CHAPTER-I:

INTRODUCTION

Antibiotics are natural products of microorganism, identical synthetic product or similar semi synthetic products that inhibit the growth of or destroy the microorganisms (Andrej Kirbis,2007) .In veterinary medicine , antibiotics are widely used as therapeutic, prophylactic and growth promoting agent and nutritive purpose in livestock and poultry production (Donoghue,2003). Various antibiotics take different time to be excreted from the body. Therefore, if animal originated food is not marketed after the recommended period to be free of residual effect, it may cause presence of residue in food and then transfer to consumers and becomes a potential hazard for human health (Kozarova, 2001). The presence of Xenobiotics especially antibiotic residue in foodstuffs of animal origin is one of the most important index for their safety. Many livestock producers treat their animals themselves. Even if they use the same drugs as the veterinarians, they have little understanding of the condition and quantities to administer or the waiting period. In addition, there are cases of veterinary medicinal products intended for ruminants being administered to other species. Indiscriminate use of antibiotics results noticeable residue in meat, milk, cheese, butter and other livestock products, causes development of resistant gene in bacteria against antibiotic which may cause non response to therapy (Lee et al,2000). The use of antimicrobials for the treatment or prevention of disease in animals closely follows their uses in human (Bowen, 1997) and today antimicrobial drugs are used to control, prevent and treat infection, and to enhance growth and feed efficiency (Tollefson and Miller, 2000).

Antibiotics were first employed in veterinary medicine for the treatment of mastitis in dairy cows (Gildow et al, 1946). This happened later stage of world war II when lyophilized penicillin preparation were available to veterinarians who reconstituted the antimicrobial with saline for intra mammary infusion (Gustafson and Bowe, 1997).

Now a days, antibiotics are being used worldwide including Bangladesh in poultry and animal production. In Bangladesh, in livestock practice Oxytetracycline, Amoxicillin, Gentamycine, Sulfur drugs are most frequently used.

Although drugs are widely used in livestock practices in Bangladesh, farmers are not aware of maintaining withdrawal period of antibiotics. They do not maintain withdrawal period before selling their animals or animal products to market. A

withdrawal period is the time require for antimicrobials residues to reach at a level that is tolerable and nontoxic. The animal cannot be sent off to slaughter until they

have been off antimicrobials for the withdrawal period that is specific for the antimicrobials they were treated with. It is a responsibility of farmers and veterinarians to enforce the rule for betterment of future public health As a Muslim country the people of Bangladesh consumes a large amount of beef. Therefore, these above mentioned factors pose a great threat to public health in Bangladesh.

Moreover, in Bangladesh, very few pharmaceutical companies actually mention the withdrawal period on their packaging. Additionally, farmers are often unaware of the dangers associated with antimicrobials residues for public health. These two factors combined can lead to animals being sent to market before they are safe for human consumption (Nisha, 2008; Chowdhury et al., 2009).

Thin layer chromatography is a simple non expensive and exact method which can execute easily in the laboratory. TLC is a reliable method for monitoring low amounts of different biological materials and chemicals. Illumination of antibiotics against UV light helps as a simple detector for this mean However, TLC is a qualitative method and does not give any idea about the amount of residue present in the sample; UHPLC is a well-known test for the quantification.

1.1 Overall aim

The overall aim of the study was to detect the antimicrobial residue in beef and cattle liver.

1.2 Specific objectives

1. To assess the status of antibiotic residue in beef and cattle liver.
2. To compare percentage of antibiotic residue in between beef and cattle liver.

CHAPTER-II:
MATERIALS AND METHODS

2.1: Study Area

Present study was conducted at Chattogram Metropolitan Area (CMA), the important port city of Bangladesh. Due to great demand of protein for the huge city population, cattle and goat business is quite remarkable here. Large number of cattle, buffalo and goat are brought to the markets from different parts of the country for sell. That is why different markets of Chattogram Metropolitan Area (CMA) were selected as the study site.

2.2: **Collection of samples:**

Meat and liver samples were collected from six different markets of Chattogram Metro Area (CMA). The first batch of samples were collected in February and then in March 2019.

A total of twenty five muscle samples (10 neck muscles, 10 thigh muscles, and 5 abdominal muscles) and 25 liver samples were collected from six different markets. Samples were collected from Jautala, Kazirdewri, Agrabad, Bahaddar hat, Pahartoli and Kornofuly market.

2.3. **Transportation of sample:**

The collected samples were transported in air tied ice box with ice bricks. These samples were stored in deep fridge at-20°c until further analysis.

2.4: Sample preparation of meat:

Samples were blended separately by a food processor for three to five minutes. The smashed samples were taken into properly sterilized Petri dishes with proper care as well as covering. From these samples four gram of aliquot sample was taken into beaker with the help of electric balance and spatula.

Then homogenization was done with addition of 10ml phosphate buffer (pH 6.5). After proper mixing protein contents were precipitated with the addition of 2 ml trichloroacetic acid(30%) maintaining sufficient care and attention. Then those mixture samples were taken into properly cleaned and sterilized test tubes for centrifugation. Then centrifugation was done at 7000 rpm for 15 minutes with the

help of automatically time regulated centrifuge machine. Then filtration of supernatant was performed with the help of filter paper and funnel. Filtration fluid was collected into beaker with sufficient care. Then equal volume of di-ethyl ether was added into the solution and mixing properly in order to perform defatation. Then mixture was kept for 10 minutes to form separate layers, upper oily layer and bottom layer. Then by using cleaned and sterilized separating funnel, these mixtures were separated from each other and upper oily layer was discarded and only the bottom layer was collected. This extraction of supernatant was repeated twice with diethyl ether. After filtration, extracts were collected into screw cap vial with proper care and kept into refrigerator for further advanced analysis.

2.5: **Selection of antimicrobials and preparation of antimicrobials standards:**

Antimicrobial standards were prepared for comparison with the extracted samples. Standards were prepared for five commonly used antimicrobials- Amoxicillin, Gentamycine, Ceftriaxone, Oxytetracycline and Ciprofloxacin. These are the commonly used antimicrobials in cattle production in Chattogram. The standards were prepared by dissolving 0.1 gm of standard (Sigma-Aldrich Co.; Fluka Analytical, USA) in 2 mL of methanol (Sattar et al, 2014).

2.6: **Preparation of silica plates:**

For TLC, plate with adsorbent is necessary which was used as stationary phase. Here we used commercial available recoated TLC plates which are 20×20 cm in size and .25mm in thickness (Merck Germany). This recoated plate is an ideal plate for research and easy to handle.

2.7: **Thin Layer Chromatography (TLC):**

Thin Layer Chromatography was run on silica plates (20 cm2) from EMD Millipore (Merck KGaA, Massachusetts, USA). Plates were cut into 8 equal parts. Then, a line was drawn with pencil 2 cm up from the bottom of the plate. For preparation of the mobile phase, 25 mL of acetone was combined with 25 mL of methanol (in a 1:1 ratio). A 2 µL of sample was spotted on the plate at the 2 cm line. The spot was then allowed to dry before placing it in the TLC chamber containing the mobile phase.

Precautions were taken to make sure that the mobile phase fell below the 2 cm line on the TLC plate before running the TLC plate. After the TLC plates were placed in the chamber, the mobile phase was allowed to run until it was approximately 1 cm away from the end of the plate (approximately 45 minutes). Then, the plate was removed and allowed to dry. After drying, the plate was placed in a UV chamber (254 nm) to expose any compounds present in the sample. Bands from chemical compounds that showed up under UV light were marked. The Retention factor (Rf) value of each sample band was measured and compared to the Rf values of the prepared standards. The Rf value was calculated by measuring the distance traveled by the sample, and dividing it by the distance traveled by the solvent. All methods were performed in accordance with the methods described by Sattar et al. (2014).

2.8: **Pointing, Running and detection**:

About 50 µl of methanol dissolved deposits were pointed on silica plates with the help of capillary tube. Treated plates were allowed to be air dry then transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. After reaching of solution front to end plates just below one cm from the top level and dried in air, chromatograms observed on UV light at 256 nm.

CHAPTER-III:

RESULT

This study identified oxytetracycline (predominantly), amoxicillin, ciprofloxacin, gentamycine and ceftriaxone residues in tissues of beef and liver samples. In total, 50 meat and liver samples were collected from 6 different markets. Of these samples collected, 3/50 (6 %) were positive for Oxytetracycline (OTC) (2 liver and 1 muscle sample), 2/50 (4%) were positive for Amoxicillin (1 muscle and 1 liver), 0/50 (0%) were positive for others (Ciprofloxacine, Gentamycine and Ceftriaxone). OTC was the most prevalent antimicrobial among the five antimicrobials studied.

Table: Number of positive and negative test sample

	Liver		**Muscle**	
	Positive	Negative	Positive	Negative
Oxytetracycline	2 (4%)	23	1(2%)	24
Amoxicillin	1 2%)	24	1(2%)	24
Ciprofloxacin	0	25	0	25
Gentamicin	0	25	0	25
Ceftriaxone	0	25	0	25

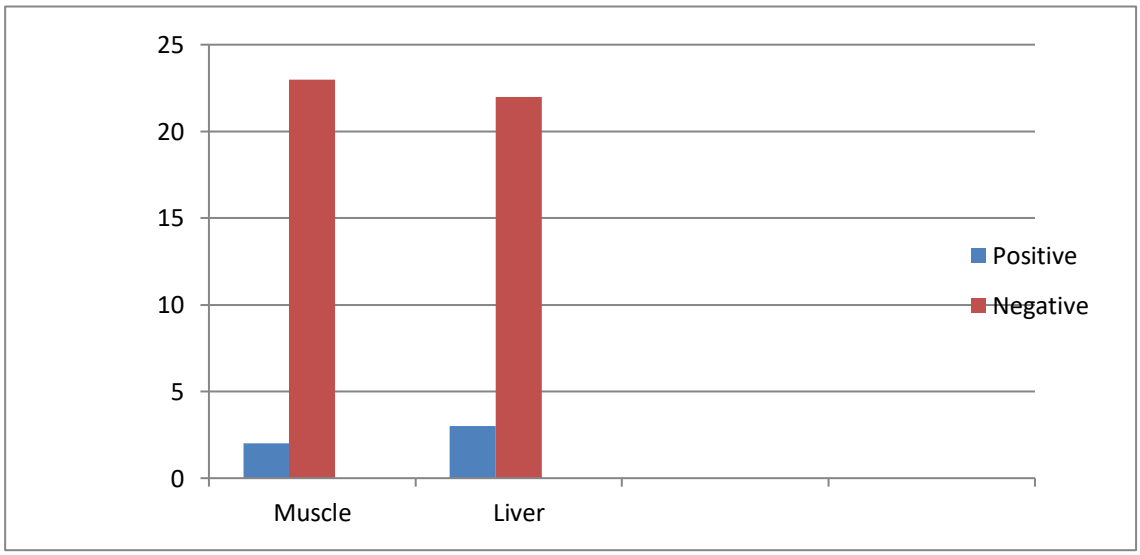

Fig1: Total positive and negative sample

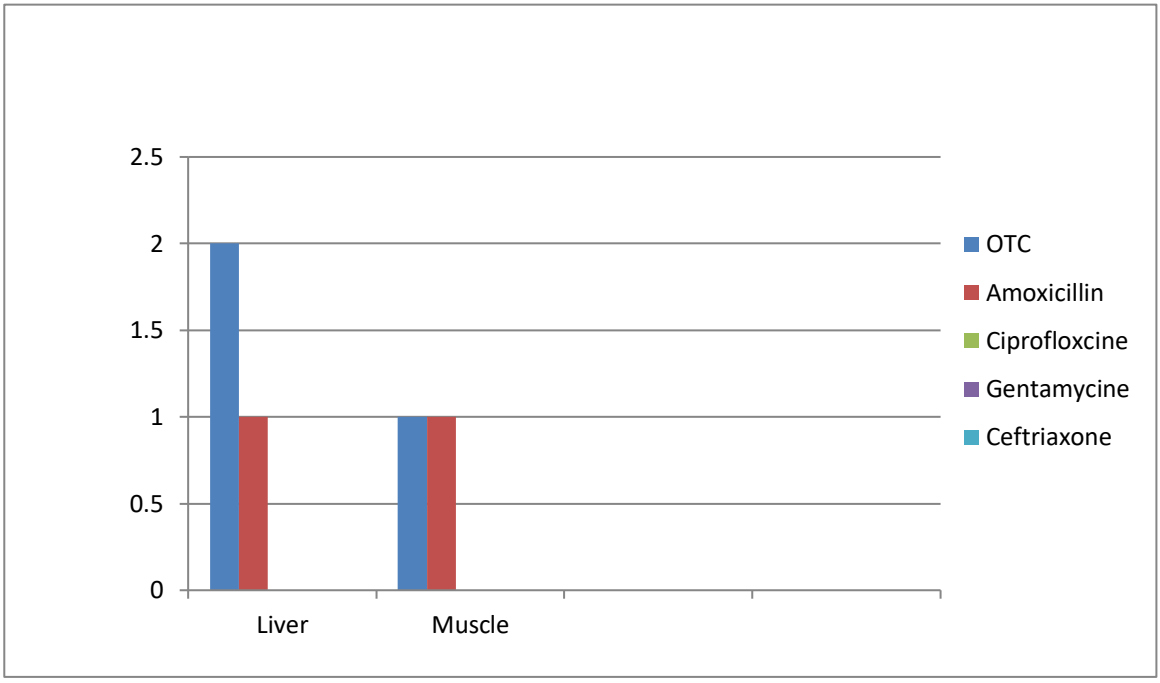

Fig2: Positive samples according to Antibiotics

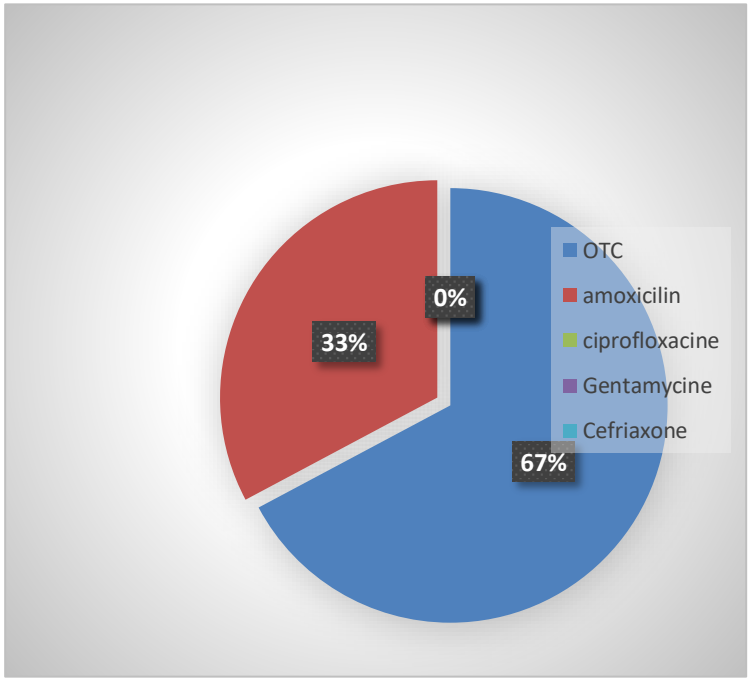

Fig3: Percentage of Residue in Positive samples (5/50)

CHAPTER-IV:

DISCUSSION

In this study a total 50 samples were collected from retail market of different parts of CMA during February, 2019 to March, 2019. After collection these samples (muscle and Liver) were tested for antimicrobial residue by Thin Layer Chromatography.

Five out of 50 samples showed positive results in TLC (3 liver and 2 muscle samples).Among 5 antibiotic standards used, we detectedonly 2 (OTC and Amoxicillin) in the samples. However, different researchers observed higher percentage of residue in meat compared to our result (Ramatla et al.,2017; Muaz et al.,2018). The difference may be due to the difference in tests and number of sample used.

The deposition and subsequent detection of residual amounts of these antimicrobial substances in consumable meat is really a matter of concern. Furthermore, emergence and co-occurrence of resistance to various and/or multiple antibiotics in commensal, pathogenic, and mutualistic microbes is also threatening human health. Therefore, strict legislations and control measures at national and local levels should be established for production, sale, and use of veterinary drugs in treatment or as feed supplements. Additionally, the recommended withdrawal periods between administration of drugs and slaughtering must be enforced by the respective food and drug administration, other regulatory authorities, and professional veterinarians. There should be regular monitoring and assessments for the presence of drug residues in edible tissues of poultry to ensure the consumers safety.

Recent decades have seen the significant progress in development of quantitative confirmatory methods for the detection of antimicrobial residues. However, microbiological inhibition method plays a vital role in antimicrobial residue analysis because of their broad-spectrum characteristics, which make them the suitable option for the screening step. According to Kuhne and Ebrechal (1994), an orientation to immunological and chromatographic method would lead to a drastic reduction of the number of analyses. Microbiological methods are nonspecific and semi quantitative in nature and therefore a positive test result should be confirmed with specific quantitative methods.

The use and sometimes misuse of antimicrobials in food animal production has resulted in the emergence and dissemination of resistant pathogens and resistance gene. Antimicrobial resistant pathogen in food can affect not only the animal health but also public health when they enter the food chain. Evidence suggest that more judicious use of antimicrobial in food animal will reduce the selection of resistant bacteria and help to preserve these valuable drugs for both human and veterinary medicine. To encourage countries to address the problem, the WHO developed global principles for the containment of antimicrobial- resistance in animals intended for food (WHO, 2000). The WHO global principles incorporate several additional recommendations to reduce the overuse and misuse of antimicrobials in food animals and protect public health. They include: (I) obligatory prescriptions for all antimicrobials used for disease control in food animals, (2) preapproval evaluation of food animal antimicrobials to assess their risk of causing resistance to human drugs, (3) identification of emerging health problems through resistance monitoring so that timely corrective actions may be taken to protect human health and (4) development of prudent use guidelines for veterinarians and producers. Addressing the issue of antimicrobial-resistance is one of the most urgent public health priorities today. National authorities should adopt a proactive approach that promotes programs aimed at reducing the need for antimicrobials in food animals and ensuring their prudent use.

The method described in this study is a simple, easy inexpensive which can be readily adopted by any laboratory for the detection antibiotic residues in tissues of food- producing animals.

Though the present study did not observe antibiotic residue in a high number of meat and liver samples, we cannot draw a conclusion from it as we covered only a very small population and samples. Moreover, more sensitive tests and tests that can detect the amount of residue might have needed to be employed. Therefore, an extended study using more samples and different screening tests might be necessary to make an inference.

Recommendation

The worrying consequences of antimicrobial residue to human health prompt a number of recommendations. These are aimed at public authorities, veterinarians, livestock producers and consumers.

The public authorities must:

1. Setting up a permanent national meat QC program (for antibiotic residue and bacterial resistance)
2. Organization of seminars on the dangers of anarchic use of substances with an antimicrobial effect on public health
3. Building the analytical capability of laboratories in the control of veterinary drug residues in food.

Livestock producers must:

1. Respect the prescribed withdrawal period and keep slaughter records to facilitate controls.
2. Comply with the rules of good animal husbandry practice.

Veterinarians must:

Veterinarians are advised to be more careful in prescribing veterinary medicinal product by making livestock producers aware of the rules for the present use of antibiotics.

Consumers must:

Be informed and reject practices that could harm their health.

CHAPTER -V**: CONCLUSION**

In the present study we detected some samples with antibiotic residues. Though we did not detect residue in a remarkable number of samples but it can be stated that probably in some cases livestock producers do not respect the regulation about withdrawal period of the centenary products. An extended study including more samples and advanced detection tests should be employed in the study area to draw conclusion regarding the presence of antimicrobial residue in meat and other food products of livestock.

Acknowledgements

All praises to almighty Allah who gave me the opportunity to be enrolled in Chattogram Veterinary and Animal Sciences University for the purpose of Achieving Doctor of Veterinary Medicine. I would like to express my veneration to honorable supervisor **Professor Dr. Sharmin Chowdhury**, Head, Department of Pathology and Parasitology for her coherent and articulated instruction. It could not be possible to complete such a laborious task without her scholastic guidelines. I found her very much dedicated to me to complete my work effectively and fruitfully. It was an exquisite experience for me to work under her supervision.

Special thanks for **Dr. MD. Sahidur Rahman**, MPH fellow, One Health Institute, Chattogram Veterinary and Animal Sciences University for his help to develop the protocol and lab work.

I am also grateful to all staff of the Dept. Pathology and Parasitology and also to the meat seller due to their effective collaboration during sample collection.

Last off all I am ever indebted to **my parents** and **my beloved wife** for their sacrifice, blessings and encourage.

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BIOGRAPHY

I am Nur Mohammad from Cumilla, have passed the Secondary School Certificate examination From Dhampti Islamia Kamil Madrasah, Cumilla in 2008 and Higher Secondary Certificate examination from Adhyapak Abdul Majid College in 2010. I have enrolled my internship program for Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and animal Sciences University (CVASU), Bangladesh. I have immense interest to work in the field of veterinary science.