ISOLATION, IDENTIFICATION, AND AMR PATTERN OF *E. COLI* ISOLATES FROM PELLET-FED AND CHICKEN-OFFAL FED AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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CERTIFICATION

This is to certify that we have read this research paper entitled '**Isolation, Identification, and AMR Pattern of *E. coli* Isolates from Pellet-fed and Chicken Offal-fed African Catfish (*Clarias gariepinus*)**' by Nur Syaza Syazwani Bte Mohd Azhar, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

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Daisy

Thank You

DEDICATION

.

To five year-old Ain – for having to burden a decision made too early.

Still in the age of lullabies, naive by design
Wide eyed and cherried cheeks, a moment unmoved forever
In my dreams, she ceased.

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Abbreviations

AMR	Antimicrobial resistance
CLSI	Clinical and Laboratory Standard Institute
E. coli	*Escherichia coli*
MDR	Multidrug resistance

ABSTRACT

An abstract of the research paper presented to the faculty of Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 - Research Project.

Escherichia coli (*E. coli*) belongs to the family Enterobacteriaceae and is a commensal bacteria of the gastrointestinal tract of African catfish (*Clarias gariepinus*). This is a preliminary study to determine the antimicrobial resistance (AMR) pattern of *E. coli* in African catfish as well as the possible relationship between antibiotic resistance and type of aquaculture feed. Five pellet-fed and five chicken offal-fed African catfishes were obtained for the collection of intestinal mucosa samples for isolation and identification of *E. coli*, and an AMR pattern of isolated *E. coli* was executed. Results revealed three *E. coli* isolates from the pellet-fed catfish group were resistant to tetracycline; one of the isolates was simultaneously resistant to chloramphenicol. All *E. coli* isolates from the chicken offal-fed catfish group were resistant to at least one selected antibiotic, and all isolates were 100% resistant against tetracycline and amoxicillin. There is an occurrence of multidrug resistance for all *E. coli* isolates from the chicken offal-fed catfish group. The study supports the notion that *E. coli* isolated from chicken offal-fed catfish are more resistant to selected antimicrobials than commercial pellet-fed catfish which could suggest influence of type of feed received.

***Keywords**: African catfish, Antimicrobial resistance, E. coli, Feed*

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 - Projek Penyelidikan.

Escherichia coli (E. coli) tergolong dalam keluarga Enterobacteriaceae dan merupakan komensal dari saluran gastrousus ikan keli Afrika (*Clarias gariepinus*). Ini adalah kajian awal untuk menentukan corak Kerintangan Antimikroba *E. coli* dalam ikan keli Afrika serta kemungkinan hubungan antara rintangan antibiotik dan makanan akuakultur. Lima ikan keli dari kumpulan diberi pelet dan jeroan ayam telah diperoleh untuk pengumpulan sampel mukosa usus untuk pengasingan dan pengenalpastian *E. coli*, dan penentuan kerintangan antimikroba telah dilaksanakan. Keputusan menunjukkan tiga pencilan *E. coli* daripada kumpulan ikan keli diberi makan pelet menunjukkan ketahanan terhadap tetrasiklin, di mana salah satu pencilan secara serentak kerintangan terhadap kloramfenikol. Kesemua pencilan *E. coli* daripada kumpulan ikan keli diberi makan jeroan ayam merintang terhadap sekurang-kurangnya satu antibiotik terpilih, dan semua pencilan merintang 100% terhadap tetrasiklin dan amoksisilin. Turut didedahkan terdapat kejadian rintangan *multidrug* bagi semua pencilan *E. coli* daripada kumpulan ikan keli diberi makan jeroan ayam. Oleh itu, kajian itu menyokong tanggapan bahawa *E. coli* yang diasingkan daripada ikan keli diberi makan jeroan ayam lebih tahan terhadap antimikroba terpilih berbanding ikan keli diberi makan pelet komersial yang berkemungkinan disebabkan oleh jenis makanan yang diberi.

Kata kunci: *E. coli, ikan keli, kerintangan antimikroba, makanan*

1.0 INTRODUCTION

African catfish is the second most harvested freshwater aquaculture fish in Malaysia with total production of 28, 464.07 tonnes in 2019 with a production value of RM128, 559, 770 (DOF, 2019). It became popular with fish farmers due to their hardiness, rapid growth rate, high fecundity, and ease of culture (Saba et al., 2020). The African catfish, *Clarias gariepinus,* is recognized by their cylindrical body which is grayish-black in colour with the underside head and body creamy-white, scaleless skin, flattened body head, elongated spineless dorsal fins and four pairs of barbels around a broad mouth (Van Oijen, 1995). It is a non-native species to Malaysia and was introduced through aquaculture from Thailand sometime between 1986-1989. Over time, it has become the highest finfish to be farmed, and the second largest constituent of aquaculture produce in Malaysia next to seaweed (Dauda et al., 2018). Based on a cross-sectional survey conducted between February 2008 and May 2009 to investigate patterns of fish consumption among Malaysian adults in Peninsular Malaysia, catfish is one of the most frequently consumed freshwater fish other than snakehead fish (Ahmad et al., 2016).

Escherichia coli (*E. coli*) is a prominent foodborne pathogen that causes serious illness in humans all over the world. It is a Gram-negative microorganism usually found in the gastrointestinal tract, on the gills, in the muscles and on the skin (Ogbondeminu, 1993). Salle (1964) reported that the most heavily contaminated parts are the intestines and the skin. Presence of *E. coli* in water or food indicates the possible presence of other causative organisms of many gastrointestinal diseases (Ampofo and Clerk, 2010).

Antimicrobial resistance occurs when bacteria, viruses, fungi, and parasites change over time and fail to respond to treatments, making illnesses become more difficult to treat and the risk of disease spread, severe illness, and higher death rate (WHO, 2021); which can be resulted from AMR transmissions to humans after consuming contaminated fishes.

Based on the Malaysian Action Plan on Antimicrobial Resistance (MyAP-AMR 2017 - 2021), AMR monitoring in fisheries is relatively naive, and although there is an on-going monitoring programme for drug residues in aquaculture farms, there is limited research and monitoring from an AMR aspect. There is also a lack of registry on the permitted antibiotics to be used by aquaculturists in Malaysia. Therefore, MyAP-AMR prioritizes public awareness and education, surveillance and research, infection, prevention, and control, and appropriate use of antimicrobials.

E. coli is a common opportunistic pathogen in African catfish (*Clarias gariepinus*), existing within the normal microflora of the intestinal mucosa. While the AMR pattern of the bacteria has been reported, it is lacking in information regarding the influence of feed in correlation to the recorded resistance. This study proposes the investigation of comparison between AMR patterns of *E. coli* isolated from chicken-offal fed catfish and pellet-fed catfish.

2.0 RESEARCH PROBLEM

Although research has identified microbial flora of African catfish and its AMR pattern, there is no research or reports of the influence of feed in the rise of AMR, particularly that of *E. coli*. This study aims to establish and compare the AMR patterns of *E. coli* isolated from chicken-offal fed and strictly pellet-fed catfish to determine correlation between feed and AMR.

3.0 RESEARCH QUESTION

3.1 Are *E. coli* isolated from intestines of chicken offal-fed catfish more resistant to selected antimicrobials than commercial pellet-fed catfish?

4.0 RESEARCH HYPOTHESIS

4.1 *E. coli* isolated from intestines of chicken offal-fed catfish are more resistant to selected antimicrobials than commercial pellet-fed catfish.

5.0 RESEARCH OBJECTIVES

5.1 To isolate *E. coli* from intestines of chicken offal-fed and pellet-fed catfish.

5.2 To determine AMR pattern of *E. coli* isolated from chicken offal-fed and pellet-fed catfish.

6.0 LITERATURE REVIEW

6.1 *Clarias gariepinus* Aquaculture in Malaysia

Aquaculture production in Malaysia has usually been ruled by aquatic plants (seaweeds), which constitute 51.5% of the total aquaculture manufacturing as of 2015. However, greater than 30 finfish species are farmed, with the maximum coming from African catfish (10%) and lower quantities from red (hybrid) tilapia, sea bass, river catfish (*Pangasius* sp.), and red snapper (Dauda et al., 2018). The African catfish, *Clarias gariepinus,* is a non-native species to Malaysia and was introduced through aquaculture from Thailand sometime between 1986-1989. Within the past two decades, aquaculture catfish has grown to become the highest produced finfish farmed in either fresh or brackish water, surpassing production of red tilapia in 2008.

The contribution of African catfish to the overall aquaculture manufacturing increased from 0.83% in 1995 to 17.73% in 2009 and was the highest finfish manufacturing for any single species ever recorded in Malaysia. In freshwater aquaculture, catfish contributed a rise from 5.99% in 1995 to an all-time highest of 54.86% in 2009. However, the overall manufacturing declined significantly from 83,727 in 2009 to 63,206 tonnes in 2010 and was stagnant until 2013. The trend increased and declined once again in 2014, and then increased slightly in 2015 with a total production of 50, 683.12 tonnes. The fluctuation of African catfish production recorded within the period which affected the aquaculture industry and is suggested to originate from emergence of infectious diseases.

6.2 Chicken Offal meal as Feed for *Clarias gariepinus*

The major protein constituent in aquaculture feed is commonly provided by fishmeal. However, due to a stagnant global supply it is used more sparingly to improve profitability, thus chicken offal is an economic alternative (Falaye et al., 2011). Chicken offal meal is acceptable, nutritionally suitable (Altan et al., 2010) and can reportedly partially replace fishmeal in diets for *C. gariepinus* (Abdel-Warith et al., 2001). Falaye et al. (2011) reported that the partial replacement of fishmeal with chicken offal by 30% significantly increased whole-body protein level, but had reduction in growth performance which may be attributed to limiting amino acids in the chicken offal. According to Hag et al. (2017), replacement of 5% to 10% of fishmeal with chicken offal in diets of *Clarias gariepinus* is possible without compromising the growth performance, feed utilization, and health status of the fish.

6.3 Antimicrobial Resistance of *Escherichia coli* in *Clarias gariepinus*

As reported by Efuntoye et al. (2012), commensal *E. coli* strains were found to be sensitive to antibiotics except for ampicillin, chloramphenicol, and tetracycline with antibiotic resistance percentage to be 82.4%. Amoxicillin, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, novobiocin, nitrofurantoin, streptomycin, and sulphamethoxazole AMR percentage ranged from 0% to 47.1%. Tilahun and Engdawork (2020) reported that commensal *E. coli* strains were 100% susceptible to ciprofloxacin, trimethoprim, gentamicin, and sulfamethoxazole. Meanwhile, the antibiotic resistance percentage ranged from 12.5% to 100% for tetracycline, doxycycline, streptomycin, ampicillin, and cefoxitin. Akande and Onyedibe (2019) isolated *E. coli* strains with high levels of resistance to cefoxitin (77.1%) and amoxicillin/clavulanic acid (74.3%).

These findings of antibiotic resistance percentage were of *E. coli* isolated from apparently healthy fishes which indicated that the bacteria itself did not pose a significant threat to the fishes used for the study. However, the organism is of public health significance in humans through consumption as strains have been found to be pathogenic and enterotoxigenic, causing life-threatening foodborne diseases, as well as a number of other infections such as gastroenteritis, osteomyelitis, cellulitis, and meningitis (Manna et al., 2008).

6.4 Antimicrobial Resistance and its Public Health Concerns

Antimicrobials include antibiotics, antivirals, antifungals, and antiparasitic; medicine used to treat and prevent infections in animals and humans. AMR occurs when pathogens evolve over time and gradually become unresponsive to antimicrobial agents thus rendering it increasingly difficult or impossible to treat (WHO, 2021). According to Thiang et al. (2021), frequently detected classes of antibiotics used in aquaculture included tetracyclines, quinolones, and sulfonamides. Due to the emergence of resistant pathogens in consumable fish which increases the risk of transmission of this resistance to consumers, it therefore raises the concerns regarding effective tools for the prevention and treatment of which is now rendered to be less effective (Lammie and Hughes, 2016). As a result, new antibiotics are necessary and urgently needed to overcome the alarming rapid global spread of resistant bacteria.

7.0 METHODOLOGY

This research will be conducted as per scheduled upon receiving approval from Final Year Project Research Committee and Animal Ethics Committee 2022, Faculty of Veterinary Medicine, University of Malaysia Kelantan.

7.1 Bacteria Isolation and Identification

Five African catfish samples were collected randomly from each source: chicken offal fed and pellet-fed, in which the former is gathered from aquafarm and latter from the Aquatic Laboratory of Faculty of Veterinary Medicine, University of Malaysia Kelantan. The intestines of each fish were isolated aseptically post-euthanization. Intestinal sample collection was conducted using a sterile inoculating loop to scrape the mucosal surface of the intestines, in which primary culture was performed on MacConkey agar and then isolated onto Eosin Methylene Blue agar. The plates were incubated for 24 hours at 37°C. After incubation, colony morphology was observed and Gram staining was performed. Next, biochemical tests including urease, catalase, oxidase, motility, indole, citrate, methyl-red, Voges-Proskauer, and Triple Sugar Iron (TSI) test were performed. *E. coli* colonies from Eosin Methylene Blue agar is to then be selected and inoculated on nutrient agar, incubated at 37°C for 24 hours later to perform the antibiotic susceptibility test.

7.2 Antibiotic Susceptibility Testing

Antimicrobial susceptibility was conducted using the disc diffusion method. A single colony was taken from a nutrient agar plate and dissolved in 10mL of 0.9% normal saline in a sterile glass tube. After that, the turbidity of the inoculated normal saline was

compared to 0.5 of McFarland standard. A sterile swab was dipped into the suspension and the dipped swab was rolled firmly against the side of the tube several times to remove excess inoculum from the swab. The entire surface of Mueller-Hinton Agar (Oxoid, UK) plate was streaked by the swab, plate was rotated and streaked repeatedly up to 3 times to ensure it formed a bacteria lawn on agar surface the next day. Using flame-sterilized forceps, the chosen antibiotic discs were placed and each disc was gently pressed to ensure that the discs adhered to the surface of the agar. The plates were then incubated at 37°C for 18 hours. Antibiotics discs included for the study were Streptomycin (10 µg/disc), Trimethoprim (5 µg/disc), Enrofloxacin (5 µg/disc), Erythromycin (15 µg/disc), Chloramphenicol (30 µg/disc), Tetracycline (30 µg/disc), Amoxicillin (10 µg/disc), and Cefoxitin (30 µg/disc).

7.3 Data Analysis

The diameter of the inhibition zone for each antibiotic disc was measured in millimeters and compared with breakpoint tables of Clinical and Laboratory Standard Institute (2020) (CLSI, 2020). Then the results were interpreted as susceptible, intermediate, and resistant as expressed in the table below (Table 7.1)

***Table 7.1: Zone diameter interpretive standards for the determination of AMR status by the Disk Diffusion method for Enterobacteriaceae* based on CLSI (2020)**

Antibiotics	Concentration (µg)/disc	Inhibition zone diameter to nearest millimeter (mm)		
		Susceptible	Intermediate	Resistant
Streptomycin	10	≥ 15	12 - 14	≤ 11
Trimethoprim	5	≥ 16	11 - 15	≤ 10
Enrofloxacin	4	≥ 21	15 - 20	≤ 14
Erythromycin	15	≥ 23	14 - 22	≤13
Chloramphenicol	30	≥ 18	13 -17	≤ 12
Tetracycline	30	≥ 15	12 - 14	≤ 11
Amoxicillin	10	≥ 18	14 - 17	≤ 13
Cefoxitin	30	≥ 18	15 -17	≤ 14

8.0 EXPECTED FINDINGS

Expected results include isolation of *E. coli* from chicken offal-fed and pellet-fed catfish. Furthermore, the AMR pattern of *E. coli* isolated from chicken offal-fed and pellet-fed catfish will be determined.

9.0 SIGNIFICANCE OF RESEARCH

The significance of this study is in the determination and comparison of AMR patterns of *E. coli* between chicken-offal fed catfish and pellet-fed catfish. AMR threatens the efficacy of antibiotics for the treatment of diseases thus causing serious implications on animal health, and subsequently humans due to consumption of AMR bacteria

contaminated food animals. The results of this research can aid in the understanding of aquaculture diet and its influence on microflora of African catfish and its resistance to selected microbials. Furthermore, it can increase public awareness about antibiotic resistance that can be found in catfish, and how it affects both humans and animals.

10.0 RESULTS

E. coli was successfully isolated and identified from all ten intestinal mucosal scraping samples collected, in which five were from pellet-fed catfish and five from chicken offal-fed catfish. Cultured MacConkey agar revealed raised, circular, smooth, moist colonies which are pink in colour, indicating fermentation of lactose. Cultured Eosin Methylene Blue agar revealed dark blue-black convex colonies showing greenish metallic sheen in bright ambience. Gram stains of isolated colonies showed rod-shaped bacteria arranged singly and in pairs, stained pink indicating a Gram-negative bacterium. In this study, several biochemical tests revealed reliable characteristic of *E. coli*, with most (eight out of ten) showing similar expected reactions including isolated colonies to be motile positive indole, negative citrate test, positive methyl red test and negative Voges-Proskauer test, negative urease test, and acidic slant and butt with presence of gas in TSI agar. Two of the isolates, both from pellet-fed groups, were motile with negative indole. All these characteristics corroborated the isolation and identification of *E. coli* based on Quinn et al. (2011). Table 10.1 shows the results of the antibiotic susceptibility test for ten *E. coli* isolates. The zone inhibition diameters were measured, allowing the categorization of *E. coli* isolated from pellet-fed catfish and chicken-offal fed catfish based on chosen antibiotics, as shown in Table 10.2 and Table 10.3 respectively.

Table 10.1: Zone inhibition diameters in millimeter of E. coli isolated from chicken offal-fed and pellet-fed catfish

Antibiotic (µg/disc)	Zone inhibition diameters of *E. coli* (mm)									
	CP1	CP2	CP3	CP4	CP5	CO1	CO2	CO3	CO4	CO5
Streptomycin (10µg)	14	16	13	15	14	13	10	9	6	12
Trimethoprim (5µg)	14	18	16	16	15	15	17	10	14	13
Enrofloxacin (4µg)	17	16	19	15	19	16	17	15	14	15
Erythromycin (15µg)	14	17	22	23	18	16	12	10	10	14
Chloramphenicol (30µg)	9	14	18	16	13	10	10	13	9	8
Tetracycline (30µg)	10	6	13	12	11	6	8	6	7	6
Amoxicillin (10µg)	15	15	17	18	18	10	11	11	8	9
Cefoxitin (30µg)	17	18	19	21	23	15	15	13	16	18

CP denotes pellet-fed catfish while CO denotes chicken offal fed catfish.

Table 10.2: Antimicrobial susceptibility profile of E. coli isolated from five pellet-fed catfish

Antibiotic (µg/disc)	Frequency of *E. coli* isolate susceptibility per antibiotic		
	Susceptible	Intermediate	Resistant
Streptomycin (10µg)	2	3	0
Trimethoprim (5µg)	3	2	0
Enrofloxacin (4µg)	0	5	0
Erythromycin (15µg)	1	4	0
Chloramphenicol (30µg)	1	3	1
Tetracycline (30µg)	0	2	3
Amoxicillin (10µg)	2	3	0
Cefoxitin (30µg)	4	1	0

Table 10.3: Antimicrobial susceptibility profile of E. coli isolated from five chicken offal-fed catfish

Antibiotic (µg/disc)	Frequency of *E. coli* isolate susceptibility per antibiotic		
	Susceptible	Intermediate	Resistant
Streptomycin (10µg)	0	2	3
Trimethoprim (5µg)	1	3	1
Enrofloxacin (4µg)	0	3	2
Erythromycin (15µg)	0	2	3
Chloramphenicol (30µg)	0	1	4
Tetracycline (30µg)	0	0	5
Amoxicillin (10µg)	0	0	5
Cefoxitin (30µg)	1	3	1

Table 10.2 shows the antimicrobial susceptibility profile of *E. coli* isolated from five pellet-fed catfish, which was categorized as susceptible, intermediate, and resistant. Most of the isolated *E. coli* were found to be either sensitive or intermediately resistant except for chloramphenicol and tetracycline in which three out five isolated *E. coli* of pellet-fed catfish was resistant to, and one of the three found to be concurrently resistant to chloramphenicol. Additionally, it is found that none of the isolated *E. coli* from the pellet-fed catfish group were sensitive to enrofloxacin and tetracycline.

On the other hand, Table 10.3 which shows the antimicrobial susceptibility profile of *E. coli* isolated from five chicken offal-fed catfish revealed that at least one isolate was resistant to all selected antibiotics. Remarkably, all five chicken offal-fed catfish *E. coli* isolates showed 100% resistance to tetracycline and amoxicillin. Additionally, it is found that none of the isolated *E. coli* from the chicken offal-fed catfish group were sensitive to selected antibiotics except for trimethoprim and cefoxitin, with one sample susceptible to each.

Overall, both groups showed that a majority of the *E. coli* isolated are categorized as intermediately resistant to most of the selected antibiotics. Furthermore, results reveal that there is also the occurrence of multidrug resistance (MDR), defined as AMR shown by a microorganism to at least one antibiotic in three or more antibiotic groups, as shown by all five samples collected from the chicken offal-fed catfish group in which each sample is resistant to three antibiotics at a minimum from the eight selected antibiotics.

11.0 DISCUSSION

Overall, there was a higher percentage of resistance of *E. coli* isolated from the intestines of chicken offal-fed catfish compared to pellet-fed catfish against the selected antibiotics. This is consistent with the hypothesis in which states so, thus suggesting the notion that chicken offal in aquaculture diet may lead to increased antibiotic resistance of the commensal gut bacteria of the catfish. The occurrence of antibiotic resistance in these findings supports the idea that an indirect source of antibiotics is the culprit, which are administered in the form of supplementary feed originated from rendered animal products such as bone meal, blood meal, or fish meal (Honert, 2020), and in this case, chicken offal; frequently used as a low-cost source of supplementary nutrition.

A study by Mund et al. in 2016 revealed that although the poultry industry has gone through immense growth as a consequence of the use of certain drugs in feed to prevent diseases and promote growth, a disadvantage emerges from the inappropriate and non-judicious use of drugs in the form of harmful antibiotic drug residues in poultry products. According to Roslee et al. 2016, a study conducted to investigate the antibiotic resistance of *E. coli* isolated from chicken in Malaysia, it was found that 99.2% of isolates had a resistance towards at least one antibiotic, while 81.6% had MDR profiles, that is against erythromycin, tetracycline, spectinomycin, trimethoprim, and flumequine.

Therefore, the findings from current study can further suggest that chicken offal fed to the catfish in this study had harmful antibiotic drug residues leading to the rise in antibiotic resistance of *E. coli* of catfish intestinal mucosal microbiome, as shown by

presence of at least one resistant isolate towards each selected antibiotics, as well as MDR profiles shown by all isolates (100% MDR profiles).

This study also revealed that isolates from both groups of catfish had intermediate resistance to most drugs, namely streptomycin, enrofloxacin, erythromycin, chloramphenicol, and amoxicillin in pellet-fed groups (60% to 100%), and trimethoprim, enrofloxacin, and cefoxitin in chicken-offal fed catfish (60%). This indicates that if the aforementioned antibiotics are to be used in cases on infection, *E. coli* would be inhibited only by the maximum recommended dosage which implies that the organism may be eliminated in body compartments easily accessible by the drug, while the same antibiotic might not be able to have the same adequate effects against the same organism when located at other sites (Rodloff et al., 2008). Also from the results, *E. coli* isolates from pellet-fed catfish showed mostly intermediate resistance thus allowing the inference that they were too ultimately susceptible to occurrence of antibiotic resistance if future use of similar antibiotics were practiced as this could accelerate development of resistance mechanisms (CDC, 2021).

However, there still exists the possibility of a direct source of antibiotic resistance development due to direct administration of antibiotics (Honert et al., 2020). Antibiotic drugs are commonly used for three purposes: therapeutic use, which involves giving high doses of antibiotics to animals (either individually or in small groups), prophylactic use, which involves giving moderate doses of antibiotics to animals for longer periods of time, and growth promotion, which involves giving antibiotics in subtherapeutic doses,

such as 10 or 100 times less than therapeutic doses, for a longer period of time (Mund et al., 2016).

These activities increase selection pressure on the fish population and the spread of resistance genes. Due to the general consequent development of acquired antibiotic resistance in fish pathogens and other aquatic microorganisms, this poses a serious threat to public health. Fish bacteria can act as reservoirs for resistance genes that could then spread to even commensal human pathogens. Therefore, aqua farmers should become more aware about the substances used in the preparation of feed for their aquacultures, as certain feed components, such as antibiotic resistant animal products, can have a negative impact on health and safety (Honert et al., 2020) and in order to prevent exacerbation of resistance, antibiotics should not be used indiscriminately to control antimicrobial transmissions, therefore hygienic management is to be optimized.

12.0 CONCLUSION

In conclusion, *E. coli* was successfully isolated and identified from all samples acquired from pellet-fed catfish and chicken offal-fed catfish. From the antibiotic susceptibility test, it was found that *E. coli* isolated from pellet-fed catfish showed 60% resistance to tetracycline and 20% resistance to chloramphenicol meanwhile *E. coli* isolated from chicken offal-fed catfish revealed resistance to all selected antibiotics with percentages ranging from 20% to 100%, thus supporting the notion that *E. coli* isolated from chicken offal-fed catfish are more resistant to selected antimicrobials than commercial pellet-fed catfish.

13.0 RECOMMENDATIONS AND FUTURE WORK

For future continuation or related study, it would be beneficial to increase the sample size (e.g., n=30) to produce a more meaningful AMR pattern in order to strengthen and validate the notion stated. Furthermore, include a selection of three isolates of *E.coli* from the same site of each sample instead of one isolate as it would increase emphasis on the interpretation of possible outcomes. Thirdly, future studies may include other antibiotics for AST for the same reason. Next, include ATCC *E. coli* to AST as a quality control. Additionally, the study can be improved by acquiring samples from a more controlled setting in order to minimize risk factors which have the potential to manipulate variables such as water quality, stocking density, and exposure to environmental conditions. Lastly, it may be beneficial to acquire *E. coli* isolated from other organs as well as skin and muscles to investigate *E. coli* AMR pattern differences and prevalence, as well as possible sources of resistance.

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