

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

# بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



# Cairo University Faculty of veterinary medicine



# Bacteria associated with early mortalities in broiler farms with regard to antibiotics and disinfectants resistance genes

A thesis submitted by

#### Mohammed Iraqi Youssef Mohammed

(B.V. Sc. Fac. Vet. Med. Cairo. Univ. 2010) (M.V. Sc. Fac. Vet. Med. Cairo. Univ. 2014) For the Ph.D. degree in Veterinary Medical sciences (Microbiology)

Under supervision of **Prof. Dr.** 

#### Mona Ibrahim Hassan El-Enbaawy

Professor of Microbiology

Faculty of Veterinary Medicine- Cairo University

Prof. Dr.

#### Soad Abd El-Aziz Abd El-Wanes

Late Chief Researcher of Poultry Diseases at Reference Laboratory for Quality Control on Poultry Production-Animal Health Research Institute -Dokki

2021

## بسم الله الرحمن الرحيم



صدق الله العظيم (سورة طه:آیه ۱۱۶)

#### **Cairo University**

#### Faculty of veterinary medicine

#### Microbiology department

Name: Mohammed Iraqi Youssef Mohammed

**Thesis title:** Bacteria associated with early mortalities in broiler farms

with regard to antibiotics and disinfectants resistance genes

**Degree:** For the Ph.D. degree in Veterinary Medical sciences

(Microbiology)

#### Supervisors: Prof. Dr. Mona Ibrahim Hassan El-Enbaawy

Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University.

#### Prof. Dr. Soad Abd El-Aziz Abd El-Wanes

Late Chief Researcher of Poultry Diseases at

Reference Laboratory for Quality Control on

Poultry Production, Animal Health Research Institute, Dokki

#### **Abstract**

Antibiotics and disinfectants' resistant Gram-negative bacteria represent a major risk on the broiler chicks especially during the first ten days of the rearing cycle, mainly *Salmonella*, *E. coli*, and *Pseudomonas aeruginosa* as they contribute as major causes of early mortalities in broiler farms.

We aimed in this study to shed light on these main three bacterial pathogens through detection of their prevalence, sensitivity range against the different antimicrobials, and resistance genes that hinder the efficacy not only of some antibiotics but also of Quaternary Ammonium Compounds that are widely used to eliminate them.

Five hundred samples (liver, yolk sac, cecum, spleen and heart) from freshly dead affected chicks (1-10 days old) were cultured on different media for the isolation of causative agents by conventional and serological methods.

PCR was used for the detection of resistance genes. The Bacteriological examination revealed the presence of *Salmonella* spp., *E. coli*, and *P. aeruginosa* in the percentages of 23, 25 and 8%, respectively. Single and mixed infections were observed as 41, and 7%, respectively. We found that 86.9% of *Salmonella* serovars were resistant to colistin sulphate, 48% of *E. coli* strains showed resistance against norfloxacin, and 87.5% of *P. aeruginosa* showed resistance against florfenicol.

The *mcr1* gene was found in 86.9% of all *Salmonella* serovar, *qnrS* gene was detected in 16% of *E. coli*, and *floR* gene was present in 100% of *P. aeruginosa* isolates. PCR screening for *qacED1* revealed that all bacterial isolates under test were positive.

The single and mixed experimental bacterial infections of twenty-five one-day-old broiler chicks classified into five groups revealed that the mixed bacterial infection represents a high risk on the broiler chicks than the single infection.

It was concluded that the existence of *mcr1*, *qnrS*, *floR*, and *qacED1* genes among (*Salmonella* spp., *E. coli*, and *P. aeruginosa*) which were isolated from early aged broiler dead chicks that represents a high risk on the poultry industry in Egypt.

**Keywords:** Salmonella serovars, E. coli serotypes, P. aeruginosa, mcr1, qnrS, floR, qacED1, dead broiler chicks, experimental infections.

## **Dedication**

To My dear Mother, My dear Father,

My sisters, My brothers, their children,

And

My friends

### **Acknowledgment**

## First and foremost, I'm extremely grateful and thankful to Allah

who gave me the ability and power to finish this work.

I wish to express my appreciation and deep gratitude to Professor Doctor **Mona Ibrahim El-Enbaawy**, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University for guidance, great efforts, advice and continuous encouragement during all stages of this work.

Very special thanks are extending to Professor Doctor **Soad Abd El-Aziz Abd El-Wanis**, Late Chief researcher of poultry diseases of central laboratory for veterinary quality control on poultry production, Dokki- Giza, for her support, provision of valuable facilities for work, guidance and advices through the practical work.

I would like to express my deep gratitude and appreciation to Professor Doctor **Mohammed Mahmoud Zaki**, Professor of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University for help and support in my work.

I would like to express my deep gratitude and appreciation to **Dr\ Neveen Rabie Bakry** Deputy Head of Epidemiology RLQP, AHRI, Dokki, Giza for help and support in my work.

I wish to express my thanks and gratitude to all members in bacteriology, and biotechnology units in RLQP for their help and for supplying me with work data.

#### **Contents**

Title		
1-Introduction	1	
2-Review of literature:		
2.1. Importance of broiler chicks:	4	
2.2. Causes of early mortality in broiler chicks:	7	
2.3. Early mortality by Gram negative bacteria:	9	
2.3-a Salmonella:	9	
2.3.b-E. coli:		
2.3.c- P. aeruginosa:	17	
2.4. Causes of antimicrobial resistance:		
2.4.1- Antimicrobial resistance in Salmonella species:		
2.4.2- Antimicrobial resistance in E. coli:		
2-4.3 Antimicrobial resistance in <i>P. aeruginosa</i> :		
2.5. Causes of disinfectant resistant Gram-negative bacteria:	37	
2.6. Early mortality by antimicrobial and disinfectant resistant Gram- negative bacteria:	43	
2.7. Early mortality by single and mixed antimicrobial and disinfectant resistant Gram-negative bacteria:		
3-Published Papers		

4-Discussion	56
5-Appendix	72
6-Conclusion	80
7-English Summary	82
8-References	84
9-Arabic Summary	114

#### • <u>List of figures:</u>

No	Title	Page No.
1	Figure (1): Typical chicks' behavior at different temperatures	5
2	<b>Figure (2):</b> Necrotic foci on the liver of broiler chick affected with salmonellosis	12
3	<b>Figure (3):</b> Peritonitis and unabsorbed yolk sac in a 3-day-old broiler chicken affected with salmonellosis	12
4	<b>Figure (4):</b> A 5 days-old-chick with omphalitis clinical signs, and peritonitis	14
5	<b>Figure (5):</b> Gram-negative bacteria structure and their resistance mechanisms	23
6	Figure (6): The horizontal gene transfer mechanisms	24
7	<b>Figure (7):</b> A graphic illustration describing intensive poultry production and its relation to the antimicrobial resistance	25
8	<b>Figure (8):</b> The genetic information exchange involved in the primary pathways conferred to antibiotic resistance	28
9	<b>Figure (9):</b> The descriptive figure of the major types of multidrug-resistance efflux pumps included in the extrusion of quaternary ammonium compounds and quinolones	41
10	<b>Fig. (10):</b> <i>Salmonella, E. coli,</i> and <i>P. aeruginosa</i> isolates isolation %	57
11	Fig. (11): The percentages of single and mixed infections.	58
12	Fig. (12): The single infections percentages.	58
11	<b>Fig.</b> (13): <i>E. coli</i> serotypes %.	60
12	Fig. (14): Salmonella serovars %.	61
13	<b>Fig. (15):</b> Antibiogram range of <i>Salmonella, E. coli</i> , and <i>P. aeruginosa</i> isolates against different groups of antibiotics.	62

14	Fig. (16): The percentage of Salmonella serovars harboring	63
	mcr1, and qacED1 resistance genes.	
15	<b>Fig. (17):</b> The percentage of <i>E. coli</i> serotypes harboring <i>qnrs</i> , and <i>qacED1</i> resistance genes	65
16	Fig. (18): The percentage of mcr1, qnrs, and floR resistance	66
	genes in Salmonella, E. coli, and P. aeruginosa isolates	
	respectively, and <i>qacED1</i> in the three isolates	
17	Figure (19): Cecal core	78
18	Figure (20): Liver enlargement	78
19	Figure (21): Pasty vent	78
20	Figure (22): Necrotic foci on liver	78
21	Figure (23): Pericarditis& perihepatitis.	78
22	Figure (24): Unabsorbed yolk sac.	78
23	Figure (25): Unabsorbed congested yolk sac.	79
24	Figure (26): Poor growth, ruffled feather, sleepy appearance.	79
25	Figure (27): Unabsorbed yolk sac.	79
26	Figure (28): Pericarditis.	79
27	Figure (29): Swollen kidneys.	79
28	Figure (30): Bronzy liver.	79

#### **List of tables:**

No	Title	Page No.
1	<b>Table</b> (1) The experimental infections protocol in each	73
	group:	13

#### • List of abbreviations:

AMC	Amoxicillin-clavulanic acid
AMP10	Ampicillin
CLSI	Clinical and Laboratory Standard Institute
CT10	Colistin sulphate
CFU	Colony forming unit
DO30	Doxycycline
E. coli	Escherichia coli
FFO30	Florfenicol
ISO	International Organization for Standardization
N30	Neomycin
NOR10	Norfloxacin
ppm	part per million
PCR	Polymerase chain reaction
QACs	Quaternary ammonium compounds
S10	Streptomycin
TSI	Triple Sugar Iron
XLD	Xylose lysine deoxycholate agar