ANTIBIOGRAM OF *Edwardsiella tarda* ISOLATED FROM DISEASED ANGLEFISH (*Pterophyllum scalare*) OF AQUARIUM SHOP

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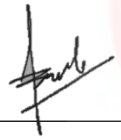
DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any institution



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I certify the report of this final year project entitled ‘Antibiogram of *Edwardsiellatarda* Isolated from Diseased *Pterophyllum scalare* of Aquarium Shop’ by Farah ‘Aliah Binti Hasan, matric number F18B0029 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry) with Honors, Faculty of Agro-Based Industry, University Malaysia Kelantan.

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Antibiogram of *Edwardsiella tarda* Isolated from Diseased *Pterophyllum scalare* ofAquarium Shop

ABSTRACT

The purpose of the present study was isolate and identified *Edwardsiella tarda* from diseased *Pterophyllum scalare* of aquarium shop. *E.tarda* is a gram-negative bacterium with facultative properties and will lead to several diseases in fish, human, and other animal. *E.tarda* can be found in fish and ornamental fish by isolate from freshwater or brackish water environments. It has also been isolated from the human stool after consuming freshwater food source such as catfish, eel and including reptiles. Therefore, this is crucial ornamental fish are rare in intensive aquaculture activity. Despite all the previous research, the study of *E.tarda* already has antibiotic yet it still not be commercialize and still under developing. In this research, diseased *Pterophyllum scalare,* anglefish were collected from aquarium shop in Jeli, Kelantan and lesion were swab on the external and internal of the ornamental fish at the abdomen, skin, gills, eyes, and kidney. The method of isolation *E.tarda* was used Xylose-Lysine-Deoxycholate (XLD) agar follow with identification by using BBL Crystal commercial kit. The selected bacteria were subjected to diffusion method. This 30 of isolates *E. tarda* was then continued into Antibiogram disk that were used in the present study. At the mean time the sensitivity of *E.tarda* was determined the characterize antibiogram with 16 types of antimicrobial susceptibility test. The result was analyzed convert as Sensitive (S), Intermediate Sensitive (IS) or Resistance (R) by using Antibiotic resistance index and MAR calculation. The antibiogram of isolates was found to be highly sensitive to flumequine and resistance to amoxycillin antibiotic. This finding is indicate that *E.tarda* presence of combination of the antibiotic resistance in angelfish isolates and has the possibility found in others ornamental fish.

Keyword: Ornamental fish,*Pterophyllum scalare, Edwardsiella tarda,* antibiogram

Antibiogram *Edwardsiella tarda* Diasingkan daripada *Pterophyllum scalare* Berpenyakit di Kedai Akuarium

ABSTRAK

Tujuan kajian ini adalah mengasingkan dan mengenal pasti *Edwardsiella tarda* daripada *Pterophyllum scalare* kedai akuarium yang berpenyakit. *E.tarda* ialah bakteria gram-negatif dengan sifat fakultatif dan akan membawa kepada beberapa penyakit pada ikan, manusia dan haiwan lain. *E.tarda* boleh didapati dalam ikan dan ikan hiasan dengan mengasingkan diri daripada persekitaran air tawar atau air payau. Ia juga telah diasingkan daripada najis manusia selepas mengambil sumber makanan air tawar seperti ikan keli, belut dan termasuk reptilia. Oleh itu, ini adalah penting ikan hiasan jarang berlaku dalam aktiviti akuakultur intensif. Di sebalik semua kajian terdahulu, kajian *E.tarda* telah pun mempunyai antibiotik namun masih belum dikomersialkan dan masih dalam pembangunan. Dalam penyelidikan ini, *Pterophyllum scalare*, anglefish yang berpenyakit telah dikumpul dari kedai akuarium di Jeli, Kelantan dan kesan luka disapu pada bahagian luar dan dalam ikan hiasan di perut, kulit, insang, mata dan buah pinggang. Kaedah pengasingan *E.tarda* telah digunakan agar Xylose-Lysine-Deoxycholate (XLD) dengan pengecaman menggunakan kit komersial BBL Crystal. Bakteria yang dipilih tertakluk kepada kaedah resapan. 30 pencilan *E. tarda* ini kemudiannya diteruskan ke dalam cakera antibiogram yang digunakan dalam kajian ini. Pada masa yang sama, sensitiviti E.tarda ditentukan untuk mencirikan antibiogram dengan 16 jenis ujian kerentanan antimikrob. Hasilnya dianalisis menukar sebagai Sensitif (S), Sensitif Pertengahan (IS) atau Rintangan (R) dengan menggunakan indeks rintangan Antibiotik dan pengiraan MAR. Antibiogram isolat didapati sangat sensitif terhadap flumekuin dan rintangan kepada antibiotik amoksisilin. Dapatan ini menunjukkan bahawa kehadiran *E.tarda* gabungan rintangan antibiotik dalam pencilan angelfish dan mempunyai kemungkinan terdapat pada ikan hiasan lain.

Kata kunci: Ikan hiasan, *Pterophyllum scalare*, *Edwardsiella tarda*, antibiogram

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LIST OF ABBREVIATIONS

DOF	Department of Fisheries Malaysia	2
USD	United State of America Dollar	3
RM	Ringgit Malaysia	4
AST	Antibiotic Susceptibility Test	17
NCCLS	National Committee for Clinical Laboratory Standards	18
MAR	Multiple Antibiotic Resistance	19
NA	Nalidixic Acid	19
OA	Oxolonic Acid	19
S3	Compound Sulphonamides	19
DO	Doxycycline	19
TE	Tetracycline	20
NV	Novobiocin	20
C	Chloramphenicol	20
K	Kanamysin	20

RL	Sulphamethoxazole	20
UB	Flumequine	20
E	Erythromysin	21
AMP	Amplicillin	21
SP	Spiramycin	21
OT	Oxytetracycline	21
AML	Amoxycillin	22
FOS	Fosfomycin	22

LIST OF SYMBOLS

µm	Micrometre	7
%	Percentage	8
°	Degree	9

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ornamental fish keeping is a centuries-old popular, as leisure companion which growing interest resulted in steady increase in globally aquarium fish trading. The economic importance of ornamental fisheries has been acknowledged by many developing countries for employment opportunities and resulting advancement in breeding, transport and aquarium technology and various species added every year (Dey, 2016). Based on the 7th International conference of Aquaculture & Fisheries, the highest exporting in Asian country ornamental fish is Singapore followed by Hong Kong, Malaysia, Thailand, Philippines, Sri Lanka, Indonesia, and India. Majority ornamental fish are native trade from Northeastern states in India and the rest is from Southern states.

Ornamental fish trade is worth more than USD10 billion with annual growth by 10%, while the entire industries that include feed, plants, accessories, aquarium, and drugs were estimated to be worth more than USD 18-20 billion (Dey, 2016).

In Malaysia, Johor is the first state as fish dealers with economic value at RM 252 million in 2013. Based on Department of Fisheries Malaysia (DOF) report in production of ornamental fish and aquatic plant by state in 2016, stated that total of ornamental fish produce with 402 million bundle of fishes includes species Cyprinids, Barb, danio, goldfish, koi, *Loricariidae*, *Osteoglossids*, Poecilids, and others.

Edwardsiella tarda or *E. tarda* (Ewing et al, 1965) is a gram-negative, rod-shaped bacterium, peritrichously flagellated and motile. *E. tarda* usually can be found in fish from freshwater, seawater, or brackish water environment. *E. tarda* can cause infection and mortality with severe liver cirrhosis to human but rarely case happen unless after consume water food source with effected *E.tarda* (Hirai, Asahata-Tago, Ainoda, Fujita & Kikuchi, 2015). Based on Park, Aoki & Jung *E. tarda* is about 2 – 3 μm in length and 1 μm in diameter and capable to survive and adapt to various host environment condition, that including temperature, pH, salinity, and other important nutritional element like phosphate.

Treatment antibiotic has been developed by scientist in China yet, the antibiotic is in on hold for commercialized. Thus, another treatment formalin-killed bacteria is not strong enough to kill *E.tarda* (Xu & Zhang, 2014). These conditions prompted this research is to investigate the occurrence of any *Edwardsiella tarda* in the diseased *Pterophyllum scalare* that was taken in aquarium shop and the characterize antibiogram of isolated *Edwardsiella tarda*

in diseased *Pterophyllum scalare*.

1.2 Problem Statement

Despite that ornamental fish is rising in aquaculture industry, bacteria and other pathogen can lead to huge losses to aquaculture industries. One of the broad host rage pathogens that infect more than 20 species fish (including seawater, freshwater, rare fish, and ornamental fish) and other animal including humans. This will cause a huge problem when fish are subjected to intensive culture farming. Thus, bacterial infection in ornamental fish caused mostly from lack of water quality, crowing, transportation, handling, and poor nutrition (Musa, Chuah, Wei & Shazili, 2011).

Therefore, this study may facilitate further investigation on identify *Edwardsiella tarda* in diseased *Pterophyllum scalare* and characterize antibiogram of *Edwardsiella tarda*. to know the least acceptability of the less resistance of antibiogram for suitability treatment antibiotics in *Edwardsiella tarda* infection.

1.3 Objectives

The objectives of the present study are:

1. To isolate and identify *Edwardsiella tarda* in diseased *Pterophyllum scalare* from aquarium shop.

2. To characterize antibiogram of isolated *Edwardsiella tarda* from disease angelfish (*Pterophyllum scalare).*

1.4 Scope of Study

This research is focused on isolation and identification of *Edwardsiella tarda* in diseased angelfish (*Pterophyllum scalare)* from aquarium shop. The bacteria isolated is identifiedas *Edwardsiella tarda* with Antibiotic Susceptibility Test (AST) with 16 types of antibiotics using disk diffusion method. Also, the determination was according to National Committee for Clinical Laboratory Standards (NCCLS) and followed by Multiple Antibiotic Resistance (MAR) to calculate the present of isolates against the antibiotic tested.

1.5 Significances of Study

In this research study, *Edwardsiella tarda* is a harmful bacterium and can havepotential cause in human health and other aquatic animal. Yet it is not much study onwhich chemical reagent can be effectively against it. According to Xu & Zhang (2014),the treatment of formalin-killed bacteria is not strong enough to kill *Edwardsiella Tarda*, this is important to understand, able to identify the type of bacteria including effective antibiotics against it. Using of treatment antibiotics always the effective ways in combat bacterial infection in aquaculture industries (Thiang, et. al, 2021).

There are a lot of studies have been reported regarding the Antibiogram of Edwardsiella *tarda* isolated in from diseases freshwater fishes in overseas and journals. However, isolate and identify *Edwardsiella tarda* in diseased ornamental fish from aquarium shop are not preferred while ornamental fish are often purchased and farmed by farmer and it also being the highest marketing aquaculture marketing in Malaysia. Therefore, the focus of this isolation and identify *Edwardsiela Tarda* in diseases *Pterophyllum scalare* is to characterize the antibiogram of isolated bacteria to investigate the least acceptability antimicrobial for sustainability treatment antibiotic in *Edwardsiella tarda* infection.

1.6 Limitation of study

Since the country Movement Control Order was issued on March 18, 2020 upuntil now. All places need to perform a standard procedure to comply with Malaysian Standard of Procedure (SOP) to prevent from spread of coronavirus. This SOP are including in laboratory activities which gives limitation on the schedule. Behalf of that, the total number of antimicrobial need to increase to have the better accurate in the determining the antibiogram. Moreover, the dead fish sampling needed to be more than 30 fishes with fully diseases effected body as it can get more accuracy. Thus, this research field in bacteria *Edwardsiella tarda* is limited where mostly only focus on farmed fish rather than ornamental fish.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to *Edwardsiella tarda*

Edwadsiella sp. consisnt of three species which are *E. tarda*, *E. ictaluri*, and *E. hoshinae* (Park, Aoki & Jung, 2012). Fish usually infected with *E.tarda* and *E. ictaluri* while the other infected on reptiles and birds. With its gram-negative, short, rod-shape and anaerobic bacterium it can survive at 0 – 4% of sodium chloride, pH of 4 – 10 and temperature at 14 - 45°C. Based on B & T (2016), *E.tarda* frequently found in polluted water with poor water quality and affect fish stressed by situation. *E.tarda* can cause death to human and other clinical symptom shown like diarrhea, gastroenteritis and wound infection. This is because when consuming partially cooked fish and unhygienic practice can have higher risk in getting infected with these bacteria.

In ornamental fish clinical sign can be seen clearly with loss of pigmentation, swelling of the abdominal surface, petechial hemorrhage in fin and skin, opacity of eyes

and rectal hernia. While in internally, there will be watery and bloody ascites in abdominal space and liver, spleen, and kidney. In liver, spleen, and kidney they will be abscesses of various sizes and bacterial colonization. For movement, will be abnormal swimming behavior, including spiral movement and floating near water (Park,Aoki & Jung, 2012). According to Bullock and Herman (1985), Internal fish that infected with *Edwardsiella tarda* most commonly on gross lesion occur of light- colored nodules on parts of kidney, spleen or at liver. The liver organ is the primary organ affected followed by kidneys.

2.2 Isolation and identification of *Edwardsiella tarda*

They are many types of isolation of bacteria *Edwardsiella tarda* can be done. *Edwardsiella tarda* can be isolate with Edwardsiella Isolation Media (EIM), Brain heart Infusion (BHI), Trypic Soya Agar (TSA), Xylose Lysine Deoxycholate (XLD) and MackConkey. On agar plate of XLD, will occur small, circular, raised, whitish with black center, while in MacConkey agar will be pale on the center. Both isolations will grow bacteria at optimum temperature ranging between 25°C -37°C, with pH of 7-8, 0.5% of NaCl, and characteristically will be shown as catalase positive, cytochrome oxidase negative, glucose fermentative, indole positive, citrate negative, lysine positive,

mannitol, dulcitol, sorbitol, inositol, xylose, rhamnose negative, produce hydrogen sulphide (B & T, 2016).

2.3 Chemical test of *Edwardsiella tarda*

The reaction of *E.tarda* will show positive in indole test production, methyl red reduction and hydrogen sulphide generation, fermentation of glucose and reduction of nitrate to nitrate (Park, Aoki & Jung, 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Material and Apparatus

3.1.1 Chemical and Reagent

All chemicals and reagent used in this laboratory works and this studywere listed in Table 3.1.

Table 3.1: List of chemicals

No.	Chemicals
1	Thiosulphate Citrate Bile Salts Sucrose (TCBS)
2	Trypticase soy agar (TSA)
3	Tryptic soy broth (TSB)
4	Lactose broth (LB)
5	Graphene oxide (GO)
6	Antibiotics disc
7	BBL Crystal kit

3.1.2 Equipment

The equipment and apparatus that was used in this experiment were listed in Table 3.2. This equipment was handling and measured carefully to reduce the inaccuracy and obtain precise result.

Table 3.2: List of Equipment and Apparatus

No.	Apparatus
1	Dissection instrument (scalper, forceps, scissors)
2	Isopropyl alcohol
3	Plates
4	Inoculate wire loop
5	Slide
6	BBL Crystal kit
7	Incubator
8	Bijou bottle
9	Durham tube
10	Vortex machine
11	petri dish
12	pipette
13	Lamina flow cabinet
14	Autoclave machine
15	Electronic balance
16	L-shape hockey sticks
18	Bunsen burner

3.2 Method

3.2.1 Sample collection and preparation

Diseased *Pterophyllum scalare* for 30 pieces was purchase and collect from available ornamental fish shop in Kelantan using plastic bag. The diseased *Pterophyllum scalare* then were dissected with disinfected scraper at along the abdomen and gently scrape with the blunt end scraper and will be scraping on the external lesions. Use forceps to open the abdomen and gills to check the available infected internal organs on kidney and guts. Collect the sample and culture on alkaline peptone water (APW) with 1% Nacl at pH of 8.6.

3.2.2 Isolation and identification of *Edwardsiella tarda*

3.2.2.1 Isolation of *Edwardsiella tarda* by using spread plate

After the external fish skin were disinfected with 70% ethyl alcohol, The sample from the test tube were streaked using wire loop on petri dish with

xylose-lysine-deoxycholate (XLD) agar for 24 hours at 35°C incubation(B & T, 2016).

3.2.2.2 Total plate count of bacteria isolates

Colony of bacteria becomes visible to the naked eye. The colony sample on the sample dish must be range between 30 to 300 colonies to prevent from overlapping (Scott, 2011). Then, mark bacteria colonies below of the petri dish with marker pen. For Record data, Colony Forming Unit (CFU) calculation is applied. The unit for colony forming are CFU/ml (Chouhan, 2015).

$$CFU\ /ml = \frac{Number\ of\ Colonies \times dilution\ factor}{Volume\ of\ culture\ plate\ (ml)}$$

3.2.2.3 Bacteria identification using BBL Crystal kit

Specimens need to label on the side of the cover of the base. Next, choose bijou bottle with suspend colonies and pour into target area of the base. While holding base with both hand, roll inoculum fluid gently along the track until full. Place whole panel inside incubate at 24 hours for 35 °C to 37 °C. The test of indole and oxidase will perform separately using indole reagent and BBL Oxidase Reagent. Record data colour changes in BBL crystal after 24hour by place down. Each test result will give value of 4, 2 or 1 corresponding where test is located. Positive reaction will be total up and 10-digit number obtain will be profile number for the test (Holmes, Costas, Thaker & Stevens, 1994).

3.2.2.4 Antibiotic susceptibility test (AST)

This method is to evaluate resistance of antibiotic. After swapping sterile cotton bud on the TSA agar plate, Kirby-Bauer disc diffusion method is used to evaluate resistance of antibiotic. By spreading bacteria on an agar plate, place paper with submerge of antibiotic on the plate. Seal the petri dish with parafilm and the

bacteria growth will be observed after 24 hours of incubation at 35°C (Jorgensen &Ferraro, 2009).

3.2.3 Antibiogram susceptibility test

3.2.3.1 Calculation of Multiple antibiotic resistance (MAR) index

After overnight of incubation, the petri dish is ready to be observing for the zone of inhibition where no bacterial growth can be seen. Measure the nearest millimetre with rule and use to determine and compare the bacteria susceptibility base on standard guideline of Clinical and Laboratory Standard Institute. Each antibiotic disc will be analysis and convert as Sensitive (S), Intermediate Sensitive (IS) or Resistance (R). Antibiotic resistance index will be calculated by using formula below(Nandi & Mandal, 2016).

$$MAR = \frac{Number\ of\ antibiotics\ to\ which\ the\ isolate\ showed\ resistance}{Number\ of\ total\ antibiotics\ exposed\ to\ the\ isolate}$$

CHAPTER 4

RESULTS

4.1 Bacteria Isolation and Identification

As external and internal lesion in isolates bacteria the angelfish examined and start swabbing on the diseased area abdomen, eyes, gills, kidney, and skin in figure 4.1. Clinical findings of the diseased angelfish, hemorrhage in the eyes and loss of scales.

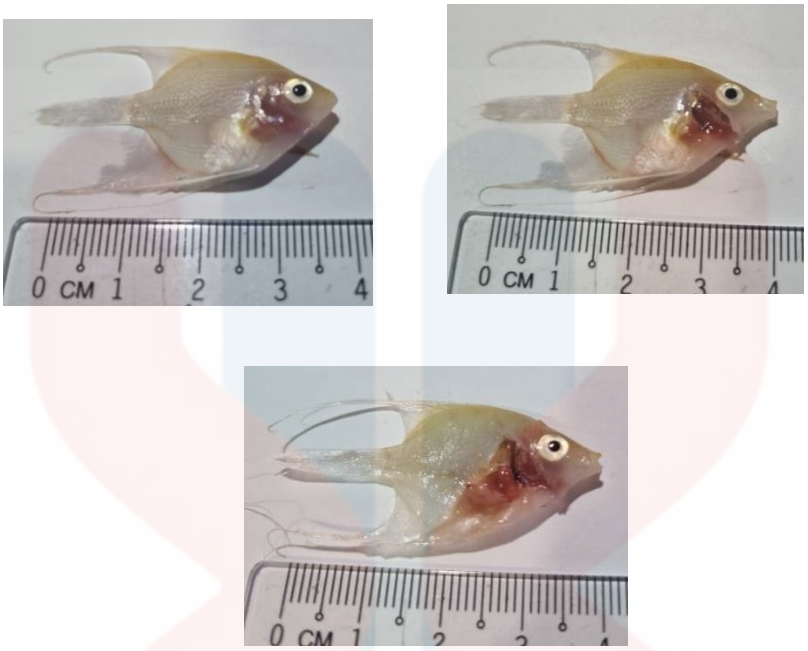

Figure 4.1: Anglefish (*Pterophyllum scalare)* isolates bacteria on diseases infected area

In this study, *Edwardsiella tarda* isolates from 30 samples disease *Pterophyllum scalare* the were screened against 16 antimicrobial agents. Antibiotic disc that already been analysis is convert as Sensitive (S), Intermediate Sensitive (IS) or Resistance (R) by using Antibiotic resistance index, MAR calculation. Table 4.1 shows the antimicrobial susceptibility test of *Edwardsiella tarda* isolated from Angelfish, *Pterophyllum scalare,* ornamental fish from aquarium shop.

Table 4.1: Table of antimicrobial susceptibility test of *Edwardsiella tarda* on angelfish (*Pterophyllum scalare)*

Types of antimicrobial agent	Concentration (µg/disk)	Bacteria Isolated (*Edwardsiella tarda*)		
		sistancecase (%)	Intermediary Sensitive case (%)	ensitivecase (%)
Amoxycillin (AML)	25	76.6	20.0	3.33
Amplicillin (AMP)	10	43.3	56.66	0
Chloramphenicol (C)	30	13.3	43.3	43.3
Compound Sulphonamides (S3)	300	90	6.6	3.3
Doxycycline (DO)	30	0	66.6	33.3
Erythromycin (E)	15	0	50.0	50.0
Flumequine (UB)	30	0	26.6	73.3
Fosfomycin (FOS)	50	10.0	26.6	63.3
Kanamysin (K)	30	0	60.0	40.0
Nalidixic Acid (NA)	30	0	33.3	66.6
Novobiocin (NV)	30	33.3	60.0	6.66
Oxolonic Acid (OA)	2	0	30.0	70.0
Oxytetracycline (OT)	30	3.33	56.6	40.0
Sulphamethoxazole (RL)	25	50.0	50.0	0
Spiramycin (SP)	100	0	70.0	30.0
Tetracycline (TE)	30	0	76.6	23.4

From the table result above, the *Edwardsville tarda* isolates were obtain from lesion external and internal on abdomen, eye, gill, kidney, and skin of *Pterophyllum scalare.* According to the result, it was determined that the isolation was highly sensitive to flumequine with 73.3% and oxolonic acid with 70%, while being resistance to Compound Sulphonamides with 90% followed by amoxycillin, Sulphamethoxazole, Amplicillin, Novobiocin with 33.3%. For intermediate sensitive isolation the highest is tetracycline antibiotics with 76.6% followed with Spiramycin and doxycycline. Kanamysin and novobiocin antibiotics having the same result with 60% of intermediate sensitive.

In Multiple antibiotic resistance (MAR) index, calculation was done by using formula set by Nandi & Mandal (2016). The value for bacterial *Edwardsiella tarda* isolates is 0.5 the result shows that *Edwardsiella tarda* in these fish might have been exposed to that antibiotic during culturing stages.

CHAPTER 5

DISCUSSION

5.1 Discussion

Esdwardsiella tarda is one of a bacteria that can cause severe economic losses because to it morbidity and mortality among various aquaculture population and age groups in many countries (Jun and Yin, 2006). This is a global challenge in the aquaculture industry where it could influence both human and aquatic wildlife. In prevent from diseases to spread wider and to inhibit the bacteria, antibiotic is used in aquaculture. Currently, the common use of antibiotic in aquaculture farming are tetracyclines, sulfonamides, oxolinic acid and erthromysin (Thiang et al., 2021). Aquaculture used antibiotics by influenced of microbial genetic ecology through mutation and gene transfer between microorganism (Matyar et al., 2008). Moreover, these antibiotics usually used by oral route and in pellet feeding.

Information about the incidence of *Edwardsiella tarda* in anglefish Malaysia are limited. According to Musa, Wei, Shaharom & Wee (2008), isolated of

Edwardsiella tarda was found in other ornamental fish at aquarium shop in Malaysia. This study proved that isolated of *Edwardsiella tarda* occurrence in angelfish, *Pterophyllum scalare* are valid. This support in one of similar studies by Turgay (2020) of isolated *Edwardsiellosis sp*. inangelfish at Turkey.

Antibiotic susceptibility test

In this study, were surprisingly observed as the most susceptible drugs with more than 70% are flumequine and oxolonic acid. The result susceptibility was unexpected, and it would indicate the efficacy of antibiotic treatment towards this bacteria disease. Adversely, Wimalasena et al. (2018) stated that the resistance isolates were highly frequency for ampicillin. Althought, flumequine and oxolonic acid in freshwater ell in Taiwan researcher Lo et al. (2014) were highly susceptibility to this antibiotic. Whereby, ampicillin and sulphamethoxazole in this study shows it is the lowest frequency in sensitivity antibiotic with 0%. This result has the same with Turgay, 2020 where ampicillin is has lowest frequency in sensitive antibiotic yet has the highest frequency in resistance antibiotic to*Edwardsiella tarda.*

Many studies have shown (Sedek et al., 2020; Lo et al., 2014) that *Edwardsiella tarda* found to be resistance in ampicillin and tetracycline where it is compatible with other studies on *Edwardsiella tarda*. Adversely, in this finding tetracycline is the highest frequency in intermediary sensitive. Whereby according Nadirah, Najiah & Teng (2012) tetracycline is the most effective antibiotic for controlling growth of *Edwardsiella tarda.*

In all, 16 antibiotic agents were identified in isolated bacteria

Edwardsiella tarda in antibiotic isolates testing result shows that more than 70% bacteria isolates were sensitive to compound which compound flumequine, fosfomycin, nalidixic acid, oxolonic acid. Which this can be support by the same antibiotic susceptibility testing on freshwater tilapia withresulting of 42% (Lee & Wendy, 2017) in isolating *A. hydrophila* and *E. tard*.

In the other hand, 90% of these isolates were resistance to compound sulphonamides, amoxycillin and sulphamethoxazole which is resulted for multiple antibiotic resistance. Where in Lee & Wendy (2017) studies that were resistance with lower percentage of 41.6%. Perhaps, during the lesion isolation bacteria on *Pterophyllum scalare* the diseases is not infected because of high resistance. According to some researchers, antibiotic resistance reservoirs can interact between different ecological systems, with the possibility of resistance bacteria or resistant genes being transferred from animals to humans through the food chain. (Van & Stobberingh, 2000; Teuber, 2001)

MAR index

In the present study, MAR index value of Edwardsiella tarda shows the value is higher than 0.2. The value of isolates from angelfish was 0.5, where it was much higher than 0.2. A MAR index value higher than 0.2 is considered that the aquatic animal has exposed to high-risk exposure to those antibiotics. Based on Baquero et al. (1998), A high value index could suggest the presence of varying pressure, which can result in bacterial strains with diverse mechanisms that can survive in a changing environment.

Antimicrobial sensitivity test

The use of antimicrobial agent in aquaculture can give benefits in increase the prevalence in bacteria resistance that can be transmitted to and cause infection to humans and animals. The impact of application antimicrobial also reduces the spread of bacterial infection that impact both human and animals.

Antimicrobial sensitivity test revealed that isolated edwardsiella tarda were sensitive to flumequine, oxolonic acid, fosfomycin and nalidixic acid, while intermediately sensitive to tetracycline, spiramycin, doxyxyxline, kanamysin, novobiocin, oxytetracycline, ampilicillin and chloramphenicol. These findings point to numerous antibiotic resistances in isolated bacteria, particularly tetracycline, which is routinely used in fish farms. (Choresca et al,2011). Moreover, it was observed that antimicrobial sensitivity test revealed resistance in compound sulphonamides and amoxycillin. Where *Edwardsiella tarda* has no longer respond amoxycillin and compound sulphonamides and increase the risk of disease spread and severe illness in the angelfish. Thus, antimicrobial resistance in aquatic pathogens emerges because of overuse and misuse of antimicrobial agents.

CHAPTER 6

CONCLUSIONS

5.1 Conclusions

Edwardsiella tarda is commonly seen as an opportunistic infection, however it can cause bacteriological septicemia and death in variety hosts including both fresh and marine water fish, reptiles, and mammalian species. Therefore, it is appropriate to control, and prevention takes measures to reduce the infection in ornamental fish. Overall, in finding isolations *Edwardsiella tarda* in *Pterophyllum scalare* has a resistance more than 90 % in compound sulphonamides and amoxycillin and found to successfully inhibit in Tetracycline, spiramycin, oxolonic Acid, Flumequine, amplicillin more than 55%. It the best ways in counter E.tarda diseases by using oxolonicacid and flumequine in freshwater *Pterophyllum scalare.*

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