UNIVERSITI PUTRA MALAYSIA

DETECTION OF ANTIMICROBIAL RESIDUES IN ASIAN SEA BASS (Lates calcarifer) FROM LOCAL MARKETS IN SELANGOR, MALAYSIA

AZYYAN LAILY BINTI ROSLAN

lp
FPV 2020 18

DETECTION OF ANTIMICROBIAL RESIDUES IN ASIAN SEA BASS (*Lates calcarifer*) FROM LOCAL MARKETS IN SELANGOR, MALAYSIA

AZYYAN LAILY BINTI ROSLAN

A project paper submitted to the
Faculty of Veterinary Medicine, University Putra Malaysia
In a partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
University Putra Malaysia Serdang, Selangor Darul Ehsan

2020/2021

CERTIFICATION

It is hereby certified that we have read this project paper entitled — Detection of Antimicrobial Residues In Asian sea bass (*Lates calcarifer*) from Local Markets in Selangor, Malaysia by Azyyan Laily binti Roslan and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

____

ASSOC. PROF. DR. SITI KHAIRANI BINTI BEJO
DVM (UPM), MSc. (UPM), PhD (UPM)
Department of Veterinary Pathology & Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

____

ASSOC. PROF. DR. MD. SABRI BIN MOHD. YUSOF
DVM (UPM), MVSc (UPM), PhD (UPM**)**
Department of Veterinary Pathology & Microbiology
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co Supervisor)

____

DR. SITI ZUBAIDAH BINTI RAMANOON
DVM (UPM), MVSc (GUELPH), PhD MURDOCH)
Department of Large Animals & Exotic Medicine & Surgery
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co Supervisor)

ACKNOWLEDGEMENT

First and foremost, Alhamdulillah I managed to finish my final year project with the help of my supervisor, Assoc. Prof. Dr. Siti Khairani binti Bejo who was always there to help me from the beginning till the end of the project. Thank you for your guidance and patience throughout this project. I would like to also thank to my co-supervisor, Assoc. Prof. Dr. Md. Sabri bin Mohd. Yusof that allow me to use aquatic lab equipment and Dr. Siti Zubaidah binti Ramanoon that helped me throughout the project. Without their supports, I would never been succeed in achieving this milestone.

Other than that, I would like to shows appreciation to my parent who always support me in each and every single little thing and give me support along this journey. Thank you also for the endless dua' and loving me for who I am.

Massive thank you to Bacteriology Laboratory staff Mr. Azri, Miss Krishnammah Kuppusamy and Miss Nur Rabiatuladawiyah Rosli, who always guide and provide materials to me during the lab work.

Not to forget to my FYP mate, Nur Fatimah, Aliaa Hanini , Ann Nur Najeehah , Nur Aqilah Rahmat and Nur Aqilah Anuar that help accompany me to buy fish at the markets and laboratory works. Also, to my close friends and my DVM 2021 classmates that are fighting and surviving these journey with me,

Thank you to all that help me to finish this study and thesis. May Allah bless you.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
^{O} C	Degree Celsius
AMR	Antimicrobial resistance
AMPG	Antimicrobial growth promoter
MDR	Multiple drug resistance
MHA	Mueller-Hinton Agar
FAO	Food and Agriculture Organization
Z.O.I	Zone of Inhibition

ABSTRAK

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

PENGESANAN ANTIMIKROB RESIDU DI DALAM SIAKAP (*Lates calcrifer*) DARIPADA PASAR TEMPATAN DI SELANGOR, MALAYSIA

oleh

Azyyan Laily binti Roslan

2020

Penyelia: Prof. Madya Dr. Siti Khairani binti Bejo

Penyelia Bersama : Prof. Madya Dr. Md. Sabri bin Mohd. Yusof
Dr. Siti Zubaidah binti Ramanoon

Kebelakangan ini, akibat faktor pertambahan populasi dan kesedaran akan pentingnya penjagaan kesihatan dalam kalangan masyarakat, maka ikan telah diperakukan sebagai sumber protein haiwan yang lebih sihat. Oleh itu, penggunaan agen antimikrobial dalam penternakan ikan adalah untuk menggalakkan pertumbuhan ikan yang pesat. Selain itu, agen antimikrobial ini juga digunakan dengan tujuan untuk mencegah pertumbuhan tumbuh-tumbuhan herba dan penyakit ikan secara berlebihan. Walau bagaimanapun, penggunaan agen mikrobial secara sewenang, kegagalan para penternak untuk mematuhi dos penggunaannya dan tempoh menghentikan pemberian ubat antimikrobial dalam akuakultur yang tidak betul boleh menyebabkan pemendapan ubat yang tidak diingini pada tisu otot ikan yang boleh dimakan. Sebenarnya, kebimbangan akan penggunaan antimikrobial ini ialah residue antimikrobial dan ketahanan antimikrobial pada bakteria akan dapat dipindahkan kepada pengguna yang boleh menyebabkan risiko kesihatan kepada masyarakat. Antara risiko kesihatan yang didokumentasikan pada manusia ialah kesan alergi, toksik, mutagenik, teratogenik atau karsinogenik akut atau kumulatif. Oleh itu, kajian ini dilakukan untuk menyiasat kehadiran residu antimikrobial pada ikan siakap yang diperoleh dari pasar-pasar di Selangor. Sejumlah 30 ekor ikan diperoleh daripada 10 pasar iaitu tiga sampel ikan dari setiap pasar. Semua sampel dianalisis menggunakan ujian perencatan mikrobiologi dan menggunakan *Eschericia coli* ATCC 25922 dan *Salmonella spp* ATCC 14028 sebagai organisma ujian. Hasil kajian ini menunjukkan tiada residu antibiotik pada mana-mana sampel kajian. Daripada kajian ini, dapat disimpulkan bahawa daging ikan siakap dari pasar tempatan di Selangor tidak mempunyai bahaya residu antibiotik dan selamat untuk dimakan oleh masyarakat umum.

KEYWORDS: *Antimikrobial residu, siakap, akuakultur, ketahanan antimikrobial*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

DETECTION OF ANTIMICROBIAL RESIDUES IN ASIAN SEA BASS (*Lates calcarifer*) FROM LOCAL MARKETS IN SELANGOR, MALAYSIA

By

Azyyan Laily binti Roslan

2020

Supervisor: Assoc. Prof Dr Siti Khairani binti Bejo

Co-Supervisor: Assoc. Prof. Dr. Md. Sabri Mohd. bin Yusof
Dr. Siti Zubaidah binti Ramanoon

Over the past years, with the growing population, increasing affluence, and the recognition that fish was actually the healthier source of animal protein. The application of antimicrobial agents in fish farming has been increasingly used to prevent the overgrowth of herbal plants and fish diseases with addition to promoting the rapid growth of the fish. However, improper usage and failure to comply with dosage and withdrawal period of antimicrobial drugs in aquaculture could cause undesirable deposition of drugs in edible tissues of fish muscle. The primary concern regarding the usage of antimicrobial was the antimicrobial residues and the emergence of antimicrobial resistance in bacteria that may be passes to consumers and cause significant health risk to public. This present study was conducted to investigate the presence of antimicrobial residues in Asian sea bass (*Lates calcarifer*) from local markets in Selangor. A total of 30 Asian sea bass were sample from 10 local markets which comprised of three samples from each market. All of the samples were analysed using microbiological inhibition test using Eschericia coli ATCC 25922 and Salmonella spp ATCC 14028 as test bacteria. The result revealed no antibiotic residues in any of the samples tested in this present study. It was suggested that Asian sea bass meat from local markets in Selangor has no antibiotic residues and considered safe for the consumption of public.

Keywords: Antimicrobial residues, Asian sea bass, aquaculture, antimicrobial resistance

1.0 INTRODUCTION

Overview

Fish is an important supply of food for human consumption and is known as a very good source of animal protein consumed by the world's population. Malaysian, in particular through a report of food consumption pattern of adult shows that fish are eaten in the quantity of one and a half medium of fish every day, at least once a day (Ahmad et al., 2008). As a matter of fact, annual per capita consumption of fish was estimated to be 56kg making Malaysian the second highest after Japan, among Asian nations, or ranked number five throughout the world (York & Gossard, 2004). Over the past year, aquaculture has grown rapidly in Malaysia which is now has become one of major production sector. It has seen as a way to increase local food production and export revenues. More culture practices are being integrated in the country in order to meet the demand for fish, with the main method is brackish water aquaculture followed by freshwater pond and marine aquaculture (FAO, 2016)

Many new species are being introduce to the country that are fit for human consumption. Asian Sea Bass or also known as Barramundi are one of the contributing species to the aquaculture sector. It is an anadromous species that can adapt to both freshwater and marine environment due to its euryhaline charateristics and is widely cultivated and marketed especially in South East Asia (Paterson et al., 2003). In order to meet local and global market demands, many sea bass hatcheries and farms are

Study Background

The used of pesticide and antimicrobial agents in fish farming has been increasingly to prevent the overgrowth of herbal plants and fish diseases with addition to promoting the rapid growth of the fish (Ibrahim et al., 2010). Antimicrobial drug is defined as an agent that is used to kill micro-organism or suppress their multiplication growth and these agents can be grouped into antibiotics, antifungals, antiprotozoal, and antiviral drugs. Antimicrobial drugs have been very beneficial for human and veterinary healthcare. These drugs are either used as therapeutic treatment individually or as prophylactic treatment against the onset of certain disease. Fluoroquinolones, tetracyclines, penicillins, sulphonamides and other antibiotics, exhibiting activity against both Gram-positive and Gram-negative bacteria, are widely used for the treatment and prevention of diseases in fish (Rasheed et al., 2014).

Over the last decades, there has been a concern over the problem of antibiotic resistance in human pathogen. The concern has been directed to the use of antibiotic in food producing animal especially due to its possible impact on human health. Improper usage and failure to comply with the instruction (dosage and withdrawal period) of antimicrobial drugs in aquaculture could cause undesirable deposition of drug residues in edible tissues of fish muscle which could create public health risks to the consumers (Mensah, 2014) even after harvesting and sold in markets. Studies have shown wide variety of antimicrobial used in large amounts could result in accumulation of residues in aquaculture products, which can eventually lead to a series of adverse effects on human health (Manage, 2018). Among the health risk documented in human is acute or cumulative allergic, toxic, mutagenic, teratogenic or carcinogenic effect (Olatoye & Basiru, 2013)

Also, indiscriminate use of antibiotic can promote the emergence of drug resistant microorganisms known as antimicrobial resistant (AMR) even if it presents at levels well below regulatory limits. Frequent antibiotic usage over long periods of time puts selective pressure on bacteria by killing susceptible bacteria, allowing antibiotic-resistant bacteria to survive and multiply. Misuse, overuse and administering antibiotics to animals without professional prescription are all linked to AMR. Moreover, monitoring programme of antibiotic residues that has been implemented by Department of Veterinary Services (DVS) since year 2013 were only extensively involved species such as poultry, bovine, porcine and goat/sheep (Hassali et al., 2018). The application of antimicrobials in aquaculture sector is largely undocumented and unregulated. The possibility of unaccepted residues in the products may be more likely to occur.

In Asia, sea bass production that are sold in local markets that are for public consumption does not limited to aquaculture only but also caught from the wild. This might lead to no antimicrobial being used directly on the fish if it is caught in the wild. Despite this, wild aquatic animal could still be exposed to resistant bacteria or antibiotic residue from the environment based on study of (Hansa & Halden, 2015), which could potentially lead to accumulation of residues in fish edible part. Hence, even though the fish are not directly exposed to the antimicrobial agents, the possibility for the occurrence of residues in wild Asian sea bass cannot rule out.

Justification

Global development and the usage of antimicrobials in livestock farming (milk, meat animals and poultry) are well documented. As for borderline information regarding the usage of these drugs in aquaculture, further investigation is required to assess the exact quantities of antibiotics were used.

Currently, there is no data regarding the antimicrobial residues of locally consumed sea bass in Selangor. The risk of drug residue effect may be higher if the trend of abuse and misuse of drugs is left unchecked. Thus, method in detecting antimicrobial residue has been developed and practiced worldwide to ensure the safety of food is essential to prevent and control problem related to health and safety of public. Accordingly, the present study is to investigate the occurrences of antimicrobial residue in edible fish part of sea bass sold from local markets that are direct for human consumption. This is to illustrate the responsibilities of farmer to strictly follow the right procedure for harvesting the fish and to raise awareness to public regarding the health threat from the consumption of these antimicrobial residues.

OBJECTIVE

To determine the presences of antimicrobial residues in Asian sea bass collected from local markets in Selangor.

HYPOTHESIS

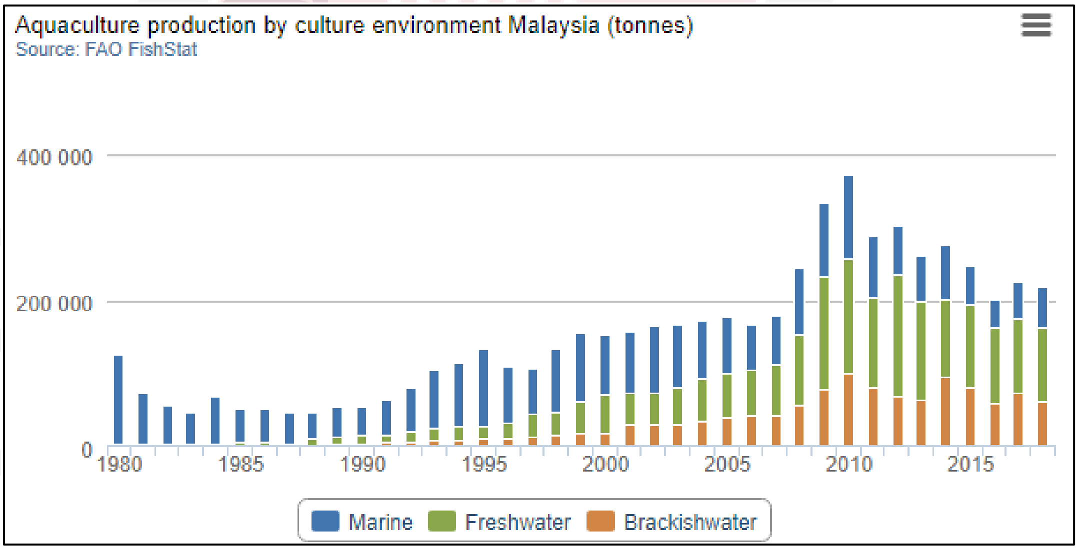
Null hypothesis: There is no antimicrobial residues in Asian sea bass sold from selected local markets in different district in Selangor.

Alternative hypothesis: There is antimicrobial residues in Asian sea bass sold from selected local markets in different district in Selangor.

2.0 LITERATURE REVIEW

2.1 Aquaculture in Malaysia

The term aquaculture refers to farming of aquatic organism including fish, mollusc, crustaceans and aquatic plants. Aquaculture in Malaysia began in 1920's, where it quickly developed and becoming important activity with annual growth rate of about 10 percent in the last 5 years. Several culture practices were being integrated in the country such as marine aquaculture and freshwater pond aquaculture but brackish water aquaculture is the main practice in Malaysia as shown in Figure 1. A large range of species, including freshwater species, shellfish and marine finfish are being cultivated (FAO, 2004)



(FAO, 2016)

Figure 1 shown types of aquaculture practices in Malaysia and their productions from (1980-2015)

2.1.1 Cage culture

In Malaysia, cage culture is a well-established grow out production system, where the marine fish are reared in floating net pens (Kechik, 1995). This type of culture system uses existing water resources in the offshore, lagoon or in brackish river water where the uses of cages allows water to pass freely between the enclosed sea bass. Most farms in Peninsular Malaysia fed the sea bass with trash fish and uses the traditional cage system where it is made from wood (Aripin et al., 2020).

2.1.2 Pond culture

Pond culture is standard intensive farming production system that were developed since the early 1980s, due to problem that arise from the traditional cage culture systems such as limited sites for cage culture production. In this intensive system, water is maintained in an enclosed area by artificially constructed pond where the sea bass is reared and were built in a way that the brackish water from river or ocean can access (Aripin et al., 2020). The ponds may be filled with canal water, rain water or from other water resources.

2.2 Asian sea bass

Lates calcarifer commonly known as giant sea perch, Asian sea bass or barramundi can be found in the tropical and subtropical region of Asia- Pacific such as Thailand, Indonesia and Singapore. It is a popular aquaculture fish that have high economic value. Due to its as hardy and euryhaline characteristics, it is deemed as suitable species for saline water and freshwater aquaculture. Sea bass production has grown exponentially over the past decade following the success of private hatcheries. In

Malaysia, Asian sea bass has been reported as the highest cultured marine fish (Kandan, 2015).

According to (FAO, 2006), the characteristics that make sea bass as an ideal species for aquaculture is due to (i) it is a relatively hardy species that have wide physiological and crowding tolerances (ii) female fish provides plenty of material for hatchery production of seed due to its high fecundity traits (iii) relatively simple hatchery production of seed (iv) they feed well on pelleted diets, and juveniles are easy to wean to pellets and (v) in six months to two years. Sea bass can grow rapidly reaching a harvestable size (350 g – 3 kg).

2.3 Bacterial disease associated with cultured sea bass

Several bacterial disease have been reported in sea bass cultured. Some of the studies are *Streptococcus iniae* infection (Bromage & Owens, 2002) that causes severe losses to marine cages barramundi in Australia , *Pseudomonas anguiliseptica* possible agent that cause mass mortality in marine caged reared sea bass in southern Peninsular Malaysia (Nash et al., 1987), *Vibrio harveyi* that causes vibriosis to open net cages sea bass in Sabah (Ransangan, Mohammad & Al-Harbi, 2012), *Aeromonas hydrophila* that causes symptoms of hemorrhagic septicaemia in Istanbul (Dinçtürk, Tanrikul & Çulha, 2018) , Columnaris disease caused by *Flavobacterium columnare* and various bacteria that could cause bacterial gill disease in sea bass (FAO, 2006). All of these disease could be link to the used of antibiotic in aquaculture as treatment.

2.4 Food borne pathogen

2.4.1 *Escherichia coli*

Escherichia coli are gram-negative bacilli belong to *Enterobacteriaceae*. They are rod- shaped and arranged in chains or pairs (Sajid et al., 2016). *Escherichia coli* is the most frequently found facultative anaerobic bacteria species in the microflora of gastrointestinal tract in human and animals. They are typically a harmless microbe however it is also a medically important species that causes a number of serious illnesses reported. Antibiotic resistance in *E. coli* is of particular concern as it could cause treatment of bacterial infections more difficult in terms of longer hospitalisation and high treatment cost (Rasheed et al., 2014).

2.4.2 *Salmonella* spp.

Salmonella spp. is a gram negative, facultative anaerobic bacillus species belonging to the *Enterobacteriaceae*. The pathogen is ubiquitously present in the human food chain, and is frequently associated with outbreaks of foodborne disease. Antibiotic resistance in foodborne pathogens such as *Salmonella* spp. is a major concern for public health safety (Nair et al., 2018). Many studies have been reported that these non-typhoidal *Salmonella* spp. causes the highest number of illnesses, hospitalizations, and deaths associated with foodborne illness (Scallan et al., 2011) which in turn cause a life threatening situation in human if already acquire the resistance bacteria.

2.5 Antimicrobial

2.5.1 Definition of antimicrobials

The main groups of veterinary medicinal products are antimicrobial that are used since 1950s to treat bacterial infectious disease in both food producing animals and also companion animals (Mensah, 2014). Antimicrobial drug is defined as an agent that is used to kill micro-organism or suppress their multiplication growth and these agents can be grouped into antibiotics, antifungals, antiprotozoal, and antiviral drugs. Antibiotics can be derived from natural sources or have synthetic origin (Asma, 2017). These drugs act by selectively inhibit the cell wall synthesis and other membranes involves, the synthesis of macromolecules and the activity of enzyme in prokaryotic cells (Guardabassi & Courvalin, 2006) . They are administering in order to prevent or to treat infectious disease that could cause significant morbidity and possible mortality.

2.5.2 Antimicrobials use in Aquaculture

Due to integrated farm system of aquaculture, the animals are reared in a group , indoors and often have very high stock densities that could increase the risk of disease outbreak (Mensah, 2014). This is because the organism is continuously stressed in terms of environment, salinity, feeding and management of the farms. In order reduce the fast spread of diseases, a wide range of medication are prescribe including vaccines, antibiotics, anti-parasites, antifungal agents, and immunostimulants (Wardle and Boetner, 2012). In the aspect of veterinary medicine and animal husbandry, the three types of treatment includes preventive therapy (prophylaxis) where the drugs is given to the animals when the chance of animals getting the disease is high, therapeutic treatment that were given to already sick animals and metaphylaxis which is giving drugs to a group of animals in advance of an expected disease outbreaks (Labro, 2012).

In aquaculture, antibiotic is usually incorporated in feeds, often as bath and a rarely through injection as it is time consuming and require labour work. Blinding antibiotic with specially formulated fish feed that are either added during manufacture or surface-coated onto pellets by the farmer itself or manufacturer is the most well-known route of antibiotic in fish. Many of these antibiotic contain stable chemical compounds that are not effectively metabolized by fish and remain active where they largely pass into the environment after being excreted in faeces and urine (Amsah, 2017). About 75 percent of the antibiotics that were fed to fish are estimated to be excreted into the water. The studies also reported that there is considerable amount of antibiotic accumulating in the sediments at the bottom of the farm as antibiotic will also be loss to environment through undigested waste feed falling (Burridge et al., 2010). Malaysian Action Plan on Antimicrobial Resistance (MyAP-AMR) 2017-2021 Report, data regarding antimicrobials usage in aquaculture is still limited. At present, there is no register or list of the permitted antibiotics to be used by the aquaculturist in Malaysia. Off-label use of antimicrobials may be significant and may also result in the use of a wider range of agents.

2.5.3 Effect of improper antimicrobials use

It is well known that in the process of food producing animals, the extensive use of antimicrobials has led to the spread of antimicrobial resistant bacteria and resistance genes due presence of residues in the animal's product (Baquero, Martínez, & Cantón, 2008). Unethical used of these drugs in animal production has led to the emergence of resistant bacteria which possess risk to public health as these genes can be transmitted to human through environment, food consumption or by direct contact with the infected meat (Vishnuraj et al., 2016). These resistant bacteria genes and antimicrobial residues have been detected in many studies in living chickens, bovines, and fish as well as in related food products.

2.6 Antimicrobials residues

Definition of drug residues is used to describe under certain circumstances the amount of medication used can be distinguished in tissue after the drug has been discontinued (Burgat-Sacaze, Delatour, & Rico, 1981). In veterinary drug contexts, antimicrobial residue is used to describe the extensive use antimicrobials drug in aquaculture for the treatment of bacterial disease that may result in deposition of their residues in edible tissues which could hamper public health to some extents (Barman et al., 2017).

2.6.1 Antimicrobials residue in seafood product

Previous studies have proven the occurrences of antimicrobial residues from aquaculture product sold from the markets. Barman et al., (2018) reported that, oxytetracycline residues were detectable from five samples out of 24 tilapia sold in local markets in Bangladesh. The reports also state 19 of the samples have detectable residues that were lower than limit of detection according to the European Commission. However, long term presence of high level oxytetracyclines could be a potential hazardous for public health. Another report from United States, five out of 47 antibiotics were detected in grocery bought seafood product (Hansa & Halden, 2014).The most commonly detected antibiotic compound is oxytetracycline. One study in Gaza , Palestine detect residues from fish in local markets that are direct for human consumption (Amsa, 2017) . The most detected antibiotic residues were aminoglycosides in sea bream, red tilapia and Nile tilapia. followed by tetracyclines in sutchi catfish fillet and negative results for β-lactams and macrolides.

In Malaysia , one study was done to assess the chemical risk assessment from farm to table of freshwater aquaculture product. The study found that only 5.8% of fish sample collected directly from farms have low level of detectable residues. Antibiotic residues were not detected in all 240 (100%) of samples taken from markets and food premises. This report conclude that there is very low risk of antibiotic residues in freshwater aquaculture fish which are sold at markets in Malaysia (Ibrahim et al., 2010). Study were also done to assess residues in catfish in Nigeria. According to (Olatoye & Basiru, 2013), the study conclude that catfish consumed in Ibadan, Nigeria has appreciable amount of residues that could cause antibiotic residue risks and food safety consequences.

2.6.2 Effect of antimicrobial residues to human

The existence of antibiotic residues in food stuff can pose hazards to human health by altering the intestinal microflora through the emergence of resistant strains to commonly used antibiotics and promoting the development of acquired resistance in pathogenic enteric bacteria (Amsah, (2017). In susceptible individuals, these residues can cause detrimental effect such as acute or cumulative allergic, mutagenic, toxic, teratogenic or carcinogenic effect when they eat meat that contain antibiotic residues. In addition , other than health problem concerning the transfer of antibiotic resistant strains of bacteria to the human beings, the major pathological effects produced by antibiotic residues in food include immunological effects, autoimmunity disorders, arcinogenicity especially due to sulphamethazine, oxytetracyclines, and furazilidone, mutagenecity, nephropathy due to gentamycin, hepatotoxicity, reproductive, disorders, bone marrow toxicity due to chloramphenicol and allergy due to penicillin (Nisha, 2008).

2.7 Withdrawal period

Unethical use of antimicrobials in animal production leads to development of resistant bacteria

and which later transmit to human through food, environment or by direct contact through affected meat. In order to protect the safety of public health, withdrawal periods for such compounds when using in animal production were established, Withdrawal period is a time between the last dose of antibiotic given to food animals and consumption of food animals or food derived from it. These period need to be mentioned and take noted for every antimicrobial used in food producing animals. (Marangunich et al., 2003)

2.8 Antimicrobial resistance

Antimicrobial resistance (AMR or AR) is the ability of a bacterium to resist the effects of the antimicrobial that previously it was sensitive to**.** Antimicrobial resistance is an increasing problem for both human and veterinary medicine (Witte, 1998). There are many factors that can lead to AMR such over-prescribing of antibiotics, the intake of antibiotics prescribed are not taken based on the regime, overuse of antibiotic in especially in aquaculture due to overcrowded fish where the outbreak of diseases can happen. These factors will lead to selection pressure of antibiotic use in aquaculture (Marshall & Levy, 2011). The effect of the increasing selection pressure is worrying as it causes only a limited number of approved drugs are available (Pham et al., 2015). The misuse, overuse and also given without professional prescription of antibiotic might be one of the driven factors for AMR emergence that results in the ability of the organism to resist the effect of the antimicrobial agent (WHO, 2015). The principle of this can be due to exposure of organism towards the different types, concentration, and frequencies to the antimicrobial agents. The misuse of prophylactic agents is popular in developing countries in particular due to the lack of information, guidance on the proper use of antibiotics to the farmers and effective rules regulating the administration of antibiotics used in aquaculture (Olatoye & Basiru, 2013).

Those resistant-antibiotic bacteria can be transmitted to human; (i) via direct contact with animals, (ii) by environment spread via water stream and soil and in most cases (iii) consumption of animal based food product (Kane, 2012). The resistant bacteria could then cause disease that is difficult to treat in humans and may also transfer the resistant gene to some other human pathogens. The main problem of antibiotic resistant in human and animal population is ineffective antibiotic therapy against causative pathogen during disease course which at times severity is hasten by intruders of more virulent agent consequent to untreatable infection (IFT, 2006).

2.9 Screening technique to detect antimicrobial residues

Screening of antibiotic residue in edible animal products are of great concern to regulatory agencies and consumers, therefore, reliable screening method for rapid, selective detection of these residues are necessary to ensure food safety. Generally, there are 6 types of methods in detecting the antibiotic presence in food product which are; (i) microbial inhibition assay (ii) microbial receptor assay (iii) enzymatic colorimetric assays (iv) receptor binding assay (v) chromatographic methods and (vi) immunoassays. (Biswas et al., 2009).

2.9.1 Microbial Inhibition assays

Microbial inhibition assay were the earliest methods used for detection of antibiotic residues and still widely used. This test is cost effective and able to cover broad spectrum of antibiotic (Pikkemaat, 2009). The two main test format consist of tube test and plate test. Tube test are integrated with dye indicator and supplemented with a pH, causing colour change when acid is produced by live test microorganism (Vermont et al., 1993). Thus, absence or delayed colour change is highly suspicious for antibiotic residues contaminant. The theory of a plate test is that the samples of interest is applied on top of a layer of inoculated agar or in wells in the agar. Bacterial growth will turn the agar into an opaque layer, which if the samples contains antimicrobial substances will result in clear growth-inhibited area around the sample. Suspected positive cases will result in formation of inhibition zone on the bacterial cultured plate due to inhibitory effect of antibiotic in sample towards the microorganism (Karrauan et al., 2009). However, the limitation of agar diffusion tests is that the results are usually obtained only after an overnight incubation. It is also necessary to make reference that all positive results were with unknown antimicrobial substance, because this method does not allow differentiation of the type of drugs (Pavlov et al., 2009).

3.0 MATERIAL AND METHOD

3.1 Sea bass (*Lates calcarifer*) fish sampling

A total of 30 Asian sea bass were sample from 10 local markets in Selangor from 18th until 27th August 2020. The sample were transported in a cooler box to Bacteriology Laboratory of Faculty Veterinary Medicine for analysis. The sample were kept in freezer at -20°C for two hours before sample processing for better handling and processing of the samples. Approximately 2 mm thick and 10 mm diameter fish meat samples were sliced into a disc shape using a scalpel blade.

3.2 Detection of antimicrobial residue in sea bass (*Lates calcarifer*) fish

The detection of antimicrobial residues in fish meat samples was done by using microbial inhibition technique describe by Babapour et al., (2012) with some modification in terms of antibiotic used as controls and the test organism. *Eschericia coli* ATCC 25922 and *Salmonella* spp ATCC 14028 were obtained from Bacteriology Laboratory and used as test organism. Bacteria suspension were made by culturing one or two colonies of the test bacteria into a 5ml of nutrient broth respectively for 24 hours at 37°C. Then the suspension were compared with 0.5 McFarland Standards with concentration of 1.5×10^{8} / ml (Soepranianondo & Wardhana, 2019). A sterile swab was dipped into the suspension tube. The swab was rotated against the side of the tube to remove excess fluid and were then inoculated the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface. The plate was rotated approximately 60 degrees each time to ensure an even distribution of the suspension.

Three fish meat were placed on the surface of inoculated agar medium. Kanamycin 30µg and doxycycline 30µg were also included as control. The plates were incubated at 37°C for 24 hours and later were visually analysed for the presence of inhibition zone. Complete inhibition zone not less than 2mm in diameter around the fish meat samples were suggested of positive result (Karrauan et al., 2009).

4. 0 RESULT

All fish meat samples tested using the microbiological inhibition test assay were absent of inhibition zone. Positive control clearly showed inhibition zone surrounding antibiotic disc.

Figure 1. Picture of microbial inhibition technique showed inhibition zone surrounded kanamycin 30µg and doxycycline 30µg on agar surface inoculated with *Escherichia coli* ATCC 25922. No inhibition zone observed around fish meat sample.

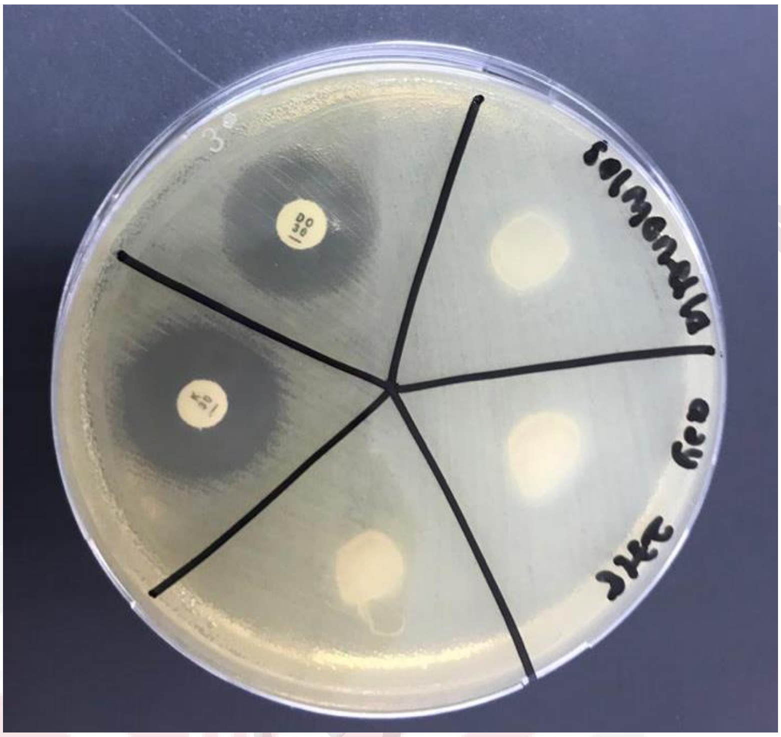


Figure 2. Picture of microbial inhibition technique showed inhibition zone surrounded kanamycin 30µg and doxycycline 30µg on agar surface inoculated with *Salmonella* spp ATCC 14028. No inhibition zone observed around fish meat sample.

5.0 DISCUSSION

Antibiotic residues presence in food primarily as a consequences of therapeutic treatment for animals or animal feed supplementation. The presence of these residues has captured the attention of local, national and international public health organisation as it possess a public health risk concern. When human consume a large quantity of the fish and fish product that have been exposed to antibiotic contaminant may lead to detrimental changes in intestinal microflora and in susceptible person could create immunological response reactions (Mottier et al., 2003).

The present study used microbiological inhibition test which were the earliest methods used to detect antibiotic residues from meat samples such as poultry and cattle but are still widely used. This is because this method is very cost effective, simple to perform and have the characteristics to cover entire antibiotic spectrum within one test (Pikkemaat, 2009). However the disadvantages of these method is that if there is a inhibition zone surrounding the meat samples, it cannot tell whether the detected residues concentration were either above or lower than maximum residue limits (MRL), which is the amount of residues permitted in animal food product . This method also unable to identify the types of drug residues presence in the samples (Pavlov et al., 2009).

In this study, the antibiotic residues of fish meat were examined to investigate the presence of antibiotic residues of Asian sea bass (*Lates calcarifer*) presented for direct human consumption in the local markets of Selangor. This study is to determine whether fish consume from local markets are safe to be consumed and do not possess public health risk. Asian sea bass was selected due to its popularity and high demands especially from hotels, catering and restaurant. From the experiment conducted, there were no antibiotic residues presence in all 30 (100%) samples taken from local markets in Selangor. This situation suggests that there is very low risk of residues in Asian sea bass fish which are sold at markets. These findings correlate with Ibrahim et al., (2010), where they also asses chemical risk in freshwater fish namely red *tilapia* (*Oreachromis* sp. red hybrids), *keli* (*Clarias* spp.) and *patin* (*Pangasius sutchii*)]. The result showed that there were no pesticide and antibiotic residues in all 240 (100%) of samples taken from markets. These study also indicate that there is very low risk of antibiotic residues in freshwater aquaculture product which are sold at markets in Selangor. The no antibiotic residues detected in these study could be due to the source of Asian sea bass in the market itself. Mainly, there were three main origin or source of Asian sea bass in the market in Selangor. The fish could be originated from aquaculture farm that are antibiotic- free as the prevalence of antibiotic usage in aquaculture Malaysia is still low. This mean that the farmers does not used any remedial drugs in the fish farms to control disease.

As Malaysia fisheries sector is still depending on caught fish to meet the demand of consumers, these Asian sea bass sold in markets could be wild caught fish which makes them less likely to be exposed to antibiotic contaminant. However, we cannot completely rule out the occurrence of antibiotic residues in wild fishes as there a study that have been reporting these phenomena.

Oxytetracycline residues have been detected in wild caught shrimp imported from Mexico Done & Halden, (2014) This could be due to possibility of cross contamination that occur during handling, processing and packaging. These residues could also originate from the uptake of drugs from coastal waters and sediments impacted by raw and treated wastewater inputs (Kim & Carlson, 2007). Wild fish could be exposed to these antibiotic residues through eating the left-over food pellets from the marine fish farm which are medicated with antibiotic (Vignesh et al., 2011). Many research has confirmed that these wild fish do ingest the residual feed pellets that lead to passing of these residues. Study found that tetracycline and quinolones residues was detected in wild fishes capture in aquaculture areas (Ten dencia et al., 2001).

Another source of Asian sea bass is from commercial farm that practiced antibiotic as prophylaxis or preventive measure of herd health. However, no positive antibiotic residues among the sea bass samples found in this study could be attributed to the local farmers follow the recommended procedures for using antibiotics in terms of dosage and routes of antibiotic at their fish farms and they are aware regarding the withdrawal period that can ensure there is no residues left in fish before harvesting the fish to be sold in markets for direct human. Antibiotic residues were not detected in this present study. Therefore, it was suggested that Asian sea bass that was sold in selected local markets in Selangor are safe to be consumed.

6.0 CONCLUSION

The result of the study showed that there were no antimicrobial residues found on all 30 Asian sea bass fish samples collected from 10 markets in Selangor. It can be concluded that the Asian sea bass that are sold in local markets in Selangor was free to antibiotic residues. However, taking into account the short duration of sample collection and limited number of samples, the total assumption of antimicrobial residual substances in this project may not truly reflecting the whole scenario. Regular and comprehensive surveillance of antibiotics in food would decrease the likelihood of getting the resistance through the digestion of contaminant in long period of time. Also, monitoring the attitude of the food producers (example the farmers) administering the antibiotics in food product is a must to control the residues and other adultery contaminant.

RECOMMENDATIONS

In the future studies, it was suggested to detect the presence of antimicrobial residues in the farm level as the small amount of antibiotic residues which may exist in fish at the farm level may be totally dissolved by the time they arrive at markets. The microbiological inhibition test method is one of the alternative ways to detect antibiotic residues. More sensitive and specific methods can be implemented in the future such as liquid chromatography or the commercial test kit to detect antibiotic residues in fish meat.

REFERENCES

Ahmad, N. I., Wan Mahiyuddin, W. R., Tengku Mohamad, T. R., Ling, C. Y., Daud, S. F., Hussein, N. C., Abdullah, N. A., Shaharudin, R., & Sulaiman, L. H. (2016). Fish consumption pattern among adults of different ethnics in Peninsular Malaysia. *Food & nutrition research*, *60*, 32697.

Aidara-Kane A. Containment of antimicrobial resistance due to use of antimicrobial agents in animals intended for food: WHO perspective. *Rev Sci Tech*. 2012;31(1):277-287. doi:10.20506/rst.31.1.2115

Ammarr Aripin, Louisa Coglan, Sean Pascoe & Viet-Ngu Hoang (2020) Productive efficiency and capacity utilization of sea bass grow-out culture in peninsular Malaysia, Aquaculture Economics & Management, 24:1, 102-121, DOI: 10.1080/13657305.2019.1661045

Alla, M. B. W., Mohamed, T. E., & Abdelgadir, A. E. (2013). Detection Of Antibiotics Residues In Beef In Ghnawa Slauterhouse, Khartoum State, Sudan. *Journal Of Veterinary Medicine And Animal Production*, *2*(1).

Babapour, A., Azami, L., & Fartashmehr, J. (2012). Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. *World Appl Sci J*, *19*(10), 1417-1422.

Cultured Aquatic Species Information Programme. Lates calcarifer. Cultured Aquatic Species Information Programme. Text by Rimmer, M.A. In: *FAO Fisheries Division* Balizs, G., & Hewitt, A. (2003). Determination of veterinary drug residues by liquid chromatography and tandem mass spectrometry. *Analytica Chimica Acta*, *492*(1), 105-131.

Bandyopadhyay, Samiran, Tapas K. Biswas, Devasis Sasmal, Monoj K. Ghosh, Tapan K. Dutta, Suresh C. Das, Debasis Bhattacharya et al. "Virulence gene and antibiotic resistance profile of Shiga-toxin-producing Escherichia coli prevalent in captive yaks (Poephagus grunniens)." *Veterinary microbiology* 3, no. 138 (2009): 403-404.

Baquero, F., Martínez, J.-L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current opinion in biotechnology*, *19*(3), 260-265

Bromage, E. S., Thomas, A., & Owens, L. (1999). Streptococcus iniae, a bacterial infection in Asian seabass Lates calcarifer. Diseases of Aquatic Organisms, 36, 177–181.

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. *Aquaculture*, *306*(1-4), 7-23.

Chuah, L. O., Effarizah, M. E., Goni, A. M., & Rusul, G. (2016). Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Current environmental health reports*, *3*(2), 118-127.

Dayan, A. D. (1993). Allergy to antimicrobial residues in food: assessment of the risk to man. *Veterinary microbiology*, *35*(3-4), 213-226.

Dinçtürk, E., Tanrikul, T. T., & Çulha, S. T. (2018). Fungal and Bacterial Co-Infection of Sea Bass (Dicentrarchus labrax, Linnaeus 1758) in a Recirculating Aquaculture System: Saprolegnia parasitica and Aeromonas hydrophila. *Aquatic Sciences and Engineering*, *33*(3), 67-71.

Done, H. Y., & Halden, R. U. (2015). Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States. *Journal of hazardous materials*, *282*, 10-17.

El Siqali, A. (2017). microbial quality and antibiotic residues of fish sold in the gaza strip, palestine. *microbial quality and antibiotic residues of fish sold in the gaza strip, palestine*.

Foodborne illness acquired in the United States--major pathogens. *Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM Emerg Infect Dis. 2011 Jan; 17(1):7-15.*

Guardabassi, L., Courvalin, P., 2006. Modes of antimicrobial action and mechanisms of
bacterial resistance. In: Aarestrup, F.M. (Ed.), Antimicrobial Resistance in Bacteria
of Animal Origin. ASM Press, Washington, D.C., pp. 1–18.

Hind, A. E., Adil, M., & Samah, A. (2014). Screening of antibiotic residues in poultry liver, kidney and muscle in Khartoum State, Sudan. *studies*, *1*, 4.

Ibrahim, A. B., Mohd, K. A., Ayob, M. Y., and Norrakiah, A. S. (2010). Pesticide and antibiotic residues in freshwater aquaculture fish: chemical risk assessment from farm to table. *Asian Journal of Food and Agro-Industry*, *3*(3), 328-334

Kandan, S. (2015). Culture of Sea bass (*Lates calcarifer*) in Cages in Ponds. In *Advances in Marine and Brackishwater Aquaculture* (pp. 89-93). Springer, New Delhi.

Karrauan B, B Bouchhrif, N ziyate, A Talrni, KI Sidiyahia, N Cohen and A Fassouaune (2009). Evaluation of multi-plate microbial assay for the screening of antibacterial residues in poultry muscles. European journal of scientific research, 35(2): 311- 317.

Kathleen, M. M., Samuel, L., Felecia, C., Reagan, E. L., Kasing, A., Lesley, M., & Toh, S. C. (2016). Antibiotic resistance of diverse bacteria from aquaculture in Borneo. *International journal of microbiology*, *2016*.

Kechik, I. A. (1995). Towards sustainable aquaculture in Southeast Asia and Japan: Proceedings of the Seminar-Workshop on Aquaculture Development in Southeast Asia,
Iloilo City, Philippines, 26–28 July, 1994-Aquaculture Department, Southeast Asian
Fisheries Development Center, pp. 125–135.

Kungvankij, Pinij, Leonardo B. Tiro Jr, Beato J. Pudadera Jr, and Ihra O. Potestas. "Biology and culture of sea bass (Lates calcarifer)." (1986).

Labro M.T. (2012). – Immunomodulatory effects of antimicrobial agents. Part I: antibacterial and antiviral agents. *Expert Rev. anti-infect. Ther*., **10** (3), 319–340

Le, T.; Munekage, Y.; Kato, S. Antibiotic resistance in bacteria from shrimp farming in mangrove areas. Sci. Total Environ. 2005, 349, 95–105

Lee, S. W., Najiah, M., & Wendy, W. (2010). Bacterial flora from a healthy freshwater Asian sea bass (Lates calcarifer) fingerling hatchery with emphasis on their antimicrobial and heavy metal resistance pattern. *Veterinarski arhiv*, *80*(3), 411-420.

Manage, P. M. (2018). Heavy Use of Antibiotics in Aquaculture; Emerging Human and Animal Health Problems–A review. Sri Lanka J. Aquat. Sci. 23(1) (2018): 13-27

Mangsi, A. S., Khaskheli, M. U. H. A. M. M. A. D., Soomro, A. H., & Shah, M. G. (2014). Antibiotic residues detection in raw beef meat sold for human consumption in sindh, Pakistan. *Inter J Res Appl Nat Soc Sci*, *2*, 15-20.

Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev. 2011;24(4):718–33.

Mensah, S. E., Koudande, O. D., Sanders, P., Laurentie, M., Mensah, G. A., & Abiola, F. A. (2014). Antimicrobial residues in foods of animal origin in Africa: public health risks. *Revue Scientifique et Technique (International Office of Epizootics)*, *33*(3), 987-96.

Mohamed, S., Nagaraj, G., Chua, F. H. C., & Wang, Y. G. (2000). The use of chemicals in aquaculture in Malaysia and Singapore. In *Use of Chemicals in Aquaculture in Asia: Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996, Tigbauan, Iloilo, Philippines* (pp. 127-140). Aquaculture Department, Southeast Asian Fisheries Development Center.

Mottier, P., Parisod, V., Gremaud, E., Guy, P. A., & Stadler, R. H. (2003). Determination of the antibiotic chloramphenicol in meat and seafood products by liquid chromatography–electrospray ionization tandem mass spectrometry. *Journal of chromatography A*, *994*(1), 75-84

Nadirah, M., Najiah, M., & Teng, S. Y. (2012). Characterization of Edwardsiella tarda isolated from Asian seabass, Lates calcarifer. *International Food Research Journal*, *19*(3), 1247.

Nash, G., Anderson, I. G., Shariff, M., & Shamsudin, M. N. (1987). Bacteriosis associated with epizootic in the giant sea perch, Lates calcarifer, and the estuarine grouper, Epinephelus tauvina, cage cultured in Malaysia. Aquaculture, 67(1–2), 105–111.

Norimah A. J., Safiah M., Jamal K. (2008). Food Consumption Patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). Malaysian Journal of Nutrition, Mar;14(1):25-39.

Olatoye IO, Basiru A. Antibiotic usage and oxytetracycline residue in African catfish (Clarias gariepinus in Ibadan, Nigeria). World J Fish Mar Sci. 2013;5(3):302–9.

Paterson, B. D., M. A. Rimmer, G. M. Meikle, G. L. Semmens (2003): Physiological responses of the Asian sea bass, Lates calcarifer to water quality deterioration during simulated live transport: acidosis, red-cell swelling, and levels of ions and ammonia in the plasma. Aquaculture 218, 717-728.

Pavlov, A., Lashev, L., Vachin, I., & Rusev, V. (2008). Residues of antimicrobial drugs in chicken meat and offals. *Trakia J Sci*, *6*(1), 23-25.

Pikkemaat, M. G. (2009). Microbial screening methods for detection of antibiotic residues in slaughter animals. *Analytical and bioanalytical chemistry*, *395*(4), 893-905.

Ransangan, J., Lal, T. M., & Al-Harbi, A. H. (2012). Characterization and experimental infection of Vibrio harveyi isolated from diseased Asian seabass (Lates calcarifer). Malaysian Journal of Microbiology, 8(2), 104–115.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo*, *56*(4), 341-346.

Sajid, A., Kashif, N., Kifayat, N., & Ahmad, S. (2016). Detection of antibiotic residues in poultry meat. *Pak. J. Pharm. Sci*, *29*(5), 1691-1694.

Singh, S., Shukla, S., Tandia, N., Kumar, N., & Paliwal, R. (2014). Antibiotic Residues: A Global Challenge. *Pharma Science Monitor*, *5*(3).

Soepranianondo, K., & Wardhana, D. K. (2019). Analysis of bacterial contamination and antibiotic residue of beef meat from city slaughterhouses in East Java Province, Indonesia. *Veterinary World*, *12*(2), 243.

Kim S. C., Carlson K. (2007) Temporal And Spatial Trends In The Occurrence Of Human And Veterinary Antibiotics In Aqueous And River Sediment Matrices, Environ. Sci.Technol. 4150–57.

Ten dencia, de la Pena (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195: 193-204.

Vignesh, R., Karthikeyan, B. S., Periyasamy, N., & Devanathan, K. (2011). Antibiotics in aquaculture: An overview. *South Asian J Exp Biol*, *3*, 114-120.

Vishnuraj, M. R., Kandeepan, G., Rao, K. H., Chand, S., & Kumbhar, V. (2016). Occurrence, public health hazards and detection methods of antibiotic residues in foods of animal origin: A comprehensive review. *Cogent Food & Agriculture*, *2*(1), 1235458.

Nair V.T., Venkitanarayanan D., Kollanoor J.(2018). Antibiotic-resistant Salmonella in the food supply and the potential role of antibiotic alternatives for control. *Foods*, *7*(10), 167.

Watkins, H. S., & Kožárová, I. (2019). Broad Spectrum Detection of Antibiotic Residues in Poultry Meat by a Multi-Plate Assay. *Folia Veterinaria*, *63*(3), 9-17.

York, R., & Gossard, M. H. (2004). Cross-national meat and fish consumption: exploring the effects of modernization and ecological context. *Ecological economics*, *48*(3), 293-302.