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# Pathological Changes Induced by Systemic Bacterial Infections of Layer Hens Suffered from Respiratory Signs

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**ABSTRACT**

**Aims:** to determine pathological changes of systemic bacterial infections with respiratory signs involvement in layer hens**.**

**Methods:** Sixty sample of internal viscera (liver, lung, kidney, trachea, ovary, oviduct) 10 sample for each of them, belong to layer chickens were collected aseptically from different flocks suffering from respiratory signs, nervous signs, diarrhea, depression, reluctant movement and ruffled feathers.

**Results:**

High rate of bacterial infection reported in liver, (43.48%), in which S. *aureus recovered* from *(*26.08%), *E. coli* (13.04%) then, Klebsiella (4.35%). The second affected organ was the ovary (21.74%), in which S. *aureus* represent (8.7%), *E. coli* (4.35%)then, *Klebsiella* (8.7%). Kidney involvement represent (17.39%), in which S. *aureus recovered* from *(*8.7%), *E. coli (*4.35%)then, Klebsiella (4. 35%).Infection of Oviduct was reported as forth level, (8.7%), in which S. *aureus represent (*4.35%) followed *by E. coli(*4.35%).Lung and trachea located at the fifth level according to the rate of infection, (4.35%) for each one , both of them were infected with *S.aureus* .

*S. aureus* develop resistant to 3out of 5 antimicrobial agent used which represent 60% ,which mean that the multidrug resistant trait bearing *S. aureus* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics on the other hand *S. aureus* was sensitive to Doripenem 10µg and Tetracycline30mcg.On the other hand *E.coli* and *Klebsiella* develop resistant to 3out of 5 antimicrobial agent used which represent 60% ,which mean that the multidrug resistant trait bearing *E.coli* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics as well as for Doripenem.

Histopathological examination for liver revealed slight dilatation of sinusoid with obvious dispersed apoptotic cells scattered associated with congestion for blood vessels. Degenerative changes for renal tubules as well as interstitial chronic inflammatory cells infiltration which is typical pathology for pyelonephritis. The ovary shows different level of follicles development and typical pathology for serous cyst adenoma formation. Luteal cysts with haemorrhages and typical appearance of histiocytes laden with iron formation also reported.

**Conclusion:** Liver was the main target for infection followed by ovary. *S. aureus* was the prominent agent followed by *E. coli*. *S. aureus* and *E. coli* have multidrug resistant trait for antibiotics commonly used in poultry medicine. The pathological changes range from apoptotic changes in liver, to degenerative changes of renal tubules and typical pathology for pyelonephritis in kidney. While in the ovary typical pathology was the formation of serous cyst adenoma.

**Key words: Layer hens, Bacterial infections, pathological changes, Multidrug resistance, Respiratory involvement**

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## Introduction:

Respiratory tract involvement are quite common in poultry farms [1]. Respiratory infections constitute an important economic challenge for owners of poultry farms who raise poultry intensively in the halls [2]. Clinical signs due to respiratory disease range from" nasal and ocular discharge, gasping or open mouth breathing, wheezing, snick with various mortalities in a flock" can be observed[3]. Bacterial infection are associated with serious diseases of chickens which cause significant morbidity and mortality and substantially attack the respiratory and digestive systems[4].

The histopathological lesions of the respiratory system are non-specific. Histopathology provides for the possible etiology and it is important that isolation, molecular techniques and other tests should be done to confirm the diagnosis[5]. Further the lesions in the respiratory tract can be complicated due to the involvement of multiple etiologic agents such as "viruses, bacteria, fungi, parasites, nutritional deficiencies, ammonia exposure", etc.[6] . In clinical practice, the respiratory involvement usually come from numerous causative agents and it is rarely to find a single cause.

Current study aims to determine pathological changes of systemic bacterial infections in layer hens suffered from respiratory signs involvement

## Materials and Methods

### Study area and study population

This study was conducted on hen farms in Baqubah city -Diyala Province 33°45'34.71"N; 44°36'23.97"E, North-east. The study extended from October 2019 to April 2020 [7, 8]

### Ethical consideration:

This study conducted according to the principles of Helsinki declaration. An Approval of an ethics committee at the pathology department, College of veterinary medicine, Diyala University, Iraq, was taken before starting of the study.[9-18].

### Clinical examination and Inclusion criteria

Sixty sample of internal viscera (liver, lung, kidney ,trachea ,ovary, oviduct) ,10 sample for each of them ,belong to layer chickens were collected aseptically from different flocks suffering from mild respiratory signs (serous nasal discharge, ocular discharge) to sever respiratory signs ( gasping, sneezing, coughing, conjunctivitis, closed eyes, purulent nasal discharge , head swelling, swelling infra orbital sinus), nerves signs, diarrhea as well as depression , reluctant movement , ruffled feathers also included in current study.

### Sample transportation

Collected samples were transported by insulating foam boxes with ice to the department of pathology, college veterinary medicine, Diyala university, immediately after collection.

### Preparation for Histopathological Investigation

Tissue specimens from (liver, kidney and ovary) were fixed with 10% buffered formalin for preparation of paraffin embedded tissue blocks to prepare

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slides for histological examination using haematoxylin-eosin[19].

**Microbiological Examination for tissue specimens.**

Brain Heart Infusion (BHI) broth was used for enrichment of bacterial population via incubation at 37°C for 24 h. Then after that they were cultured aerobically in "Brian heart infusion agar (BHIA), MacConkey's agar and eosin methylene blue agar (EMB), Mannitol Salt Agar(MSA)". Bacterial isolates were identified on the basis of " morphology, growth characteristics, sugar fermentation and biochemical characteristics such as indole productivity, methyl red, motility, Simon citrate, sulphur reduction and sugar utilization".

**Antibiotic Sensitivity Test**

Kirby- Bauer method was used to determine the susceptibility of the bacterial isolates to antibiotic agents[20]. Five different antibiotic discs ,Danofloxacin25mcg , Ciprofloxacin 5mcg, Cefixime 5mcg, Tetracycline30mcg,and Doripenem 10µg were obtained from (Oxoid Ltd, Baringstoke, Hampshire, England).The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute[21]

**Statistical Analysis:**

Data were statistically described in terms of frequencies and relative frequencies (percentages)[22, 23] . T test used for evaluation the differences[24, 25] . All statistical calculations were done using Microsoft Excel 2010 (Microsoft Corporation, New York,USA) and SPSS version 17[26, 27].The level of significance was 0.05 (two-tail) [19, 28] .

## RESULTS

**Isolation and Identification of Bacteria form Internal Organs**

E. coli was reported in ,6/23 (26.09%)of samples with green metallic sheen EMB media and positive indole test. On the other hand, *S. aureus was isolated from* 13/23 (56.52%) on MSA and confirmed by "Gram staining, biochemical tests, catalase, and coagulase activity assays". *Klebsiella* spp. was recovered from 4/23, (17.39%)showed opaque, mucoid colonies that were pink in color on MacConkey agar and exhibited urease activity and positive for citrate utilization.

The results revealed that high rate of bacterial infection reported in liver , (43.48%) ,in which *S.aureus* represent (26.08%)followed *by E.coli* (13.04%) then ,*Klebsiella* (4.35%) .The second affected organ was the ovary(21.74%) ,in which *S.aureus* represents (8.7%)followed *by E.coli* (4.35%)then ,*Klebsiella* (8.7%).The third affected organ was the kidney(17.39%) , in which *S.aureus* represents (8.7%)followed *by E.coli* (4.35%)then ,*Klebsiella* (4.35%) .infection of Oviduct was reported as forth level , (8.7%) , in which *S.aureus* represents (4.35%) followed *by E.coli* (4.35%).Lung and trachea were located at the fifth level according to the rate of infection, (4.35%) for each one , both of them were infected with *S.aureus* .

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**Antibacterial activity against *S. aureus, E. coli*, and *Klebsiella spp.***

Current study revealed in table(2 ),that *S. aureus* develop resistant to 3out of 5 antimicrobial agent used which represent 60% ,which mean that the multidrug resistant trait bearing *S. aureus* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics on the other hand *S. aureus* was sensitive to Doripenem 10µg and Tetracycline30mcg.On the other hand *E.coli* and *Klebsiella* develop resistant to 3out of 5 antimicrobial agent used which represent 60% ,which mean that the multidrug resistant trait bearing *E.coli* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics as well as for Doripenem.

**Pathological changes in liver, ovary and kidney of hens**

Histopathological examination for liver revealed a slight dilatation of sinusoid with obvious dispersed apoptotic cells scattered associated with congestion for blood vessels (x40) as shown in fig.1-A. Figure2 revealed degenerative changes for renal tubules as well as interstitial chronic inflammatory cells infiltration which is typical pathology for pyelonephritis. (x40). Figure 3-A represent a section of ovary shows different level of follicles development and typical pathology for serous cyst adenoma formation while for other section B shows luteal cysts with haemorrhages and typical appearance of histiocytes laden with iron formation (Figure 3-B).

**Table (1): Bacterial Isolates from internal Organs of Layer Chicken**

| ***Pathogens*** | ***Internal organs*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Trachea** | **Lung** | **Liver** | **Kidney** | **Ovary** | **Oviduct** | Total |
| *S.aureus* | 1(4.35%) | 1(4.35%) | 6(26.08%) | 2(8.7%) | 2(8.7%) | 1(4.35%) | 13(56.52%) |
| *E.coli* | 0( 0%) | 0( 0%) | 3(13.04%) | 1(4.35%) | 1(4.35%) | 1(4.35%) | 6(26.09%) |
| *Klebsiella spp* | 0( 0%) | 0( 0%) | 1(4.35%) | 1(4.35%) | 2(8.7%) | 0( 0%) | 4(17.39%) |
| *Total* | 1(4.35%) | 1(4.35%) | 10(43.48%) | 4(17.39%) | 5(21.74%) | 2(8.7%) | 23(100%) |

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**Table2: Antimicrobial susceptibility test against *S. aureus, E. coli* and *Klebsiella***

| *Bacterial spp* | *Antibiotic* | *Antibiotic Group* | *Mean Diameter of inhibition zone (mm)* | *Reference value* | | | *Interpretation* | *Frequency of Multidrug resistant isolates* |
|---|---|---|---|---|---|---|---|---|
| | | | | *Resistant ≤ mm* | *Intermediate mm* | *Sensitive ≥ mm* | | |
| *S. aureus* | ***Cefixime 5mcg*** | **3rd generation cephalosporins** | ***0*** | ***15*** | ***16-18*** | ***19*** | ***Resistant*** | 60% |
| | ***Danofloxacin25mcg*** | **Synthetic fluoroquinolone** | ***10.66*** | | | ***22*** | ***Resistant*** | |
| | ***Ciprofloxacin 5mcg*** | **fluoroquinolones** | ***0*** | ***15*** | ***16-20*** | ***21*** | ***Resistant*** | |
| | *Doripenem 10µg* | **β-lactam family of antibiotics (Carbapenem)** | *29* | *19* | *22-20* | *23* | *Sensitive* | 0% |
| | *Tetracycline 30mcg* | **Tetracyclines** | *30.33* | *11* | *12-14* | *≥15* | *Sensitive* | 0% |
| *E.coli* | ***Cefixime 5mcg*** | **3rd generation cephalosporins** | ***0*** | ***15*** | ***16-18*** | ***≥ 19*** | ***Resistant*** | 40% |
| | ***Danofloxacin25mcg*** | **Synthetic fluoroquinolones** | ***0*** | | | ***22*** | ***Resistant*** | |
| | *Ciprofloxacin 5mcg* | **fluoroquinolones** | *20* | *15* | *16-20* | *21* | *Intermediate* | 0% |
| | ***Doripenem 10µg*** | **β-lactam family of antibiotics (Carbapenem)** | ***17*** | ***19*** | ***22-20*** | ***23*** | ***Resistant*** | 20% |
| | *Tetracycline 30mcg* | **Tetracyclines** | *31* | *11* | *12-14* | *≥15* | *Sensitive* | 0% |
| *Klebsiella* | ***Cefixime 5mcg*** | **3rd generation cephalosporins** | ***12.66*** | ***15*** | ***16-18*** | ***19*** | ***Resistant*** | 40% |
| | ***Danofloxacin25mcg*** | **Synthetic fluoroquinolones** | ***0*** | | | ***22*** | ***Resistant*** | |
| | *Ciprofloxacin 5mcg* | **fluoroquinolones** | *28* | *15* | *16-20* | *21* | *Sensitive* | 0% |
| | ***Doripenem 10µg*** | **β-lactam family of antibiotics (Carbapenem)** | ***19*** | ***19*** | ***22-20*** | ***23*** | ***Resistant*** | 20% |
| | *Tetracycline 30mcg* | **Tetracyclines** | *25* | *11* | *12-14* | *≥15* | *Sensitive* | 0% |

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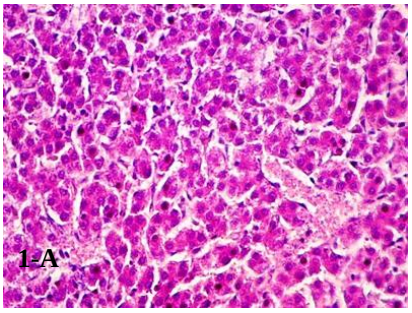


**Figure 1-A:**section of liver showing slight dilatation of sinusoid ;dispersed apoptotic cells and congestion for blood vessels .(x40)

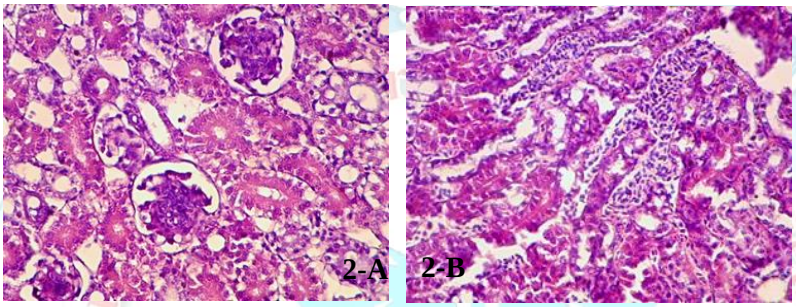


**Figure 2:**
**A:** section of the kidney showing few renal tubules with degenerative changes. .(x40)
**B:** section of the Kidney Showing interstitial chronic inflammatory cells infiltration, atypical pathology for pyelonephritis .(x40)

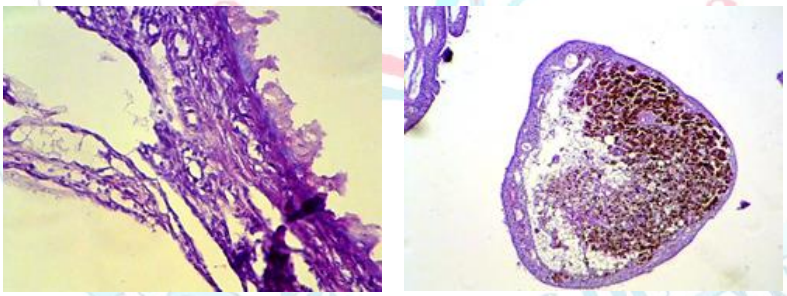


**Figure 3: A:** Section of ovary showing different level of follicles development and typical pathology for serous cyst adenoma formation**. (X10)**
**B:** A section of ovary showing luteal cyst with haemorrhages and typical appearance of histiocytes laden with iron formation**. (X10)**

spread, highly mortality and difficult in prevention and control [29]

The results revealed that high rate of bacterial infection reported in liver , (43.48%) ,in which *S.aureus* represent (26.08%)followed *by E.coli*

## Discussion:

The respiratory diseases are largest importance problem bring extensive economically harm to poultry industry worldwide due to highly

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represent (8.7%)followed *by E.coli* (4.35%)then ,*Klebsiella* (8.7%).The third affected organ was the kidney(17.39%) , in which *S.aureus* (8.7%)followed *by E.coli* (4.35%),then ,*Klebsiella* (4.35%) .Infection of oviduct was reported as forth level , (8.7%) , in which *S.aureus* represent (4.35%) followed *by E.coli* (4.35%).Lung and trachea located at the fifth level according to the rate of infection, (4.35%) for each one , both of them were infected with S.aureus . These results were less than that reported by [30] who stated that *E.coli* infection for internal viscera range from 17.7% to 38.6% in broilers and layers and less than that reported by [4] who stated that *E.coli* was isolated for internal viscera in 40.38% of broilers .On the other hand current study reported Klebsiella spp. in (17.39%) of samples which was higher than that reported in Egypt by [4] who recover the pathogen from 2.1% of examined poultry samples.

The quinolones include Ciprofloxacin; Danafloxacin commonly used in poultry industry as growth promoter and for treatment of various bacterial infections. In current study , *S.aureus* isolated from internal organs of hens develop resistant to extended-spectrum Cephalosporins,( 3rd generation cephalosporins), Cefixime 5mcg; Danofloxacin25mcg and Ciprofloxacin 5mcg which come in line with [31]. Current study revealed that *S. aureus* develop resistant to 3out of 5 antimicrobial agent used which represent 60% (13.04%) then ,*Klebsiella* (4.35%) .The second affected organ was the ovary(21.74%) ,in which *S.aureus* ,which mean that the multidrug resistant trait bearing *S. aureus* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics on the other hand *S. aureus* was sensitive to Doripenem 10µg and Tetracycline30mcg which come in line with [31].While *E.coli* and Klebsiella spp. develop resistant to 3out of 5 antimicrobial agent used which represent 60% ,which mean that the multidrug resistant trait bearing *E.coli* was present in 60% of isolates for third generation cephalosporins ,Cefixime 5mcg, second generation of quinolone antibiotics as well as for Doripenem. This come in line for that reported by[4, 32] for general criteria of multidrug resistant bacteria . Current result come in contrary with that mentioned by [33] "Danofloxacin, like other fluoroquinolones, has activity against a broad spectrum of bacteria, including gram-negative bacilli, especially Enterobacteriaceae (Escherichia coli, Klebsiella, and Salmonella spp.) and some gram-positive cocci, such as Staphylococcus spp".

Multi drug resistance(MDR), trait refers to" simultaneous resistance in one bacterium to three or more classes of antibiotics by various resistance mechanisms generally encoded by different genes is defined as multi drug resistance(MDR)" . In current study MDR S.aureus was reported in 60% of isolates which come

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in line with that reported by [31] who stated that 52% of *S.aureus* isolates have MDR phenotype .

Multi-drug-resistance-genes usually encoding for different resistance phenotypes that use the same mechanism (e.g. efflux). While "Cross-resistance" is defined as "resistance to different antibiotics belong to the same class by the same resistance mechanism". MDR generally due to gaining of transposons, integrons and/or plasmids bearing genetic determinants for dissimilar mechanisms of resistance[34]. The genetic determinant MDR for certain antibiotic ,the bacteria will not simply forfeited its resistance , even after stop of usage [32]. The possible cause was the antibiotic resistance gene, as a result of the use of other antibiotics to which the determinant is genetically linked (co-selection). Another explanation would be that the plasmid encoding the gene is not counter-selected in the absence of the antibiotic. Because of the intensive use of antibiotics in animal production, bacteria in farm animals tend to "collect" resistance genes. As a result, larger number of MDR bacteria can be detected [35] ,which agree with results of current study .

In current study , histopathological changes for liver revealed that there was slight dilatation of sinusoid with obvious dispersed apoptotic cells scattered associated with ;with congestion for blood vessels which come in line with that reported by [36, 37].Figure2 revealed degenerative changes for renal tubules as well as interstitial chronic inflammatory cells infiltration which is typical pathology for pyelonephritis which come in concordance with[36, 37] who reported a similar histopathological changes such as " interstitial nephritis, tubular degeneration, and infiltration by heterophils; necrotic and dilated tubules are filled with urates and casts " . Experimental studies have shown necrosis of the proximal convoluted tubule and distension of distal convoluted tubule. In addition, necrotic foci, heterophils, and lymphocytes are observed in the interstitial spaces. Oedema of Bowman's capsule and granulocytic infiltration ".Figure 3-A represent A section of ovary showing different level of follicles development and typical pathology for serous cyst adenoma formation which come in agreement with [36] while for other section B showing luteal cysts with haemorrhages and typical appearance of histiocytes laden with iron formation.

**Conclusions:**

Liver was the main target for infection followed by ovary. *S. aureus* was the prominent agent followed by *E. coli*. *S. aureus* and *E. coli* have multidrug resistant trait for antibiotics commonly used in poultry medicine. The pathological changes range from apoptotic changes in liver, to degenerative changes of renal tubules and typical pathology for pyelonephritis in kidney. While in the ovary typical pathology was the formation of serous cyst adenoma.

**Conflict of Interest:** not applicable

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