Antibiogram Of *Salmonella Spp.* Isolated from Diseased Blood-Red Parrot Cichlid Fish (*Amphilophus citrinellus X Paraneetroplus Synspilus*) of Aquarium Shop

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DECLARATION

I hereby declare that the work embodied here is the result of my research except for the excerpt as cited in the references.

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Antibiogram Of *Salmonella Spp.* Isolated from Diseased Blood-Red Parrot Cichlid Fish (*Amphilophus citrinellus x Paraneetroplus synspilus*) of Aquarium Shop

ABSTRACT

Ornamental fish production is increasing, infectious diseases are becoming more common among ornamental fish, resulting in a lot of economic loss because of high fish mortality. In the ornamental fish industry, there is a lot of concern about bacterial infections. Salmonella spp. is the most commonly isolated bacteria from infected ornamental fish purchased from an aquarium shop, according to bacterial species surveillance. The idea has been raised that ornamental fish getting infected by *Salmonella spp.* is through contaminated water and feeding by fishermen, sellers, and hobbyists. Thus, an antibacterial compound is being used to minimize bacterial disease problems. However, the water treatment and the most effective antibiotic for *Salmonella spp.* treatment is still unknown. This study aims (1) to isolate and identify *Salmonella spp.* from diseased Blood-Red Parrot Cichlid fish and (2) to characterize antibiogram of isolated *Salmonella spp.* A total of 30 samples were taken from unhealthy Blood-Red Parrot Cichlid fish which consists of four different parts of fish which are the abdomen, eye, gill, kidney, and skin. Salmonella spp. were isolated using the spread plate method and identified using a commercial BBL Crystal kit. Disk diffusion was used to conduct the antibiotic susceptibility test on the present bacterium. Nalidixic Acid, Oxolonic Acid, Compound Sulphonamides, Doxycycline, Tetracycline, Ampicillin, Novobiocin, Chloramphenicol, Kanamycin, Sulphamethoxazole, Erythromycin, Flumequine, Oxytetracycline, Spiramycin, Fosfomycin, Amoxycillin are among the 16 antibiotics utilized. Results of antimicrobial testing were categorized as sensitive, intermediately sensitive, or resistant to several antimicrobial agents. Based on the findings of the antibiotic sensitivity tests, the multiple antibiotic indexes (MAR) were calculated. Oxolonic acid was shown to be the most effective in suppressing current bacterial isolates, with 83.3 percent sensitivity. The most responsive antibiotics were doxycycline (73.33%) and erythromycin (60%) respectively. On the contrary, all bacterial isolates were ampicillin-resistant.

Keywords: Ornamental fish, antibiogram, *Salmonella spp.,* Blood-Red Parrot Cichlid fish

Pengambilan Antibiogram *Salmonella spp.* daripada Ikan Parrot Merah (*Amphilophus citrinellus x Paraneetroplus synspilus*) Berpenyakit di Kedai Akuarium.

ABSTRAK

Pengeluaran ikan hiasan meningkat, penyakit berjangkit menjadi hal biasa dalam kalangan ikan hiasan, mengakibatkan banyak kerugian ekonomi kerana kematian ikan yang tinggi. Dalam industri ikan hiasan, penyakit bakteria adalah masalah yang serius. Menurut pengawasan spesies bakteria, *Salmonella spp.* adalah bakteria yang paling kerap diasingkan daripada ikan hiasan berpenyakit yang dibeli dari kedai akuarium. Keberangkalian menyatakan bahawa ikan hiasan dijangkiti oleh *Salmonella spp.* adalah melalui air yang tercemar dan semasa pemberian makanan oleh nelayan, penjual, dan penggemar ikan. Oleh itu, ubat antibakteria telah digunakan untuk meminimumkan masalah penyakit bakteria. Walau bagaimanapun, terdapat kekurangan maklumat mengenai rawatan air dan antibiotik yang paling sesuai untuk merawat *Salmonella spp*. Kajian ini bertujuan (1) untuk mendapatkan dan mengenal pasti *Salmonella spp*. daripada ikan Parrot Merah yang berpenyakit dan (2) untuk mengelaskan antibiogram *Salmonella spp*. yang didapati. Sebanyak 30 sampel telah diambil daripada ikan Parrot Merah yang tidak sihat yang diambil daripada empat bahagian ikan yang berbeza iaitu perut, mata, insang, buah pinggang dan kulit. Kaedah "spread plate" digunakan untuk mendapatkan *Salmonella spp* manakala pengenalpastian *Salmonella spp*. menggunakan kit komersial BBL Crystal. Bakteria yang didapati telah menjalani ujian kerintangan antibiotik menggunakan kaedah resapan cakera. Sebanyak 16 antibiotik digunakan iaitu Asid Nalidixic, Oxolonic Acid, Compound Sulphonamids, Doxycycline, Tetracycline, Novobiocin, Chloramphenicol, Kanamysin, Sulphamethoxazole, Flumequine, Erythromycin, Ampicillin, Spiramycin, Oxytetracycline, Fosfomycin. Keputusan ujian antimikrob diklasifikasikan sebagai sensitif, sederhana sensitif atau kebal. Indeks pelbagai antibiotik (MAR) ditentukan berdasarkan keputusan sensitiviti antibiotik. Asid oksolonik telah ditunjukkan sebagai yang paling berkesan dalam menyekat bakteria, dengan sensitiviti 83.3%. Antibiotik yang paling responsif ialah doxycycline (73.33%) dan erythromycin (60%). Sebaliknya, semua bakteria Salmonella yang didapati adalah tahan ampicillin.

Kata Kunci: Ikan hiasan, antibiogram, *Salmonella spp*, Blood-Red Parrot Cichlid fish

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		Page
%	Percentage	16
°C	Degree Celsius	7
µg	Microgram	27
mm	Millimetre	28
>	More than	28
>	Less than	28

LIST OF ABBREVIATIONS

		Page
XLD	xylose lysine desoxycholate	13
MAR	Multiple Antibiotic Resistance	12

LIST OF EQUATIONS

CHAPTER 1

INTRODUCTION

1.1 Research Background

Ornamental fish are often imported exclusively from the United States, with the European Union constituting the largest market for ornamental fish. The ornamental fish trade is estimated to be worth approximately USD 278 million globally (FAO, 2005), while the aquarium industry is predicted to be worth more than USD 1,000 million (Cato & Brown, 2003). In the 1950s, ornamental fishes gained popularity in Malaysia after recovering from the wreckage of World War II. Entrepreneurs in Johor began raising species found in peat bogs and marshes, and soon the commerce reached other countries. There is RM353 million worth of Johor fish dealers since the state got out early. A wide range of ailments can be contracted by ornamental fish (Department of Fisheries, 2016). In this case, the cost of testing and managing the fish outweighs its monetary worth. Because they are small and the expense of replacing ill or dead fish is low, this is a good choice for aquariums. Infectious diseases frequently affect the majority, if not all, of the fish in an aquarium because of the little volume of water and high concentration of fish in most tanks.

Ornamental fish in aquariums can be harmed or stressed by a variety of factors, including inadequate aquarium plants, a dysfunctional nitrogen cycle, and potentially harmful freshwater invertebrates. Many fish illnesses can be avoided or prevented if proper water conditions and a well-balanced aquarium atmosphere are maintained.

Gram-negative bacillus *Salmonella spp*. belongs to the Enterobacteriaceae family and is non-sporulated. One isolate was found in aquarium water imported from Asia by Shotts et al. To reduce the danger of bacterial infection among aquatic animals, antibacterial chemicals may be introduced in shipping waters that convey ornamental fish (Newaz et al., 2006). Increased usage of antimicrobial medications without regard for efficacy may result in selection pressure, resulting in the development of drug-resistant microorganisms (Newaz et al., 2006). *Salmonella spp.* were isolated from diseased Blood-Red Parrot fish using a spread plate streaked over xylose lysine desoxycholate (XLD) agar in the current study (Oxoid, Basingstoke U.K). The commercial BBL Crystal kit is then used to identify *Salmonella spp*. The disc diffusion method was used in the antibiogram test to assess *Salmonella spp*. exposure to the following antibiotics: Nalidixic Acid, Oxolonic Acid, Compound Sulphonamides, Doxycycline, Tetracycline, Novobiocin, Chloramphenicol, Kanamycin, Sulphamethoxazole, Flumequine, Erythromycin, Ampicillin, Spiramycin, Oxytetracycline, Amoxycillin. As a result, an appropriate antibiotic for future use were identified.

1.2 Problem Statement

When antimicrobial compounds are employed indiscriminately, they have the potential to create a selection pressure that exposes microorganisms that are resistant to antimicrobial treatments. Numerous studies have shown resistance to *Salmonella spp.* in ornamental fish water. It is possible that antibiotic-resistant R-plasmids found in ornamental fish could have serious consequences for human health, as they've been found in resistant strains of bacteria. In this investigation, antibiotics for *Salmonella spp.* infection is identified. As a result, eradicating *Salmonella spp.* from aquariums can help keep much sick ornamental fish off the market.

1.3 Significance of Study

According to the findings of research investigations, there are *Salmonella spp.* that are resistant to antibiotic in the aquatic environment in which ornamental fish are kept. As a result, it is critical in this research that *Salmonella spp.* be isolated and classified from diseased Blood-Red Parrotfish. This study investigated the antibiogram of *Salmonella spp.* According to the findings, a more appropriate antibiotic for the treatment of Salmonella spp. infectious disease was identified and shall be used in the future. Also feasible is that the highly successful antimicrobial agent was able to be used in medical applications in the future.

1.4 Research Objectives

i) To isolate and identify *Salmonella spp*. from diseased Blood-Red Parrot Cichlid fish.
ii) To characterize antibiogram of isolated *Salmonella spp* using BBL Crystal kit.

1.5 Scope of Study

The scope of this study is microbiology.

1.6 Limitation of the study

The limitation of this study is the movement control order (MCO) that was announced due to the COVID-19 pandemic has interfered with the progress in the middle of this study. This leads to limited access to the laboratory because of the standard operating procedure (SOP) that we need to follow. Additionally, the absence of a relevant database on the connected current study would affect the outcome. Few research on the antibiogram of Salmonella spp. isolated from unwell ornamental fishes have been undertaken. As a result, additional studies on antibiogram testing can be undertaken to aid in prophylaxis and prevention, as well as increasing awareness of the condition among ornamental fish growers.

CHAPTER 2

LITERATURE REVIEW

2.1 The Ornamental Fish Industry in Malaysia

An estimated 325 million pieces of ornamental fish have been produced over the past few years, with a total worth of RM350 million (Department of Fisheries, 2016). The Department of Fisheries reports that Malaysia is the eighth greatest producer of ornamental fish in the world, with more than 70% of the ornamental species produced being sold to foreign countries. There are now 259 exporters of ornamental fish from Malaysia (Department of Fisheries, 2016). The world's second-largest exporter, Malaysia, accounts for 9% of global trade.

According to Chiew et al. (2019), as an expanding sector, present procedures have resulted in disease outbreaks, which have been identified as one of the most serious problems encountered by aquaculture farms. Several bacterial and viral infections have been discovered to persist in Malaysian farms during 20 years of disease reporting. Aside from that, numerous farms have been found to be infected with new worldwide diseases. These disease epidemics cost a great deal of money. Finally, the aquaculture industry faces a serious threat from a combination of long-standing and new infections.

2.2 Ornamental Fish

Ornamental fish are tranquil, colourful fish kept as pets in enclosed environments like aquariums or garden pools for the sole purpose of admiring their aesthetic appeal. They're also called "living diamonds" because of their vibrant colours and playful natures. An attractive aquarium was first enjoyed by people from the Far East (Chinese and Japanese), Egypt, and Rome (Swann, 1992). Early aquarists not only collected fish from the wild but also learned how to breed them and create unique hybrids. Attractive colour and patterning are important in the ornamental fish trade, as is a non-aggressive nature that lets them live with other species. Popularity can be boosted by fish with interesting names. Live-bearers include guppies, mollies, platies, and swordtails. Everything else in the aquarium is an egg-layer, with barbs, tetras, gouramis, danios, and various cichlids being the most common. Outdoor swimming pools are used nearly exclusively to raise livebearers.

According to Grether (2000), ornamental fish in the wild rely on body design and coloration to attract partners and conceal from predators. It is possible that fish may not be able to produce their colour pigments. Natural colours are found in their food chain. Species can feed on insects, flowers, and fruits that flourish in their habitats. Colorants are added to the fish's diet in commercial fish farms to alter the animal's look. More unique tactics and trade secrets are hidden in this sector than can be listed here. Primitive and ineffective fishing gear is used by traditional subsistence fishermen, according to

Chao (2001). Overfishing has been detected in several areas where aquarium fish have been harvested.

Scientists have discovered that 90 % of freshwater ornamentals may be obtained through cultivation, with the remaining 10 percent deriving from naturally occurring sources. It is common to practice raising ornamental fish on a bigger scale in locations like Israel and the Far East, where mortality and illness are major problems for the industry. Approximately 95 % of marine species are harvested from the wild, with only 5 % of marine species being grown in captivity (Oliver, 2001, 2003; Sea Shepherd, 2014). Because of the potential for habitat damage, conservation efforts for tropical marine fish are put in danger.

2.2.1 Blood Parrot Cichlid Fish

Figure 2.1: Blood Red parrot Cichlid Fish (Source: The Aquarium Guide, 2021)

When it comes to breeding fish, breeders believe that they have come up with something new, but they cannot say for sure which parent species is responsible for the creation of the blood parrot or the Midas or Gold Severum. Blood Parrot Cichlids are gorgeous, albeit controversial, freshwater fish that can be found in rivers and lakes. These fish cannot be found in the wild because they are endangered. Instead, they are a hybrid fish species that was originally discovered in Taiwan in 1986 and has since spread throughout the world (Yang et al., 2021). Because the blood parrot is a hybrid cichlid with a variety of anatomical defects, there is some debate about the ethics of creating this creature. One of its deformities is that it has a tiny vertical hole at the back of its mouth. In turn, blood parrots are more difficult to feed and may be more susceptible to malnutrition as a result. For a healthy Blood Parrot Cichlid, the average lifespan is 10 to 15 years.

2.3 Introduction to *Salmonella spp.*

According to Santana (2012), *salmonella* was not found in fish for the first time, but it was introduced by contaminated water or unsafe handling of soil and water, the bacteria can be spread to fish. When it comes to the type of illness as well as the anticipated route of propagation, this information is crucial. It allows for traceability of the source of this bacterium in the fish market (Setti et al., 2009).

According to a study of Brazil (2011), *Salmonella* is an Enterobacteriaceae family non-sporulated gram-negative bacillus . In the current *Salmonella* categorization scheme, two primary species of *Salmonella* are being considered: *Salmonella bongori* and *Salmonella enterica* (Dekker & Frank, 2015). It is more appropriate for some serogroups to specific animals than others. *S. enterica Typhi, S. enterica,* and *S. Paratyphi*, for example, are pathogens that only affect humans and cause respectively typhoid and paratyphoid fever (Holt et al., 2009).

Salmonella, according to Jarvis et al., (2016) is a mobile, facultatively anaerobic, oxidase-negative bacteria that generates gas from glucose in its environment . It can grow at temperatures ranging from 7°C to 46°C. The minimum water activity necessary for growth is 0.84 at a maximum salt concentration of 4%. Fish guts, flesh, and gills, as well as muscles, have all been proven to be contaminated with *Salmonella.* (Nwiyi & Onyearbor, 2012). It is easier to spread *Salmonella* during processing because of the presence of *Salmonella* on fish's surface and internal organs (Heinitz et al., 2000).

2.4 Isolation and Identification of *Salmonella spp.*

According to Cetinkaya (2008), *Salmonella spp*. multi-drug resistance in food has become another serious issue in recent years. The goal of this study was to determine the frequency of *Salmonella spp*. in curries and the antibiotic sensitivity of the *Salmonella* isolates. A sterile swab was used to inoculate each sample onto *Salmonella*-Shigella (SS) agar before the samples were appropriately mixed and incubated for 48 hours at 35°C. The urease test and the triple-sugar-iron (TSI) agar test were both employed to confirm the presence of Salmonella. *Salmonella* was found in only seven of fifty curry samples. This study also shows that *Salmonella* strains differ in their ability to resist antibiotics.

Salmonella infections in ducks, according to Kumar et al. (1988), in ducks are a major cause of clinical disease as well as a source of foodborne disease transmission to people. Duck mortality and morbidity, as well as poorer egg and meat output, have a significant economic impact on the poultry industry. T. Mondal (2008) revealed that each cloacal swab was contaminated with selenite broth that had just been made. After that, the tubes were correctly labelled and incubated aerobically at 37 ° C for 24 hours in a bacteriological incubator. Bacterial growth was then seen in the tubes that had been incubated. When gram-negative rods were detected in the smears, the materials from the tube matching the smears were streaked into three different agars: MacConkey agar, SS agar, and bright green agar. SS agar was used to subculture the likely *Salmonell*a plates, which allowed for the establishment of a pure culture.

When it comes to *Salmonella* detection in an ambient water sample, Kuo (2013) discovered that it takes time to identify the bacteria using conventional culture methods and selective media, as well as to characterize questionable colonies using biochemical tests and serological assays. The analysis employed membrane filtration, non-selective pre-enrichment, and selective enrichment of *Salmonella*. Using selective culture plates, they isolated *Salmonella* strains. Finally, biochemical testing and serological assays were used to confirm the *Salmonella* serovars. In this study, PCR was utilized to identify *Salmonella* in 15 water samples. A culture procedure were used to determine the serotypes of the positive water samples. It has been demonstrated that waterborne transmission can occur in the drinking watershed by the presence of *Salmonella spp*. in ambient water.

2.5 Antibiogram of *Salmonella spp.*

Increased usage of antimicrobial medications in aquaculture has increased the number of fish and crustaceans that are available for consumption around the world. The potential of pathogen resistance spreading to humans is also a concern for consumers (Amagliani & Schiavano 2012). Sulfonamide compounds, ampicillin, nalidixic acid, and tetracycline (49.1%) are not effective against *Moroccan Salmonella* isolates. Six isolates were found to be resistant to two antibacterial drugs (Setti et al., 2009). For freshwater fish farming in Brazil, there have been limited studies on the prevalence of bacterial resistance. These investigations discovered microbial resistance in a variety of environments, including aquaculture operations, and demonstrated that resistance was transmitted across organisms.

According to M. Boaventura (2006), shrimp produced in both saltwater and freshwater is becoming increasingly susceptible to bacterial infection because of the ease with which diseases are transferred in aquaculture. This has resulted in the spread of antimicrobial-resistant bacteria in aquatic settings because farmers have been using antibiotics inappropriately. A study by S Carvalho (2013) examined the presence of

Salmonella spp. and antibiotic tolerance among shrimp farming communities in Northeast Brazil. In water and sediment samples collected from two farms where the freshwater-acclimated *Litopenaeus vannamei* was being farmed, *Salmonella* was identified. *Salmonella spp.* was found in 30 out of 186 isolates, with 5 serovars. A total of 23% of the isolates tested positive for at least one antibiotic, and 20% tested positive for two or more. Resistance to ampicillin, oxytetracycline, nitrofurantoin, and tetracycline was discovered in three bacteria recovered from water samples. One of them carried a plasmid containing genes that gave resistance to nitrofurantoin and ampicillin, and the other did not. In this study, the presence of harmful bacteria in shrimp aquaculture and their drug-resistance patterns emphasized the need for more research in this area.

CHAPTER 3

METHODOLOGY

3.1 Materials and Apparatus

3.1.1 Sample, Chemical and Reagents

This study used an unhealthy Blood-Red Parrot Cichlid Fish, sterile distilled water, ethyl3-aminobenzoate methanesulfonate (MS-222), xylose lysine desoxycholate (XLD) agar (Oxoid, Basingstoke U.K), Tryptone Soy Broth, Nalidixic Acid, Oxolonic Acid, Compound Sulphonamides, Doxycycline, Tetracycline, Ampicillin, Novobiocin, Chloramphenicol, Kanamycin, Sulphamethoxazole, Erythromycin, Flumequine, Oxytetracycline, Spiramycin, Fosfomycin, Amoxycillin, antibiotics disc, and BBL Crystal kit.

3.1.2 Apparatus and Equipment

This study used sterile plastic bags, a loop of inoculation, sterile swab, incubator (Jeiotech, Korea) spatula, filter papers (Smith, UK), scalpel (Albion, UK), Petri dishes, forceps, incubator, laboratory film (Parafilm M, USA).

3.2 Procedures

3.2.1 Preparation and Sample Collection

One diseased Blood-Red Parrot Cichlid Fish from the aquarium shop was used in this study. The fish showing at least one clinical sign of disease (hemorrhages, skin ulcer, fin rot, loss of appetite, or abdominal distension) were collected from an aquarium shop located at Kelantan, Malaysia. The fish was deoxygenated before being placed in sterile plastic bags filled with water from the same aquarium. The sample was then transported into a cooler within 1 hour. The fish was killed humanely using an overdose of ethyl3-aminobenzoate methanesulfonate (MS-222).

3.2.2 Isolation and Identification of *Salmonella Spp.*

3.2.2.1. Isolation of *Salmonella spp.*

A total of 30 samples from a fish were collected from one diseased Blood-Red Parrot Fish that consists of a different area of the swab which is the abdomen (A), eye (E), kidney (k), skin (S), and gills (G) using sterile swab then streaked on xylose lysine desoxycholate (XLD) agar for determination of the presence of *Salmonella spp*. After that, the plates were incubated at 35 °C for 48 h. Unless a homogenous cell culture was established after at least two subcultures, the strains were considered to be complete.

3.2.2.2. Typical Single Colonies Selection and Inoculation into Tryptone Soy Broth (TSB)

In each colony, Tryptone Soy Broth (TSB) was injected and vortexed for a few seconds before being incubated at 27°C for 24 hours (Jeiotech, Korea).

3.2.2.3. Identification of bacteria using BBL Crystal kit

The inoculum fluid was put into the Tryptone Soy Broth (TSB) base of the target location and allowed to fill the well. After being snacked into place, the lid was replaced. Using the colour response map, the kit's colour changed after 24 hours of incubation in an incubator at 27°C. The data was generated by manually entering the codes into the manufacturer's application.

3.2.3 Antibiogram Susceptibility Test

The agar diffusion method was used to determine antibiotic sensitivity (Kirby-Bauer Test). This study employed the following antimicrobial susceptibility discs (Biolab Inc., Budapest, Hungary) to evaluate the antibiotic sensitivity of isolated bacteria: Nalidixic Acid (30µg), Compound Sulphonamides (300µg), Oxolonic Acid (2µg), Kanamycin (30mcg), Doxycycline (30µg), Amoxycillin (25µg), Tetracycline (30µg), Novobiocin (30µg), Ampicillin (10µg), Chloramphenicol (30µg), Sulphamethoxazole (25µg), Flumequine (30µg), Oxytetracycline (30µg), Erythromycin (15µg), Fosfomycin (50µg), Spiramycin (100µg). To ensure that selected bacteria were inoculated onto Mueller-Hinton agar dishes in three distinct directions, the plates had to be rotated at 60° angles after each streaking procedure was completed. The streaking was carried out in three distinct directions throughout the entire agar surface. Antimicrobial discs were taken from the cartridge and placed on the test plates using sterile forceps after drying the Petri dishes at room temperature for 15 to 20 min.

According to Clinical and Laboratory Standards Institute (CLSI) guidelines for evaluating isolated bacteria, the generated zones were evaluated in terms of the minimum inhibitory diameter after overnight incubation at 22 °C. The zone of inhibition was classified as resistant (< 15mm), intermediate sensitive (16-17mm), and sensitive (>18mm). Antibiotics restrict microbial growth in a specific area, known as the inhibition zone.

The MAR index is used to determine if bacteria isolated are from a high- or low-antibiotic-use location, which is connected with the assessment of health risk. The percentage of antibiotics to which an organism is resistantly divided by the total antibiotics exposed determines a MAR index. (Mthembu, 2008). (Krumperman, 1983). In places where antibiotics are commonly used, MAR index values more than 0.2 suggest a significant risk of infection. The following formula will be used to determine the MAR index (Nandi, Mandal, I M, et al., 2016):

$$MAR = \frac{Amount\ of\ antibiotics\ that\ showed\ resistance}{Amount\ of\ total\ antibiotics\ used}$$

CHAPTER 4

RESULT AND DISCUSSION

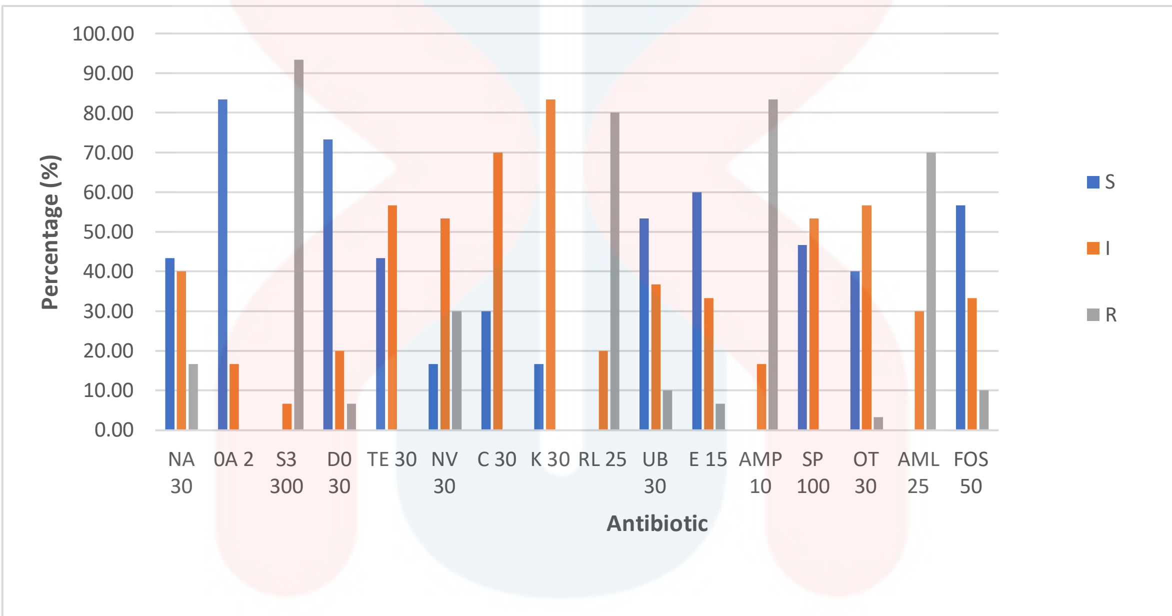
4.1 Results

In this present study, a total of 30 samples from a fish were collected from one diseased Blood-Red Parrot Fish that consists of a different area of the swab which is the abdomen (A), eye (E), kidney (k), skin (S), and gills (G). From the table, there are 16 types of antimicrobial agents used to evaluate the antibiotic sensitivity of isolated bacteria: Nalidixic Acid (30 mcg), Oxolonic Acid (2 mcg), Compound Sulphonamides (300 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), Novobiocin (30 mcg), Chloramphenicol (30 mcg), Kanamycin (30mcg), Sulphamethoxazole (25 mcg), Flumequine (30 mcg), Erythromycin (15 mcg), Ampicillin (10 mcg), Spiramycin (100 mcg), Oxytetracycline (30 mcg), Amoxycillin (25 mcg), Fosfomycin (50 mcg). The zone of inhibition was classified as resistant (< 15mm), intermediate sensitive (16-17mm), and sensitive (> 18mm)

Table 4.1: Antibiotic resistance of *Salmonella spp.* of diseased Blood-Red Parrot Fish.

Types of antimicrobials Agent (mcg/disk)		**Bacteria isolated (*Salmonella spp.*)**		
		Sensitive (%)	**Immediate sensitive (%)**	**Resistance (%)**
Nalidixic Acid	NA 30	43.33	40	16.67
Oxolonic Acid	OA 2	83.33	16.67	-
Compound Sulphonamides	S3 300	-	6.67	93.33
Doxycycline	DO 30	73.33	20	6.67
Tetracycline	TE 30	43.33	56.67	-
Novobiocin	NV 30	16.67	53.33	30
Chloramphenicol	C 30	30	70	-
Kanamycin	K 30	16.67	83.33	-
Sulphamethoxazole	RL 25	-	20	80
Flumequine	UB 30	53.33	36.67	10
Erythromycin	E 15	60	33.33	6.67
Ampicillin	AMP 10	-	16.67	83.33
Spiramycin	SP 100	46.67	53.33	-
Oxytetracycline	OT 30	40	56.67	3.33
Amoxycillin	AML 25	-	30	70
Fosfomycin	FOS 50	56.67	33.33	10

Based on table 4.1, *Salmonella* antibiotic sensitivity testing found that thirty (100%) of the thirty *Salmonella* isolates tested were resistant to at least one and possibly all of the 16 antimicrobial agents examined. Resistance was high to S3 (93.33%), RL (80%), AMP (83.33%), AML (70%), but low to NA (16.67%), DO (6.67%), NV (30%), UB (10%), E (6.67%), OT (3.33%), FOS (10%) and no resistance (0%) for OA, TE, C, K, SP. Sensitive was high to OA (83.33%), DO (73.33%),E (60%) but low to UB (53.33%), NA (43.33%), TE (43.33%), NV (16.67), C (30%), K (30%), SP (46.67%), OT (40%) and no sensitive to S3, RL, AMP, AML. While immediate sensitive was highly to C (70%), K (83.33%).



S = Sensitive, I = Immediate Sensitive, R = Resistance

Figure 4.1: Graph of the percentage of antibiotic resistance of *Salmonella spp.* of diseased Blood-Red Parrot Fish

According to the figure 4.1, the overall percentage of antibiotic susceptibility test, 25% was recorded as antibiotic resistance, 37.5% was recorded as immediate resistance and sensitive case. Compound Sulphonamides, Sulphamethoxazole, and Ampicillin have the highest resistance to *Salmonella* which is not suitable to use as an antimicrobial agent while Oxolonic Acid, Doxycycline, and Erythromycin have the highest sensitivity which is suitable to use for antimicrobial agents. The MAR value of the present study was 0.69.

4. 2 Discussion

Ornamental fish are a unique type of pet that may be found all around the world. Aquaria holding ornamental fish can be found in a variety of settings, including hospitals, schools, and even restaurants, in addition to private residences. A certain amount of contact with either the fish or the water is always there, whether it is due to children and adults wanting to touch or catch the fish owing to their curiosity with the fish or contact that occurs as a result of cleaning the aquariums. Therefore, human interaction with *Salmonella*-positive fish and pond water could serve as a possible source of the infection to people, potentially posing a health risk to those infected. Antibiotics are routinely used in animal husbandry and aquaculture for treatment and growth promotion, resulting in the development of antibiotic resistance among environmental species (Serrano, 2005). Since the fast rise of the ornamental aquaculture business in recent decades, the use of pharmaceuticals to prevent infectious diseases has increased as a result of the accompanying increase in the usage of these drugs. Srum et al., (1993) discovered that repeated antibiotic usage increases the emergence of bacteria resistant to antibiotics and may potentially result in bacterial species extinction.

Budiati et al. (2013) showed in their study that *Salmonella* isolates have developed increased resistance to antibiotics, particularly chloramphenicol and tetracycline. Antibiotic usage in fish farming and poultry farming has been linked to the development of acquired resistance to tetracycline and chloramphenicol in various Asian nations (Mohamed et al., 2000). Oxytetracycline and similar antibiotics are classified as broad-spectrum antibiotics, which are antibiotics that have a wide range of activity (meaning they are effective against a large range of bacteria). They perform well when coupled with food and are deemed safe. Bath treatments, on the other hand, may not be as helpful for all species (Yanong, 2003). Among the top 13 countries producing aquaculture, according to Sapkota et al. (2008), excluding Egypt and North Korea, oxytetracycline was utilized in 92% of the cases, and chloramphenicol in 69% of the cases. However, Oxytetracycline (56.67%), tetracycline (56.67%), and chloramphenicol (70%) were recorded as immediate sensitive in the present study. From the study conducted by (Wei & Wee, 2011), among the bacterial isolates tested, more than 70% were found to be susceptible to nitrofurantoin, tetracycline, and oxolinic acid, among other antibiotics. A brownish colour results from the decomposition of tetracyclines since they are light-sensitive. This further degrades the water's quality and poses a risk to the fish's well-being. It is necessary to replace the water as soon as possible after the bath treatment session has ended. As a result of years of improper use, various microorganisms in a variety of settings have developed resistance to tetracyclines (Yanong, 2003).

According to the findings from Yanon (2003), oxolonic acid was shown to be the most efficient antibacterial agent in controlling current bacterial isolates, with 85.3% of the current bacterial being sensitive to it. Oxolonic acid is an antibacterial medicine that is difficult to come by. It is referred to as a "last chance treatment" because it frequently produces positive results after other, more usual treatments have failed. This study is equivalent to the result of the present study. In the present study, oxolonic acid has the highest level of sensitivity which showed that it is the most suitable antibacterial agent that can be used.

According to Gunasegaran et al. (2011), an examination of the drug sensitivity of *Salmonella* isolates to commercial antibiotics revealed that all (100%) of the isolates were resistant to ampicillin, tetracycline, and chloramphenicol. Susceptibility to kanamycin was determined to be 100% for all isolates. *Salmonella* in the present study also showed the same result as the finding of Gunasegaran et al. (2011), high resistance to ampicillin (83.33%) however there was no resistance observed for kanamycin. It is possible that kanamycin, when used in bath treatments, will be useful against external infections. It is also claimed that the antibiotic kanamycin is useful when added with feed to treat gastroenteritis (gastrointestinal bacterial infections).

Salmonella isolates were reported to be the least susceptible to nalidixic acid and ampicillin in 2005 and 2010 (Mijovi, 2012). This was true for all antibiotics examined. *Salmonella* isolates had the second-lowest level of ampicillin susceptibility (91.9 percent) in 2005 and the second-lowest level of nalidixic acid susceptibility (95.7 percent) in 2010. When comparing 2010 to 2005, the researchers discovered a statistically significant increase in susceptibility to nalidixic acid from 95.7% to 73.1%. However, *Salmonella* in the present study showed high resistance to ampicillin (83.33%) and Amoxycillin (70%). Due to their ineffectiveness against gram-positive bacteria such as *Streptococcus spp*., penicillin's including penicillin, Amoxycillin, and ampicillin are not recommended as first-line treatments for the majority of fish bacterial infections (Yanong, 2003). While nalidixic acid (43.33%) showed a high percentage of sensitivity by *Salmonella* which is suitable to use as an antimicrobial agent.

There are two key issues to address when it comes to antibiotic resistance: misuse of antibiotics for fish sickness treatment and human exposure to antibiotic-resistant microorganisms in the environment are both contributing factors to antibiotic resistance (Akinbowale et al., 2006). To stimulate animal growth and treat animal infections in big, confined animal feeding operations (CAFOs) around the world, antibiotics are routinely administered to the animals. For example, up to 75 percent of antibiotics are excreted unmetabolized through the animal's faeces and urine, allowing the medicines to remain in the environment in an unmetabolized state for long periods (Kumar et al., 2005). The

MAR index values less than or equal to 0.2 are regarded to suggest that antibiotics were used only infrequently, if at all, in the samples examined. The presence of a MAR index value of more than 0.2 is regarded to show that the contamination came from sources that have a high risk in which antibiotics are frequently utilized. From the present study, the MAR value was 0.69 which showed that the fish from the aquarium shop were also contaminated by the bacteria and the antimicrobials were frequently utilized as antibiotics or growing agents in animal feed (Krumperman, 1983; Singh et al., 2010).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

In conclusion, the results of the antibiogram exposed that all the *Salmonella* were susceptible to Oxolonic Acid, Doxycycline, and Erythromycin and resistant to Compound Sulphonamides, Sulphamethoxazole, and Ampicillin. According to the findings, Oxolinic acid was shown to be the most efficient antibacterial agent in controlling current bacterial isolates. With adequate care, many bacterial illnesses in ornamental fish can be avoided. All stressors must be eliminated or minimized if a population becomes afflicted. Consult a fish health specialist for help with diagnosis, culture, and sensitivity testing, as well as the most accurate dosage and treatment interval recommendations. Quality of the aquaculture environment, as well as microbial contamination of fish, are linked. Antibiotic resistance can limit the therapeutic options available to doctors in clinical situations where therapy with an antibiotic is necessary. Any antibiotic misuse can lead to the development of antibiotic-resistant microorganisms in a healthcare facility. Rotating antibiotics every few months or a year is one way some farms try to avoid this. As a result, the best course of action would be to undertake tests to confirm the bacteria's identity and prevent any therapies that would be ineffective, costly, or even hazardous. the continuation of active surveillance for salmonella-resistant strains at the global, regional, and intersectoral levels of surveillance is critical for the prevention of outbreaks.

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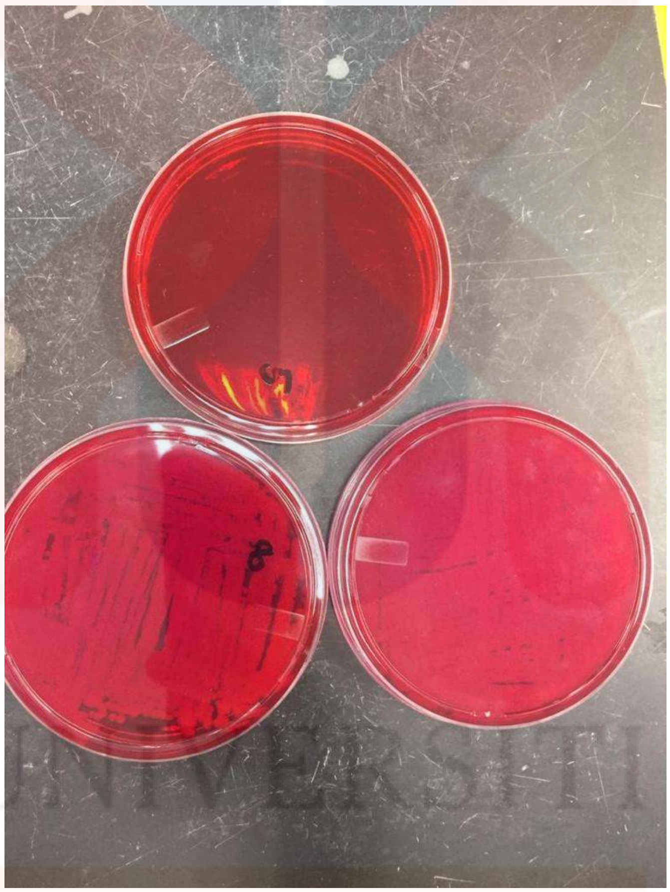
APPENDIX A

SALMONELLA SPP. BY STREAKED ON XYLOSE LYSINE DESOXYCHOLATE (XLD) AGAR



APPENDIX B

IDENTIFICATION OF *SALMONELLA SPP.* USING BBL CRYSTAL KIT

APPENDIX C

SAMPLE COLLECTION AND PREPARATION