



UNIVERSITI PUTRA MALAYSIA

**DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF
ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM
CHICKEN MEAT AND CHICKENS IN MALAYSIA**

TIN TIN MYAING

FPV 2003 17

**DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF
ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM
CHICKEN MEAT AND CHICKENS IN MALAYSIA**

TIN TIN MYAING

**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2003



**DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF
ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM
CHICKEN MEAT AND CHICKENS IN MALAYSIA**

By

TIN TIN MYAING

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

January 2003



**DEDICATED TO MY PARENTS,
MY HUSBAND AND MY SONS**



Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

**DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF
ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM
CHICKEN MEAT AND CHICKENS IN MALAYSIA**

By

TIN TIN MYAING

January 2003

Chairman: Associate Professor Dr. Saleha Abdul Aziz

Faculty: Veterinary Medicine

Public health is intrinsically related to food animal production. Antibiotic residues in the food of animal origin and antibiotic resistant bacteria threaten human health. There is an increase in population and the demand for chicken meat in Malaysia is also increasing. Not much data is available on antibiotic residues and antibiotic resistant *E. coli* from chickens and foods of animal origin in Malaysia.

In this study, a total of 400 chicken meat samples were subjected to antibiotic residues screening tests. The prevalence of antibiotics residues in chicken meat was between 11.1% to 21.7%, using three microbial growth inhibition tests; namely, fast antimicrobial screening test (FAST), *Bacillus stearothermophilus* disc assay (BSDA) and a commercial test kit (TAT) with reference to four plate test (FPT). The test performances were evaluated on sensitivity, specificity, positive predictive value and negative predictive value. The sensitivity of these tests ranged



from 55.6 to 65% and specificity, from 82 to 90.6%. Kappa agreement was between 0.5 to 0.8. Based on the above performance parameters, as well as the cost, simplicity and incubation period, BSDA is a screening test of choice.

A total of 182 *E. coli* isolates from these chicken meat samples were found resistant to twelve antibiotics; vancomycin (99.4%), trimethoprim (98.9%), nalidixic acid (97.2%), tetracycline and cephadrine (96.7%), ampicillin (94.5%), enrofloxacin (83.5%), erythromycin (82.9%), ciprofloxacin (81.3%), cefoperazone (80.2%), chloramphenicol (74.4%) and kanamycin (68.6%). Forty-six antibiotypes and nine antibiograms were observed.

Escherichia coli isolated from antibiotic residues positive samples and antibiotic residues negative samples were tested for antibiotic susceptibility to twelve antibiotics. A higher percentage of antibiotic resistance was observed in *E. coli* isolates from antibiotic residues positive samples compared to those from antibiotic residues negative samples. 58.3% and 29.2% of *E. coli* isolates from antibiotic residues positive samples and 25% and 17.1% of *E. coli* isolates from antibiotic residues negative samples were resistant to 12 and 11 antibiotics, respectively.

Plasmid isolation was conducted in 132 of the *E. coli* isolated. Plasmid occurrence rate of 81.8% were observed in this study with high diversity of plasmids profiles among *E. coli* isolates from different sources. The number of plasmids ranged from 0 to 8 and the sizes of plasmids ranged from 1.2 MDa to 118.6 MDa. Forty-five different plasmid profiles were observed. No apparent correlation was

found between the plasmid profiles of the strains and their antibiotic resistance patterns.

In another study, the occurrence of antibiotic resistant *E. coli* was determined in four flocks where in three flocks, chickens were given commercial feed containing antibiotics and in one flock the feed given were without antibiotics. *Escherichia coli* isolates from chickens given feed without antibiotics showed low resistance to all antibiotics even at one day old. The screening of antibiotic residues was done in 20 of these chickens at the age of 42 days old. The occurrence of antibiotic residues was 10% in chickens given feed containing antibiotics. It was observed that *E. coli* isolates from the antibiotic residues positive samples were also resistant to 12 antibiotics while those from antibiotic residues negative samples were resistant to 2-8 antibiotics. This observation requires further investigation.

Klebsiella spp., *Pseudomonas* spp. and *E. coli* were isolated from the feeds and water of the flock where chickens given feed without antibiotics. All these bacteria were resistant to 4-10 antibiotics. Antibiotic resistant *E. coli* was observed in day-old chicks without any selective pressure, such as even when no antibiotic was added to the ration. Thus, proliferation of antibiotic resistant *E. coli* is less dependent on the use of antibiotics/antimicrobials in poultry farms and most likely that chickens obtain antibiotic resistant *E. coli* from the environment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk ijazah Doktor Falsafah.

**MENGESAN RESIDU ANTIBIOTIK DAN PENGASINGAN
ESCHERICHIA COLI YANG TAHAN TERHADAP ANTIBIOTIK
DARIPADA DAGING AYAM DAN AYAM DI MALAYSIA**

Oleh

TIN TIN MYAING

Pengerusi: Profesor Madya Dr. Saleha Abdul Aziz

Fakulti: Perubatan Veterinar

Kesihatan awam berkait rapat dengan pengeluaran haiwan ternakan. Kehadiran patogen bawaan makanan dan juga dalam makanan mengancam kesihatan manusia. Terdapat peningkatan dalam populasi ayam dan permintaan daging ayam juga meningkat. Data yang berkaitan dengan residu antibiotik and *E. coli* tahan antibiotik dalam ayam adalah kurang di Malaysia.

Dalam kajian ini, sejumlah 400 sampel daging ayam telah diuji kehadiran residu antibiotik. Prevalens residu antibiotik dalam daging ayam adalah antara 11.1% hingga 21.7%, dengan menggunakan tiga ujian perencatan pertumbuhan mikrobial, iaitu “fast antimicrobial screening test (FAST), *Bacillus stearothermophilus* disc assay (BSDA) commercial test kit (TAT)” dengan dirujuk kepada “four plate test (FPT)”. Prestasi ujian yang dinilai meliputi sensitiviti, spesifisiti, nilai ramalan positif dan nilai ramalan negatif. Sensitiviti ujian tersebut berkisar antara 55.6 hingga 65% dan spesifisiti daripada 82 hingga 90.6%.

Persetujuan kappa adalah antara 0.5 hingga 0.8. Berdasarkan parameter prestasi diatas, serta kos, kemudahan dan tempoh pengeraman, didapati BSDA adalah yang terpilih sebagai ujian penyaringan.

Sejumlah 182 isolat *E. coli* yang diasingkan daripada sampel daging ayam didapati tahan terhadap 12 jenis antibiotik- vankomisin (99.4%), trimetoprim (98.9%), asid nalidik (97.2%), tetrasiklin dan sefaridin (96.7%), ampisilin (94.5%), enrofloxasin (83.5%), eritromisin (82.9%), ciprofloxasin (81.3%), cefoparazon (80.2%), khlorampenikol (74.4%) dan kanamisin (68.6%). Terdapat 46 antibiotik dan sembilan kumpulan antibio.

Esherichia coli yang diasingkan daripada sampel yang positif dan yang negatif residu untuk diuji ketahanan terhadap dua belas jenis antibiotik. Keputusan ujian menunjukkan peratusan *E. coli* tahan antibiotik yang tinggi daripada sampel yang positif untuk antibiotik berbanding dengan sampel yang negatif untuk residu antibiotik. Terdapat 58.3% dan 29.2% *E. coli* daripada sampel positif untuk residu antibiotik tahan terhadap 12 dan 11 antibiotik manakala 25% dan 17.1% *E. coli* daripada sampel negatif untuk residu antibiotik yang tahan terhadap 12 dan 11 antibiotik.

Pengasingan plasmid dilakukan ke atas 132 strain *E. coli* Kadar kehadiran plasmid adalah 81.8% dan terdapat kepelbagaian tinggi dalam profil plasmid di antara isolat *E. coli* yang diperoleh daripada pelbagai sumber Bilangan plasmid berkisar antara 0 hingga 8 dan saiz plasmid berada pada julat 1.2 MDa hingga 118.6 MDa. Empat puluh lima profil plasmid yang berbeza telah diperhatikan. Tiada

korelasi dijumpai antara profil plasmid strain *E. coli* dengan pola ketahanan antibiotik.

Pada kajian selanjutnya, kewujudan *E. coli* tahan antibiotik ditentukan dalam empat kelompok ayam yang mana tiga kelompok ayam diberi makanan dengan penambahan antibiotik dan dalam satu kumpulan makanan tiada penambahan antibiotik. *E. coli* yang diasingkan daripada ayam yang diberi makanan tanpa penambahan antibiotik menunjukkan ketahanan yang rendah terhadap semua antibiotik, walaupun pada umur satu hari. Penyaringan residu antibiotik terhadap 20 sampel pada umur 42 hari telah dilakukan. Terdapat 10% positif untuk residu antibiotik. Juga didapati bahawa *E. coli* yang diasingkan daripada sampel positif untuk residu antibiotik didapati tahan terhadap 12 antibiotik, sedangkan ayam daripada sampel negatif untuk residu antibiotik negatif tahan terhadap 2-8 antibiotik. Pemerhatian ini perlu dikaji.

Klebsiella spp., *Pseudomonas* dan *E. coli* diasingkan dalam makanan dan air daripada ayam diberi makanan tanpa penambahan antibiotik. Semua bakteria ini didapati tahan terhadap 4-10 antibiotik. *Escherichia coli* tahan antibiotik didapati pada anak ayam umur satu hari, tanpa ada sebarang tekanan pemilihan seperti tiada antibiotik ditambah pada makanan. Oleh itu, perkembang biakan *E. coli* tahan antibiotik kurang bergantung pada penggunaan antibiotik dalam ladang ayam dan diperkirakan bahawa ayam peroleh *E. coli* tahan antibiotik daripada persekitaran.

ACKNOWLEDGEMENTS

First and foremost, my utmost appreciation, heartfelt thanks and gratitude to Associate Professor Dr. Saleha Abdul Aziz, Chairman of Supervisory Committee, for her kind guidance and close supervision that enable me to complete my Ph.D study.

I would like to express my sincere gratitude to Malaysian Technical Cooperation Programme (MTCP) for the award of scholarship to pursue Ph.D study in Universiti Putra Malaysia, Malaysia.

My special thanks to Associate Professor Dr. Raha Abdul Rahim, Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, for her close supervision while doing molecular biology work in her laboratory and Dr. Arifah Abdul Kadir also a member of The Supervisory Committee, for her invaluable suggestions and comments.

My close appreciation and grateful to Brigadier General Maung Maung Thein (Minister, Ministry of Livestock Breeding and Fishery), U Aung Thein (Deputy Minister), U Kyaw Lwin (Director General), U Maung Maung Nyunt (Director General), Dr. Maung Maung Sa (former Rector), Dr. Min Soe (former Rector), Dr. Saw Plae Saw (former Pro-Rector), Dr. Tin Htwe (Pro-Rector) and Dr. Yin Yin Thein (Assistant Director) for their kind encouragement and appreciation through out my studies.



My sincere thanks to Dr. Maznah Ahmad (Director), Puan Marnie and Puan Norzila, in Veterinary Public Health Diagnostic Laboratory, Petaling Jaya, for their generous support while doing my research work in the laboratory.

My special thanks to Dr. Ganapathy Kannan, for his guidance and kindness in the samples collection from the poultry farms.

I am grateful to Dr. Zulkifli Idrus, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, for his kind permission to use the poultry experimental facilities.

My special and sincere thanks to Encik Kamarzaman Ahmad in Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, who willingly assist me in my works.

I am also grateful to Encik Zaid Othman and Encik Ghazali Md. Yusoff for providing the transport to various places in samples collection.

My sincere thanks to all colleagues and staff of Faculty of Veterinary Medicine, Universiti Putra Malaysia, for their contribution in the completion of my study.

My heartfelt and special thanks to Dr. Myint Thein (Faculty of Veterinary Medicine), U Win Myint (Faculty of Forestry), Dr. Kyaw Zay Ya (Faculty of Food Science and Biotechnology), Dr. Than Da Min (Faculty of Agriculture), Dr. Nwe



Nwe Htin (Faculty of Agriculture) and Miss Chua Lee Chuan (Faculty of Education)
for their kind moral support and encouragement.

Finally, my heartfelt thanks and appreciation to my husband Professor Dr.
Aye Cho, my sons, Zayar Aye Cho, Tay Zar Aye Cho and Yar Zar Aye Cho for
their love, support and encouragement to strive harder.



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

SALEHA ABDUL AZIZ, Ph.D.

Associate Professor,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia.
(Chairperson)

RAHA ABDUL RAHIM, Ph.D.

Associate Professor,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia.
(Member)

ARIFAH ABDUL DAKIR, Ph.D.

Lecturer,
Faculty of Veterinary Medicine,
Universiti Putra Malaysia.
(Member)

AINI IDERIS, Ph.D,
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



TABLE OF CONTENTS

	Page
DEDICATION.....	ii
ABSTRACT.....	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS.....	xii
DECLARATION	xiv
LIST OF TABLES	xix
LIST OF FIGURES	xxiv
LIST OF ABBREVIATIONS	xxix

CHAPTER

I	INTRODUCTION	1
	Objectives of the Present Study	5
II	LITERATURE REVIEW	6
	Antibiotic and Antibiotic Residues in Farm Animals	6
	The Use of Antibiotic in Farm Animals	6
	The Aim of Antibiotic Use in Farm Animals....	7
	Benefits and Risks of Antibiotic Use in Farm Animals	7
	Mode of Action of Antibiotics	9
	The Occurrence of Antibiotic Residues in Food of Animal Origin.....	11
	Public Health Aspects of Antibiotic Residues in Food of Animal Origin	13
	Screening Methods for Detection of Antibiotic Residues.....	15
	Maximum Residues Limit (MRLs)	16
	<i>Escherichia coli</i>	17
	Taxonomy of <i>Escherichia coli</i>	17
	Typing Schemes of <i>Escherichia coli</i>	19
	Prevalence of <i>Escherichia coli</i>	19
	Public Health Significance of Poultry Meat Contaminated with <i>Escherichia coli</i>	21



	Page
Antibiotic Resistance Pattern and Plasmid Profile.....	24
Nature of Antibiotic Resistant Gene.....	25
Transfer of Antibiotic Resistance.....	26
Transfer of Antibiotic Resistant Gene.....	27
Plasmids.....	29
Antibiotic Resistance Mechanism.....	30
Resistance to β -lactam Antibiotics.....	31
Resistance to Quinolones	31
Resistance to Tetracycline.....	32
Resistance to Aminoglycosides.....	33
Resistance to Glycopeptides Antibiotics	33
Resistance to Macrolides and Lincosamide.....	33
Resistance to Trimethoprim and Sulfonamides.....	34
Relationship between Antibiotic Residues and Antibiotic Resistant Organisms	34
 III DETECTION OF ANTIBIOTIC RESIDUES IN CHICKEN MEAT AND EVALUATION OF SCREENING METHODS	 36
Introduction	36
Epidemiological Considerations for Effective Interpretation of Test Results.....	38
Objectives of This Study	39
Materials and Methods	39
Samples Collection	39
Antibiotic Residue Screening Methods	39
Four Plate Test (FPT)	40
Fast Antimicrobial Screening Test (FAST)	40
<i>Bacillus stearothermophilus</i> Disc Assay (BSDA)..	41
A Commercial Antibiotic Test Kit (TAT).....	42
Identification of Tetracycline Residues by ELISA.	43
Test Interpretation of the Four Screening Tests.....	43
Test Agreement and Decision Making.....	45
Results	46
Test agreement between FPT and FAST.....	52
Test agreement between FPT and BSDA.....	54
Test agreement between FPT and TAT.....	55
Discussion	69

	Page
IV ANTIBIOTIC RESISTANT PATTERN OF <i>ESCHERICHIA COLI</i>	79
Introduction.....	79
Objectives of This Study.....	82
Materials and Methods	83
Bacterial Strains	83
Isolation of <i>Escherichia coli</i>	83
Antibiotic Sensitivity Test	83
Antibiotic Disc	84
Interpretation of Inhibitory Zone.....	84
Multiple Antibiotic Resistance Indexing of Isolates (MAR)	84
Detection of Antibiotic Residues and percentage of Antibiotic Resistant <i>Escherichia coli</i>	85
Results	85
Discussion	105
 V PLASMID PROFILES OF <i>ESCHERICHIA</i> <i>COLI</i> ISOLATES FROM CHICKEN MEAT.....	 115
Introduction	115
Objectives of This Study.....	117
Materials and Methods.....	117
Sample Collection.....	117
Bacterial Isolation	117
Antibiotic Susceptibility Test	117
Plasmid Isolation	118
The Modified Alkaline-Lysis/PFGE precipitation Procedure	118
Agarose Gel Electrophoresis	120
Determination of Molecular Weight of Plasmid.....	121
Results.....	123
Correlation between Antibiotic Resistance and Plasmid carriage among <i>Escherichia coli</i> Isolates.....	147
Discussion.....	152

	Page
VI	
COMPARISON OF ANTIBIOTIC RESISTANT <i>ESCHERICHIA COLI</i> ISOLATED FROM CHICKENS GIVEN FEED WITH AND WITHOUT ANTIBIOTICS	156
Introduction	156
Objectives of This Study	158
Materials and Methods	159
Sample Collection	159
Bacterial Isolation and Identification	159
Antibiotic Susceptibility Test	159
Control Farm (Flock D)	160
Isolation of <i>Escherichia coli</i> from Feeds and Water in Control Farm (Flock D)	160
Statistical Analysis	161
Results	161
Discussion	223
VII	
GENERAL DISCUSSION AND CONCLUSION.....	228
BIBLIOGRAPHY.....	237
APPENDIX.....	265
VITA.....	266



LIST OF TABLES

Table		Page
1	Impression of antibiotic use in humans and animals.....	7
2	Mode of action of antibiotics.....	9
3	Occurrence of antibiotic residues in farm animals.....	20
4	Biochemical tests for identification of <i>Escherichia coli</i>	18
5	Serogroups and disease associations of six virulent types of <i>Escherichia coli</i>	22
6	Characteristic of <i>Escherichia coli</i> - related illness in human.....	23
7	Evaluation of a test using a 2 × 2 table	44
8	Kappa agreement.....	46
9	Comparison in performance parameters of FAST, BSDA and TAT standardized against FPT.....	50
10	Test performance of FPT against ELISA.....	51
11	Test performance of FAST against FPT.....	52
12	Test performance of FAST against FPT at (95%) confidence interval.....	53
13	Test performance of BSDA against FPT.....	53
14	Tests performance of BSDA against FPT at (95%) confidence interval.....	54
15	Test performance of TAT against FPT.....	55
16	Tests performance of TAT against FPT at (95%) confidence interval.....	56
17	Comparison in test performance of FAST, BSDA and TAT against FPT at (95%) confidence interval.....	56



18	Tests performance of FPT, FAST, BSDA and TAT at (95%) confidence interval.....	59
19	Relationship between prevalence and predictive value of FAST.....	63
20	Relationship between prevalence and predictive value of BSDA	64
21	Relationship between prevalence and predictive value of TAT.....	65
22	Comparison of bacterial inhibition test.....	67
23	Different parameters of FPT, FAST, BSDA and TAT.....	68
24	Number and percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotics	88
25	Percentage of <i>Escherichia coli</i> isolates resistant to a number of antibiotics	91
26	MAR index of <i>Escherichia coli</i> isolates	93
27	Antibiogramme of <i>Escherichia coli</i> isolates from chicken meat....	96
28	Antibiogroups of <i>Escherichia coli</i> isolates from chicken meat	98
29	Comparison of <i>Escherichia coli</i> isolates from antibiotic residue positive and antibiotic residue negative samples resistant to individual antibiotic.....	100
30	Comparison of <i>Escherichia coli</i> isolates from antibiotic residue positive and antibiotic residue negative samples resistant to number of antibiotic.....	103
31	Percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotics	124
32	Plasmid DNA fragments harboured by <i>Escherichia coli</i> isolates	126
33	Plasmid profiles and antibiogramme of <i>Escherichia coli</i>	143
34	Antibiotic resistance pattern of <i>Escherichia coli</i> isolates without plasmid.....	148
35	Resistant to antibiotics among <i>Escherichia coli</i> isolates with and without plasmids.....	149

	Page
36	Number and percentage of plasmids harboured by <i>Escherichia coli</i> isolates in relation to number of antibiotics they are resistant to 150
37	Occurrence of <i>Escherichia coli</i> isolates in one- day - old chicks, 21 days old and 42 days old chickens in flocks A, B, C and D.... 163
38	Percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic at different ages in flock A 164
39	Antibiotic resistance pattern of <i>Escherichia coli</i> isolated from one - day - old chicks, 21 days old and 42 days old chickens in flock A 166
40	Antibiogroups of <i>Escherichia coli</i> isolates in flock A 167
41	<i>Escherichia coli</i> isolates resistant to number of antibiotics at the age of one - day - old chicks, 21 days and 42 days old chickens in flock A..... 169
42	Percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic at different ages in flock B 171
43	Antibiotic resistance pattern of <i>Escherichia coli</i> isolated from one - day - old chicks, 21 days old and 42 two days old chickens in flock B 173
44	Antibiogroups of <i>Escherichia coli</i> isolates in flock B 175
45	<i>Escherichia coli</i> isolates resistant to number of antibiotics at the age of one - day - old chicks, 21 days old and 42 days old chickens in flock B..... 178
46	Percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic at different ages in flock C 180
47	Antibiotic resistance pattern of <i>Escherichia coli</i> isolated from 21 days old and 42 days old chickens in flock C 182
48	Antibiogroups of <i>Escherichia coli</i> isolates in flock C 184



	Page
49	<i>Escherichia coli</i> isolates resistant to number of antibiotics at the age of 21 days and 42 days old chickens in flock C 186
50	Percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic at different ages in flock D 188
51	Antibiotic resistance pattern of <i>Escherichia coli</i> isolated from one - day - old chicks, 21 days old and 42 days old chickens in flock D 190
52	Antibiogroups of <i>Escherichia coli</i> isolates in flock D 193
53	<i>Escherichia coli</i> isolates resistant to number of antibiotics at the age of one - day - old chicks, 21 days and 42 days old chickens in flock D..... 195
54	Total number and percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic in one - day - old chicks in flocks A, B, C and D 198
55	Total number and percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic isolated from 21 days old chickens in flocks A, B, C and D 299
56	Total number and percentage of <i>Escherichia coli</i> isolates resistant to individual antibiotic isolated from 42 days old chickens in flocks A, B, C and D 200
57	Comparison of <i>Escherichia coli</i> isolates resistant to number of antibiotics isolated from one - day - old chicks in flocks A, B, C and D 204
58	Comparison of <i>Escherichia coli</i> isolates resistant to number of antibiotics isolated from 21 days old chickens in flocks A, B, C and D..... 205
59	Comparison of <i>Escherichia coli</i> isolates resistant to number of antibiotics isolated from 42 days old chickens in flocks A, B, C and D..... 206



	Page
60	Antibiotic residues positive and negative samples from flocks A, B, C and D 210
61	Antibiotic resistance pattern and antibiotic residues positive and negative results of <i>Escherichia coli</i> isolates in 42 days old chickens in flocks A, B, C, and D 210
62	Antibiogramme of <i>Escherichia coli</i> isolated from antibiotic residues positive and negative samples in 42 days old chickens in flocks A, B, C and D 212
63	Mean value of antibiotic resistant <i>Escherichia coli</i> isolates in one - day - old chicks in flocks A, B, C and D using Duncan's multiple range test 213
64	Mean value of antibiotic resistant <i>Escherichia coli</i> isolates in 21 days old chickens in flocks A, B, C and D using Duncan's multiple range test..... 214
65	Mean value of antibiotic resistant <i>Escherichia coli</i> isolates in 42 days old chickens in flocks A, B, C and D using Duncan's multiple range test..... 215
66	Antibiotic susceptibility test of <i>Klebseilla</i> isolated from feed in flock D 216
67	Antibiotic susceptibility test of <i>Pseudomonas</i> isolated from feed in flock D 217
68	Antibiotic susceptibility test of <i>Escherichia coli</i> isolated from drinking water in flock D 218

