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Studying of Multi Drug Resistant Bacteria Isolated From Broilers Infected by Viruses

A thesis submitted by

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M.V.Sc., Fac. Vet. Med., Benha University (Microbiology) (2016)

For the degree of PHD in veterinary medicine
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(2022)

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List of abbreviations

Abbreviation	Full Name
AI	Avian Influenza
CIA	Chicken infectious anemia
<i>E.coli</i>	Escherichia coli
FC	fowl cholera
HPAIV	Highly pathogenic avian influenza
IB	Infectious bronchitis
IBDV	infectious bursal disease virus
MDR	multi-drug resistant
MDV	Marek's disease virus
ND	Newcastle disease
NDV	Newcastle disease virus
PCR	Polymerase chain reaction
PD	pullorum disease
QACs	Quaternary ammonium compounds
REV	reticuloendotheliosis virus
RT-PCR	Real time PCR
<i>S.</i>	Salmonella

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Abstract

Title:	Studying of Multi Drug Resistant Bacteria Isolated From Broilers Infected by Viruses
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Abstract	<p>The small broilers flocks help in obtaining a clear picture of the real situation of circulating pathogens among the poultry production sector in Egypt. This study was conducted on total 283 diseased broilers collected from small broilers flocks in Giza and El-Qalubia governorate, Egypt. The clinical signs and autopsy findings were highly suggestive for: Infectious Bronchitis (IB), Avian Influenza (AI), Newcastle Disease (ND), and Chicken Infectious Anemia (CIA). Trachea, lungs, and kidney were collected during the autopsy and examined using molecular tests: polymerase chain reaction and real time polymerase chain reaction (PCR & RT-PCR) for rapid diagnosis of the viral pathogen revealing a high incidence of IB and CIA (71.4 and 61.3% respectively). The 165 liver and intestine samples of the virally infected broilers were subjected to bacteriological examination and all were positive for <i>Escherichia coli</i> (<i>E. coli</i>) or <i>Salmonella</i> or both. <i>E. coli</i> isolates were serotyped into O125, O158 and O111 while <i>Salmonella</i> were serogrouped into: <i>S. Entraitidis</i>, <i>S. Gaille</i> and <i>S. Altona</i>. The multidrug resistance (MDR) pattern was identified by disk diffusion method using 12 different antimicrobial discs: (nalidixic acid, neomycin, trimethoprim, streptomycin, norfloxacin, sulfamethazine, chloramphenicol, tetracycline, doxycycline, oxytetracycline, gentamycin, and fosfomycine). The results showed complete resistance to sulfamethazine, nalidixic acid and oxytetracycline. High resistance to chloramphenicol, trimethoprim, tetracycline, and streptomycin, low resistance to gentamycin, and all isolates were sensitive to fosfomycine. A farther investigation using polymerase chain reaction (PCR) used to determine the prevalence of a pool resistance genes including antimicrobial & disinfectant resistance and mobile genetic element genes (<i>bla_{TEM}</i>, <i>bla_{SHV}</i>, <i>bla_{OXA}</i>,</p>

aadA1, *qacEΔ1* and *intI1*) within the bacterial isolates. The results revealed: 100%/80% , 90%/100%, 0%/0% prevalence of *bla_{TEM}*, *bla_{SHV}*, *bla_{OXA}* in *E.coli*, *Salmonella* isolates respectively. While the *aadA1* gene was detected in 100% of *E.coli* and *Salmonella* isolates as well as the *qacEΔ1* gene .Finally, *intI1* gene was detected in 100% of *E.coli* and 80% of *Salmonella* isolates.

Key words: Broiler, Viral, Bacterial, Multi-drug resistant, genotypic, genes, drug resistant, disinfectant resistant

SUMMERY

MDR bacteria has been a serious concern for animal care sector, human health and environment. This study was conducted to obtain a clear picture of circulating pathogens and its multi-drug resistant (MDR) patterns. Total 283 diseased broilers collected from small broilers flocks in Giza and El-Qalubia province, Egypt. Presumptive diagnosis based on history of disease and clinical signs were suggestive for: Infectious Bronchitis (IB), Avian Influenza (AI), Newcastle Disease (ND), and Chicken Infectious Anemia (CIA). Several organs collected : Trachea, lung and kidney, liver and intestine during the autopsy and according to signs the collected samples were examined by PCR and RT-PCR for these viral diseases revealing a high incidence of IB and CIA (71.4and 61.3% respectively), after wards liver and intestine samples of virally infected broilers subjected to bacteriological examination. All samples were positive for at least one bacterial pathogen (*E. coli*, *Salmonella* or both). *E. coli* were serotyped into O125, O158 and O111 and *Salmonella* into: *S. Entraitidis*, *S. Gaille* and *S. Altona*. 12 different antimicrobial agents (nalidixic acid, neomycin, trimethoprim, streptomycin, norfloxacin, sulfamethazine, chloramphenicol, tetracycline, doxycycline, oxytetracycline, gentamycin, and fosfomycin) used to determine the MDR pattern. Showing high drug resistant phenotypically. A further investigation using polymerase chain reaction (PCR) used to identify genotypic characterization of isolates. Inspection of *bla_{TEM}*, *bla_{SHV}*, *bla_{OXA}* genes (ESBL resistance genes) in *E.coli* & *Salmonella* isolates revealed: 100%/80%, 90%/100%, 0%/0% prevalence respectively. While the *aadA1* gene (streptomycin resistance gene) was detected in 100% of *E.coli* and *salmonella* isolates as well as the *qacEΔ1* gene (the quaternary ammonium compounds ((QACs)) resistance genes). Finally, *intI1* gene (integron classI gene) detected in of *E.coli* and *Salmonella* isolates as followed 100%, 80%.

These findings clearly shows that *bla_{TEM}*, *bla_{SHV}*, *aadA1*, *qacEΔ1* and *intI1* are highly prevalent among the MDR bacteria here in Egypt. This study revealed MDR bacterial pathogens are highly prevalent among the small poultry flocks and greatly interacts with the viral avian diseases here in Egypt. So, Proper hygienic measures and appropriate restrictions actions on the antimicrobial agent usage either for treatment nor as growth promoter is required. Those recommendations are good strategy for controlling the MDR bacteria in poultry production.