



**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**

**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.

## Contents

Title	Page
<b>1-Introduction</b>	1
<b>2-Review of literature:</b>	4
<b>2.1. Importance of broiler chicks:</b>	4
<b>2.2. Causes of early mortality in broiler chicks:</b>	7
<b>2.3. Early mortality by Gram negative bacteria:</b>	9
<i>2.3.a Salmonella:</i>	9
<i>2.3.b-E. coli:</i>	13
<i>2.3.c- P. aeruginosa:</i>	17
<b>2.4. Causes of antimicrobial resistance:</b>	21
<b>2.4.1- Antimicrobial resistance in <i>Salmonella</i> species:</b>	26
<b>2.4.2- Antimicrobial resistance in <i>E. coli</i>:</b>	29
<b>2-4.3 Antimicrobial resistance in <i>P. aeruginosa</i>:</b>	35
<b>2.5. Causes of disinfectant resistant Gram-negative bacteria:</b>	37
<b>2.6. Early mortality by antimicrobial and disinfectant resistant Gramnegative bacteria:</b>	43

<b>2.7. Early mortality by single and mixed antimicrobial and disinfectant resistant Gram-negative bacteria:</b>	47
<b>3-Published Papers</b>	50
<b>4-Discussion</b>	56
<b>5-Appendix</b>	72
<b>6-Conclusion</b>	80
<b>7-English Summary</b>	82
<b>8-References</b>	84
<b>9-Arabic Summary</b>	114

- **List of figures:**

No	Title	Page No.
1	<b>Figure (1):</b> 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	<b>Figure (6):</b> 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</i> , <i>E. coli</i> , and <i>P. aeruginosa</i> isolates isolation %	57
11	<b>Fig. (11):</b> The percentages of single and mixed infections.	58
12	<b>Fig. (12):</b> The single infections percentages.	58
11	<b>Fig. (13):</b> <i>E. coli</i> serotypes %.	60
12	<b>Fig. (14):</b> <i>Salmonella</i> serovars %.	61

13	<b>Fig. (15):</b> Antibioqram range of <i>Salmonella</i> , <i>E. coli</i> , and <i>P. aeruginosa</i> isolates against different groups of antibiotics.	62
14	<b>Fig. (16):</b> The percentage of <i>Salmonella serovars</i> harboring <i>mcr1</i> , and <i>qacEDI</i> resistance genes.	63
15	<b>Fig. (17):</b> The percentage of <i>E. coli</i> serotypes harboring <i>qnrS</i> , and <i>qacEDI</i> resistance genes	65
16	<b>Fig. (18):</b> The percentage of <i>mcr1</i> , <i>qnrS</i> , and <i>floR</i> resistance genes in <i>Salmonella</i> , <i>E. coli</i> , and <i>P. aeruginosa</i> isolates respectively, and <i>qacEDI</i> in the three isolates	66
17	<b>Figure (19):</b> Cecal core	78
18	<b>Figure (20):</b> Liver enlargement	78
19	<b>Figure (21):</b> Pasty vent	78
20	<b>Figure (22):</b> Necrotic foci on liver	78
21	<b>Figure (23):</b> Pericarditis& perihepatitis.	78
22	<b>Figure (24):</b> Unabsorbed yolk sac.	78
23	<b>Figure (25):</b> Unabsorbed congested yolk sac.	79
24	<b>Figure (26):</b> Poor growth, ruffled feather, sleepy appearance.	79
25	<b>Figure (27):</b> Unabsorbed yolk sac.	79
26	<b>Figure (28):</b> Pericarditis.	79
27	<b>Figure (29):</b> Swollen kidneys.	79
28	<b>Figure (30):</b> Bronzy liver.	79

**List of tables:**

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