0.85% sterilized saline solution was added and ground, before centrifugation at 500 rpm for 5 minutes. Then the supernatant was diluted with 0.85% sterilized saline solution to obtain $10^{-4}$, $10^{-5}$ and $10^{-6}$ solution. Then 100 ul of each solution were used to culture bacteria. Modified Shieh's Agar media mixed with 1 ug per ml of tobramycin was used to grow *Flavobacterium columnare*. Nutrient agar media + 1% NaCl was for growing *Aeromonas hydrophila.* Tryptic Soy Agar media was used as media to grow *Streptococcus* spp. All the plate bacterial cultures were incubated at 37°C for 18-24 hours afterwards.

Single colony of the three bacteria was selected for gram strain test to identify bacteria group (positive and negative). Then biochemical characteristics were determined according to Buchanan and Gibbon (1974) by using the commercial kit API 20E (Özoğul, Küley & Özoğul, 2010). Then they would be cultured in NA media to obtain single colony of the bacteria. After that, 5 ml of each bacterium was transferred onto NB media and placed in an incubator shaker at 37°c for 18-24 hours. After that, bacterial cells from NB media were extracted with Genomic DNA mini kit. The DNA from all samples was stored at -20°C before use.

**3. Confirmation of Bacteria Strain with Molecular Technique**

Confirmation of pathogenic bacteria in Nile tilapia with molecular technique and electrophoresis was used to find DNA patterns of specific bacteria. PCR was employed to examine bacterial infection by using primer of each specific bacteria such as *S. agalactiae* (Laith et al., 2017). *Aeromonas hydrophila* (Aboyadak et al., 2015; Surya et al., 2014) and *Flavobacterium columnare* (Darwish & Ismaiel, 2005).

Briefly, one reaction contained 25 uL mixture, including 1 uL of DNA template, 1 uL of 10 uM concentrated forward primer, 1 uL of 10 uM concentrated reverse primer, and 5 uL of 5x concentrated PCR master mix. The thermo cycling conditions included a first denaturation step at

94°C for 3 min; 35 amplification cycles of denaturation at 94°C for 30 s, annealing 50°C for 30 s, and extension at 72°C for 30 s; and final extension at 72°C for 7 min. Then the mixture was stored at 4°C before being tested with 1.5% agarose gel electrophoresis.

**4. Determination of antibiotic susceptibility of selected bacteria**

Disk diffusion technique was used for sampling culture growing on TSB to increase the number of bacteria for 24 hours at 37-40°C. The bacteria were washed with sterilized saline. Optical density was measured with a spectrophotometer (wavelength = 550nm, OD = 0.125). Then 0.1 mL of bacterium solution was spread on Muller Hinton media and antibiotic resistant sensitivity was tested by disk diffusion assay.

After confirmation with PCR technique, each bacterium was used in disc diffusion method (Hudzicki, 2009) to perform against eight antibiotics including Amoxicillin-clavulanate (30 µg), Cephalexin (30 µg), Gentamicin (10 µg), Enrofloxacin (5 µg), Ciprofloxacin (5 µg), Sulfamethoxazole (25 µg), Oxytetracycline (30 µg), and Tetracycline (30 µg) (Chitmanat et al., 2011; Arungamol et al., 2017) . The resistance test was done in Muller Hinton agar in triplicate and incubated at 37°C for 24 hr. Then the diameter of inhibition zone was measured and compared with the standard value according to Clinical and Laboratory Standards Institute (2008) and Andrews (2009).

**Results**

**1. Fish Farming Practice from Farmers**

Results of data surveying on cage culture of Nile tilapia farms in Tapi river in Nakacha subdistrict and Thungyai subdistrict in Nakhon Si Thammarat province are as follow. The samples were collected from 10 (out of 20) farms and used for screening for antibiotic resistant bacteria. Data from the questionnaire was summarized and it showed that sick fish had various signs of symptoms such as bulging and cloudy eyes, fin or gill rot, fin hemorrhage, wounded body, enlarged stomach, inactive floating, sediment coated body, and sluggish or imbalanced swimming. The farmers used several antibiotics as medical treatment, including Oxytetracycline, Enrofloxacin, Sulfonamind, and Amoxicillin.

**2. Examination and Confirmation of Bacteria from Nile tilapia Samples**

*Streptococcus agalactiae, Flavobacterium columnare* and *Aeromonas hydrophila* were detected with microbiology technique and found that there were 93 colonies giving both positive and negative gram result. After that the 93 colonies was tested with PCR technique (Table 1). The result indicated that 14 colonies were as Nile tilapia pathogenic bacteria (Table 2).

**Table 1** Olgoneucleotide primers sequence for bacteria detection with PCR technique

| Primer name | Oligoneucleotide primer sequence | Size | Target gene |
|---|---|---|---|
| AH – F2 | 5' – CCA AGG GGT CTG TGG CGA CA – 3' | 200 Bp. | aerA |
| AH – R2 | 5' – TTT CA CGG TAA CAG GAT TG – 3' | | |
| SA – F2 | 5' – TGG TAG TCG TGT AGA AGC CTT AAC – 3' | 220 Bp. | Cfb |
| SA – R2 | 5' – TCC AAC AGC ATG TGT GAT TGC – 3' | | |
| FC – F2 | 5' – TGC GGC TGG ATC ACC TCC TTT CTA GAG ACA – 3' | 400 Bp. | 16S-23S rRNA |
| FC – R2 | 5' – TAA TCA CTA AAG ATG TTC TTT CTA CTT GTT TG – 3' | | |

**Table 2** Colonies of bacteria detected with PCR technique

| Name of Bacterial | Numbers of Detected by PCR |
|---|---|
| *Aeromonas hydrophila* | 10 |
| *Streptococcus agalactiae* | 4 |
| *Flavobacterium columnare* | 0 |

Eight antibiotics against 14 bacteria colonies (10 *Aeromonas hydrophila* and 4 *Streptococcus agalactiae*) were determined for antibiotics resistance (Table 3). Ten strains of *Aeromonas hydrophila* were resistant to Cephalexin (80%); Amoxicillin-clavulanate (30%), Oxytetracycline (30%), Tetracycline (30%), Enrofloxacin (20%), Gentamicin (10%), Ciprofloxacin (0%), and Sulfamethoxazole (0%) approximately.

Four strains of *Streptococcus agalactiae* were resistant to Cephalexin (75%), Tetracycline (50%), Amoxicillin-clavulanate (25%), Oxytetracycline (25%), Gentamicin (25%), Enrofloxacin (0%), Ciprofloxacin (0%), and Sulfamethoxazole (0%) approximately.

**Discussion**

Generally, Nile tilapia are highly resistant to diseases, can be culture intensively, and feed on either natural or commercial feed (Chitmanat, 2013). Health management in Nile tilapia is crucial in preventing them from being affected by diseases since it is difficult to cure disease affected ones and using antibiotics also increases cost which could be burdensome for farmers. Additionally, excessive use of antibiotics may cause antimicrobial resistance and would be harmful to consumers. Carrier of diseases in Nile tilapia includes parasite, bacteria, fungi, and viruses. Damage levels depend on susceptibility to carrier species and number of living carriers. Confirmed by PCR technique, this paper found that dominant bacteria were *Streptococcus agalactiae* and *Aeromonas hydrophila*. The two bacteria are in normal flora strain and this strain can be usually found in water resources as Ashiru et al. (2011) pointed out that *Aeromonas hydrophila*, a normal flora, was presented in all water resources.

**Table 3** Antimicrobial susceptibility of selected bacteria

| Antimicrobial agent | Number of resistant isolates (% of each species)[1,2,3,4,5,6 and 7] | | Total resistant (% of each species) |
|---|---|---|---|
| | *Aeromonas hydrophila* (n=10) | *Streptococcus agalactiae* (n=4) | |
| Amoxicillin-clavulanate 30 µg | 3 (30) | 1 (25) | 4 (28.57) |
| Enrofloxacin 5 µg | 2 (20) | 0 (0) | 2 (14.28) |
| Cephalexin 30 µg | 8 (80) | 3 (75) | 11 (78.57) |
| Gentamicin 10 µg | 1 (10) | 1 (25) | 2 (14.28) |
| Ciprofloxacin 5 µg | 0 (0) | 0 (0) | 0 (0) |
| Oxytetracycline 30 µg | 3 (30) | 1 (25) | 4 (28.57) |
| Sulfamethoxazole 25 µg | 0 (0) | 0 (0) | 0 (0) |
| Tetracycline 30 µg | 3 (30) | 2 (50) | 5 (35.71) |
| $MDR_1$ | 0 (0) | 0 (0) | 0 (0) |
| $MDR_2$ | 2 (20) | 0 (0) | 2 (14.28) |

*$MDR_1$ : Enrofloxacin/ Sulfamethoxazole/ Oxytetracycline (antibiotics commonly used in aquaculture animals)

$MDR_2$ : Amoxicillin-clavulanate / Cephalexin / Tetracycline (antibiotics commonly used in humans)

**Reference sensitivity result : [1](Arungamol et al., 2017), [2](Samal, Das & Pal, 2014.),[3](Andrews, 2009),[4](Andrews, 2001),[5](Vanita et al., 2013) and [6](Members of the Society for Microbiology Antibiogram Committee, 2003), [7](European committee on antimicrobial susceptibility testing, 2017)

Moreover, it has been reported that *Aeromonas* spp. could be used in aquatic environments for indicating antibiotic susceptibility (Baron et al., 2017; Penders & Stobberingh 2008; Usui et al., 2016) and group of Streptococci (Vanita et al., 2013).

Enrofloxacin, Oxytetracycline and Sulfamethoxazole are commonly used in medical treatment for bacterial diseases in aquaculture animals while Amoxicillin-clavulanate, Cephalexin, Gentamicin, Ciprofloxacin and Tetracycline are rarely applied and some of them are prohibited. The resistance to antibiotics in bacteria living in natural settings has been developed due to mutually contact between the bacteria and antibiotics which provoke the bacteria to develop themselves in order to survive (Al-Bahry et al., 2009).

Even though Cephalexin is the most resisted by the studied bacteria; it is not used as medical treatment for aquaculture animals by farmers. There is a possibility that this antibiotic might have been released from household or polluted by sewage from other facilities (Rhodes et al., 2000). This is in accordance with Goni-Urriza et al. (2000) who stated that antibiotic resistance in *Aeromonas* spp. was higher in water source contaminated by sewage in housing areas.

There are two antibiotics (Ciprofloxacin and Sulfamethoxazole) that were not resisted by the bacteria. Ciprofloxacin is not commonly used as a cure to aquaculture animals while Sulfamethoxazole is still effective to treat aquaculture animals affected by bacteria.

It would be necessary to avoid using medicine or chemical substance and initiate preventive means and safe treatment such as biosecurity system, strain selective and strain improvement for disease resistant fish, use of probiotics, and immune stimulation. (Chitmanat, et al., 2011)

**Conclusion**

Culturing Nile tilapia in cage culture is prominent financially for farmers living by Tapi riverside. A sudden environmental change in river, water contamination, and bacterial infection can lead to fatality of Nile tilapia in cage culture and a business failure. Detection of antibiotic bacteria from gill, kidney, skin, and brain of fish can clearly indicate that excessive use of antibiotics had been performed, so this would lead to drug resistance in cage culture Nile tilapia.

*Aeromonas hydrophila* were highly of resistant to Cephalexin (80%) and non-resistant as Ciprofloxacin (0%), and Sulfamethoxazole (0%), while *Streptococcus agalactiae* were highly resistant to Cephalexin (75%), and non-resistant as Enrofloxacin (0%), Ciprofloxacin (0%), and Sulfamethoxazole (0%).

Strain selective and strain improvement for disease resistant fish, use of probiotics, immune stimulation, environment quality control, and vaccine development might be promoted to partly substitute treatment with antibiotics for bacteria-caused diseases. Unfortunately, antibiotic resistance in bacteria in water sources can be caused by human activities. Therefore, a further study on an effect of bacterial contamination in the environment and community sanitary would be essential.

For antibiotics selection for pathogenic bacteria treatment, farmers should considerably consult fishery scientists or fish veterinarians to determine the necessity of the drugs, even though they are allowed to be used (Chitmanat et al., 2011).

Unfortunately, dealing with pathogenic bacterial infections in Nile tilapia in cages cultured is not easy. Nevertheless, selecting strong fish species, using vaccine, providing feed with immune stimulants and probiotics supplementation can substitute use of antibiotics. This would lead to prevention of pathogenic bacterial infections (Chitmanat et al., 2011).

## Recommendations

Water samples in the Nile tilapia culture area should be tested with the total plate count method for microbiological water assessment to help support information on antibiotic resistance in tilapia in the culture area in the Tapi river.

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**References**

Aboyadak, I.B., GMA, N., Goda, A.A., Aboelgalagel, W.H. & Alnokrashy, A. (2015). Molecular detection of *Aeromonas hydrophila* as the main Cause of outbreak in tilapia farms in Egypt. *Journal of Aquaculture & Marine Biology, 2*(5),1-4.

Alanis, A.J. (2005). Resistance to antibiotics: Are we in the post-antibiotic era. *Archives of Medical Research, 36*(6), 697-705.

Al-Bahry, S.N., Mahmoud, I.Y., Al-Belushi, K.I.A., Elshafie, A.E. Al-Harthy, A. & Bakheit. C.K. (2009). Coastal sewage discharge and its impact on fish with reference to antibiotic resistant enteric bacteria and enteric pathogens as bio-indicators of pollution. *Chemosphere, 77*(11), 1534-1539.

Andrews, J.M. (2001). BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy, 48* (Suppl.S1), 43–57.

Andrews, J.M. (2009). BSAC standardized disc susceptibility testing method (version 8). *Journal of Antimicrobial Chemotherapy*, *64*(3), 454-489.

Arungamol, S., Jitjak, T., Patchanee, N. & Jensiriwong, S. (2017). The testing of antibiotic sensitivity from isolated *Aeromonas hydrophila* from infected lowland frog (*Hoplobatrachus rugulosus*) in Sakon Nakhon province. *Prawarun Agricultural Journal*, *14*(2), 238-246.

Ashiru, A.W., Uaboi-Egbeni, P.O., Oguntowo, J. E., & Idika, C.N. (2011). Isolation and antibiotic profile of *Aeromonas* species from tilapia fish (*Tilapia nilotica*) and catfish (*Clarias betrachus*). *Pakistan Journal of Nutrition*, *10*(10), 982-986.

Baron, S., Granier, S.A., Larvor, E., Jouy, E., Cineux, M., Wilhelm, A., Gassilloud, B., Le Bouquin, S., Kempf, I. & Chauvin, C. (2017). *Aeromonas* diversity and antimicrobial susceptibility in freshwater an attempt to set generic epidemiological cut-off values. *Frontiers in Microbiology*, 8, 503.

Buchanan, R.E. & Gibbon, N.E. (1974). *The shorten Bergey's manual of determination bacteriology*. Baltimore: The Williams & Wilkins Co.

Chitmanat, C. (2013). Diseases of tilapia. *Chiang Mai Veterinary Journal*, *11*(1), 75-86.

Chitmanat, C., Whangchai, N., Priktong, T., Itayama, T. & Kawabata, Z. (2011). Drug resistance of bacteria in cage-cultured red tilapia from the Upper Ping river, Chiangmai. *In 49th KUCON* Full paper at 2011. p.383-390.

Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Riccardi, G. & Banat, I.M. (2003). Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture*, *219*(1-4), 83-97.

Clinical and laboratory standards institute. (2008). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals*: Approved Standard-Third Edition M31-A3. Wayne, PA, USA.

Darwish, A.M. & Ismaiel, A.A. (2005). Genetic diversity of *Flavobacterium columnare* examined by restriction fragment length polymorphism RNA gene and the and sequencing of the 16S ribosomal 16S-23S rDNA spacer. *Molecular and Cellular Probes*, *19*(4), 267-274.

European committee on antimicrobial susceptibility testing. (2017). *Streptococcus agalactiae calibration of zone diameter breakpoints to MIC values*. EUCAST. Verson 2.

Goni-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P. & Quentin, C. (2000). Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp.. *Applied and Environmental Microbiology*, *6*(1) , 125-132.

Hudzicki, J. (2009). *Kirby-Bauer disk diffusion susceptibility test protocol: American Society for Microbiology*. Retrieved from: http://www.asmscience.org/content/education/protocol/protocol.

Laith, A.A., Ambak, M.A., Hassan, M., Sheriff, S.M., Nadirah, M., Draman, A.S., Wahab W., Wan Ibrahim, W.N., Aznan, A., Jabar, A. & Najiah, M. (2017). Molecular identification and histopathological study of natural *Streptococcus agalactiae* infection in hybrid tilapia (*Oreochromis niloticus*). *Veterinary World, 10*(1), 101-111.

Members of the SFM antibiogram committee. (2003). Comite´ de l'Antibiogramme de la socie´te´ franc¸aise de microbiologie report 2003. *International Journal of Antimicrobial Agents, 21*(4), 364-391.

Özoğul, F., Küley, E. & Özoğul, Y. (2010). Usefulness of API test strips for identification of bacterial flora in blue crab (*Callinectes sapidus*) caught from Akyatan lagoon (ADANA-TURKEY). *Journal of Fisheries Sciences, 4*(1), 1-7.

Pathak, S.P. & Gopal, K. 2005. Occurrence of antibiotic and metal resistance in bacteria from organs of river fish. *Environmental Research, 98*(1), 100-103.

Penders, J. & Stobberingh, E.E. (2008). Antibiotic resistance of motile aeromonads in indoor catfish and eel farms in the southern part of The Netherlands. *International Journal Antimicrobial Agents*, *31*(3), 261-265.

Rhodes, G., Huys, G., Swings, J., McGann, P. Hiney, M. & Pickup., R.W. (2000). Distribution of oxytetracycline resistance plasmids between Aeromonads in hospital and aquaculture environments: Implication of Tn*1721* in dissemination of the tetracycline resistance determinant Tet A. *Applied and Environmental Microbiology, 66*(9), 3883-3890.

Samal, S.K., Das, B.K. & Pal, B.B. (2014). Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish. *International Journal of Current Microbiology and Applied Sciences, 3*(12), 259-267.

Shoemaker, K.L., Parson, N.M. & Adamo, S.A. (2006). Egg-laying behaviour following infection in the cricket *Gryllus texensis*. *Canadian Journal of Zoology, 84*(3), 412-418.

Usui, M., Iwasa, T., Fukuda, A., Sato, T., Okubo, T. & Tamura, Y. (2016). Use of *Aeromonas* spp. as general indicators of antimicrobial susceptibility among bacteria in aquatic environments in Thailand. *Frontiers in Microbiology, 7*, 710.

Vanita, D., Priyanka, C., Devinder, T., Rajesh, K. & Anuradha, C. (2013). Antimicrobial susceptibility pattern of betahaemolytic group A, C and G streptococci isolated from North India. *Journal of Medical Microbiology, 62*(3), 386-393.

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