UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILE OF BACTERIA FROM CATFISH, *Clarias* spp. OBTAINED FROM SELECTED MARKETS IN SELANGOR

ILIA IRYANI BINTI ROSMAN

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IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILE

OF BACTERIA FROM CATFISH, *Clarias* spp.

OBTAINED FROM SELECTED MARKETS IN SELANGOR

ILIA IRYANI BINTI ROSMAN

A project paper submitted to the

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In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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MARCH 2017

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CERTIFICATION

It is hereby certified that we have read this project paper entitle “Identification and Antibiotic Sensitivity Profile of bacteria from Catfish, *Clarias* spp obtained from selected markets in Selangor”, by Ilia Iryani Binti Rosman and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4901 – Project.

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DEDICATION

This project paper is specially dedicated to

My mom, Firza Hussain

A strong and gentle soul who taught me to trust Allah, be strong, and independent.

My dad, Rosman Abdullah Sani,

For earning an honest living for us and encouraging me to believe in myself.

My sisters and brothers,

Azra Aina, Qistina Qaisara, Azrif Aqil & Azril Aqif

For always being supportive through my ups and down in completing this project.

My beloved cats,

Milky Blue & Mocha. I love you.

And to all my teachers who have committed themselves towards the noble cause of education.

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In the name of Allah, The Most Gracious, the Most Merciful.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Tahun Akhir.

IDENTIFIKASI DAN KERENTANAN PROFIL ANTIBIOTIK TERHADAP BAKTERIA YANG DIASINGKAN DARIPADA IKAN KELI, *Clarias* spp.

Oleh

Ilia Iryani Binti Rosman

2017

Penyelia: Profesor Madya Dr Zunita Binti Zakaria

Penyelia Bersama: Dato' Dr Mohamed Shariff Bin Mohamed Din

Clarias sp. juga dikenali sebagai ikan keli adalah ikan air tawar yang sangat popular di Malaysia. Ikan keli liar sering ditemui di dalam air berlumpur atau berpaya yang mempunyai kekeruhan yang tinggi. Untuk kegunaan manusia, spesis ikan keli biasanya diternak dalam kolam dan sering tercemar oleh pelbagai mikroorganisma kerana cara ikan keli diternak serta faktor alam sekitar. Oleh itu, ikan ini mempunyai potensi untuk membawa patogen dan boleh menjadi tidak selamat untuk kegunaan manusia sekiranya ikan ini tidak dimasak dengan betul. Objektif kajian ini dijalankan adalah untuk mengasing dan mengenalpasti jenis bakteria yang dijumpai dalam ikan keli, *Clarias* spp. serta menentukan corak kerentanan bakteria terhadap antibiotik yang terdapat dalam ikan keli, *Clarias* spp.

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Sejumlah dua puluh empat sampel ikan keli, *Clarias* spp. telah diperoleh dari 8 pasar terpilih di sekitar kawasan Selangor untuk tempoh dua minggu. Insang dan usus telah diproses untuk pengasingan dan pengenalpastian bakteria. Kerentanan bakteria juga telah ditentukan terhadap antibiotik yang biasa digunakan dalam akuakultur. Dua puluh Gram negatif bakteria (41.67%) dan tujuh Gram positif bakteria (14.58%) telah berjaya diperoleh daripada sampel. Gram negatif bakteria yang diasingkan adalah *Aeromonas* species (37.5%), *Vibrio cholarae* (31.25%), *Escherichia coli* (16.66%), *Klebsiella pneumonia* (12.5%), *Vibrio parahaemolyticus* (10.41%), *Proteus vulgaris* (10.41%), *Pantoea agglomerans* (10.41%), *Proteus mirablis* (10.41%), *Enterobacter aerogenes* (6.25%), *Photobacterium damsela* (6.25%), *Edwardsiella Tarda* (6.25%), *Shigelloides Pleisiomonas* (6.25%), *Citrobacter freundii* (6.25%), *Salmonella* species (2.08%), *Vibrio alginolyticus* (2.08%), *Chromobacterium* (2.08%), *Achromobacter* (2.08%), *Shewanella putrefaction* (2.08%) dan *Alcaligenes fecalis* (2.08%). Manakala, Gram positif bakteria pula adalah *Staphylococcus* species (6.25%), *Streptococcus viridans* species (4.16%), *Actinomyces* species (4.16%), *Staphylococcus hyicus* (2.08%), *Corynebacterium* species (2.08%), *Corynebacterium kutcheri (*2.08%), dan *Listeria monocytogenes* (2.08%). Tambahan pula, 4 spesis Gram negatif bakteria dan 1 spesis Gram positif bakteria yang rentan terhadap antimikrob pelbagai telah dikenalpasti di dalam ujian ini. Kesimpulannya, kajian ini menunjukkan bahawa ikan keli mempuyai daya kerentanan terhadap pelbagai antibiotik yang boleh membawa risiko kepada pengguna.

Kata kunci: *Akuakultur, Ikan Keli, Insang, Usus, Kerentanan, Antibiotik, Pengguna.*

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Final Year Project.

IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILE OF BACTERIA ISOLATED FROM CATFISH, *Clarias* spp.

by

Ilia Iryani Binti Rosman

2017

Supervisor: Associate Professor Dr Zunita Binti Zakaria

Co- Supervisor: Dato' Dr Mohamed Shariff Bin Mohamed Din

Clarias sp. also known as catfish is a very popular freshwater fish in Malaysia. Wild catfish are most commonly encountered in muddy or swampy water of high turbidity. For human consumption, catfish species are normally reared in ponds and often get contaminated by a variety of microorganism because of the way they are being reared and the environment. Thus, these fish are highly likely to carry pathogens and can be unsafe for human consumption if the fish is not cooked well. This study was conducted to isolate and identify the types of bacteria found in catfish, *Clarias* spp. and to determine the antibiotic susceptibility pattern of the common bacteria found in the fish, *Clarias* spp.

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Twenty-four *Clarias* spp. samples were purchased from 8 selected markets around the areas of Selangor for a period of two weeks. The gills and intestines were processed for isolation and identification of bacteria. The antibiotic susceptibility of the obtained isolates was also determined against the commonly used antibiotics in aquaculture. A total of twenty Gram negative (41.67%) and seven Gram positive bacteria (14.58%) were isolated from the samples. The gram negative bacteria isolated were *Aeromonas* species (37.5%), *Vibrio cholarae* (31.25%), *Escherichia coli* (16.66%), *Klebsiella pneumonia* (12.5%), *Vibrio parahemolyticus* (10.41%), *Proteus vulgaris* (10.41%), *Pantoea agglomerans* (10.41%), *Proteus mirabilis* (10.41%), *Enterobacter aerogenes* (6.25%), *Photobacterium damsela* (6.25%), *Edwardsiella tarda* (6.25%), *Pleisiomonas shigelloides* (6.25%), *Citrobacter freundii* (6.25%), *Salmonella* species (2.08%), *Vibrio alginolyticus* (2.08%), *Chromobacterium* (2.08%), *Achromobacter* (2.08%), *Shewanella putrefaction* (2.08%) and *Alcaligenes fecalis* (2.08%). While, the Gram positive bacteria species isolated were *Staphylococcus* species (6.25%), *Streptococcus viridans* species (4.16%), *Actinomyces* species (4.16%), *Staphylococcus hyicus* (2.08%), *Corynebacterium* species (2.08%), *Corynebacterium kutcheri* (2.08%), and *Listeria monocytogenes* (2.08%). Multidrug resistant traits were presented for 4 Gram negative bacteria species and 1 Gram positive bacteria species. As a conclusion, this study suggested that the catfishes harbours pathogens that exhibited multidrug resistant trait which may bring risk to the consumers.

Keywords: *Aquaculture, Catfish, Gill, Intestine, Antibiotic sensitivity test, Consumer*

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1.0 INTRODUCTION

Clarias sp belongs to the Phylum of Chordata, Class of Actinopterygii, Family of Clariidae, Order of Siluriformes, and locally known as catfish or ikan keli in Malaysia (Kottelat, 2013). These catfish are most commonly found in muddy or swampy water of high turbidity (Allen, 2011). It is considered as one of the delicacies in Malaysia. For human consumption, catfish species are normally reared in ponds and often get contaminated by a variety of microorganism because of the way they are being reared and the environment.

There has been a dramatic recent rise in the use of trash fish in aquaculture with the expansion of freshwater culture of catfish in cages and ponds (Edwards *et al.,* 2004). Consuming fish that are fed with trash fish made from marine fishes may also bring risk to the health of consumers as it may expose consumer to bacteria such as *Vibrio* spp. which are commonly found and considered as human pathogens (Feldhusen, 2000). Therefore, these fish are highly likely to carry pathogens and can be unsafe for human consumption if the fish is not cooked and handled well.

As reported by FDA (2014), the judicious use of antibiotic in aquatic farming is a part of good veterinary practice. Exposure of antibiotic residue from aquaculture exposes freshwater aquatic animals and may cause antibiotic resistance and it should be considered as a public health concern.

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The objectives of present work are:

1) to isolate and identify the types of bacteria found in catfish, *Clarias* spp.
2) to determine the antibiotic sensitivity of the common bacteria found in catfish, *Clarias* spp.

While, the hypotheses of this project are:

Null hypothesis is there will be no bacterial pathogens contamination in catfish, *Clarias* spp. whereas Alternative hypothesis is there will be presence of bacterial pathogens contamination in catfish, *Clarias* spp.

This project is approved by the Institutional Animal Care and Use Committee (IACUC)

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2.0 LITERATURE REVIEW

2.1 Aquaculture Industry in Malaysia

Aquaculture industry in Malaysia begins in the 1920's, whereby several culture practices are used such as brackish water aquaculture, freshwater pond aquaculture and marine aquaculture. A wide variety of species is cultured, including shellfish, freshwater species and marine finfish (FAO, 2017). In Malaysia, of the commercially cultured freshwater species, Nile tilapia (*Oreochromis niloticus*), was first introduced in 1944 from Indonesia (Ang *et al.*, 1989), accounts for (44.7 percent) of the total freshwater aquaculture production, followed by catfish (36.7 percent) and carps (10.08 percent). In terms of value of production, tilapia contributes (49.37 percent), followed by catfish (37 percent) and carps (10 percent). The catfish that is widely cultured now is the hybrid between *Clarius batrachus,* which is indigenous, and *Clarias gariepinus,* an exotic African catfish which was introduced in the early 1980's. The success in the induced breeding and seed production of the local walking catfish (Thalathiah, 1986) and the African catfish (Thalathiah and Ibrahim, 1992) paved the way for commercial seed production of the hybrid catfish nowadays.

2.2 Public Health Significance

According to Schmidt et al., (2000) fishes are susceptible to a wide variety of potentially pathogenic bacteria. Among the common fish pathogens are *Staphylococcus* sp., *Aeromonas* sp., *Salmonella* sp., *Shigella* sp., *Enterococcus faecalis, Escherichia coli, Yersinia* sp., *V. cholarae* and other vibrios (Kam et al., 1995; Lehane and Rawlin, 2000; Isonhood and Drake, 2002). Bacterial infection of fish may influence human health by inducing disease/infection and cause clinical signs such as abdominal pain, acute gastroenteritis, bloody/mucoid diarrhoea, nausea, vomiting and fever upon ingestion of insufficiently heat-treated fish contaminated during the processing and the presence of these bacteria harmful to human generally indicates poor sanitation in handling and processing (Kam *et al.*, 1995; Han *et al.,* 2001). Most outbreaks of food poisoning associated with fish derived from the consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from the water environment (*Vibrio sp., C. botulinum*) or terrestrial sources (C. *perfringens, Salmonella* sp*., Shigella* sp*., Staphylococcus* sp.*, V. cholarae*) (Khatib *et al.,* 1994; Novothy *et al*., 2004).

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3.0 MATERIALS AND METHODS

3.1 Sampling method

A total of twenty-four live catfish were purchased from 8 selected local markets at separate locations in Selangor for a period of two weeks. Three live catfish were collected from each local markets and transported in an ice box to the Aquatic Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Samples were processed within 24 hours for bacterial isolation and identification.

3.2 Sample processing

Pithing method was performed for the collection of samples (gill and intestine) from catfish, *Clarias* spp. In the laboratory, the samples were obtained using a sterile knife and a pair of sterile dissecting tools as shown in **Appendix 1**.

For isolation of bacteria other than Vibrio spp., the gills and intestines were placed separately in sterile plastic bags, labelled and homogenized with 10 ml of sterile peptone water using stomacher for 1 minute. After homogenization using stomacher, a loopful of each sample (gill / intestine) was streaked onto blood agar and McConkey agar respectively. The plates were incubated at 30°C for 24 hours under aerobic condition as showed in **(Figure 1).**

For Vibrio spp. isolation, the gills and intestines were then separately placed in a sterile plastic bag, labelled and homogenized with 10 ml of alkaline peptone water using stomacher for 1 minute. The homogenized sample was then incubated at 30°C for 24 hours under aerobic condition. After incubation, a loopful of each enriched sample (gill / intestine) was streaked onto Thiosulfate-Citrate Bile Salt (TCBS) agar

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respectively. Then the plates were incubated at 30°C for 24 hours under aerobic condition.

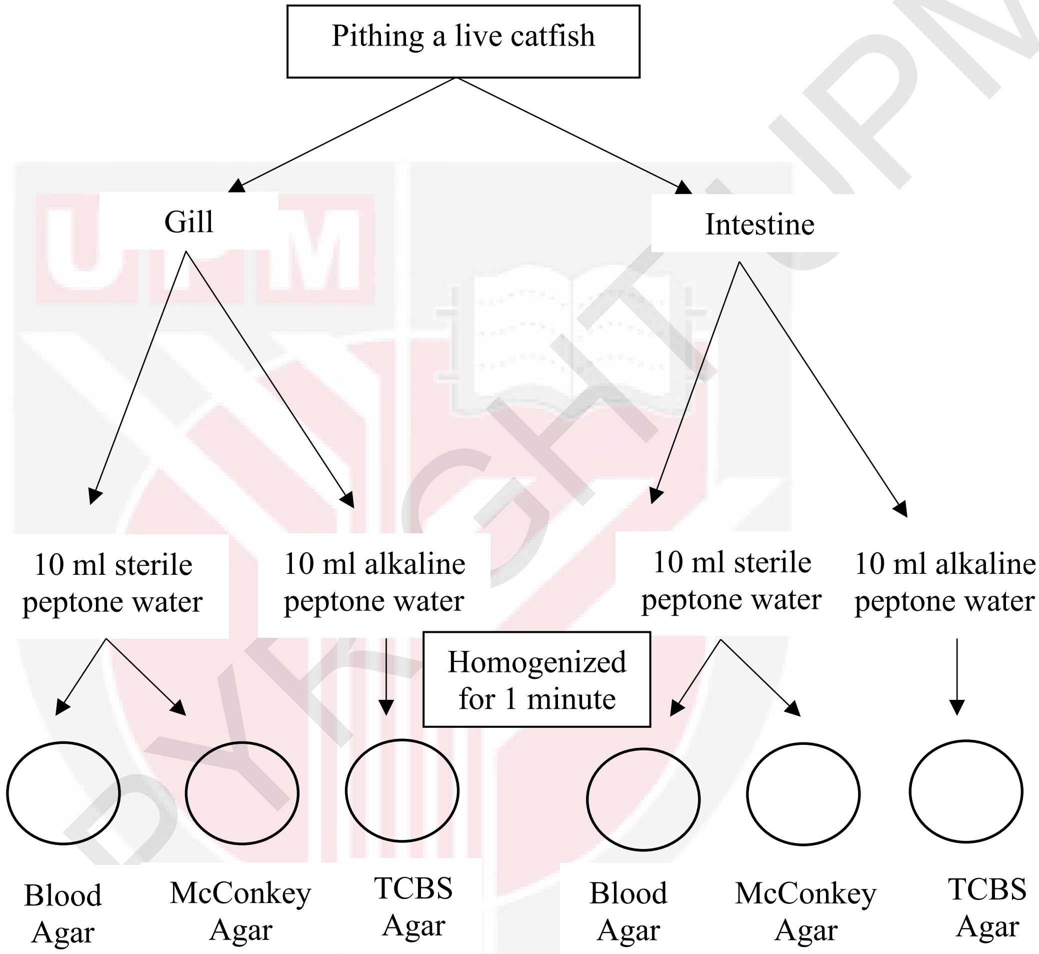

Figure 1: Each sample were inoculated onto three different agars and incubated at 30°C for 24 hours under aerobic condition.

3.3 Bacterial isolation and identification procedure

3.3.1 Primary culture

All the samples were inoculated into three different agars which are Blood agar, McConkey agar and Thiosulfate-Citrate Bile Salt (TCBS) agar and incubated at 30°C for 24 hours under aerobic condition as explained in **(Figure 1).**

3.3.2 Purification of culture

The visible colony on the blood and McConkey agar after 24 hours of incubation respectively was examined. The morphology of each of the visible colonies was recorded based on their shape, size, color, surface texture, hemolytic activity (only on the blood agar) and smell. Each of the visible colonies from the blood agar and McConkey agar was taken by using a sterile inoculating loop and was streaked onto a blood agar and were incubated aerobically for 24 hours at 30°C.

The visible colony on the TCBS agar after 24 hours of incubation was examined. Typical colonies on TCBS are green and yellow in colour. Single colonies were selected and gram stained. Purification was done on Tryptic Soy (TSA) agar.

3.3.3 Gram staining

Gram staining was done for each identical bacterial colony to differentiate Gram negative bacteria and Gram positive bacteria. The detail steps for gram staining are described in **Appendix 2.** The stained smear was examined for Gram staining reaction, cell morphology and cell arrangement.

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3.3.4 Biochemical tests

Series of biochemical tests were performed to identify the identity of bacteria. Colonies presenting each bacterial species were identified and characterized using standard biochemical tests method according to the methods describe by Jang, Biberstein & Hirsh (2008). The detail steps for biochemical tests are described in **Appendix 2**. Among tests carried out are Oxidase, Sulfur Indole Motility (SIM) test, Triple sugar iron (TSI) test, Urease test, Citrate test, Catalase, Coagulase and other additional test.

3.4 Antimicrobial susceptibility test

Testing for antimicrobial susceptibility of bacteria against selected antimicrobials was done using Kirby Bauer method. Two ml of sterile saline was dispensed into sterile test tube by using sterile pipette. Several isolated colonies form the subculture were collected by using inoculating loop and transferred into the test tube of sterile saline. The bacteria were diluted to obtain a turbidity equivalent to 0.5 MacFarland test standard. A sterile swab was dipped into the inoculum and streaked onto entire surface of the Mueller Hinton agar 3 times with the swab by turning the plate 60 degrees between streaking to obtain an even inoculation. Eight (8) antibiotics were selected based on the commonly used antibiotics in aquaculture. The antibiotics selected were Ampicillin (10µg), Penicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Gentamycin (10µg), Streptomycin (10µg), Sulfamethoxazole/trimethoprim (25µg). The antibiotic discs were placed onto the Mueller Hinton agar by using a disc dispenser and it was then lightly pressed down with the sterile forceps to make contact with the agar surface. The plate was then

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incubated at for 24 hours. Zones showing complete inhibition by gross inspection were measured in millimeters (mm) by using calipers and the values obtained were compared to acceptable limits in Clinical and Laboratory Standard Institute (2010) to determine the susceptibility level of the antibiotic used.

Plate 1: Pure culture of bacteria isolate grown on blood agar. *Escherichia coli* (left) and *Aeromonas* sp. (right).

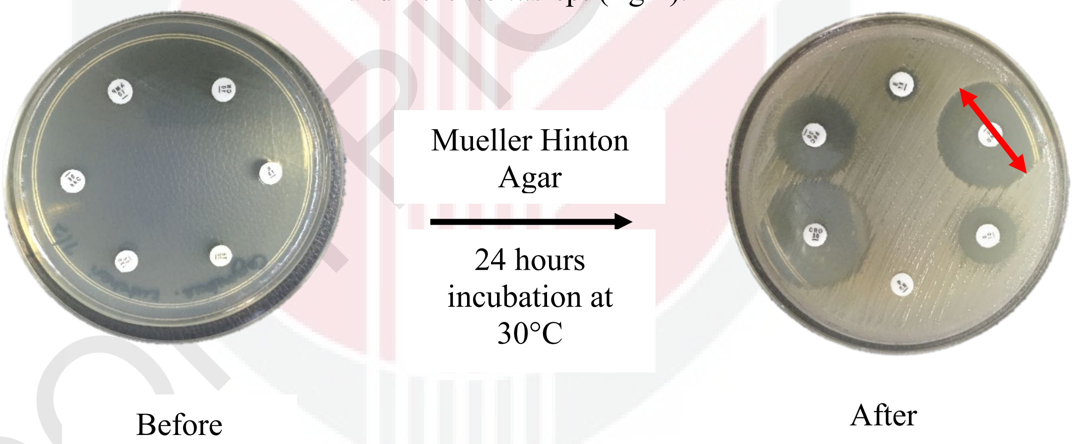

Plate 2: Antimicrobial susceptibility test. *Proteus vulgaris* before (left) and after incubation (right). Red arrow indicates the diameter of zone inhibition.

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4.0 RESULTS

4.1 Isolation and Identification of bacteria

Out of 48 samples (24 gill & 24 intestine), 20 Gram negative (41.67%) and seven Gram positive bacteria (14.58%) were successfully isolated as shown in **Figure 2**.

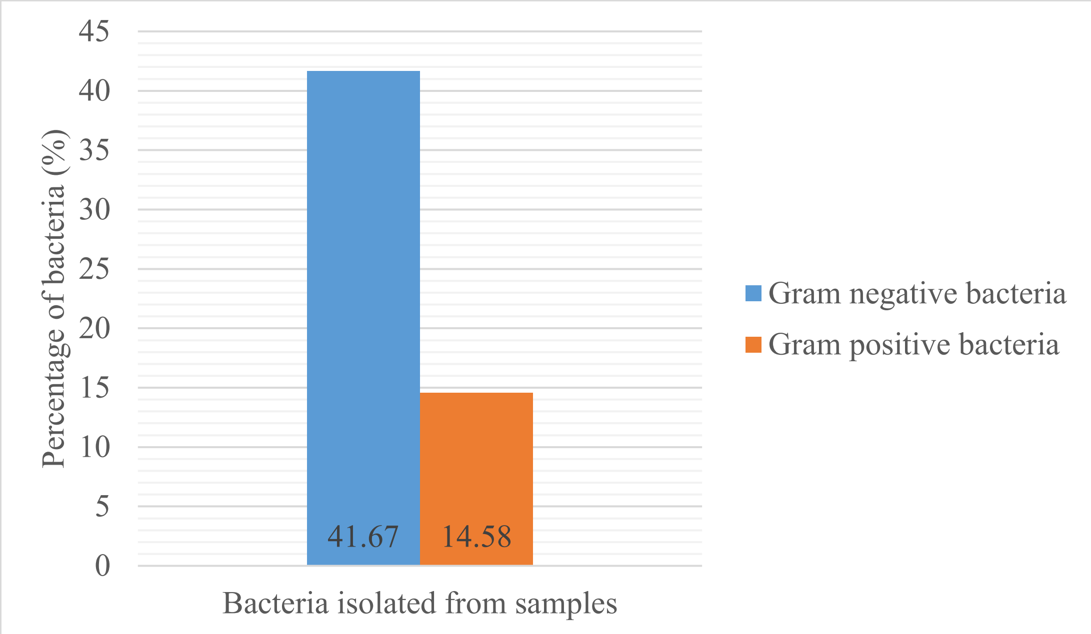

Figure 2: The isolation and identification of bacteria isolated from the samples of gill and intestine of twenty-four catfish.

The gram negative bacteria isolated were *Aeromonas* species (37.5%), *Vibrio cholarae* (31.25%), *Escherichia coli* (16.66%), *Klebsiella pneumonia* (12.5%), *Vibrio parahemolyticus* (10.41%), *Proteus vulgaris* (10.41%), *Pantoea agglomerans* (10.41%), *Proteus mirablis* (10.41%), *Enterobacter aerogenes* (6.25%), *Photobacterium damsela* (6.25%), *Edwardsiella tarda* (6.25%), *Pleisiomonas shigelloides* (6.25%), *Citrobacter freundii* (6.25%), *Shigella species (4.16%), Salmonella* species (2.08%), *Vibrio alginolyticus* (2.08%), *Chromobacterium* (2.08%), *Achromobacter* (2.08%), *Shewanella putrefaction* (2.08%) and *Alcaligenes fecalis* (2.08%) as shown in **Figure 3**.

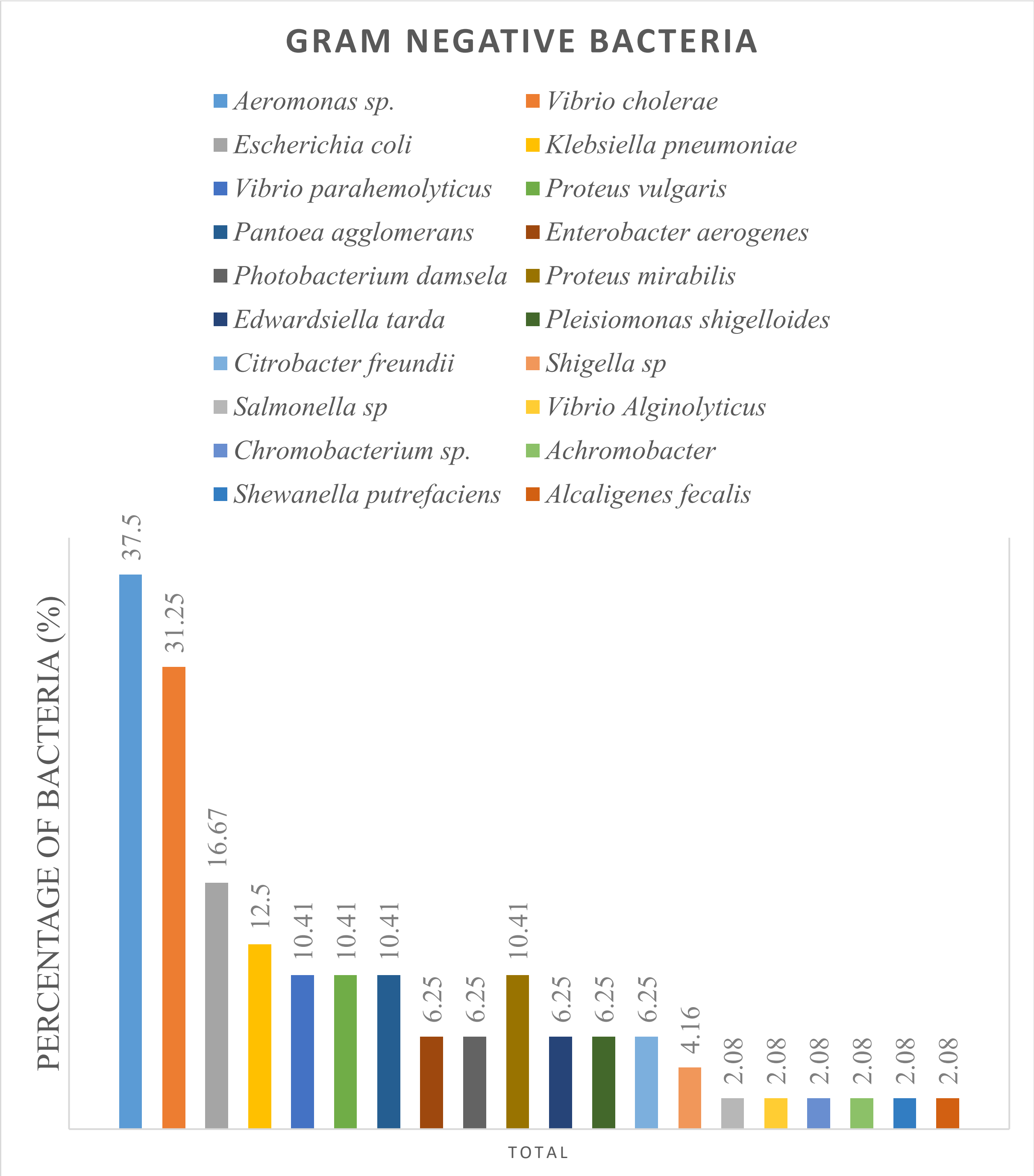

Figure 3: Gram negative bacteria isolated from the samples of twenty-four catfish.

While, Gram positive bacteria species isolated were *Staphylococcus* spp (16.67%), *Corynebacterium* spp (8.33%), *Streptococcus viridans* spp (8.33%), *Actinomyces* spp (4.16%) and *Listeria monocytogenes* (4.16%) as shown in **Figure 4**.

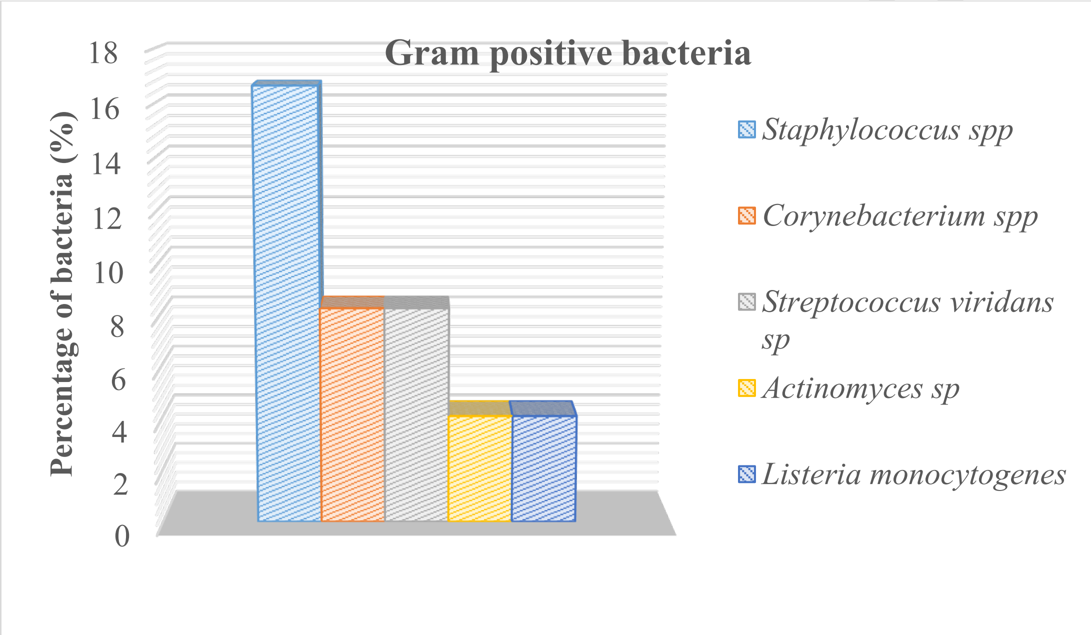

Figure 4: Gram positive bacteria isolated from the samples of twenty-four catfish

Figure 5 shows Gram negative bacteria isolated from the gill were *Aeromonas* species (58.3%), *Escherichia coli* (16.67%), *Vibrio cholarae* (41.60%), *Vibrio parahemolyticus* (12.50%), *Klebsiella pneumonia* (29.17%), *Salmonella* species (4.16%), *Shigella species (4.16%), Pleisiomonas shigelloides* (0%), *Edwardsiella tarda* (0%), *Enterobacter aerogenes* (12.5%), *Vibrio alginolyticus* (4.16%), *Proteus vulgaris* (4.16%), *Proteus mirablis* (8.33%) and Gram negative bacteria isolated from the intestine were *Aeromonas* species (37.5%), *Escherichia coli* (29.17%), *Vibrio cholarae* (33.33%), *Vibrio parahemolyticus* (12.50%), *Klebsiella pneumonia* (8.33%), *Salmonella* species (0%), *Shigella species (0%), Pleisiomonas shigelloides* (12.5%), *Edwardsiella tarda* 12.5%), *Enterobacter aerogenes* (4.14%), *Vibrio alginolyticus* (0%), *Proteus vulgaris* (16.67%), *Proteus mirablis* (4.16%)

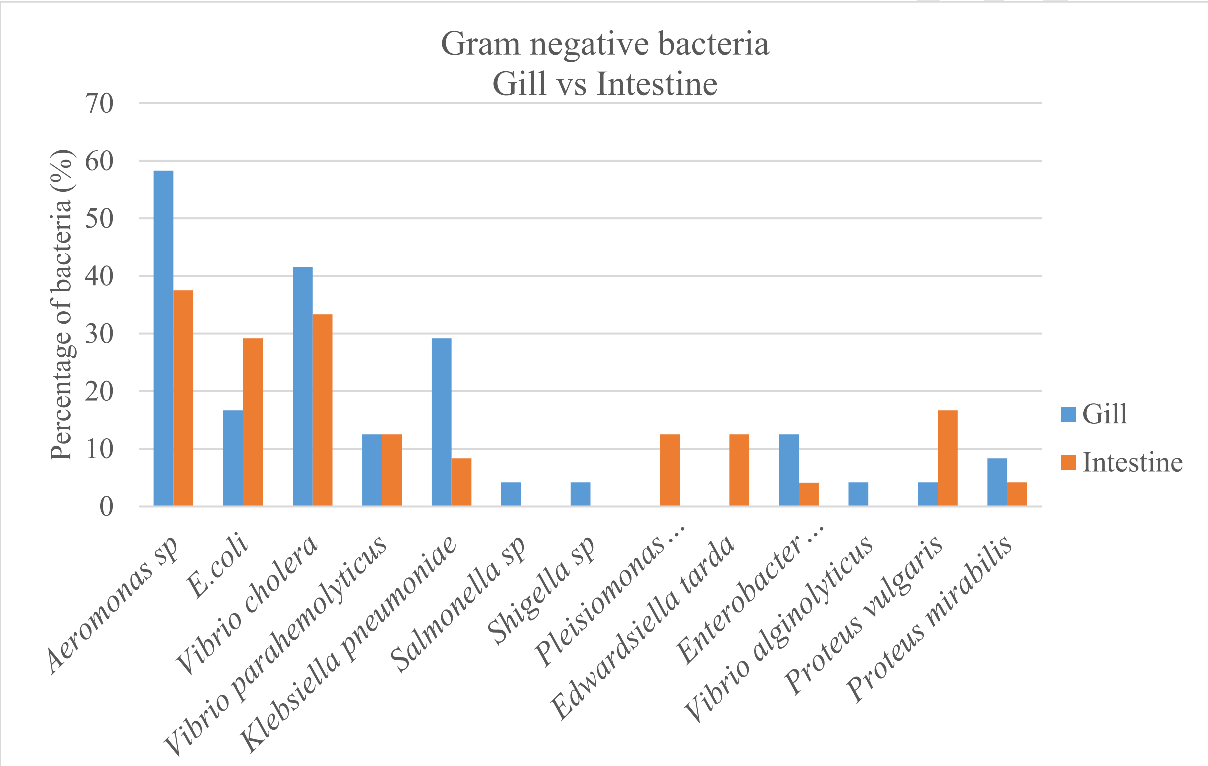

Figure 5: Gram negative bacteria isolated in gill versus intestine respectively from twenty-four catfish.

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Figure 6 shows Gram positive bacteria isolated from the gill were *Staphylococcus* species (12.50%), *Staphylococcus hyicus* (4.16%), *Streptococcus viridans* species (8.33%), *Corynebacterium* species (4.16%), *Corynebacterium kutcheri* (0%), *Actinomyces* species (0%), and *Listeria monocytogenes* (0%) and Gram positive bacteria isolated from the intestine were Staphylococcus species (4.16%), *Staphylococcus hyicus* (0%), *Streptococcus viridans* species (0%), Corynebacterium species (0%), *Corynebacterium kutcheri* (4.16%), Actinomyces species (8.33%), and *Listeria monocytogenes* (4.16%).

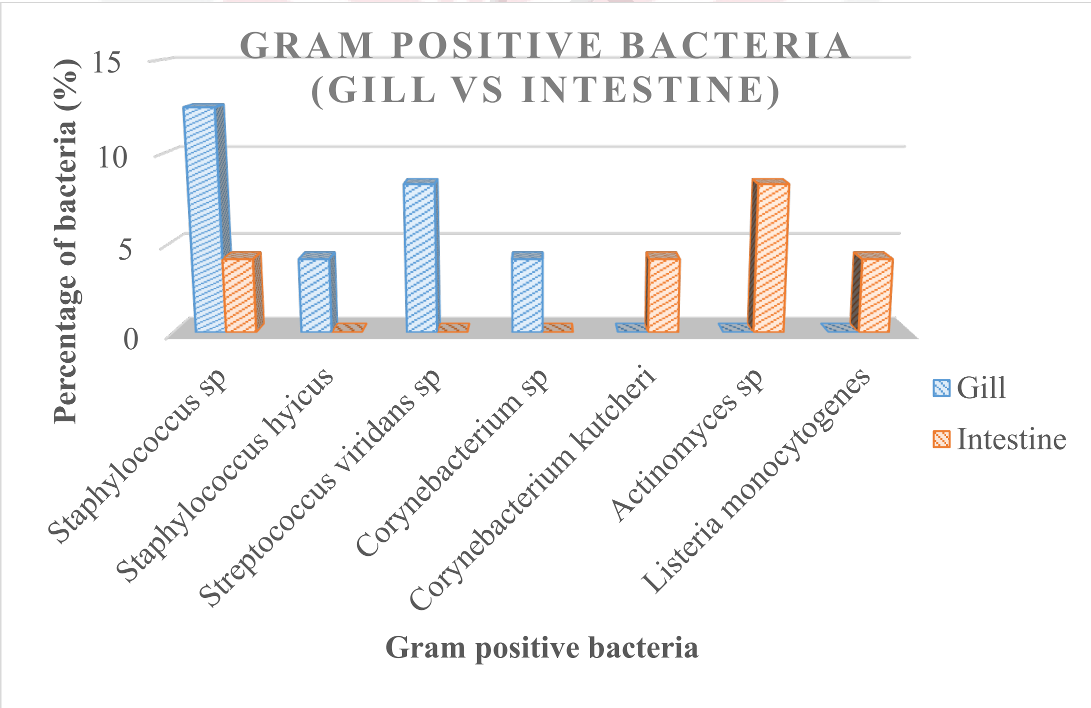

Figure 6: Gram positive bacteria in gill versus intestine respectively from twenty-four catfish

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4.2 Antimicrobial susceptibility test

All Gram negative and Gram positive bacteria isolated were subjected to eight types of antibiotics and the results of the multi-drug resistant (MDR) bacteria are tabulated as in Table 2 and Table 3 respectively. There are three Gram negative multi-drug resistant bacteria and one multi-drug Gram positive bacteria were detected from this study.

	BL		TC	Q	M	AG		S	MDR
GRAM NEGATIVE BACTERIA	Penicillin	Ampicillin	Tetracycline	Ciprofloxacin	Erythromycin	Gentamycin	Streptomycin	Sulfamethoxazole / trimetroprim	
Vibrio cholarae	Resistant	Resistant	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	**YES**
Proteus vulgaris	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Intermediate	Sensitive	**YES**
Proteus mirabilis	Resistant	Resistant	Resistant	Sensitive	Intermediate	Sensitive	Resistant	Sensitive	**YES**

Resistant Intermediate Sensitive

Beta- Lactam (BL), Tetracycline (T), Quinolone (Q), Macrolides (M), Aminoglycoside (AG), Sulfonamides (S).

Table 1: Antimicrobial susceptibility test result for Gram negative bacteria.

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	BL		TC	Q	M	AG		S	MDR
GRAM POSITIVE BACTERIA	Penicillin	Ampicillin	Tetracycline	Ciprofloxacin	Erythromycin	Gentamycin	Streptomycin	Sulfamethoxazole / trimetroprim	
Staphylococcus species									YES

Resistant Intermediate Sensitive

Beta- Lactam (BL), Tetracycline (T), Quinolone (Q), Macrolides (M), Aminoglycoside (AG), Sulfonamides (S).

Table 2: Antimicrobial susceptibility test result for Gram positive bacteria.

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5.0 DISCUSSION

In this study, twenty Gram negative and seven Gram positive bacteria were successfully isolated from the gill and intestine of catfish, *Clarias* spp. All of these bacteria may be pathogenic and potentially pathogenic bacteria associated with fish include, *Vibrio* spp., *Aeromonas spp*, *Salmonella* spp. and others (Lipp and Rose, 1997; Zlotkin *et al.,* 1998; Chattpadhyay, 2000). People most often get infected either by through contact with infected fish while handling them, or orally by consumption infected/raw fish or related products or food contaminated with water or other constituents of water environment (Acha and Szyfres, 2003).

Aeromonas sp (37.5%) were found to be the highest gram negative bacteria isolated from the total samples followed by *Vibrio* spp, *Escherichia coli (16.67%)*, and other Enterobactericiae (Figure 3). As reported by (Isonhood and Drake, 2002), Aeromonads are ubiquitous in fresh water, fish and shellfish and it is commonly reflects the distribution of bacteria within the water environment. *Aeromonas* sp. also has been recognized as potential foodborne pathogens for more than 20 years.

The presence of *Vibrio* spp. and *V. parahaemolyticus* in samples of freshwater fish in this study suggests that foodborne illness could arise if these fish are consumed in the uncooked or undercooked state. Based on this study, three types of *Vibrio* spp are obtained which are *Vibrio cholarae* (31.25%), *Vibrio parahemolyticus* (10.41%) and *Vibrio alginolyticus* (2.08%). This high incidence

probably reflects the nature of *Vibrio* spp. which is known as a halophilic waterborne bacterium that commonly inhabits environmental water sources worldwide. It has been found that freshwater rivers as well as brackish water and marine environments may support the growth of these organisms which are also pathogenic to humans

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(Janda, 1987). Previous study suggested that fish may act as reservoir and vectors of *Vibrio cholarae* (Senderovich *et al.,* 2010).

Other than *Aeromonas* sp and *Vibrio* spp, Enterobactericiae such as *Escherichia coli* and *Shigella* sp are also important as they are one of the potential source of bacterial pathogen to humans. *Escherichia coli* is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcu*s spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. For instance, such organisms should not be present on fresh-caught fish (Chattopadhyay, 2000). The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process (Ayulo *et al*., 1994; Asai *et al*., 1999). *Shigella* sp has nearly always been as a result from contamination of raw or previously cooked foods during preparation by an infected, asymptomatic carrier with poor personal hygiene.

The presence of *Listeria monocytogenes* in this study as shown in Figure 4 and Figure 6 is highly likely due to contamination during processing. As reported by Huss *et al*., (2000), *L. monocytogenes* is widely distributed in the general environment including fresh water and coastal water.

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Uncontrolled usage of antibiotics in aquaculture contributes to the emergence of resistant bacteria. Since most prescribing in aquaculture sector is done by untrained salesperson of little knowledge regarding disease, the products prescribed may have little to do with the disease and led to the emergence of bacteria into the environment such as the swamps. Resistant bacteria carried by food-producing animals can spread to people, mainly via the consumption of inadequately cooked food, handling of raw food or cross-contamination with other food, but also directly from the environment (Health Action International Asia Pacific, 2013)

Health implications of consuming food contaminated with resistant bacteria are transfer of resistance gene to human pathogens. Human exposed to antibiotic resistant bacteria have higher chances of getting infection from bacteria that are resistance. The implication of antimicrobial resistance are increased number of infections, increase frequency of treatment failure and increase severity of infection. (Heuer *et al.*, 2016).

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6.0 CONCLUSION AND RECOMMENDATION

As a conclusion, this study suggests that the catfish *Clarias* spp harbour many pathogenic bacteria such as *Vibrio cholarae, Proteus vulgaris, Proteus mirabilis* and *Staphylococcu*s sp that exhibited multidrug resistant trait which may bring risk to the consumers.

It is recommended that highly sensitive, rapid and specific methods such as polymerase chain reaction (PCR) be used to detect specific bacteria species for better results. Other than that, good sanitation and hygiene during handling, processing and proper cooking might reduce the possibilities of getting infection since most of the pathogenic bacteria can be killed by using high temperature.

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APPENDICES

APPENDIX 1: SAMPLE TRANSPORTATION, COLLECTION & PROCESSING

1.0 Transportation of catfish in icebox

2.0 Pithing Method

3.0 Equipment used for pithing/collection of samples
(Sterile knife, 70% alcohol, scisssor, forceps & bunsen burner)

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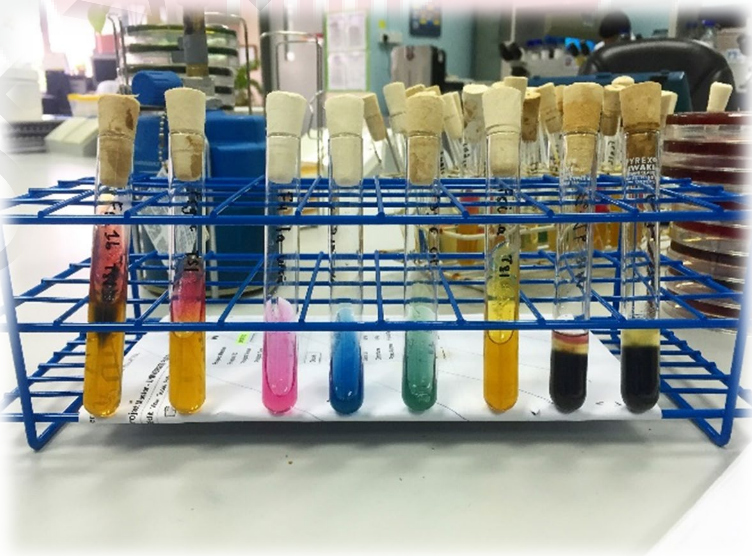
Gill

Intestine

4.0 Gill and Intestine (Sample collected for processing)



5.0 Biochemical Test

APPENDIX 2: GRAM STAINING TECHNIQUE

Place a drop of normal saline onto a clean glass slide

⇩

Using a wire loop, mix a loopful of isolated bacteria colonies onto the glass slide

⇩

Heat-fix the slide and allow to dry for few seconds

⇩

Drain with crystal violet for one minute and then wash with distilled water

⇩

Drain with iodine for one minute and wash with distilled water

⇩

Drain with acetone for few seconds and wash with distilled water

⇩

Drain with diluted carbol fuschin for one minute and wash with distilled water

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APPENDIX 3: BASIC BIOCHEMICAL TEST FOR GRAM POSITIVE BACTERIA

Biochemical test	Description	Reaction
Catalase test	A drop of 3% H_20_2 was placed onto clean glass slide and the colony of tested agar was picked with wore loop and added to the drop of H_2O_2.	Positive reaction was indicated by present of gas (air bubble)
Coagulase test	A drop of normal saline was placed onto the clean glass slide. Few colonies which are positive for catalase test was picked with wire loop and mixed well with the drop of saline. Then a drop of rabbit plasma was added.	Positive reaction was indicated by the present of immediate clumping effect.

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APPENDIX 4: BASIC BIOCHEMICAL TEST FOR GRAM NEGATIVE BACTERIA

Biochemical test	Description	Reaction
Oxidase test	A drop of oxidase reagent was placed on the filter paper. By using the wire loop, the tested colony was streak onto the filter paper.	The positive reaction was indicated by the change into purple colour.
Triple Sugar Iron (TSI) test	The medium was inoculated by stabbing with straight wire and streaking at the slant area which contains the bacteria colony. Then the tube was incubated aerobically for 24 hours at 30°C	The reaction of media was observed for any colour changes at the butt and slant of the test tube. The present of yellow and red colour indicates of acid and alkaline respectively. Present of black precipitate indicates of H_20 production and bubbles in test tube indicates of production of gas.
Sulfur-Indole Motility (SIM) test	The medium was inoculated by stabbing with straight wire which contains the bacteria colony. Then the tube was incubated aerobically for 24 hours at 30°C. The reaction of media was observed. One drop of Kovac's reagent was added to the test tube for indole production test.	The positive reaction was indicated by the present of pink colour in the reagent layer within one minute. (Indole test) The present of black precipitation in the test tube indicated positive for the H_20 production. If present of turbidity inside the test tube indicated positive for motility test.

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Urease test	The tested colony was inoculated onto the slope of the urea agar medium, incubated aerobically for 24 hours at 30°C	The positive reaction was indicated by presence of the pink colour.
Citrate test	The tested colony was inoculated onto citrate agar medium, then incubated aerobically for 24 hours at 30°C	The positive reaction was indicated by presence of the blue colour.

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APPENDIX 5: ADDITIONAL BIOCHEMICAL TEST

Biochemical test	Description	Reaction
Voges-Proskauer (VP) test	A loopful of isolated colonies from pure culture was collected and mixed into the broth. It was then incubated for 24 hours at 30°C. After incubation, six drop of alpha-naphthol solution and two drops of 4% Potassium 'hydroxide solution (KOH) aqueous solution were added and it was well shaken for 1 minute. It is allowed to stand for 10-20 minutes if there is no change.	The positive reaction was indicated by the presence of the red colour.
Mannitol and Maltose test	A loopful of isolated colonies from pure culture was collected and mixed into the broth. It was then incubated for 24 hours at 30°C.	The positive reaction was indicated by the change into yellow colour.
Arginine Dihydrolase (ADH) test	A loopful of isolated colonies from pure culture was collected and mixed into the broth. Mineral oil was added to cover the broth and incubated for 24 hours at 30°C.	The positive reaction was indicated by the change into turbid to purple colour.
6.5% NaCl	A loopful of isolated colonies from pure culture was collected and mixed into the broth. It was then incubated for 24 hours at 30°C.	The positive reaction was indicated by the change into turbid .
Bile esculin test	The tested culture was streaked into the bile esculin agar and incubated aerobically for 24 hours at 30°C.	The positive reaction was indicated by present of black colour on the agar.

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Soluble heamolysin	A loopful of isolated colonies from pure culture was collected and mixed into the broth. It was then incubated for 24 hours at 30°C.	The positive reaction was indicated as the blood broth is hemolysed.
Lactose, Sorbitol, Arabinose, Maltose, Dulcitol and Trehalose test.	A loopful of isolated colonies from pure culture was collected and mixed into the broth. It was then incubated for 24 hours at 30°C.	The positive reaction was indicated by the change into yellow colour.
Nitrate Reduction Test	The tested culture was inoculated into the solution and incubated aerobically for 24 hours at 30°C. After incubation, the nitrate reagent 1 (Sulfanilic acid) and Nitrate Reagent 2 (Naphthylamine) was added.	The positive reaction was indicated by changes into red colour.
O-nitrophenyl-β-D-galactopyranoside (ONPG) Test	The tested culture was inoculated into the nutrient broth and the ONPG reagent was added. Then incubated aerobically for 24 hours at 30°C.	The positive reaction was indicated by the change into yellow colour.
Ornithine Decarboxylase (ODC) Test	The tested culture was inoculated into ODC media and glycerol oil was added to cover the layer of solution. Then incubated aerobically for 24 hours at 30°C.	The positive reaction was indicated by the change into purple colour.
Lysine Decarboxylase Test (LDC) test	The tested culture was inoculated into LDC media and glycerol oil was added to cover the layer of solution. Then incubated aerobically for 24 hours at 30°C	The positive reaction was indicated by the change into purple colour.

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