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Profile of Fish farms and Bacterial quality of farmed Tilapia in the Province of Cagayan, Philippines 2021

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Abstract

This study evaluated the microbial quality of farmed tilapia from seven fresh water farms of Cagayan which were endorsed by the Bureau Fisheries and Aquatic Resources Provincial Office of Cagayan. The study utilized a Mixed Method of analysis where the profile of fish farms and microbial quality of tilapia harvested from the fish farms were quantitatively described while a Focus Grouped Discussion was done to determine farm practices of the fish farm owners. Interview, observation techniques and document reviews were also done to validate some responses provided by the participants in the FGD. Results of the bacterial culture conducted revealed the presence of different bacterial isolates from the fish samples which were found to be pathogenic and may be due to poor fish farm practices. Further, the results of the study showed that most fish farm owners do not comply to practice basic sanitary and hygienic protocols as endorsed by BFAR. While the bacteria load levels detected from the fish samples were within the acceptable limit as prescribed by the Food and Drug Administration of the Department of Health, some fish farms were detected to have high bacterial loads in as much as the harvested fishes from these sites are concerned because of the use of contaminated source of water supply. The study strongly recommends BFAR to conduct stricter monitoring and implement sanitary and hygienic practices in the fish farms of Cagayan to avoid food poisoning and intoxication once fishes like tilapia are consumed. Consumers are likewise advised to cook properly fishes cultured in fish farms.

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Introduction

Fishes like tilapia are one of the essential foods for people. Aside from being cheap, fishes provide a very good source of animal protein. (Houlihan, Boujard, & Jobling, 2008).The high nutritional value of tilapia is credited to the essential nutrients that it contain such essential minerals like sodium, potassium, calcium, magnesium, phosphorus, sulfur, iron, manganese, zinc, copper, and iodine (Mogobe, Mosepele, & Masamba, 2015). It also has eicosapentenoic acid and docosahexenoic acid which are significant for healthy growth and normal maintenance of the human body (Hossain, 2011).

In order to meet the growing demand of fresh cultured fishes in the Philippines, there had been increased interest in aquaculture focused on production, genetic engineering and species diversification. In their natural habitat, fishes like that of tilapia are exposed to a wide variety of bacteria, of which some post global public health concern such as Streptococcosis, Columnaris, Francisellosis, etc. Detection of bacterial quality in fish produced by fish farms is an essential strategy to recognize and prevent problems related to health and safety. Some studies delved on determining the levels of microbiological indicators of the edible parts of fishes in order to highlight gaps in the hygienic quality of the fishes. This is also imperative so as to predict the hazard which endangered the health of consumers. Not forgetting to mention that farmed fish may be exposed, legally or illegally to a wide range of chemicals because of the high stocking densities or preservation. Most of these chemical treatments contain antibiotics which are known to prolong the viability of the stocked fish commodities. In addition to their therapeutic action and preservation inputs in the veterinary field, antibiotics when introduced to fishes also have prophylactic and growth promoting actions.

In local areas of the Philippines such as here in Cagayan, Tilapia is one of the most consumed fishes because it is relatively cheap and abundant. Tilapia is rampantly cultured all year round and can be stable in man-made lakes. It is an ideal fish for farming because this specie does not mind being crowded and it even grows quickly. Moreover, it consumes a cheap vegetarian diet. These qualities translate to a relatively affordable product compared to other types of seafood. However, the benefits and threats of tilapia consumption depend largely on poor farming or aquaculture practices, and this issue is observed among fish farms in Cagayan. Currently, there is no data regarding the microbial quality of locally farmed and consumed fish and products in Cagayan, most specifically that of tilapia hence, this study.

Materials and methods

This research made used of the Mixed Method research design where the profile of fish farms in Cagayan were quantitatively described with the bacterial quality of tilapia derived from the fish farms through colony counting and profiling of the size or span of fish farm. The qualitative part of the study is the Focused Group Discussion where each of the participants were asked about the profile of the fish farm as to Location, Span, Farm Care Management Aquatic Veterinary practices that affects sanitary practices applied in the fish farms in order goes beyond merely gathering and tabulation of data.

Materials

Equipment

Equipment used include the following: incubator, Biosafety Cabinet, freezer, weighing balance, Quebec colony counter with magnifying lens, Laminar Flow Hood, pH meter, Autoclave, Water Bath , Refrigerator, Vitek 2 Analyzer, Hot Plate and magnetic stirrers.

Glassware and disposables

The glasswares and disposables used in the study include the micropipettes with suitable tips, sterile scalpels, Eppendorf tubes, Erlenmeyer flasks 100, 250 and 500 ml, Plastic Petri dishes, 90 x 15mm, and Media bottles. All the reagents

solvents used, are of analytical grade. The Nutrient Agar and was prepared according to manufacturer's recommendation. K2HPO4, Oxidase test kit, Absolute Ethanol, Vitek 2 test kits were as utilized in the study.

Data Gathering Procedures

The researcher sought permission to conduct the study from the Bureau of Fisheries and Aquatic Resources Regional office 2, Municipal Agriculture Office and the Department of Agriculture Regional Animal Disease and Diagnostic Laboratory. The floating of questionnaires sample collection and interview of the respondents who themselves are both the owner and the farm worker were conducted by the researcher with the assistance of the personnel of BFAR fishery laboratory and the representative of Municipal Agriculture Office.

For ethical considerations, the researcher devised a code of the respondent fish farms to safeguard the interest of the fish farms. Fish Collection, Processing and Transport. Tilapia fishes were harvested at the actual site of the fish farm through the assistance of the employees of the regional office of the Bureau of Fisheries and Aquatic Resources fish laboratory and the Department of Agriculture and municipal Agriculture Office. They were immediately placed in sterile transport bags and were brought to the laboratory following transportation protocols (cooler boxes maintained at 20 degrees Celsius and transport duration must be within less than 4 hours after collection).

Sample Preparation

The tilapia samples were examined under aseptic conditions. Biometric data such as the standard length (cm) and the body weights (g) of each fish were measured. After which, the muscle of the tilapia was prepared for microbiological quality testing. Only the dorsal part of each tilapia was chosen to be analyzed for microbiological quality.

Ten-grams for each sample of the tilapia (flesh or muscle with skin which was aseptically cut from dorsal side of each fish with a sterile blade and weigh in sterile petri dish) were homogenized with 90 ml of sterile phosphate buffer solution using a stomacher (AES, Laboratoire, France). Tenfold dilutions of the homogenates up to 10-9 was prepared in normal saline using automatic micropipette (Kumar *et al*., 2014)

Microbial Analysis

From the homogenized samples of tilapia flesh, bacterial cultivation was made simultaneous with serial dilution using the homogenized samples. Colony counting was made in a mixed culture. During the study, total viable count, of microorganisms were counted and the species. While the isolation of pure culture was made by differentiating the morphological characteristics of the colonies grown from the bacterial cultivation and was re streaked in a culture media in order to acquire a pure culture so to identified through VITEK Machine.

The total bacteria/ viable count of the fish samples was determined following the conventional pour plate method (Anon, 1992). Ten serial dilutions of fish samples were prepared in test tubes and one ml of each dilution was transferred into sterile glass petri dishes. Approximately 10 ml of melted nutrient agar medium (45-50C) was poured into each place and was mixed thoroughly. It was left for 10 minutes for solidification. The plates were incubated at 30 C for 48 hours. After bacterial colonies have grown, the total colonies count per gram of sample was calculated as follows:

Total viable bacterial count = average number of triplicate plates of the same dilution x reciprocal of the dilution used colony forming unit (CFU)/g sample. This was done in triplicate for each fish sample and the average result was taken.

The Automated Microbial Identification (VITEK 2 SYSTEM) which is last test describes preparing plates seeded with sensitive strain of bacteria

(gram negative and a gram-positive bacteria). It determined if the bacterial isolates through its biochemical analysis by the said machine.

Analysis of Data

The profile of fish farms which were derived through a questionnaire as well as their bacterial quality were analyzed using two way annova. The bacterial load means were also derived for comparison if it falls within the range of the acceptable bacterial load by the Food and Drug Administration. The Analysis of Variance (ANOVA) was used to detect significant difference on bacterial quality of farmed fresh water fish when grouped by profile variables.

Results and Discussion

Profile of fish farms in Cagayan

Table 1 presents the profile of the fish farms in Cagayan in relation to their location based on actual observation conducted by the researcher. As of 2019, 4 fish farms are located near households namely that of Farm B, Farm A, Farm E and Farm D. The general condition in the said fish farms is poor. They are very near residential houses and are in depressed communities. Aside from that, they are in low- lying areas which have a tendency to be flooded during the rainy seasons. Since the said fish farms are near houses, it was also observed by the researcher that household wastes, animal and human excreta and some garbage are washed into or dumped into the fish ponds.

Septic tanks were also noticed to be so near the fish farms such as that of Farm B. All of the other fish farms were located near septic tanks less than ten meters as prescribed by the code of good aquaculture practices by BFAR and DA. Farm G is also near a factory and as per interview with the fish farm owner, it was revealed that, factory water are washed into the pond.

It was also noted that Farm G has a direct access to the national road and is elevated. The farms of A and E are located at a secluded mountainous area and take their water source from an upstream creek that drains water from a pasture land which made these farms prone to be contaminated by pasture debris and waste.

Another observation was that, Fish Farm F operates a piggery which is located just at the upper portion of the pond were pig manures are eroded into the fish farm during rainy season.

Table 1. Frequency distribution of identified neighboring location of selected fish farms in Cagayan.

Neighboring Location	Municipality	Frequency
Household	Sanchez mira Allacapan Tuao	4
Factory	Enrile Tuao	1
National Roads	Tuao	1
Mountainous Farm Location	Allacapan Sanchez mira	2
Forested Area	Sanchez mira Allacapan	2
Rural Ares	Tuao Sanchez mira	5
N		7

Span

Table 2 presents the span of the farms. The average span of the seven farms that were included in the study is 7,050 sq.m while the total productive index is at 4,775 sq. m. One of the main factors that affects the ability of the farm to cater to large number of fishes is the productive area which refers to the the sum of overall span of land in the farm that is solely dedicated for the propagation and growth of fishes. Farm F has the largest total area accounting to 20,000 sq m. total area and have numerous buildings that contribute to the optimization of the productivity of the farm. However, it has only as 8, 000 sq. m. allotted for the productive area. On the other hand, Farm G has the largest productive area with a 10,000 sq.m total area and a 9000 sq.m production area among all the farms considered in the study and has 2 buildings utilized for optimization of fish

production. Truly, the assessment of the total area of the farm compared to their productive area is imperative to be analyzed because this will indicate as to how many structures and establishment can be built within the proximity of the farm that may contribute and serve as a determinant of the quality of fish produced which can be a source of biological contaminants . No standards prescribed a size of an total area of fish farm. However productive area should be appropriate to the population of tilapia in the ponds. Crowded fishes or over populated fishes in the ponds may contribute in the rate of transmission of a probable disease that may occur among tilapia.

Table 2. Area of the selected fish farms in Cagayan as to their total land area and productive area in Cagayan.

Farm Code	Total Area (sq. m)	Productive Area (sq. m)
Farm A	7,500	5,000
Farm B	4,500	4000
Farm C	7,000	6,000
Farm D	2,500	2,200
Farm E	2,500	2,000
Farm F	20,000	8,000
Farm G	10,000	9,000

Table 2.1 Describes the areas/section which are present in the fish farms that may occupy the total area of fish farm. The infrastructures present in the fish farms are also determinants of bacterial and chemical contamination of the fish products. In fact, it can also affect the overall management of the fish farms. Gleaning from the table, it can be noticed that most of the fish farms store feeds and chemicals in the same room. It was also observed by the researcher that most of the fish farms have been keeping gasoline stocks, fertilizers and feeds all together in one storage room. The farm owners do not practice separation of food for the fishes from those which were utilized in the operation of the fish farm. It was also noted that not one of the fish farms maintain a space where carcasses of dead tilapia and other animals present in the fish farm may be thrown. In fact, when there was dead tilapia, the farm owners cook them for their food. Some dead tilapia was also dried and preserved for their family's consumption. There were no areas devoted for the treatment and isolation of sick/ unwell tilapia which is also important for infection control. In fact, the farm owners do not give cognizant attention in doing investigations for the cause of fish kill and had not been reporting the same to BFAR. This practice may endanger the health of tilapia fish caused by poor farm management practice.

Table 2.1. Frequency distribution of Areas/ sections present in the fish farms.

Areas/ sections present in the farms	Absent	Present
Feed area storage area	0	7
Chemical storage area	0	7
Area for disposal of dead fish	7	0
Isolation/observation area	7	0
Treatment/recovery area	7	0

Farm care management

Farm care management of fish farms demonstrated poor administration of the fish farms which includes essential processes but not limited to monitoring of fish ponds regularly, keeping good records, planning ahead for the operation of fish farm especially focused on ensuring that adequate and standardized sanitary and safety practices are followed. Based on the Focused Group Discussion none of the fish farms keeps records of the said practices that may contribute to poor fish quality.

Pond and water management

Pond and water management were not properly documented as well. The dissolve level of oxygen in water was not observed and changing of water in the ponds are only done on a post-harvest basis. This may result to poor environment of tilapia and may cause their untimely death. Proper preparation for pond was not likewise implemented. Most of the farms' water sources are deep well, although some also rely on rainfalls. A few exceptions to this are the farms of Panes and Cristobal which uses water from a

creek from the upstream. Having a proper water management is crucial in maintaining the sanitation and hygienic index of a farm's pond. A good source of water plays a contributory factor on the quality of tilapia produce due to the fact that water is a good habitat of pathogenic bacteria that could harbor in the ecosystem of tilapia. The farm managers did not conduct any soil analysis for the reason that their ponds were before a rice paddy. The soil analysis should be done to test the normal flora present on the soil which may contribute on the presence of bacteria in tilapia. The farm owners conducted a water analysis but they do not know the type of water analyses were conducted. In fact, most of the fish warm owners did not have much knowledge as regards water analysis. Since this test are not readily available in the municipalities where farms are located. Based on the interview made by the researcher among the respondents, it was found out that the concentration of dissolved oxygen in the fish farms was not tested every two months and is occasionally changed four months after harvest. A main concern also is that farm F which has a piggery and a cock pit directly above the pond's land area. Hence the waste of other animals are washed into the pond thus, it increases water contamination and risk of food borne diseases. This contamination directly affects the water and so as to the tilapia living in the water ponds. Contamination of fecal manures from the piggery may cause bacterial contamination of both the tilapia environment and tilapia itself.

Table 3. The Pond preparation and water management.

Pond and water management	YES	NO
Soil analysis	0	7
Water analysis	0	7
Test for dissolve oxygen	0	7
Change of water pond	0	7 (but 3- 4 months post-harvest)

Testing soil and water is part of the standards set in the Code of good Aquaculture practices by the Department of Agriculture through BFAR in order to practice good aquaculture. By doing so, this test may provide information of probable contaminants such as bateria naturally present on both soil and water that may be acquired by tilapia and its effect to its bacterial quality.

Hygiene of Farm Workers

Table 3 presents the overall hygiene and health practices of the fish farm workers which are vital in maintaining a sanitary work area as the number of pathogens will be greatly reduced. The farm owners are the fish farm workers themselves and according to the interview conducted, they have not been undergoing medical examinations although they knew that it is relatively important because it assures that individuals working in the farms are free of inflictions that may affect the productive capacity. Medical Examinations are not necessary according to the respondents since they are the employees of their own fish farms. Hiring of fish farm workers are not needed for tilapia farming do not need much of maintenance.

The farm owners also claimed that they are not wearing the proper work clothing which include having to wear rubber industrial grade gloves and boots, protective caps and textile of clothing. That these work clothing are not needed since they're just living within the compound of the fish farm and the fact that they operate a small-scale fish farm businesses. While the fish farm owners strictly follow regulatory requirements to seek a business permit and BFAR registration all of them agree that they have not been following minimum sanitary and hygienic requirements as to the standards of the code of good aquaculture practices. In addition, all of the seven farms included in the study do not have a designated area for washing or showering themselves before and after working as prescribed in the said standards. The said wash areas must be located at the main entrance of the production area or in any conspicuous place like that of the resting area of the fish farm workers for better

accessibility. Wash area is in order to sustain good hygienic practices of farm workers to avoid zoonotic diseases that may originate from humans. Despite setting a good standard on hygienic practices, the fish farms owners failed to comply with such requirement. Also, results reveal that daily screening or checking of farm workers physical condition whether they are fit to work were not observed. According to BFAR guidelines, checking of the health conditions of fish farm workers is imperative as human infections can be zoonotic as this shall ensure that the quality of fishes produced are healthy which in turn will not introduce risks to consumers.

Zoonotic infections are caused by pathogenic bacteria that infects both humans and animals, the very reason that a healthy hygienic practice should be strictly observed and implemented as per standards requirement and the provisions of the food safety act as to production of food producing animals set by the Department of Health and Department of Agriculture under the jurisdiction of the Bureau of Animal Industry specifically delegated to the Bureau of Fisheries and Aquatic Resources in line with aquatic fish farms. These hygienic practices may contribute to the bacterial quality of the fish produce that may threatens the food safety of consumers in regards to zoonotic diseases are concerned.

Table 4. Frequency distribution of hygiene management of fish Farm workers.

Personnel Hygiene Management	Yes	No/not observe
1. Fish farm workers undergo medical examination prior to work	0	7
2. Fish farm workers wear suitable and clean working clothing	7	0
3. Employee/ farmworkers have designated area for washing before and after working.	0	7
4. Daily screening or checking of farm workers physical condition fit to work is observed.	0	7
5. Farm Workers with communicable disease are not allowed to work until treated.	0	7
6. Boots and face mask are used by farm workers when feeding and harvesting	0	7

Aquatic Veterinary Practices

Antibiotic used

All of the fish farm owners claimed that they are not using antibiotics as they are relatively expensive and is impractical. They too are not familiar as to how they shall apply the antibiotics to their fish farms. As per the result of the interview that the researcher had with the respondents, they said that using antibiotics may be incorporated anyway to the feeds given to the fishes. In terms of the drugs utilized to treat fish ailments, farm workers said that they sometime use organic based preparations such as lime and leaves extracts. A fish warm owner said *"We rather buy feeds for the fishes than allotting our money for the antibiotics which will not guarantee that our fishes will remain alive. We instead catch and eat those which are sick".* Another farm worker stressed out that "*Instead of antibiotics, we incorporate substances like lime and plant extracts in our pond when we observe fish death and after that, we are happy that the condition of our fishes are better".*

Frequency of consulting veterinarians for disease infection management

Gleaning from the table, the fish farm owners had been consulting veterinarians as to how they can effectively manage their farms. Even when there had not been epidemics for fish kill in their respective farms, they had been consulting veterinarians endorsed or authorized by BFAR only that at some times, they are unavailable and would even say that they are not quite familiar as to pathology of fishes. This allows the fish farm owners to channel most of their concerns are attended by the Municipal Agriculture Office however, only the graduates of B.S. Fisheries or related fields are those that attend to them. Participant 3 of the FGD said that *"while we consult veterinarians, we still have problems as to how to implement their recommendations. That is why most of the time, we manage our fish farms using trial and error, mostly listening to the best practices of other farmers."*

Nutrition given to fishes

Type of food

In terms of the type of food given to the tilapia, five (5) farms namely that of Farm A, Farm B, Farm D, Farm E and farm F uses commercial fish (Robina Brand) feeds added with a little amount of natural feeds (cracked corn or sorgum). The fish farms of Farm F and Farm E prefer feeding the tilapia solely with commercial feeds (Meg SD-90 Brand). Farm G on the other hand gives additional food for the raised tilapia which is called Azolla which is an immune booster consisting of (mosquito fern, duckweed fern, fairy moss, water fern). They source this material from Cagayayan State University Piat Campus - Research and Extension office. The choice of feeds given by the farm workers to their fishes may have been recommended to them by veterinarians or those which have helped them have huge harvest in their previous fish farming experiences.

Type of vitamins

All of the seven fish farms included in the study reported to be using vitamins which were prescribed and distributed by BFAR- RO2. The Vitamin (Mix T90) are given weekly except when the vitamins supply is halted. According to all of the fish farm owners, they solely rely on BFAR for the supply of vitamins as they find it to be expensive and claim that the tilapia are growing healthily even without vitamins. It was also reported by the owner of Farm A that they use lactobacillus in addition with feeds to ensure the health of tilapia and to ensure good harvest and boosting tilapia immune system according to the fish farm owner. Part of the objectives of BFAR to promote tilapia Farming is to provide extensive aid.

Bacterial isolates detected from the fish samples

Out of the seven fish farms, only three (3) fish farms allowed the researcher to collect fish samples. These are Farm A in Allacapan, Farm E and farm F in Sanchez Mira. Other fish farms did not participate in this phase due to unavailability of tilapia fingerlings and other underlying issues especially that there were reports of fish kills during the time of sample collection.

Bacteria were inoculated in a general culture media from the homogenized fish muscle and was streak in a nutrient agar plate. Each of the collected homogenized samples showed different characteristics of colonies grew in the nutrient agar. The researcher was able to isolate each of the colonies in order to obtain a pure culture from a mixed culture colony grown in the nutrient agar. Colonies were differentiated according to their color, shape texture and elevation. A pure culture was re-stricted in a nutrient agar plate each of every type of colony grew from the mixed culture. Samples from fish farm E manifest two types of colonies. One has a colony characteristic of one is creamy white irregular shape and has a flat elevation coded as cf2. The other colony isolated coded cf1 from Farm E has a characteristics of small round grayish white color colony. Samples from Farm A fish farm had only one type of colony manifested coded as pf yellow with a small round or pipoint yellowish color. While the greatest number of mixed cultures is in the samples of fish farm F with four isolates. Code as rf cream, rf dry, rf green and rf yellow. Rf cream has a cream color colony slightly elevated, rf dry is rhizoid grayish form lastly rf - green appears green in color round and flat. The last but not the list was rf yellow that appears to be yellow and slightly elevated. Those characteristics were the basis of the isolation of pure cultures from the mixed culture.

Table 4 shows the bacteria isolated from the tilapia samples. Bacterial smear was prepared from pure colonies and undergone gram's staining prior to bacterial identification for biochemical testing through vitek machine. The stained smears showed morphology of the bacteria isolated reveal the presence of gram-positive and gram-negative bacteria. Gram positive bacteria shows violet color microscopically and are cocci in shape. Gram

negative bacteria are pink in color or pale red. Five out of the eight isolated colonies from the three fish farms were gram positive. The farms with gram positive bacteria are Farm E with two isolates the cf 1 and cf 2 and Farm A with one isolate coded pf yellow and one gram-positive from the Farm F coded with rf cream while the remaining three isolates from farm F are gram negative rf green, rf dry and rf yellow respectively. The fish culture labeled cf-2 showed the presence of *Staphylococcus gallinarium* with a characteristic of member of the bacterial genus Staphylococcus consisting of single, paired, and clustered cocci.

The infection rate and morbidity to humans is relatively low and its effects, limited. The bacterial isolate from the sample labeled cf-1 showed the presence of *Staphylococcus pseudointermedius* and are Gram-positive cocci in clusters. This type of bacteria is responsible for severe and necrotizing infections in humans and dogs and must not therefore be consumed through contaminated food. The bacterial colonies labeled sample Pf- yellow presented the presence of *Staphylococcus sciuri* a gram-positive coccus.

It is an important human pathogen responsible for endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections. The bacteria samples taken from colonies labeled rf-cream confirmed the presence of *Granullicatella elegans* belongs to gram positive from a family of streptococcus, a bacterium which can be found in normal human oral flora but is generally associated with infective endocarditis. The pure culture from RF-Dry displayed the presence of *Rhizobium radiobacter a* gram-negative bacterium.

This is a tumorigenic plant pathogen that rarely causes infections in humans. On the samples of RF-Green, the colonies isolated were that of *Pseudomonas aeruginosa* is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium which significantly causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections. Lastly, stemming from the pure culture labeled rf-Yellow a *Pantoea* spp is a genus of Gram-negative bacteria of the family Erwiniaceae which causes opportunistic human infections especially in people whose health conditions are immunocompromised.

These identified bacteria through Vitek were tested by their Biochemical Characteristics. Bacteria identified were mostly from domestic animal origin that are present within the proximity of the ponds. Farm E which has two identified bacterial species from tilapia are near households' area and pasteur land contributory to mechanical vectors such as pet dogs, ducks, goats and cows including household waste contaminants.

Farm A on the other hand has one identified bacterial specie where farm A depends on rainfall that water cascades on the ponds where the pasteur land is located at the upper portion of the pond, that animal manures flood down the ponds causing biological contamination that results the presence of these identified bacteria.

While Farm F which has the greatest number of isolated and identified bacteria has a piggery within the ponds and ducks swimming in the ponds and dogs roaming around the ponds. As the waters and feces from the piggery erodes in the ponds these give the bacterial load to be at the greatest number due to its contaminated water ponds.

Ducks directly swims in the ponds might as well leave their feces in the ponds that increases the contaminants leading to poor quality of tilapia in terms of the presence of bacteria among the inhabitant fishes in the ponds.

Table 5. Species identified from Bacteria isolated from the tilapia flesh samples. (see appendix C for official result).

Farm code	Colony isolated code	Colony characteristics	Stained bacterial smear Characteristic	Species Identified
FISH FARM E	cf-2	Creamy white flat elevation with irregular shape	Gram positive bacteria	*Staphylococcus gallinarium*
	cf-1	Small round grayish white colony	Gram positive bacteria	*Staphylococcus pseudintermedius*
FISH FARM A	pf-yellow	Small round pinpoint yellowish color	Gram positive bacteria	*Staphylococcus sciuri*
	rf cream	Cream color colony slighted elevated	Gram positive bacteria	*Granullicatella elegans*
FISH FARM F	rf dry	Rhizoid form in a grayish white color	Gram negative bacteria	*Rhizobium radiobacter*
	rf -green	Greenish colony round an flat elevation	Gram negative bacteria	*Pseudomonas aeruginosa*
	rf-yellow	Yellow color colony slightly elevated	Gram negative bacteria	*Pantoea* spp

Table 6 presents the cfu/g of the samples. The acceptable limits set by the FDA on the total coliforms and fecal coliforms of Fish meat is $<10^5$ CFU/g. The presence of TC is an indicator of waste contamination which may also occur during different processing steps such as transport and handling. Almost all of the samples tested from the farms presented to have numerous and above the prescribed limit. One of the samples that was tested from Farm E is within the acceptable limit but the other colony count are slightly above the acceptable limit set about by the FDA. Farm A had also one valid count with a slightly above the acceptable limits and one sample is within normal limit. Farm F had all of its sample above the set standard. Even having a sample that was too numerus to count. One of the reasons was the fact that the farm's water came from a creek that drains a pasture land upstream and presence of pigpens at the upper portion of the fish ponds.

The data also describes whether the colony of the organisms isolated from the homogenized flesh of tilapia is within the normal limit set by BFAR using the issuance consistent with FDA circular No. 10 series of 2013 stating that microbes found in fishes should not exceed $<10^5$ colony forming units. Each of the fish farms were taken four to six samples of fishes approximately 1 kilogram which were collected in a sterile zip lock and was homogenized subsequently homogenized samples undergone serial dilution and later were subjected to colony counting. Samples which did not show colony growth were not included in the study and those dilution with less than 30 colonies were not valid counts only those 30 above to 300 colonies are considered valid counts, while those above 300 are considered too numerous to count. However, for the researcher to acquire the absolute value, 300 and above counts were still considered. Results reveal that in each of the three fish farms, there were colony counts which were higher than the expected normal limit. The other dilutions were not included for they were not countable and a valid count. The result implies that indeed, the tilapia harvested from the fish farms have the potential to cause infection when they are consumed raw or when not properly cooked.

Colony counts greatly affects the bacterial quality of tilapia, these method quantitatively assess the presence of bacteria that provides us the acceptable limit of their population in the flesh of tilapia that causes harm or disease among consumers regardless if it is a mixed bacterium when it is exceeded the acceptable limit as concentrated homogenized sample are concerned. Actual populations are estimated directly from the flesh. It is clear from the data that colonies counted are not of the same kind

which it significantly implies the poor bacterial quality of tilapia having multiple isolates. Findings also reveal that these bacteria are unique and are not usually common isolate from tilapia and not even a common soil normal flora. Their presence in accordance with their numbers are alarming and potentially biohazardous among consumers.

Table 6. Comparison of colony count from concentrated homogenized assay from tilapia flesh in tilapia in the fish farms compared to FDA standard.

Farmcode	Dilution	cfu/g	FDA cfu/g standard ($<10^5$)
Farm E	$x10^3$ (1: 1000)	3.65 X 10^5	Within the Normal Limit
Farm E	$x10^4$ (1:10,000)	2.05 X10^6	Above the Normal Limit
Farm A	$x10^3$ (1:1000)	1.98 X 10^6	Above the Normal Limit
Farm A	$x10^4$ (1:10,000)	1.93 X 10^5	Within the Normal Limit
Farm A	$x10^4$ (1:1000)	4.35 X 10^6	Above the Normal Limit
Farm F	$x10^5$ (1:100,000)	3.85 X 10^7	Above the Normal Limit
Farm F	$x10^6$ (1:1,000,000)	2.93 X10^8	Above the Normal Limit

Based on the data gathered and analysis conducted utilizing a two-way ANOVA, results reveal that there no significant difference on the bacterial isolates detected on fish farms when they are grouped according to location, span, and farm care management. The bacterial isolates had inevitably come from disposing factors which may include the unsanitary and unhygienic practices of the farm workers. As highlighted by the previous data, the locations of the fish farms and their source of water are crucial variables in growing and producing uncontaminated fishes. Another reason why there was an increased level of bacterial load in the fish farms of A and E is the fact that both of them draw water creek that drains water from a pastureland. On the other hand, the fish farm owned by the Farm F had the greatest number of bacterial isolates due to the pigpens present near the farm which were observed to be draining manures to the pond.

Table 7. Test of association between bacterial isolates detected and fish farms group according to dilution factor and farm location.

Source of Variation	Df	F	P-value
Dilution Factor	2	0.981653	0.4499 NS
Farm Location	2	1.514087	0.3239 NS
Error	4		
Total	8		

Conclusion

Based on the findings of the study, the researcher therefore concludes that the tilapia derived from the different fish farms in Cagayan are harbouring bacteria, most of which are uncommon, that post great threat to public health especially when the tilapia are not properly cooked before eating them. The bacterial contamination is due to of the poor health conditions and poor fish farm practices. While the Bureau of Fisheries and Aquatic Resources have issued guidelines to ensure food safety and security, in particular for fishes and aquatic produce, these guidelines were not strictly observed/fpllowed by the fish farm owners because of financial constraints and issues on complacency of institutions in the implementation of regulatory rules for fish farms which do not comply.

Recommendations

Based on the conclusions, the following recommendations were drawn:

1. The fish farm managers must be encouraged by BFAR and DA to utilize sources of water that are free from fecal contaminants and debris like that of deep well. The water from the pond which must be regularly checked for microbial quality and must be drained every after harvest.
2. There is a need to strengthen the LGU, DA, DENR and BFAR collaboration for a more stringent mechanism to monitor fish farms in Cagayan in terms of implementing sanitary and hygienic guidelines, regulatory standards which

fish farms must abide with before issuance of a Certificate to Operate.

3. The Department of Health and the Philippine Information Agency must initiate measures to inform the public as to what bacteria may be present in tilapia and other aquatic products so as to avoid epidemic of food-borne disease brought about by consuming tilapia and other types of fishes which are grown in contaminated ponds.

4. Veterinary experts, both in the industry and the academe, should reinforce the support for tilapia growers and fish farm employees by capacitating them through trainings and seminars as to how they can properly manage their fish farms and to yield a better harvest.

5. The City Agriculture Office must consider hiring aqua culturists so as proper attention will be given to the plights of fish framers. Adequate supply of vitamins and antibiotics for fishes need to be sustained by the agency. The same office must extend services like provision of cheap source of fingerlings, microbial analysis of water in the ponds, testing of water pH and dissolved oxygen as these are the common plights of the tilapia growers who participated in the study.

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Appendix A

INFORMED CONSENT

Informed Consent Form

Thesis Title: Microbial Quality and Antibiotic Residue of Farmed Fresh Water Fishes in Cagayan

Researcher: Mary Ann m. Santos, RMT, Faculty Member, College of Veterinary Medicine, +639758776210

Adviser: **Dr. Julius T. Capili**

1. Purpose of the Study:

Generally, this study aims to assess the food safety of Fishes (Tilapia) in terms of microbial quality and antibiotic residue in selected fish farms in Cagayan. The study will also profile the fish farms in terms of, location, span, antibiotic used, frequency of the used of antibiotic , nutrition, and consultation to the veterinarians. Also include the perception of farm owners, consumers and aqua culturist as to the effects of the presence of microorganism and antibiotic residue on both fish and public health.

2. Procedure to be followed:

The questionnaire composed of two sets.The first set of the questionnaire is to profile the different farms of Cagayan, this portion will only answer by farm owners or operators. The firstset of questionnaire can be answered by checking the box or if the choices are not included you may choose the others category and specify your answer. For those items without choices, please provide your answers**.** For the second part it will assess the perception of the farm owner's, consumers and aqua culturist in terms of the different effects of the presence of antimicrobial agents and antibiotic residue in fish meaton fish and public health. The second part will answer by both fish farm owner, consumer and aqua culturist. The study is also incorporated with experimental procedure on the microbial quality and antibiotic residue of fish samples from the farm. The collection of samples in the study is assisted by the BFAR Laboratory Division Regional Office 2.

3. Interview :

Aside from the questionnaire, a short interview with you as the respondent is also requested. This is necessary to collect other important information that may be helpful in supporting the possible results of the information in the questionnaire.

4. Duration/time:

The questionnaire may take 10-15 minutes to complete.

5. Statement of confidentiality:

Your participation in this research is confidential. The results that will be obtained from the questionnaire will be statistically processed so that a specific individual and farms cannot be identified.in the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

6. Voluntary participation :

Your decision to be respondents in this study will be voluntary. Your completion and submission of the survey imply that you are consenting to take part in the research study.

THANK YOU FOR YOUR COOPERATION

QUESTIONAIRE GUIDE

Instruction: Write a check (/) in the box that corresponds your answer. For the items without choices kindly write your answer**.**

NAME OF FISH FARM: _____

NAME:_____**SEX:** ___ **Female** ___ **MaleAGE:** _____

Part I PROFILE OF FISH FARM AS TO :

A. LOCATION OF THE FARM/ADDRESS: _____

_____

1. What are the nearby/neighboring areas in your fish farms?(CHECK ALL POSSIBLE ANSWERS)

☐ OCEANS ☐ RIVER BANKS ☐ DAMS ☐ HOUSEHOLDS

FACTORY specify_____ ☐ NATIONAL ROADS ☐ OTHERS :_____

2. The fish farms is located in the _____

☐ RURAL AREA ☐ URBAN AREA ☐ FORESTED AREA

B. SPAN OF THE FISH FARM:

1. What is the total area of the fish farm? _____
2. What is the total area and volume of the fish ponds in the farm? _____
3. Check the following areas/sections if they are present in your farm. (check all possible answers)

☐ Feed storage area ☐ Isolation /observation area

☐ Chemical storage area ☐ Treatments/ recovery area

☐ Area for disposal of dead fishes Others specify:_____

4. Please check the infrastructure present in the farms. (Check all possible answers)

☐ Septink tank ☐ household

☐ Toilet Facilities ☐ Drainage others:_____

C. FARM CARE MANAGEMENT

C.1 POND AND WATER MANAGEMENT

1. Did you conduct soil analysis? ☐ YES ☐ NO
2. What type of soil analysis? _____
3. Do you conduct water analysis? ☐ YES ☐ NO
4. What type of water analysis? _____
5. How often do you test for the dissolve oxygen in water?

☐ Daily ☐ Weekly ☐ Once a month ☐ Every after two months

6. How often do you change water from fish ponds?

☐ Every month ☐ every two months ☐ every three months

Others:_____

C.2. PERSONNEL HYGIENE MANAGEMENT

1. Does farm workers undergo medical examination prior to work?

☐ YES ☐ NO

2. Does your workers wear suitable and clean working clothing?

☐ YES ☐ NO

3. Employees/farmworkers have designated area for washing before and after working.

☐ YES ☐ NO

4. Daily screening or checking of farm workers physical condition fit to work is observed.

☐ YES ☐ NO

5. Farm Workers with communicable disease are not allowed to work until treated.

☐ YES ☐ NO

6. Boots and face mask are used by farm workers when feeding and harvesting

☐ YES ☐ NO

D. AQUATIC VETERINARY PRACTICES

D.1 ANTIBIOTIC USED

1. Do you treat your fishes with antibiotics? ☐ YES ☐ NO
2. Why do you use antibiotics? (check all possible answers)

☐ FOR TREATMENT OF DISEASES ☐ FOR PREVENTION OF DISEASES ☐ FORGROWTH ENHANCEMENT

3. What antibiotic/s do you use?

☐ Amoxicillin ☐ Penicillins ☐ Chloramphenicol

☐ Furazolidone ☐ Oxytetracycline ☐ Ciprofloxacin

Others: specify,_____

4. How do you administer antibiotics?

☐ Into the water pond medication ☐ Gavage

☐ In feeds medication ☐ injection

D.2 Frequency of antibiotic utilization in between harvest

1. How often do you give antibiotics in between harvest?

☐ Once ☐ Twice ☐ Thrice ☐ Four Times

☐ More Than 5 Times ☐ All Through Out others:_____

2. How many days do you wean out the antibiotic use prior to marketing or harvesting of fishes?

☐ One day before ☐ three days before ☐ one week before

☐ No widrawal at all Others:_____

D.3. frequency of consulting with veterinarian for disease infection management

1. Do you consult Veterinarian to prescribe medication?

☐ Sometimes ☐ Always ☐ Never

2. How often do you consult Veterinarians in disease management and control?

☐ Sometimes ☐ Always ☐ Never

D.4.Food/Nutrition

1. What food do you give your fishes?

☐ Commercial fish feeds + Natural feeds ☐ Natural feeds alone

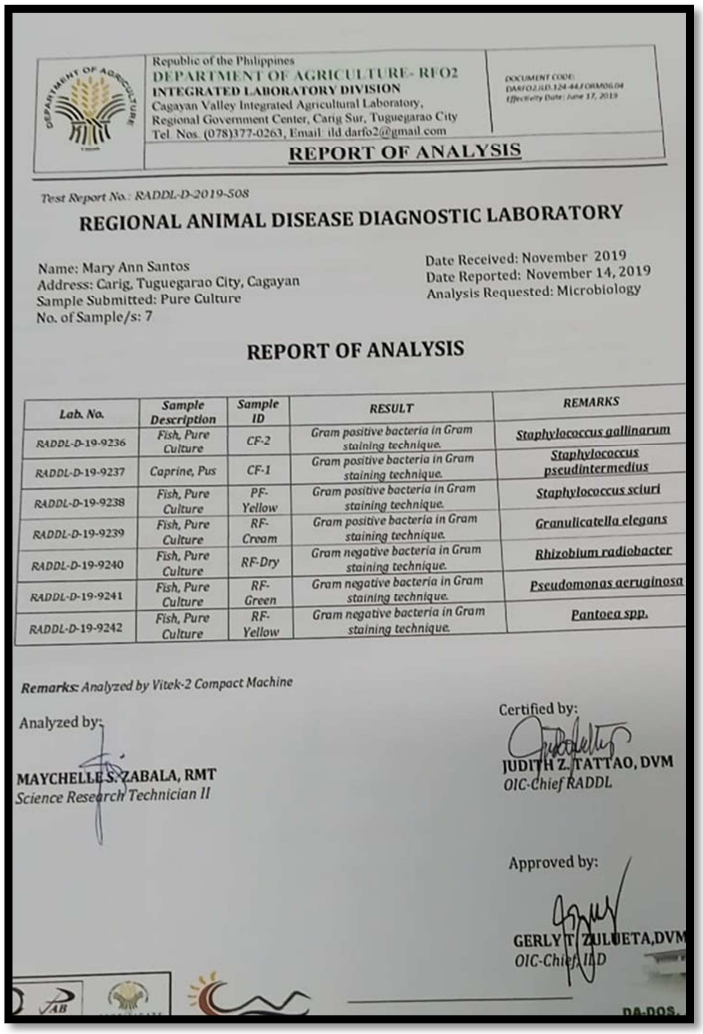
☐ Commercial feeds alone

2. What vitamins do you give your fishes?

☐ Vitamin C ☐ Vitamin A ☐ Vitamin K Others:_____

Appendix B

Data report and Statistical Analysis



Republic of the Philippines
DEPARTMENT OF AGRICULTURE- RFO2
INTEGRATED LABORATORY DIVISION
Cagayan Valley Integrated Agricultural Laboratory,
Regional Government Center, Carig Sur, Tuguegarao City
Tel Nos. (078)377-0263, Email: ild darfo2@gmail.com

DOCUMENT CODE:
DARFO2.ILD.124-44.FORM06.04
Effectivity Date: June 17, 2019

REPORT OF ANALYSIS

Test Report No.: RADDL-D-2019-508

REGIONAL ANIMAL DISEASE DIAGNOSTIC LABORATORY

Name: Mary Ann Santos
Address: Carig, Tuguegarao City, Cagayan
Sample Submitted: Pure Culture
No. of Sample/s: 7

Date Received: November 2019
Date Reported: November 14, 2019
Analysis Requested: Microbiology

REPORT OF ANALYSIS

Lab. No.	Sample Description	Sample ID	RESULT	REMARKS
RADDL-D-19-9236	Fish, Pure Culture	CF-2	Gram positive bacteria in Gram staining technique.	Staphylococcus gallinarum
RADDL-D-19-9237	Caprine, Pus	CF-1	Gram positive bacteria in Gram staining technique.	Staphylococcus pseudintermedius
RADDL-D-19-9238	Fish, Pure Culture	PF-Yellow	Gram positive bacteria in Gram staining technique.	Staphylococcus sciuri
RADDL-D-19-9239	Fish, Pure Culture	RF-Cream	Gram positive bacteria in Gram staining technique.	Granulicatella elegans
RADDL-D-19-9240	Fish, Pure Culture	RF-Dry	Gram negative bacteria in Gram staining technique.	Rhizobium radiobacter
RADDL-D-19-9241	Fish, Pure Culture	RF-Green	Gram negative bacteria in Gram staining technique.	Pseudomonas aeruginosa
RADDL-D-19-9242	Fish, Pure Culture	RF-Yellow	Gram negative bacteria in Gram staining technique.	Pantoea spp.

Remarks: Analyzed by Vitek-2 Compact Machine

Analyzed by:
MAYCHELLE S. ZABALA, RMT
Science Research Technician II

Certified by:
JUDITH Z. TATTAO, DVM
OIC-Chief RADDL

Approved by:
GERLY T. ZULUETA,DVM
OIC-Chief, ILD

DA-DOS

Appendix C

Fig. 3. Farm location.

Neighbouring Location	Frequency	Percent
Household	4	42.86
Factory	1	14.29
National Roads	1	14.29
Mountainous	2	28.57
Farm Location		
Forested Area	2	28.57
Rural Ares	5	71.43
N	7	100

Fig. 4. Farm span.

Farm Code	Total Area (sq. m)	Productive Area (sq. m)
R2-CAG-066	2,400	2,000
R2-CAG-067	2,500	2,000
R2-CAG-068	2,500	2,200
R2-CAG-063	4,500	4000
R2-CAG-065	7,500	5,000
R2-CAG-064	7,000	6,000
R2-CAG-069	20,000	8,000
R2-CAG-061	10,000	9,000
Average	7,050	4,775

Fig. 5. Personnel hygiene management.

Personnel Hygiene Management	Yes	No/not observe
1. Does farm workers undergo medical examination prior to work?	0	7
2. Does your workers wear suitable and clean working clothing?	7	0
3. Employee/ farmworkers have designated area for washing before and after working.	0	7
4. Daily screening or checking of farm workers physical condition fit to work is observed.	0	7
5. Farm Workers with communicable disease are not allowed to work until treated.	0	7
6. Boots and face mask are used by farm workers when feeding and harvesting	0	7

Fig. 6. Farm management.

Frequency of consulting with veterinarian for disease infection management	*Sometimes*	*Always*	*Never*
1. Do you consult Veterinarian to prescribe medication?	3	0	4
2. How often do you consult Veterinarian in disease management and control?	0	7	0

Fig. 7. Farm bacterial isolates.

Farm	Dilution	Colony forming unit/ ml
	-3	TOO NUMEROUS TO COUNT
E	-4	4.35 x 10 ^6
	-5	3.85 x10 ^7
	-6	2.75 x 10 ^8
A	-4	1.93 x10^ 6
	-3	3.98 x 10 ^5
	-5	1x10 3
	-6	0
F	-3	3.65 X 10^ 5
	-4	2.05 X10^ 6
	-5	5.0 X 10 ^5
	-6	1X10^6

Fig. 8. ANOVA-Two Factor Analysis without Replication.

Source of Variation	SS	df	MS	F	P-value	F crit
Dilution Factor	1.43063E+16	2	7.15E+15	0.981653	0.4499 NS	6.944272
Farm Location	2.20658E+16	2	1.1E+16	1.514087	0.3239 NS	6.944272
Error	2.91474E+16	4	7.29E+15			
Total	6.55195E+16	8				

APPENDIX D

DOCUMENTATIONS

1. The collection of samples, ocular inspection of location of the fish farms and interview was assisted by the Bureau of Fisheries and Aquatic Resources.

2. Collection of samples was coordinated with the Municial Agricultur Office and the Muniipal Mayor of Sanchez Mira Cagayan.

3. Ocular observation of the fish farm location as to near household, forested area and rural areas.

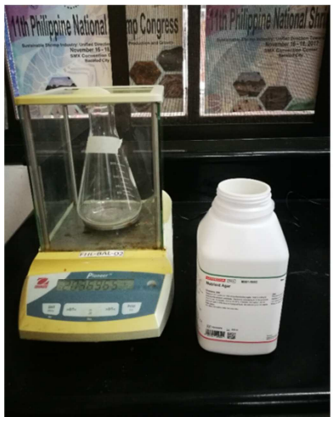
4.Stockroom and Storage of fish farms. Where chemicals and feeds are in the same stockroom.

5. Domestic animals found near the ponds.

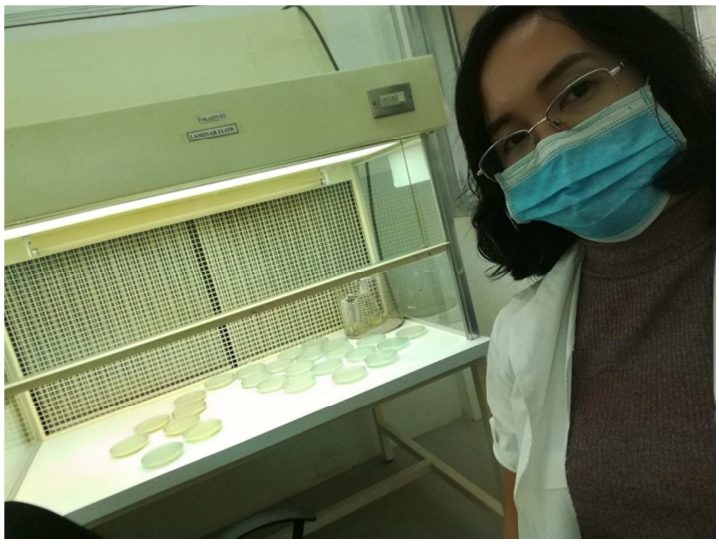
6. Collected samples were in placed in sterile sealed plastic containers to ensure sterility so samples.

7. Dingil's fish farm partnered with CSU-Piat

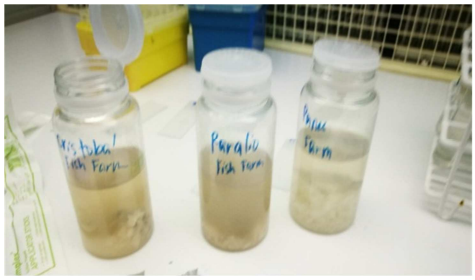
PROCESSING OF SAMPLES



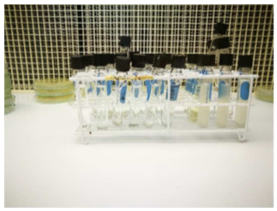
2. Preparation of materials

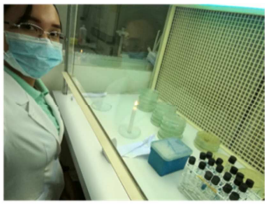


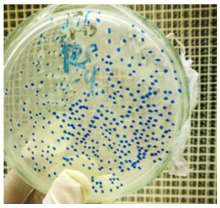
3. Sterility test for the prepared agar to ensure that there was no contamination along the preparation of samples. 24 hours incubation of blank nutrient agar plates and nutrient broth.

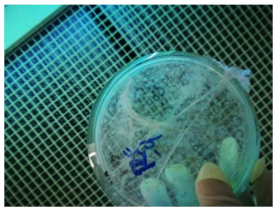


4. Bacterial culture of homogenized fish muscles of tilapia samples.

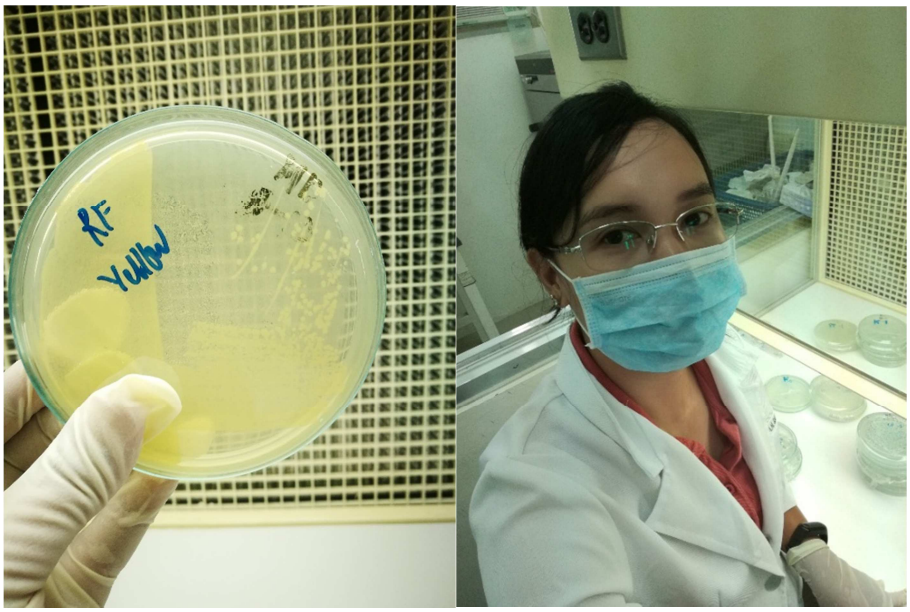






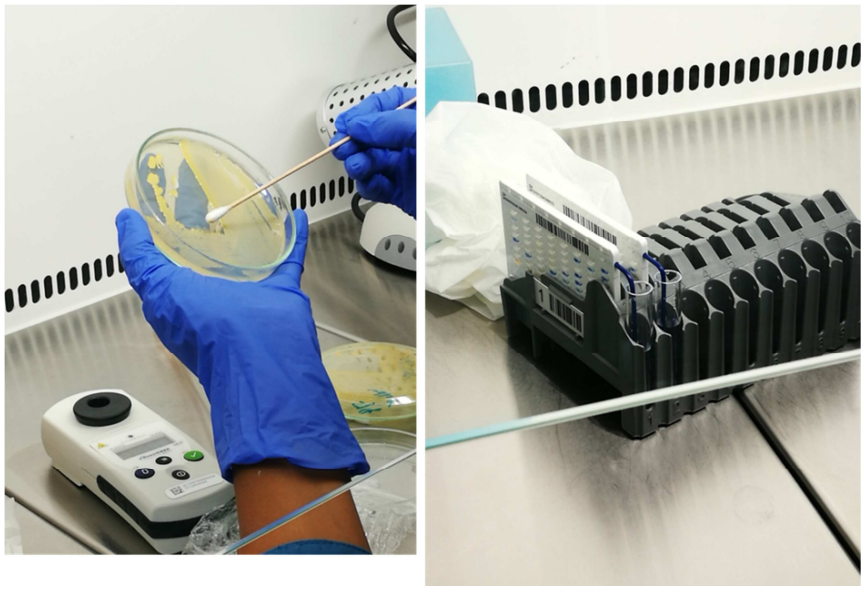


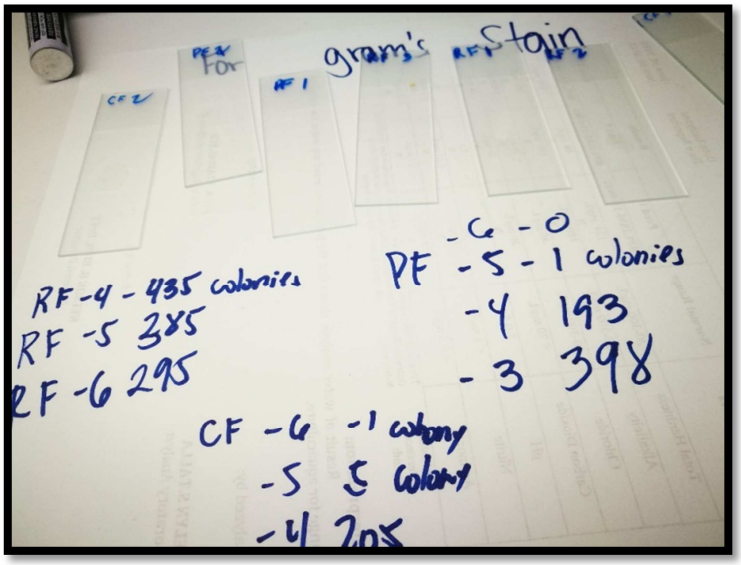
5. Serial dilution of homogenized samples fish muscle meat of tilapia for colony count.



6. Bacterial isolation for pure culture for the bacterial identifications.

7. Vitek processing. Sample preparation for bacterial suspension and cartridge loading for bacterial identification at the Department of Agriculture Regional Animal Disease And Diagnostic Laboratory.




gram's stain
RF-4-435 colonies
RF-5 385
RF-6 295
PF -6 -0
-5 -1 colonies
-4 193
-3 398
CF -6 -1 colony
-5 5 colony
-4 205