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Detection of Antibiotic Residues in Broiler Chicken Meat

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Abstract
The oxytetracycline (OCT) and chloramphenicol (CAP) residues were isolated from broiler chicken meat. Two different extraction procedures were used to extract two types of antibiotics residues in 13 different meat samples marketed in Yangon. The identification was done based on antibiotic sensitivity test, UV, and FTIR. The UV analysis showed that 69.2% of meat samples contained the OCT residues and 76.9 % of meat samples contained the CAP residues. The IR analysis also confirmed the presences of OTC residues and CAP residues. In antibiotic sensitivity test, bacterial growth inhibitions were found for four samples of OTC residues. But no inhibition zones were detected for CAP residues.
Keywords: *Antibiotic Residues, Oxytetracycline, Chloramphenicol, Broiler Chicken Meat.*

Introduction

Antibiotics are widely used in food-producing animals for various purposed. The types of antibiotics used in agriculture animals are the sampe type as used in humans for therapeutic purposed. The presence of antibiotics are thus of toxicological hazards for humans directly or indirectly[1].

Moreover, antibiotic resistance is becoming a serious problem in the world. The use of antibiotic in food-producing animals increases the risk of antibiotic resistance and allergic reactions to antibiotic. (Gard and Johansson 2004) When an animal is treated with an antimicrobial drug, a selective pressure is applied to all bacteria associated with the animal. The emerged resistant bacteria may not be harmful to the animal, but can be pathogenic to human and may complicate treatment[2].

In Myanmar poultry-industry, many kinds of antibiotics are still widely used for different purposes. The antibiotics used in poultry-industry are oxytetracycline, doxycycline, chloramphenicol, amoxicillin, norfloxacin, trimethoprim, sulfafurazol, penicillin, gentamycin, and so on. Antibiotics were used as growth-promoter, egg-enhancer, prophylaxis and preservation etc. The poultry feeds that are sold by some companies also contain antibiotics. On the other hand, the farmers have lack of knowledge about antibiotics and they do not use according to the label directions. Therefore antibiotics could be over used in the farms and the withdrawal periods of antibiotics may not observed. So the antibiotic residues may be detected in meat.

In 1990, the World Health Organization issued guidelines for acceptable levels of antibiotic residues in human foods, which contained acceptable daily intake (ADI) and maximum residue limits (MRL). The residue level should not exceed MRL[2].

Oxytetracycline is commonly used for the prevention and/or treatment of diseases in livestock production. As a feed additive in sub-therapeutic dose, it contributes to the maintenance of optimal health and thus promotes growth in food-producing animals. However, the use of this compound may result in residues in animal derived food products, especially if proper withdrawal times for treated animals have not been used[1].

Oxytetracycline can produce toxic effects in humans such as nausea, vomiting, diarrhea, abdominal cramps, anorexia, flatulence, bulky loose stool, oral lesions, pellagra, dermatitis, edema, serum sickness, thrombocytopenia, and etc[3].

Chloramphenicol residues can give rise bone marrow depression in human being. So, absolutely no use for any purpose is allowed for chloramphenicol, a highly toxic antibiotic. This drug is potentially harmful to humans who come into incidental physical contact. Of particular concern is the development of aplastic anemia from minute doses that can cause leukemia and death[4].

The residues of antibiotic can be determined by High performance Liquid Chromatography (HPLC), liquid Chromatography-mass Spectrophotometery (LC-MS) and Gas Chromatography-mass Spectrophotometery (GC-MS). However, the apparatus and chemicals used in the above methods are very expensive.

In this work, oxytetracycline (OTC) and chloramphenicol (CAP) residues were isolated from broiler chicken meat and confirmation was done by spectroscopic methods such as Ultraviolet spectroscopy (UV) and Fourier Transform Infrared spectroscopy (FTIR). *In vitro* antibiotic activities of isolated residues were evaluated against three different bacteria namely *Escherichia coli* (*E.coli*), *Bacillus subtilis* (*B.subtilis*), and *Salmonella tophi* (*S.typhi*).

Materials and Methods

Media and Chemicals

Media and chemicals used in this research were purchased from Australia Medical Diagnostics (AMD) co. Ltd and Myanmar Analytical Grade chemicals.

Microorganisms

E.coli, B. subtilis, S.typhi provided from Microbiology Laboratory under Pharmaceutical Research Department (PRD) was utilized in Antibiotic sensitivity test.

Sample Collection

Broiler chicken meats were collected from six different markets in Yangon. The names of the market were Bo Kalay Zay, Hlaing Yadanar Zay, Shwe Hinnthar Zay, Than Zay, Thait Pan Zay, and Thirimingalar Zay.

Extraction of Oxytetracycline Residues from broiler chicken meats

Extraction of Oxytetracycline Residues from broiler chicken meats was carried out according to the methods described by Hamide SENYUVA, Tuncel OZDEN, and Deniz Yurtsever SARICA 2000. Briefly, 2 g of broiler chicken meat was homogenized in a blender for 2 min and then 0.1 g citric acid was added. To this mixture, 1 ml nitric acid (30%), 4 ml methanol and 1 ml deionized water were added, respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and then centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 µm nylon filter, the solution was kept in the refrigerator and used for further analysis[1].

Extraction of Chloramphenicol Residues from broiler chicken meats

Extraction of Chloramphenicol Residues from broiler chicken meats was carried out by modifyting the methods described by Yang D, Jiang D, Wang Z, Fang C. 2004. Briefly, 2 g of broiler chicken meat was homogenized in a blender for 2 min and then ethyl acetate was added. The suspension was put in a vortex for good mixing, kept in an ultrasonic bath for 1 hr. The procedure was repeated twice and then

centrifuged for 10 min at 5300 rpm. After filtering, the solution was kept in the refrigerator and used for further analysis[5].

Antibiotic Sensitivity Test

Agar well diffusion method (Nathan's agar well diffusion technique) was used in this study[6]. The plates were filled with Muller-Hinton agar medium. Single colony from bacterial stock was streaked evenly in 6 directions over the entire surface of agar plate with a sterile wire loop to obtain uniform inoculums. After the plate was inoculated, 6-mm wells were made on the Muller-Hinton agar medium by using a 6-mm punch. The standard drug, solvent control and the neutralized extracted solutions were introduced into each well. Then the plates were placed in the incubator at 35·C for 18 hours. After 18 hours of incubation, the plates were examined and the diameter of each zone of complete inhibition were measured and recorded in millimeter by a ruler. Then the results were interpreted as referred to standard diameters of interpretative chart.

Identification of extracted solutions by UV spectroscopy

For the identification of extracted solutions, the ultraviolet absorption spectra of these compounds and the standard antibiotics i.e. oxytetracycline (3ppm concentration) and chloramphenicol (3ppm concentration) were recorded and examined by using the Perkin Elmer Lambda 40.

Identification of extracted solutions by FTIR spectroscopy

For the identification of extracted solutions, the infrared spectra of extracted residues and standard antibiotics were also recorded and examined by using the Maston, Genises II (USA) whether the respective functional groups were present or not.

Results

OTC and CAP residues were extracted from the broiler chicken meat and their presence was evaluated by Antibiotic sensitivity test, UV and FTIR.

To detect the antibiotic activity of the extracted residues, agar well diffusion method was used. The activity of OTC residues against E.coli, B.subtilis, S.typhi would be seen in Fig.1. Inhibition zone diameters in mm were showed in Table 1.

Fig.1: Inhibition zones caused by OTC Residues Extracted from Broiler Chicken Meats (samples F1, G1, H1 from Shwe Hinthat Zay, samples I1, J1from Thait Pan Zay, S-solvent control, OTC- standard oxytetracycline)

Table 1 Antimicrobial activity of OTC residues

	Zone Diameter (mm)		
Sample ID	**E.coli**	**B.subtilis**	**S.typhi**
OTC	**33**	**30**	**30**
Solvent control	**-**	**-**	**-**
F1	**24**	**20**	**20**
G1	**23**	**19**	**18**
H1	**-**	**-**	**-**
I1	**18**	**16**	**18**
J1	**21**	**20**	**19**

The OTC has an absorption maximum at wavelength 360nm. Table 2 represents the data from UV spectra for OTC residues. The CAP has found an absorption maximum at wavelength 331nm. This mentioned fact is in agreement with those cited in the literature. Table 3 shows the data from UV spectra for CAP residues. Fig.2 and 3 represent the histograms of OTC and CAP residues from ordinate values.

Table 2 UV spectra data of OTC residues

Sample ID	Wavelength(s)(nm)	Ordinate value
OTC(standard)	359	0.0958
A1	359	1.668
B1	-	-
C1	-	-
D1	-	-
E1	-	-
F1	360	2.301
G1	360	1.42
H1	360	0.796
I1	360	2.301
J1	360	1.796
K1	359	0.821
L1	359	0.182
M1	359	0.59

Table 4, 5, 6 and 7 represent the infrared spectra data of chloramphenicol (standard), CAP residue, oxytetracycline (standard) and OTC residue respectively. Fig.4, Fig.5, Fig.6 and Fig.7 are the infrared spectra of CAP (standard), CAP residue, OTC (standard) and OTC residue.

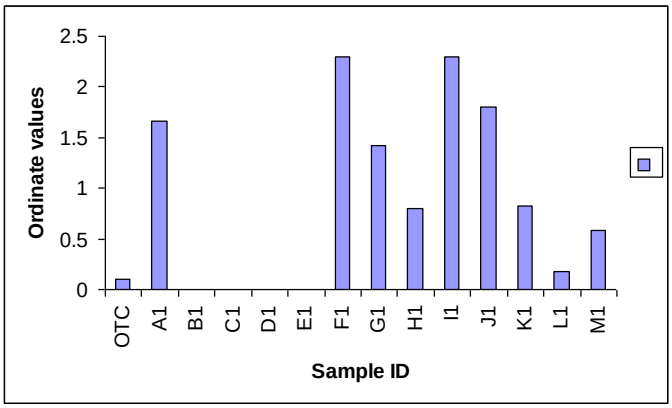

Fig. 2: Histogram of OTC residues from ordinate values

Table 3 UV spectra data of CAP residues

Sample ID	Wavelength(s)(nm)	Ordinate value
CAP (standard)	331	0.2758
A2	331.3	0.387
B2	331.3	1.1141
C2	-	-
D2	-	-
E2	-	-
F2	331	0.851
G2	331	0.478
H2	331	2.523
I2	331	0.316
J2	331	2.523
K2	331	0.215
L2	331	0.975
M2	331	0.032

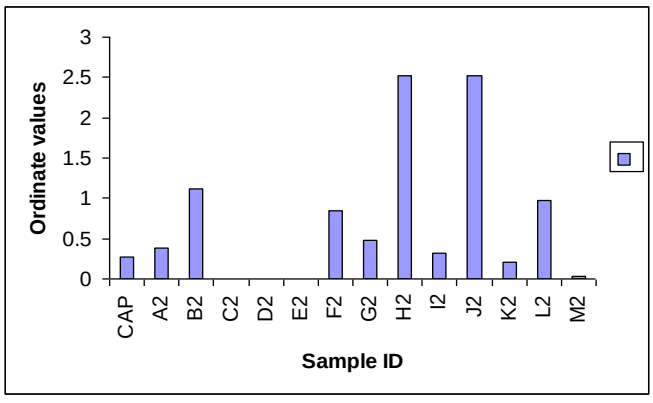

Fig. 3: Histogram of CAP residues from ordinate values.

Table 4 Functional Group Assignment for CAP (standard)

Wave number(cm^{-1})	Vibrational Mode	Group Assignment
3456 1687 1583	N-H Stretching C=O Stretching N-H Bending	} Amide Group
3434	O-H Stretching	Alcohol Group
3079 1667	CH Stretching for Alkene C···C Ring Stretching	}Aromatic Group
2953	C-H Stretching for Alkane	C-H Group
2850	C-H Stretching for CH_2 Group	Methylene Group
767	C-Cl Stretching	-CCl Group
1349	-NO_2 Stretching	Nitro Group

Table 5 Functional Group Assignment for CAP Residue

Wave number(cm^{-1})	Vibrational Mode	Group Assignment
3547 1743 1594	N-H Stretching C=O Stretching N-H Bending	} Amide Group
3435	O-H Stretching	OH Group
3016 1643	C-H Stretching for Alkene C···C Ring Stretching	} Aromatic Group
2925	C-H Stretching for Alkane	C-H Group
2854	C-H Stretching for Methylene Group	-CH_2 Group
758	C-Cl Stretching	-C-Cl Group
1370	-NO_2 Stretching	Nitro Group

Table 6 Functional Group Assignment for OTC (Standard)

Wave number(cm^{-1})	Vibrational Mode	Group Assignment
3580 1584 1230	N-H Stretching of Amine N-H Bending C-N Stretching	} Amine Group
3421 1225 1120 1633	N-H Stretching of Amide C-O Stretching C-N Stretching C=O Stretching for Amide	}Amide Group
1677	C=O Stretching	Carbonyl Group
3436	O-H Stretching of Alcohol	Alcohol Group
2930	C-H Stretching of CH_3 Group	Methyl Group
2893	C-H Stretching of CH_2 Group	Methylene Group
3135 1459 1431	Aromatic C-H Stretching C ··· C Ring Stretching C-H Bending of CH_2	} Aromatic Group

Table 7 Functional Group Assignment for OTC Residue

Wave number(cm^{-1})	Vibrational Mode	Group Assignment
3594 1590 1226	N-H Stretching of Amine N-H Bending C-N Stretching	} Amine Group
3423 1227 1077 1656	N-H Stretching of Amide C-O Stretching C-N Stretching C=O Stretching for Amide	}Amide Group
1745	C=O Stretching	Carbonyl Group
3478	O-H Stretching of Alcohol	Alcohol Group
2964 2924.90	}C-H Stretching of CH_3 Group	Methyl Group
2853.92	C-H Stretching of CH_2 Group	Methylene Group
3145	Aromatic C- H Stretching	}Aromatic Group
1590 1456.45	}C ··· C Stretching	
1441.68	C-H Bending of CH_2	Aromatic Group

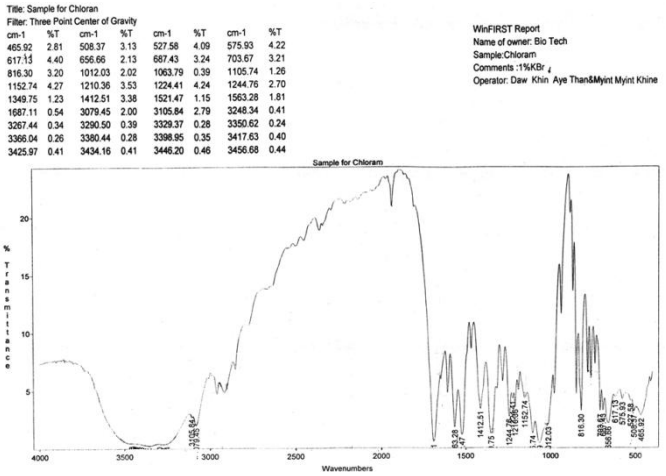

Fig.4: The Infrared Spectrum of CAP (standard)

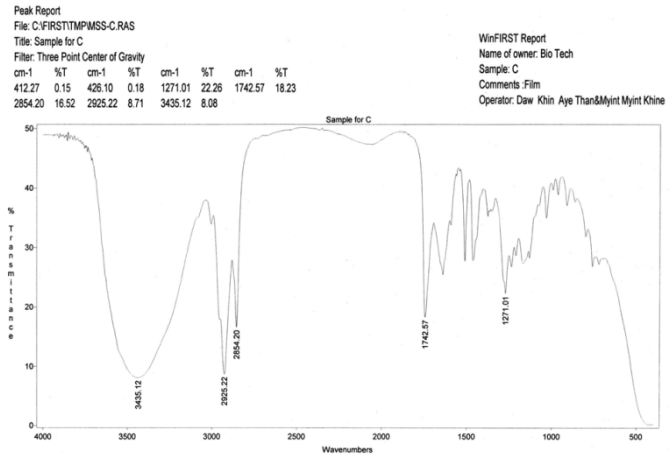

Fig.5: The Infrared Spectrum of CAP residue

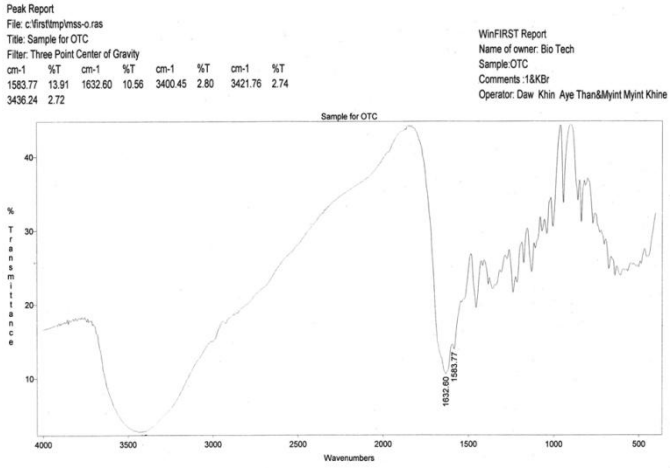

Fig.6: The Infrared Spectrum of OTC (standard)

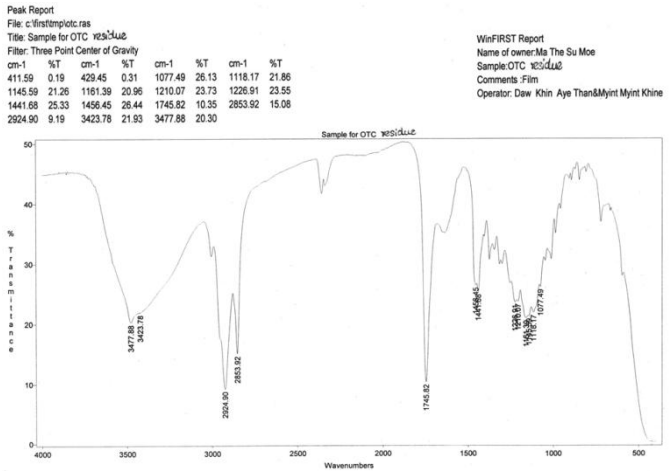

Fig.7: The Infrared Spectrum of OTC residue

Discussion

Animal breeding for food production has led to an increasing use of antibiotics for prevention, preservation, growth-promotion and so on. Antibiotic residues in animal-derived food products threaten human health by being acutely or cumulatively allergic, toxic, mutagenic, teratogenic or carcinogenic.

Senyuva, Ozden, and Sarica 2000 studied a sensitive automated method for residue control of oxytetracycline (OTC) in cured meat. The method involved the extraction of OTC from cured meat products and the determination by high performance liquid chromatography (HPLC). Ten different cured meats were used and eight of the cured meat product samples exceeded current legislation for the tolerance level in meat[1].

Gard and Johansson 2004 studied determination of chloramphenicol (CAP) in shrimp feed and sample

work up with liquid-liquid extraction and solid phase extraction by analyzing with HPLC. Yang D et al.2004 also studied the determination of chloramphenicol residues in muscles and viscera of the livestock and poultry and in the shrimp[7].

In this study, the extraction and identification of OTC and CAP has been made. The two different extraction methods were used with 13 different broiler chicken meat samples marketed in Yangon. Antibiotic Sensitivity Test, UV and FTIR were used to identify the antibiotics.

E.coli, B.subtilis and S.typhi were used as tested organisms for antibiotic sensitivity test. Bacterial growth inhibitions were found for four OTC residues only. No inhibition zones were found for other OTC residues and all CAP residues. That would be due to the trace amount of antibiotics may be present or the presented antibiotics may be destroyed during the experimental period.

Nine OTC residues and ten CAP residues have been identified at UV analysis. In this study, the ordinate values of extracted residues are much larger than that of standard oxytetracycline and chloramphenicol at the concentration of 3ppm. So we can estimate that nine samples were assumed to contain OTC residues exceeding the MRL, the amount of residue of a substance like an antibiotic that is allowed to be present in food sold for human consumption. For the detection of CAP residues, eight samples were observed the presence of CAP residues more than 3ppm.

The IR analysis also showed the presence of OTC residues and CAP residues. The structure of OTC contains amine group, amide group, carbonyl group, alcohol group, methyl group, methylene group and aromatic group. The IR spectra of OTC residue contain all these functional groups according to the specific wave numbers. So, we can confirm the extracted solutions contain the OTC residues.

The IR spectra of CAP residues contain amide group, alcohol group, aromatic group, methyl group, methylene group, chloro group and nitro group presented in the structure of CAP molecule. For this reason, we can confirm the extracted solutions contain the CAP residues.

Conclusions

In this study, OTC and CAP residues were extracted and identified using the antibiotic sensitivity test, UV and FTIR. We found that 69.2% of meat samples contained OTC residues and 76.9 %of meat samples contained CAP residues. Antibiotic residues from broiler chicken meat were not detected from some markets such as Than Zay, Bo Kalay Zay and samples from other markets were found to contain antibiotic residues. That would be due to the freshness of meat samples and their storage condition.

There are different extraction procedures for various antibiotics. Therefore, other antibiotics used in agriculture production should be detected and measured the concentration of the detected antibiotic residues. Other advanced techniques such as High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass spectrometry (GC-MS) should be used for precise concentration and rapid determination.

Dependence on medically important antibiotics used in animals should be reduced. The alternatives such as in-feed enzymes, competitive exclusion products, probiotics and infection control measures should be used instead of medically important antibiotics.

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Conflict of interest statement

We declare that we have no conflict of interest.

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