# Multidrug Resistance of isolated *Gallibacterium anatis* from Layers

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**Abstract**

*Gallibacterium anatis* (*G. anatis*) is a member of family *pasteurellaceae* which present normally in the reproductive and respiratory tracts in poultry. it causes oophorities, peritonitis, lowered egg production, salpingitis, in addition to high mortalities in layers. The purpose of the current work was to detect the prevalence of *G. anatis* in layer chickens and antimicrobial sensitivity testing of the recovered isolates.

A total of 400 samples (100 from cloacal swabs, 100 from tracheal swabs, 100 from lung, 100 from ovary and oviduct) were collected randomly from private commercial layers flocks with high mortality at El-Sharkia Governate, Egypt. Collected samples were subjected for distinct bacterial examination for identification of *G. anatis* bacteria. Recovered isolates were tested for antimicrobial sensitivity testing and detection of the prevalence of multidrug-resistant (MDR) strains.

From 400 diseased examined samples, 120 were positive for *G. anatis* as follows (cloacal swabs 27/100; 27%, tracheal swabs 35/100; 35%, lung 35/100; 35% and ovary and oviduct 23/100; 23%) of layer chickens. The overall incidence of *G. anatis* was 30% in the recovered samples. Recovered isolates were highly resistant to doxycycline, amoxycillin and gentamycin with 98%, 96% and 95%, respectively. All isolates were MDR. Isolates were sensitive to florfenicol (90%), erythromycin (96%), difloxacin (44%) and sulfamethoxazole- trimethoprim (57%).

The current study revealed the serious and wide prevalence of multi drug resistance of *G. anatis* in layers. Retrieved isolates were highly resistant for amoxycillin, doxycycline and gentamycin which make a serious problem in poultry industry with dangerously public health threat.

**Keywords:** layers, *G. anatis*, MDR, antimicrobial sensitivity testing.

## Introduction

One of the most essential dietary components all over the world is poultry meat and eggs. It is found that poultry diseases caused by resident microbiota affect animal welfare and resulted in destructive losses in poultry industry as reduced growth and decreased egg production, hence poultry infections affecting meat and egg production in addition to give rise to mortalities **(*AVEC, 2011; AVEC, 2014*)**.

Oophoritis, perihepatitis, air sacculitis, peritonitis, pericarditis, liver necrosis, tracheitis, enteritis salpingitis, and septicemia are the most reported diseases produced by *G. anatis*, biovar hemolytica in chickens (***Bojesen et al., 2004; Bojesen et al., 2007; Neubauer et al., 2009; Paudel et al., 2013***).

*G. anatis*, *G. trehalosifermentans* sp. *nov.*, *G. salpingitidis* sp. *nov.*, *G. melopsittaci* sp. *nov.* and three *G. genomospecies* are the four species allocated in the genus *Gallibacterium* **(Bisgaard *et al.*, 2009)**. According to *16S rRNA* gene sequencing, Genus *Gallibacterium* was belonged to *Pasteurellaceae* family **(*Christensen et al., 2003*)**. Genus *Gallibacterium* is a facultative anaerobic Gram-negative bacilli or pleomorphic organism, capsulated, arranges singly or in pairs, non-motile and non-sporulated bacterium **(*Singh et al., 2016; Elbestawy et al., 2018*)**.

*G. anatis* was isolated from digestive tracts (rectum), lower genital (cloaca and vagina) and upper respiratory (nasal and tracheal passages) as a matter of fact presence of *G. anatis* as normal microbiota in clinically healthy chickens (***Bojesen et al., 2003***). Several etiological and epidemiological factors are controlling *G. anatis* pathogenicity in chickens as route of infection, bacterial strain and physiological status of host (***Bojesen et al. 2008***). There are several factors related to host were found to increase disease severity like age, stress, hormones and immune status. Additionally, changes in environmental factors exaggerate disease severity such as poor ventilation, lack of biosecurity, overcrowding, deficient nutrition, seasonal variations and cold stress (***Paudel and Hess 2017; Paudel et al., 2017***). The ability of *G. anatis* to produce systemic infection is increased in case of *G. anatis* coinfection with infectious bronchitis virus (***He-ping et al., 2012; Mataried 2016***), *G. anatis* mixed bacterial infection with *E.*

*coli, A. paragallinarum* and *Mycoplasma gallisepticum*. Consequently, the increase in disease severity and increased morbidity and mortality in chickens (***Neubauer et al., 2009; El-Hamid et al., 2018***).

There are several problems during treatment of *G. anatis* by antimicrobials also during prevention of *G. anatis* disease by vaccination as a result of the antigenic variations observed among *G. anatis* strains and the widely incidence of multidrug/antibiotic resistant *G. anatis* (***Jones et al. 2013; Chavez et al., 2017; Hess et al., 2019***). Previous several studies showed multiple resistance of *G. anatis* to different antimicrobial classes such as (β-lactams, aminoglycosides, sulfonamides, and tetracyclines) (***Singh et al., 2016***).

The current work was done to assess *G. anatis* incidence in layers, antimicrobial sensitivity testing of the recovered isolates and detection of the prevalence of multidrug-resistant (MDR) strains.

## Materials and Methods

### Samples

About 400 samples including lung, ovary and oviduct and also from tracheal swabs and cloacal swabs, (100 sample for each) were recovered from 100 infected layers from commercial flocks with average mortality rate 35-50% at El-Sharkia Governate, Egypt. Whole organs were collected in sterile containers and stored in cooler with ice packs from the site of collection to the Department of Bacteriology, Animal Health Research Institute, El-Sharkia Governate for distinct bacterial examination of *G. anatis.*

### Isolation and Identification

Examined spicemens (swabs) were cultured onto trypticase soya agar (Difco, USA), also streaked onto MacConkey agar medium and went for incubation under aerobic condition for 24 hrs at 37 °C. In addition to making gram stain, detection of motility, culture characters and the biochemical identification (catalase, urease test, gelatinase, sugar fermentation tests, indole production, methyl red, Voges-Proskauer, citrate utilization,) according to **Christensen et al. (2003)**.

### *G. anatis* antibiotic sensitivity testing

Antimicrobial susceptibility testing of *G. anatis* was performed against eight antibacterial agents including gentamycin (10 µg), amoxycillin (30 µg) , florfenicol (30 µg), doxycycline (30 µg), difloxacin (10 µg), erythromycin (15 µg) and sulfamethoxazole-trimethoprim (30 µg) on Muller-Hinton agar medium (Oxoid, UK) by disc diffusion method. Procedures and interpretation were the same as ***CLSI (2018)***.

## Results

**Phenotypic detection of *G. anatis***

The recovered *G. anatis* colonies were shiny and circular, slightly raised with an entire margin and a size of 1–2 mm in diameter after incubation at 37C for 24 hrs. hemolytic isolates give clear hemolytic area (1–2 mm) on blood agar. The colonies were small pin point pink on MacConkey agar. They were Gram-negative pleomorphic bacilli. The recovered strains were positive for catalase, nitrate reduction, sucrose, and mannitol fermentation tests. But were negative for and Voges-Proskauer tests, methyl red, citrate utilization, indole, urease and gelatinase.

**Incidence of *G. anatis* from diseased layers**

About 120 *G. anatis* isolates were obtained from 400 samples. Thirty-five strains (35%) were collected from tracheal swabs, 23 strains (23%) from reproductive organs, 27 strains (27%) were collected from cloacal swabs, 35 strains (35%) from lung. Thirty-five diseased chickens with *G. anatis*, the microorganism was obtained from ovary and oviduct, trachea and lung and organs of the same chicken in twenty-three diseased chicken. The incidene of *G. anatis* was 30% (30/120) in the examined diseased layer chickens as shown in Table **1** and Figure **1**.

**Antimicrobial susceptibility testing**

The retreived strains showed resistance to doxycycline (98%), amoxicillin (96%) and gentamycin (95%). But susceptible to florfenicol (90%), erythromycin (96%), difloxacin (44%) and sulfamethoxazole- trimethoprim (57%) as shown in Table **2** and Table **3**

It was clear to note that whole *G. anatis* strains showed multiple resistance to 3-5 antibiotics (multidrug resistance).

**Table (1):** *G. anatis* incidence of diseased layers

| No. of diseased examined birds | Types of samples | No. of samples | No. of positive samples | percentage of positive samples (%) |
|---|---|---|---|---|
| 100 | Cloacal swabs | 100 | 27 | 27% |
| | Lung | 100 | 35 | 35% |
| | Tracheal swabs | 100 | 35 | 35% |
| | Ovary and oviduct | 100 | 23 | 23% |
| **Total/percentage** | | 400 | 120 | 30% |

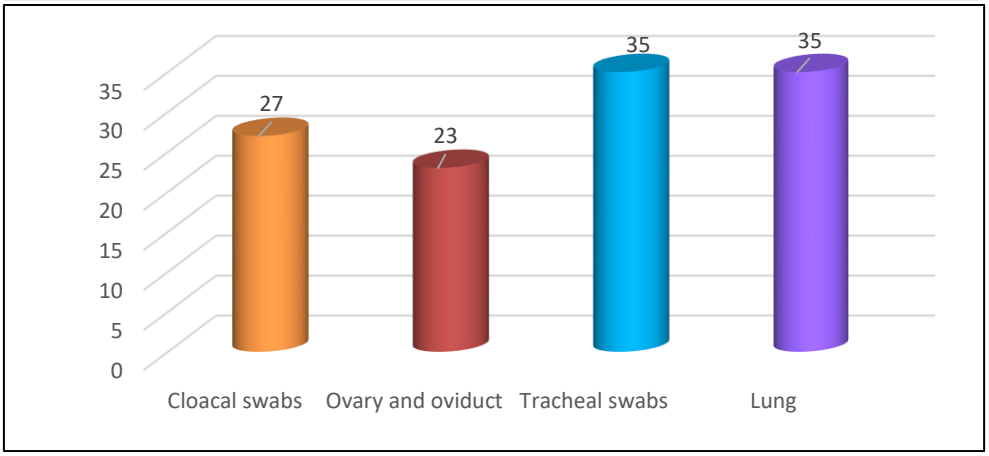


**Figure (1):** Incidence of *G. anatis* isolated from various organs

**Table (2):** *The antibiotic susceptibility testing of G. anatis against different antibacterial agents (No. of positive samples= 120)*

| Antimicrobial agents | Disc concentration (µg) | sensitive | | intermediate | | resistance | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Amoxycillin | 30 | 0 | 0 | 5 | 4.17% | 115 | 96% |
| Doxycycline | 30 | 0 | 0 | 2 | 1.67% | 118 | 98% |
| Florfenicol | 30 | 108 | 90% | 6 | 5% | 6 | 5% |
| Erythromycin | 15 | 115 | 96% | 5 | 4.17% | 0 | 0 |
| Difloxacin | 10 | 53 | 44% | 34 | 28% | 33 | 27.5% |
| Gentamycin | 10 | 0 | 0 | 6 | 5% | 114 | 95% |
| Sulfamethoxazole-Trimethoprim | 30 | 69 | 57.3% | 18 | 14.7% | 33 | 27.5% |

**Table 3:** *Multidrug resistance pattern of G. anatis against different antimicrobial agents*

| β-lactams | Phenicols | Macrolides | Floro-quinolones | Tetracyclines | Amino-glycosides | Total No. of resistance | MDR |
|---|---|---|---|---|---|---|---|
| Amoxicillin (30µg) | Florfenicol (30µg) | Erythromycin (15µg) | Difloxacin (10µg) | Doxycycline (30µg) | Gentamycin (10µg) | | |
| R | S | S | S | R | R | 3 | + |
| R | S | I | R | R | R | 4 | + |
| R | S | S | S | R | R | 3 | + |
| R | S | S | S | R | R | 3 | + |
| R | R | I | R | R | R | 5 | + |
| R | S | S | S | R | R | 3 | + |
| R | S | S | S | R | R | 3 | + |
| R | I | S | R | R | R | 4 | + |

R: resistant, S: sensitive, I: intermediate

**Discussion**

Tracheitis and salpingitis were appeared in infected chickens with *G. anatis* in the last years in different areas through the whole world. The reproductive and respiratory tracts of infected chickens were the predilection sites of *G .anatis* hence, G. *anatis* infection is reflected badly on poultry industry and causes severe economic losses **(*Elbestawy et al. 2018*)**. The purpose of the current work was to detect the incidence of *G. anatis* in chickens, the antibiotic susceptibility testing of the detected isolates.

In the recent study, *G. anatis* was found in layers with respiratory signs and decrease in egg industry. In addition, tracheitis peritonitis, oophoritis and salpingitis were investigated at postmortem examination. The current work, the incidence of *G. anatis* was 30% from diseased layers, where the lung and trachea affected mostly. Those results were agreed with those obtained by ***Johnson et al. 2013*** and ***Van Driessche et al., 2020*.** In the current study, the incidence of *G. anatis* among 400 organs obtained from 100 examined diseased layer chickens was 30%. *G. anatis* was isolated and identified from 35% of lung, 33% of tracheal swabs, 27% of cloacal swabs and 23% of ovary and oviduct samples which was in agree with other studies **(*Bojessen et al. 2003 and Elbestawy et al., 2018*)**. The present study found that presence of *G. anatis* in organs collected from layer chickens showing respiratory tract affection with decrease in egg production. Conventional methods of diagnosis based on the hemolysis of blood agar and carbohydrates fermentation was agreed with ***Christensen et al., 2003***. The antibiotic susceptibility pattern of whole recovered *G. anatis* strains revealed that all strains were resistant to amoxicillin (96%), doxycycline (98%) and gentamycin (95%) those were to some extent related to the finding of ***Bojesen et al. (2011)*** who found that *G. anatis* showed resistance to sulfamethoxazole (97%) and tetracycline (92%). But in the current study *G. anatis* showed sensitivity to florfenicol (90%), erythromycin (96%), difloxacin (44%) and sulfamethoxazole (57%). These data was disagreed with ***Bojessen et al. (2007)*** who reported that all strains was sensitive to amoxicillin + clavulanic acid, cefotaxime, colistin, florfenicol, erythromycin but showed resistance to tetracycline, ciprofloxacin and nalidixic acid. The antibiotic sensitivity testing of the isolated *G. anatis* has revealed MDR.

In conclusion, The recovered isolates were showed higher multi drug resistance to 3–5 different antibiotics. Due to the bad use of antibiotics in both veterinary and health fields this lead to antimicrobial resistance which considered the main problems to affect public health and gaining of

antibiotic resistance genes. Also, establishes a public threat that has affected badly on the poultry industry. So, we recommend for the essential routine of antibiotic sensitivity test regularly and careful use of antimicrobial agents in both health section and veterinary field.

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## مقاومة الجاليبكتريم اناتس المعزولة من الدجاج البياض المتعددة للادوية

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**الملخص العربى**

الجاليبكتريم اناتس هي بكتيريا سالبة الجرام من عائلة الباستراليسى تعيش بشكل طبيعي في الجهاز التنفسي والجهاز التناسلي في الدواجن. وهى سبب رئيسي لالتهاب المبيض وتقلل إنتاج البيض وزيادة نسبة الوفيات في الدجاج البياض. هدفت هذه الدراسة إلى الكشف عن انتشار بكتيريا الجاليبكتريم اناتس في الدجاج البياض واختبار حساسية العزلات لمضادات الميكروبات المختلفة. تم جمع 400 عينة (100 من مسحات القصبة الهوائية و 100 من مسحات الرئة و 100 من مسحات فتحة الشرج و 100 من المبيض وقناته) بشكل عشوائي من مزارع دجاج البياض التجارية الخاصة ذات الوفيات المرتفعة في محافظة الشرقية ، مصر. تم إخضاع العينات التي تم جمعها للفحص البكتريولوجي لعزل بكتريا الجاليبكتريم اناتس وتم اختبار حساسية العزلات لمضادات الميكروبات والكشف عن انتشار السلالات متعددة المقاومة لمضادات الميكروبات. أسفرت النتائج على ان اجمالى معدل انتشار بكتيريا الجاليبكتريم اناتس كان 30% حيث تم عزل 120 عزلة جاليبكتريم أناتيس من 400 عينة من الدجاج البياض (100/27 من مسحات من فتحة الشرج بنسبة 27٪ ، 100/35 من مسحات القصبة الهوائية بنسبة 35٪ ،100/35 من الرئة بنسبة 35٪ و100/23 من المبيض وقناته بنسبة 23 ٪). كانت العزلات شديدة المقاومة للدوكسيسيكلين والأموكسيسيلين والجنتامايسين بنسبة 98٪ و 96٪ و 95٪ على التوالي. وكانت جميع العزلات متعددة المقاومة لمضادات الميكروبات مما يشكل مشكلة خطيرة في صناعة الدواجن ويعتبر إنذارًا للصحة العامة .أما العزلات فكانت حساسة للفلورفينيكول (90٪) والإريثروميسين (96٪) والديفلوكساسين (44٪) والسلفاميثوكسازول-تريميثوبريم) (57٪).