CHAPTER 1

INTRODUCTION

An antibiotic is a drug used to treat bacterial infections. They work by killing or stopping the growth of harmful bacteria. Since the 1940s, antibiotics have been given to farm animals like cows, pigs poultry, and shrimps to treat infections or prevent an illness from spreading. Low doses of antibiotics are also added to animal feed to promote their growth. This means a greater production of meat, milk, or other products in shorter periods. These low doses may also reduce animal death rates and improve reproduction (Boyd, 2001). For these reasons, antibiotic use has become widespread in agriculture. In 2011, 80% of all antibiotics sold in the US were for use in food-producing animals. Antibiotics are generally fine when used properly for treating or preventing infections. However, excessive or inappropriate use is a problem. When antibiotics are overused, they end up becoming less effective for both humans and animals. This is because bacteria that are frequently exposed to antibiotics develop a resistance to them. As a result, antibiotics are no longer as effective at killing harmful bacteria. This is a great concern for public health (Landers *et al.,* 2012).The US Food and Drug Administration (FDA) has recognized this concern, updating its regulations to reduce the unnecessary use of antibiotics in livestock. The debate on antibiotic use in animals continues. According to the study of Van Boeckel *et al*. (2015), between 2000 and 2010, consumption of antibiotic drugs increased by 36% worldwide (from 54, 083, 964, 813 standard units to 73, 620, 748, 816 standard units). Although there is no evidence that antibiotics in foods harm people directly, most agree that the overuse of antibiotics in food-producing animals is a problem. It can contribute to the development and spread of drug-resistant bacteria, which is a potential risk to public health. Nitro furans are synthetic broad-spectrum antibiotics, which are frequently used in animal production due to their excellent antibacterial and pharmacokinetic properties. They have also been used as growth promoters during the production of shrimp, poultry, and pigs. Long-term animal experiments have shown that the parent compounds and their metabolites have carcinogenic and mutagenic characteristics. This led to the prohibition of nitro furans for the treatment of animals used for food production. In 1993, the EU banned the nitro furans furaltadone, nitrofurantoin, and nitrofurazone for use in animals used as sources of food, and in 1995 the use of furazolidone was also prohibited. The analysis

of nitro furans is based on the detection of the tissue-bound metabolites of nitro furans. The parent compounds are difficult to detect accurately since they are metabolized very rapidly after treatment. The tissue-bound nitro furan metabolites however are present for a long time after administration and they are used to detect nitro furan abuse. There are different types of nitro furans:

- **Nitrofurantoin:** 1-Aminohydantoin (AHD)
- **Furaltadone:** 3-Amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)
- **Furazolidone:** 3-Amino-2-oxazolidinone (AOZ)
- **Nitrofurazone:** Semicarbazide (SEM)

Due to the potentially carcinogenic and toxic properties of antibiotic residues and their allergic potential, the consumption of contaminated food poses a direct risk to public health. Furthermore, the inappropriate use of antibiotics in animal husbandry and food production promotes the multi-drug resistance of pathogen bacteria for antibiotics used in human medicine.

The purpose of this study is to find a better understanding of the current situation of the shrimps and if the shrimps are overdosed with the antibiotics. The antibiotics remain as residues which when get inside the human body through the consumption of shrimps can cause serious damage to human health.

1.1. Aim and objectives

1. To analyze the quality of shrimp that are consumed daily in the local and international markets.
2. To acquire a better overview of the current shrimp production sector in our country.
3. To make people aware of the bad health effects of antibiotic residues that are consumed through commercial products and aqua products every day.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Shrimp:

The term shrimp refers to decapod crustaceans. Usually, shrimps are groups with elongated bodies and primarily swimming mode of locomotion. Shrimps can be synonymous with prawns, covering stalk-eyed swimming crustaceans with long narrow muscular tails or abdomens, long whiskers named antennae, and slender legs (Fisheries technical paper 395, FAO, Rome).

2.2 Habitats:

Shrimps can be found near the seafloor of many coasts and estuaries, as well as in rivers and lakes. Most of them are marines, although some are found in fresh waters. Although some are entirely aquatic, the two species of merguia are semi-terrestrial and spend a significant part of their life on mangrove lands.

2.3 Shrimp and Bangladesh:

Shrimp farming is growing drastically in Bangladesh due to the suitable agro-climatic conditions, immense water resources, low labor cost, and involvement of multinational agencies (Ocean and Coastal Management 54 (3), 201, 2011).

The economic contribution of shrimp in Bangladesh: The shrimp sector in Bangladesh is unique. More than 95% of shrimp and prawns are produced in extensive polyculture ponds which were formerly known as “ghers”. The culture of *Penaeus monodon* or black tiger shrimp is referred to as “Bagda”. The culture of *Macro brachiumrosenbergii* or giant freshwater prawn also takes place in smaller ponds. *M. rosenbergii*, over 95%, is produced by small-scale farmers.

The southern part contributes about 70% of the total shrimp production, which is about 55,513mt of Bangladesh bazaar (BBS 2010-2011). The rest production is mostly concentrated in the coastal region in Chattogram and Cox’s Bazar area. It is estimated that there are 60 operational shrimp hatcheries in Bangladeshi coastal areas. Currently, the European Union is the largest importer of shrimp accounting for 60% of the total production and the export of shrimp from Bangladesh (BFFEA, January-April 2012). Data from the Department of Fisheries (DoF) revealed that the volume of export of shrimp reduced from 40860mt to 35678mt from 2010-11 to 2011-2012.

2.4 Antibiotics:

Antibiotics are antimicrobial substances that work against bacteria and bacterial infection. They may either kill or inhibit the growth of bacteria. Antibiotics are not effective against the virus.

2.5 Antibiotic Residues:

Deposition of antibiotics in meat, milk, or eggs after their bacterial effect is done is referred to as antibiotic residues. The potential health hazard of the antibiotic residue is beyond one's imagination. The possible hazards from the use of antibiotics, including the development of resistance are crucial and complex. The substances have adverse effects on shrimp farming, on adjacent ecosystems, e.g., through toxic effects on aquatic plants and animals, and on human health, e.g., through contact with skin or the development of resistant pathogens in humans. To make a risk assessment, the level of occurrence of the harmful substance in the environment should be calculated and must be compared with concentrations of the substance for which biological effects are supposed to occur (Suter, 1993). However, several of the antibiotics used by the farmers are commonly applied, which potentially persist in the environment and are known to have adverse biological effects (e.g. cause toxic effect or resistance development) and thereby may constitute potential risks to the environment and human health. These groups of antibiotics, e.g. Nitro furans, chloramphenicol, and novobiocine must be used in a way so that there are no antibiotic residues left in the shrimp because the environmental fate and biological effects in shrimp pond environments and subsequently subject to risk assessments.

2.5.1. Chloramphenicol(CAP) :

CAP is an antimicrobial substance that is naturally produced by the growth of the soil bacterium *Streptomyces venezuelae* but also can be produced synthetically nowadays. This particular antibiotic works against both gram-positive and negative bacteria as well as rickettsiae, chlamydiae, and mycoplasmas. Due to the increase of more intensive aquaculture husbandry in our country, the use of CAP has been gradually increasing. Reportedly, CAP was used in shrimp culture in Latin America and Asiato export to European, Japanese, and North American markets(Serrano, 2005). CAP is carcinogenic and can cause severe aplastic anemia, leukemia, bone marrow suppression, and gray baby syndrome in humans.

2.5.2 Furazolidone:

Furazolidone is a nitrofuran derivative with antiprotozoal and antibacterial activity. Furazolidone has been shown to exhibit antibiotic and anti-microbial functions. Furazolidone is also used as a poultry food additive. Furoxone has a broad antibacterial spectrum covering many gastrointestinal tract pathogens including *E. coli, staphylococci, Salmonella, Shigella, Proteus, Aerobacter aerogenes, Vibrio cholerae,* and *Giardia lamblia*. Its bactericidal activity is based upon its interference with DNA replication and protein production. Furazolidone binds bacterial DNA which leads to the gradual inhibition of monoamine oxidase (From Martindale, The Extra Pharmacopoeia, 30th ed, p514). Furazolidone and its related free radical products are believed to bind DNA and induce cross-links. Bacterial DNA is particularly susceptible to this drug leading to high levels of mutations (transitions and transversions) in the bacterial chromosome. Furazolidone belongs to the family of Nitrofurans. These are compounds containing a furan ring which bears a nitro group. The JECFA review of the toxicity data found that furazolidone induced a variety of tumors in rats and was positive in *vitro* genotoxicity tests. No conclusion could be made regarding *in vivo* genotoxicity - one *in vivo* mouse micronucleus test was negative while the other was equivocal. The available data indicated that furazolidone induces malignant tumors (mammary adenocarcinomas, basal cell carcinomas, and neural astrocytomas) in rats at doses of 25 mg/kg bw/day and above. A range of benign tumors was also observed. Based on this data, furazolidone should be regarded as a potential carcinogen in humans, although there is insufficient data to conclude that the tumor formation is initiated through a genotoxic mechanism. Whether there is a threshold for the observed tumor formation therefore remains unclear.

2.5.3. Furazolidone or 3-Amino-2oxazolidinone (AOZ):

The presence of the residues of nitrofuran metabolite AOZ in animal-origin foods has been also reported by McCracken and Kennedy (1997) whosuggested that using LC-MS/MS seventeen of one hundred pork samples analyzed contained the residues of this drug. Another study also reported that residues of nitrofuran metabolites by LC-MS/MS were confirmed in 12 of 1500 pork samples of which two contained AOZ at concentrations of 0.3 and 3.0μg kg^{-1}(O'Keeffe *et al*., 2004). A similar observation has been made by showing LC-MS/MS method AOZ was detected in 15% of the meat-basedproducts (Mottier *et al*., 2005). In a study performed, the content of nitrofuran

metabolite AOZ in *Tilapia* tissue was determined using both the ELISA and LC-MS/MS methods (Tsai *et al*., 2009).

2.5.4. 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ):

Furaltadone is a banned drug for use in food-producing animals and the marker residue of furaltadone in edible tissues is its metabolite, 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ). In this study, a novel polyclonal antibody of furaltadone was produced by using the conjugate of furaltadone-bovine serum albumin as an immunogen. The obtained antibody showed good specificity and sensitivity toward AMOZ with an IC_{50} value of 2.3ng/mL. Then, an indirect competitive immunoassay (ELISA) with heterologous coating antigen was developed to directly determine AMOZ in animal meat with simple sample preparation.

2.5.5. 1-Aminohydantoine (AHD):

1- Aminohydantoine, is the moiety of nitrofurantoin which has structural relationships to the identified carcinogenic 5-nitrofuran compounds. There were no toxicological data available for AHD but toxicological data from a study of nitrofurantoin led to the conclusion that there was some evidence of carcinogenic activity of the parent drug for male F344/N rats (increased incidences of uncommon kidney tubular cell neoplasms). Uncommon osteosarcomas of the bone and neoplasms of the subcutaneous tissue were observed in dosed male rats. There was clear evidence of carcinogenic activity of nitrofurantoin for female B6C3F1 mice as shown by increased incidences of tubular adenomas, benign mixed tumors, and granulose cell tumors of the ovary.

2.5.6. SEM:

Semicarbazide hydrochloride (or hydrazine-carboxamide monohydrochloride) belongs to a family of hydrazines that are known to cause cancer in laboratory animals. SEM itself demonstrated weak genotoxic activity in vitro and weak carcinogenic activity in female but not male mice when given via diet or drinking water. The types of tumors found with semicarbazide were lung and vascular tumors, which have also been found with other hydrazines. However, SEM was found to be one of the least potent carcinogens among several hydrazines.

The European Food Safety Authority (EFSA) concluded that there is limited evidence that SEM at high levels may be carcinogenic. There is no scientific evidence that

SEM is carcinogenic to humans, it is therefore not possible to conclude whether SEM may pose a carcinogenic risk to humans. There is no risk of immediate illness to adults, children, or infants from consumption of foods containing semicarbazide. The concern relates to health in the long term because of the possibility that semicarbazide may cause cancer.

2.6 Structural view of nitrofuran compounds with derivatives:

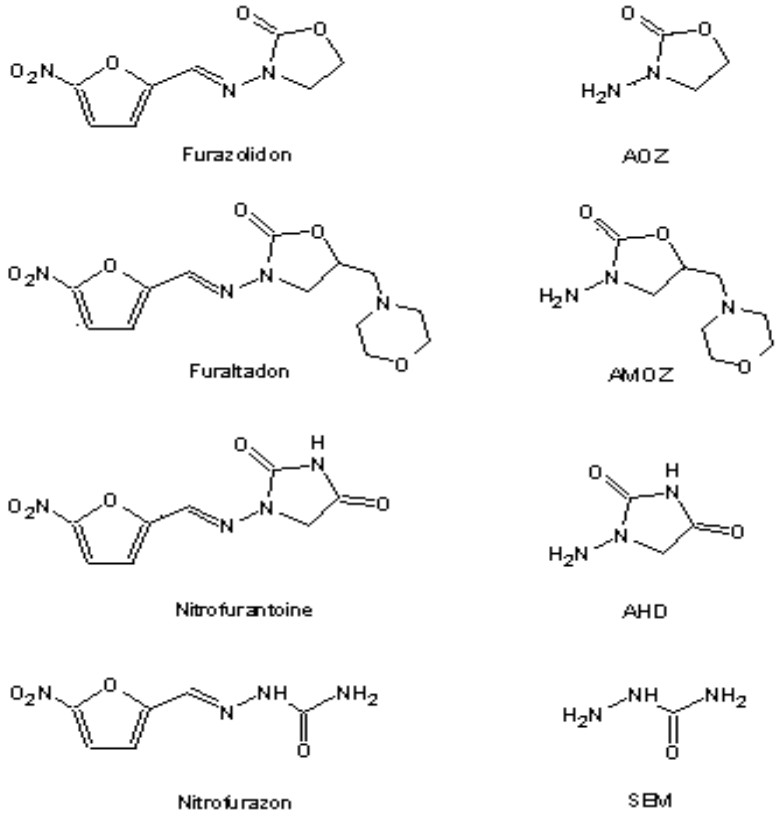

Figure 1: Structure of different nitrofuran derivatives

2.7 Previous History of Research:

According to the interference of Nisha A.R.in Antibiotic residuals –A global health hazard : A withdrawal period is established to safeguard humans from exposure to of antibiotic-added food. The withdrawal time is the time required for the residue of toxicological concern to reach a safe concentration as defined by tolerance. It is the interval from the time an animal is removed from medication until the permitted time of slaughter.

Darwish W.S., Eldaly, E.A., Ishizuka M., *et al*. also studied the antibiotic residues in food. Antibiotic residues in animal-derived foods have been extensively recorded in

many countries of Africa in their study. These residues have exceeded the WHO maximum residue levels in many cases. It has been reported that tetracyclines are the most predominantly prescribed antibiotics in Africa and of all antibiotic-associated residues they represent 41% of cases, followed by beta-lactams at 18%.

Redo-Sanchez A., Salvatella G., *et al*. assessed terahertz spectroscopy to detect antibiotic residues in food and feed matrices. These preliminary results indicated that THz spectroscopy could be suitable for screening applications to detect the presence of antibiotic residues in the food industry, with the prospect of allowing inspections directly on the production lines. THz spectroscopy is a non-destructive, non-contact, and real-time technique that requires very little sample preparation. Moreover, THz radiation can penetrate plastic and paper, which enables the detection of antibiotics in packaged food.

Donoghue (2003) measured the antibiotic residue in poultry tissues and eggs and also indicated the major health concern due to the consumption of antibiotic residue through our food intake.

Another research had been done on analyzing the residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam (Le *et al*., 2004). Surveys on residues of trimethoprim (TMP), sulfamethoxazole (SMX), norfloxacin (NFXC), and oxolinic acid (OXLA) in water and mud in shrimp ponds in mangrove areas were conducted in the north as well as in the south of Viet Nam in July and August 2002. The results showed that these antibiotics were found in all samples in both shrimp ponds and surrounding canals.

Research on acetylcholinesterase activity as a biomarker of exposure to antibiotics and pesticides in the black tiger shrimp (*Penaeus monodon*) has been done (Tu *et al*.,2006). It also emphasized ecotoxicology and environmental safety.

CHAPTER 3
MATERIALS AND METHODOLOGY

3.1 Sample collection:

For the analysis of antibiotic residue in the shrimp, three samples of Black tiger shrimps (*Penaeus monodon*) and four kinds of feeds (F1-F4) were taken.

3.2 Sample transportation:

The samples were transported to the laboratory on day one to perform the test. The samples were transported in the ice box aseptically to impede any kind of potential contamination.

3.3 Method:

The ELISA (Enzyme-Linked Immunosorbent Assay) method was applied to determine the level of antibiotic residue in the collected samples. The reason was to minimize cost, easily portable and high throughput screening method which is capable of sensitive nitrofuran metabolite determination. ELISA is a technique that is based on the emulation of the analyze (sample) with an enzyme-labeled component which is called a tracer for the binding site of an antibody in the wells of a microtitre plate. Extremely sensitive and specific immunoassays allow qualitative and quantitative detection of nitrofuran metabolites.

3.3.1. ELISA kit and other reagents:

AOZ (type R3703) and AMOZ (R3711), AHD (NF3463), SEM (NF3461), and ELISA kit with chloramphenicol were purchased. The other required reagents were: 6 ml of ethyl acetate; a stream of nitrogen; 2 ml of isooctane: chloroform (2:3).

3.3.2. Standard solutions:

Standard solutions of Chloramphenicol, AOZ, AMOZ, AHD, and SEM were prepared in the following way: Stock solutions of 100 ng/ml were prepared for all the standards in which methanol was used as a solvent. Working standard solutions (10 ng ml-1) were prepared from the stock solutions by diluting them with the buffer of peroxides which is provided with the test kit.

3.4 Sample preparation:

The samples were placed into 50 ml reaction tubes. Then 6 ml of ethyl acetate was added and homogenized for one minute with the help of a vortex mixture. The tube was then centrifuged at 2000 rpm for about 15 minutes. After the centrifugation process, the supernatant was discarded, and the precipitate was dried on a hot plate at 70°C with the help of a stream of nitrogen. The dried residue was then dissolved in 2 ml of isooctane: chloroform (2:3) mixture and it was further placed into the vortex for one minute. After that 0.5 ml diluted tissue extraction buffer provided with the ELISA kit was added and vortex for another two minutes. Then it was centrifuged at 2000 rpm for another 15 minutes. A volume of 25 µl well-1 was used in the assay to proceed further.

3.5. Procedure of ELISA:

Each standard solution at the concentration of 10 ng/mL, blank solution, and fortified samples were added to separate duplicate wells in a 25 µl volume. Then 100 µl of diluted enzyme conjugate was added to the bottom of each well. Then the solutions were mixed gently for a few seconds. Then the solutions were incubated for about 60 minutes at 19^0 C to 25°C in the dark.

The non-bound enzyme-conjugate reagent was then removed and washed 6 times accurately with diluents or wash buffer over a 10-15 min period to ensure that every well was filled properly. After the final wash, all the liquid was discarded and tapped onto tissue paper until completely dry. The amount of CAP enzyme conjugate was visualized by adding 125 µl of one-shot substrate or chromogen and incubating it for 20 min in the dark to transform it into a product colored by the bound enzyme conjugate itself. The substrate reaction was stopped by the addition of stop buffer (1N sulphuric acid) provided with the kit followed by a color change from blue to yellow. The resulting color intensity was measured at the spectrophotometer at 450 nm using an ELISA reader/micro plate reader (ELx 800, BioTek, USA).

3.6. Statistical analysis:

All the results expressed are the mean of three measurements. Data were presented as mean ± standard deviation. To test the differences between different grades, one-way ANOVA was performed. Significance was established at $P < 0.05$. Statistical analyses were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL, USA) and Microsoft Excel 2007.

CHAPTER 4

RESULTS

The results of the antibiotic analysis conducted on shrimp samples collected from the local market, export-oriented company, and feed manufacturing companies were presented below:

Table 4.1: Local Grade Shrimp Antibiotic Analysis Result:

Parameters	Chloramphenicol	AOZ	AMOZ	AHD	SEM
Reference point of action (µg/kg)	0.3	1.0	1.0	1.0	1.0
Zone 1	0.35	0.5	0.7	0.5	1.2
Zone 2	0.25	0.7	1.2	0.45	1.1
Zone 3	0.2	0.55	0.5	0.7	0.9
Zone 4	0.4	1.2	0.4	0.5	0.7
Concentration(µg/kg) Mean±SD	0.3±.09	0.73±.32	0.7±.35	0.53±.11	0.97±.22
Level of significance	<0.05	<0.05	<0.05	<0.05	<0.05

*SD=Standard Deviation

From the testresults, it was evident that, after the ELISA test on the four samples of the local grade shrimp *Penaeus monodon* contained the residue of some of the antibiotics that these shrimps had been exposed to Chloramphenicol was present in the sample collected from zone 1 and zone 4. AOZ (Furazolidone or 3-Amino-2oxazolidinone) was present in the sample collected from zone 4. AMOZ (3-amino-5-morpholino-methyl-1, 3-oxa- zolidinone) was present in the sample of zone 2. SEM (semicarbazide) was present in both samples of Zone 1 and Zone 2.

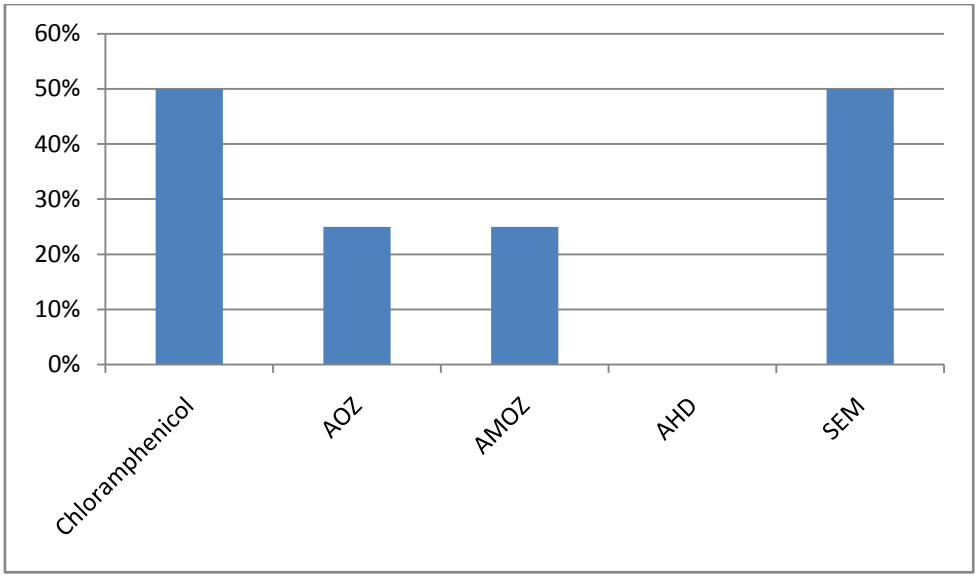

Figure 2: Distribution of antibiotics in local-grade shrimp

The bar diagram above shows that Chloramphenicol and SEM were found in 2 shrimp samples (50%) out of 4 samples. On the other hand, AOZ and AMOZ were evident in the single sample of 2 different markets representing 25% and AHD was under the maximum residue limit in all shrimp samples.

Table 4.2: Export Grade Shrimp Antibiotic Analysis Result:

Parameters	Chloramphenicol	AOZ	AMOZ	AHD	SEM
Reference point of action (µg/kg)	0.3	1.0	1.0	1.0	1.0
Sample 1	0.15	0.6	0.5	0.5	0.25
Sample 2	0.15	0.5	0.5	0.7	0.7
Sample 3	0.2	0.55	0.5	0.5	0.5
Sample 4	0.25	0.5	0.4	0.55	0.5
Sample 5	0.15	0.5	0.55	0.4	0.5
Sample 6	0.25	0.7	0.8	0.5	0.7
Mean±SD	0.19±.05	0.55±.08	0.49±.05	0.52±.09	0.73±.04

*SD=Standard Deviation

From the analyzed data, it was evident that export-quality shrimps contain antibiotic residue, but the concentration level was within the permissible limits.

Table 4.3: Distribution of antibiotics in export-grade shrimp

Sample Number	Chloramphenicol	Nitrofuran (AOZ,AMOD, AHD, SEM)	Level of Significance
Sample 1	Negative	Negative	NS
Sample 2	Negative	Negative	NS
Sample 3	Negative	Negative	NS
Sample 4	Negative	Negative	NS
Sample 5	Negative	Negative	NS
Sample 6	Negative	Negative	NS

*NS= Not Significant

The average concentration of CAP, AOZ, AMOZ, AHD, and SEM was 0.3±.09, 0.73±.32, 0.7±.35, 0.53±.11 and 0.97±.22 µg/kg ($P<0.05$) respectively in the shrimp sample of the local market as that of CAP, AOZ, AMOZ, AHD, and SEM was 0.19±.05, 0.55±.08, 0.49±.05, 0.52±.09 and 0.73±.04 µg/kg respectively in the Export grade shrimp sample.

Table 4.4: Comparison of Local-grade and export-grade shrimp

Parameters	t	Significance
Chloramphenicol	2.465036	0.039011
AOZ	1.348	0.214
AMOZ	1.458	0.183
AHD	0.187	0.856
SEM	2.682484	0.027819

Student's independent t-test was used to compare the mean value of antibiotics between local and export-grade shrimp where chloramphenicol and nitrofuran ($p<0.05$) were significant.

Table 4.5:Feed Analysis Result:

The test results of collected shrimp feed samples are presented in the following table:

Sample	Chloramphenicol	Nitrofurans
Feed 1	Not Detected	Not Detected
Feed 2	Not Detected	Detected
Feed 3	Detected	Not Detected
Feed 4	Not Detected	Not Detected

From the above table, chloramphenicol and nitro furan were detected in 2 different feed samples out of 4 samples that were collected from different feed factories.

Table 4.6: Distribution of antibiotics in feed

Test Parameter	Number of Samples	Number of Positive Samples	Prevalence
Chloramphenicol	4	1	25%
Nitrofuran	4	1	25%

CHAPTER 5
DISCUSSION

Shrimps are comparatively a good source of protein. From 100 grams of shrimp, we get approximately 19.4 ± 0.56 grams of protein (Dayal *et al*., 2013). Butto increase the growth rate of shrimp very fast, a high amount of antibiotics is given to the shrimp along with its feed. Before the washing or withdrawal period of these antibiotics from shrimps, they are sold to the markets and customers. So, the antibiotics remain in the shrimps as residues.

A massive earth is required and a reduction in the use of antibiotics in animals is one of eight issues listed in the call to action of WHO (WHO, 2000). Regarding fisheries and aquaculture, FAO has developed a Code of Conduct for Responsible Fisheries (Hosch *et al*.,2011). The Code and the connected guidelines state that preventive use of antibiotics in aquaculture should be avoided as far as possible and the use of antibiotics should be preferably under veterinary supervision. Additionally, it has declared that states should regulate the input of chemicals in aquaculture that are hazardous to human health and the environment and that marketing and use of drugs that have not been certified for aquatic use should be strictly regulated (Holmström *et al*., 2003).

A total of 228 RASFF notifications were notified of which 76% were because of the presence of antibiotic residues. The notifications showed an increasing trend from 2003, peaked in 2008 and 2009 followed by a decreasing trend. However, in 2014 there was a spurt in RASFF notifications due to antibiotic residues. The period between 2005 and 2009, was a turbulent period with two-thirds of the total notifications due to the presence of antibiotic residues; of which 35% of the complaints were recorded in 2008 and 2009. Two-thirds of notifications were notified by the United Kingdom (36%) and Belgium (33%). Nitrofuran (metabolite) furazolidone (AOZ) (44%), and nitrofurazone (SEM) (37%), were the major causes of notifications, followed by Chloramphenicol (6%) and Oxytetracycline (2%). Of the total RASFF notifications due to antibiotic residues, 39% were from black tiger, 31% from scampi, and 6% from vannamei. 94% of the complaints involving scampi were due to SEM, 85% of the complaints involving black tigers were due to AOZ and 90% of the notifications involving vannamei were due to AOZ. The reported level of SEM

in shrimp ranged between 1.1 and 170ppb and AOZ ranged from 1.1 to 150 ppb. The data shows that the trend in vannamei complaints might progress similar to that of black tiger (Rao*et al*., 2015).

Among the analyzed 160 feed samples, 38 were found contaminated with CAP and/or nitrofuran metabolites (AMOZ, AOZ, AHD, and SEM), where 11, 10, 8, and 9 samples were for shrimp feed, fish feed, poultry feed and feed ingredients. Imported feed ingredients contained with protein concentrates of improper quality were found contaminated with higher levels of SEM. Although hatcheries were found free from contamination, whereas sediment and water samples of many shrimp farms were found contaminated with high levels of SEM and CAP (Islam *et al*., 2014).

A large proportion of shrimp farmers along the Thai coast used antibiotics in their farms. Of the seventy-six farmers interviewed, 74% used antibiotics in shrimp pond management. Most farmers used them prophylactically, some daily, and at least thirteen dierent antibiotics were used (Holmström *et al*., 2003).

For the local grade shrimps, after the ELISA test wasperformed on the four samples of the local grade shrimp - Penaeus monodon contained the residue of some of the antibiotics. These shrimps had been exposed to Chloramphenicol. Chloramphenicol was present in the samples collected from Zone 1 and Zone 4. AOZ (Furazolidone or 3-Amino-2oxazolidinone) was present in the sample collected from zone 4. AMOZ (3-amino-5-morpholino-methyl-1, 3-oxazolidinone) was present in the sample of Chawkbazar. SEM (semicarbazide) was present in both samples of zone 1 and zone 2.In short, Chloramphenicol and SEM were found in 2 shrimp samples (50%) out of 4 samples. On the other hand, AOZ and AMOZ were evident in the single sample of 2 different markets representing 25% and AHD was under the maximum residue limit in all shrimp samples. Inthe case of the export-grade samples, after the ELISA was performed on the black tiger samples, it was found that all the shrimp samples had no antibiotic residues left in them. In the case of the feed testing, chloramphenicol and nitrofurans were detected in 2 different feed samples out of 4 samples that were collected from different feed factories. However, according to FDA 2017, chloramphenicol, and nitrofurans are prohibited from being used in aquaculture farming (FDA, 2017). So, there is an alarming concern regarding the use of antibiotics in aquaculture farming.

CHAPTER 6

CONCLUSION

The study shows that black tiger shrimp from zone 1 have a high concentration of Chloramphenicol and SEM antibiotic residue whereas zone 2 shows maximum antibiotic residue in AMOZ and SEM. However, in export-grade shrimp antibiotic residue was found under the maximum residue limit. Also, out of four feeds tested, two of them had antibiotic residue in them. The result indicates a concern for the common people in Bangladesh who consume shrimp from the local market daily. These antibiotic residues that were found in the Black tiger shrimp can seriously pose a risk to these people. The residual antibiotics can deteriorate human health when consumed. So, strict policies should be made and laws and regulations should be applied to those who are behind this heinous act. Also, it is mandatory to let people know about the antibiotic residue in shrimp and their harmful health effects so that they can minimize the consumption of these shrimps.

CHAPTER 7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

The main purpose of this study is to find out the conditions of the local and export-grade shrimps in Bangladesh and the accumulation of antibiotic residues in their bodies. The result is clear evidence of antibiotic residues in the local grade shrimp. So, there are some recommendations to minimize the effect. The legal basis for the planning and implementation of the annual national residue control plan (NRCP) is laid out in the Fish and Fish Products (Inspection and Quality Control) Rules 1997. These Rules are in the process of being amended - one of the actions promised in response to the 2005 FVO mission. This plan should be strictly followed in our country. Also, it is to be ensured that appropriate information is sent regularly from the regional to the central competent authorities to facilitate the effective implementation of the residue plan by the requirements. We also need to ensure that appropriate quality control and quality assurance measures are implemented across the laboratory network to increase confidence in the reliability of analytical results. Notwithstanding the Department of Fisheries' recommendation not to use antibiotics in aquaculture, it is to be ensured that in cases where antibiotics must be used, appropriate withdrawal periods are observed which will satisfy the nationally proposed MRLs and/or Community MRLs for aquaculture consignments exported to the EU by the requirements of Article 11 of Regulation (EC) 178/2002.

The study only showed only five types of antibiotic residues in shrimp. In the future, the residual effect of other human antibiotics on shrimp should be studied further.

CHAPTER 8

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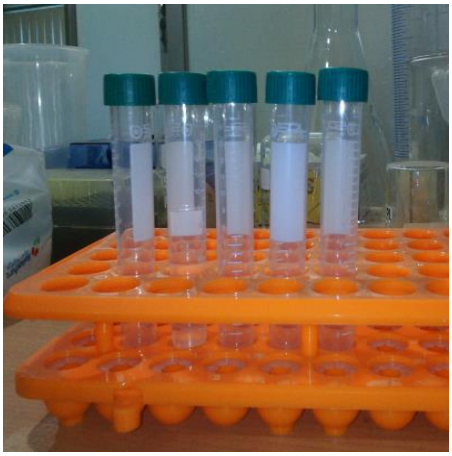
APPENDIX

Picture Gallery

Local Grade Shrimp Sample

Export Grade Shrimp Sample

Weighing of sample



Sample Preparation

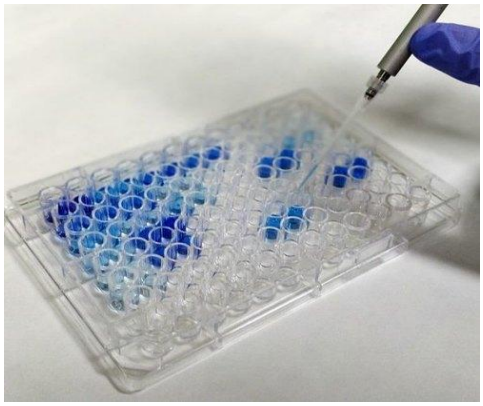
Chloramphenicol ELISA kit

Nitrofuran (AHD) ELISA kit

Nitrofuran (SEM) ELISA kit

Nitrofuran (AOZ) ELISA kit

Nitrofuran (AMOZ) ELISA kit



ELISA Diagnostic Kit

Sample Analysis in Spectrophotometer

BRIEF BIOGRAPHY

Mr. Sharjil Mahmood passed the Secondary School Certificate Examination in 2008 and then the Higher Secondary Certificate Examination in 2010. Mr. Sharjil Mahmood obtained his B.Sc. (Hons.) in Food Science and Technology in 2015 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in the Department of Applied Chemistry and Chemical Technology in Food Chemistry and Quality Assurance under the Food Science & Technology Faculty; at CVASU. He has an immense interest in working on food safety issues including food chemistry and microbiology, product development, malnutrition, reduction of nutritional changes in food processing, etc.