Preliminary Study for Investigation of *G. Anatis* in Broilers

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Abstract

Gallibacterium anatis is a widely distributed species of bacteria isolated from birds. The purpose of this preliminary investigation is to detect the prevalence of *G. anatis* infection in ill hens from private commercial broiler farms in Egypt's ELsharkia governorate. A total of 80 tracheal swabs were collected from broiler chickens with respiratory symptoms. Bacteriological investigation of the collected specimens revealed a prevalence of *G.anatis* of 35%. All isolated bacteria passed the oxidase, catalase, nitrate reduction, sucrose, sorbitol, and mannitol fermentation tests. However, the acquired isolates failed to ferment citrate, indole, urease, gelatinase, or maltose. Additionally, the recovered isolates failed the motility test. The gold standard method of bacterial isolation and identification (which includes culture characteristics, hemolytic activity on blood agar, morphological characteristics, motility test, and biochemical characteristics) is critical for determining the presence of *G. anatis* in birds.

Keywords: *G. anatis*, broilers, respiratory manifestations, prevalence.

Introduction

Chicken infections, particularly those caused by resident microbiota, are damaging to animal welfare and result in catastrophic losses in the poultry business that are often difficult to identify. Among these losses are lower development and egg production, as well as mortalities. On a global scale, chicken meat and eggs have become vital dietary components, resulting in a dramatic growth in demand for poultry goods ***(AVEC 2011; AVEC 2014).***

Approximately every year about 120 million tons of poultry meat are produced worldwide. Nevertheless, demand for animal

G. genomospecies ***(Bisgaard et al., 2009).***

Gallibacterium anatis (*G. anatis*) has two phenotypically diverse biovars: *haemolytica* and the nonhemolytic biovar *anatis* ***(Christensen et al., 2003a).*** *G. anatis* isolates were formerly strains of the avian *Pasteurella haemolytica–Actinobacillus salpingitidis* complex or *Pasteurella anatis*. Salpingitis with or without peritonitis and septicaemia, pericarditis, hepatitis, respiratory tract lesions, and enteritis are all caused by haemolytic *Pasteurella*-like bacteria (*Gallibacterium anatis*) ***(El-Adawy et al., 2018).***

Gallibacterium is a genus of bacteria that is a member of the Pasteurellaceae family. It is classified as a Gram-negative, capsulated, facultative anaerobic bacteria, a rod-shaped or pleomorphic organism that grows alone or in pairs, and a bacterium that does not produce spores or is non-motile. This bacteria is typically cultivated on blood agar plates under microaerophilic conditions containing 5% to 10% carbon dioxide ***(Singh et al., 2016b; Elbestawy et al., 2018).*** *G. anatis* is a widespread worldwide bacterium that has been isolated from chicken in several European nations ***(Bisgaard et al.,2009).***

This study aimed to find and identify G. anatis strains in broiler products such as meat and eggs continues to grow in lockstep with humans' global population and income. Despite significant growth in the chicken sector, production remains constrained by a range of factors, the most serious of which is disease, which results in high morbidity and mortality. Undiagnosed diseases result in large economic losses that go unnoticed due to the existence of generic clinical markers and/or pathological changes associated with numerous infections ***(Krishnegowda et al., 2020; Algammal et al., 2020a; ALgammal et al., 2020b).***

Gallibacterium was assigned to the *Pasteurellaceae* family based on 16S rRNA gene sequences ***(Christensen et al., 2003a).*** The genus includes strains belonging to *G. anatis*, *G. genomospecies* ***(Christensen et al., 2003b)*** and unnamed group V ***(Bisgaard et al., 2009; Algammal et al., 2021).*** Taxon 1 was assaigned to the third group of strains and named *P. anatis,* which was also found closely associated with *Actinobacillus salpingitidis* and avian *P. haemolytica* ***(El-Adawy et al., 2018; Algammal et al,2020c).*** The genus *Gallibacterium* includes four species, namely *G. anatis*, *G. melopsittaci* sp. nov., *G. trehalosifermentans* sp. nov., and *G. salpingitidis* sp. nov., and three

A freshly prepared solution of (tetramethyl-pphenylenediamine dihydrochoride) was used to wet a filter paper placed in a clean sterile petri dish or its cover. Using a glass rod or swab stick, a part of a seperated colony was streaked on the soaked paper. A positive test result was indicated by the rapid development of a dark purple color over a few seconds.

Catalase test:

This test was carried out by taking a single colony from the BA plate and mixed with 2-3 drops of 3% H_2O_2 on a clean grease-free glass slide with the help of a platinum wire loop. A positive test was thought to be the immediate production of gas bubbles.

Indole production:

Peptone water (Oxoid) was inoculated by tested isolate and incubated at 37°C for 48 hrs, then 0.5 ml of kovacs' reagent was added, shaken and examined after one minute. A red color ring indicated indole production.

Methyl red reaction:

Glucose phosphate broth (Oxoid) was inoculated by the tested isolates and incubated at 37°C for two days. Two drops of MR solution were added; the tube was shaken and examined. The development of a red color indicates positive reaction.

Voges Proskauer (VP) test:

chicken flocks in El-Sharkia governorate, Egypt.

Materials and methods

1. Sampling

Eighty tracheal swabs were randomly collected from diseased broiler chickens suffering from respiratory manifestations at the private commercial broiler flocks. All samples were collected in sterile plastic bags and kept in a cooler with ice packs from the collection site and transported to the microbiological laboratory to the microbiological lab for isolation and identification.

2. Isolation of *G. anatis*

Tracheal swabs were inoculated in pre-enriched non-selective medium as buffered peptone water (Oxoid) and incubated aerobically for 24 hr at 37° C. A loopful of incubated broth was streaked out on 5% blood agar (Oxoid) and MacConkey agar (Oxoid), incubated for 24 hr at 37 °C aerobically according to ***El-Adawy et al. (2018)***.

3. Identification of *G. anatis*:

- Suspect colonies were identified using culture characteristics, Gram staining, motility testing, and biochemical reactions as described by ***Christensen et al. (2003) and Singh et al. (2016)***.

The following biochemical reactions were carried out **(*Singh et al., 2016):***

Oxidase test:

A slope of Christensen's urea medium (Oxoid) was highly inoculated before incubation at 37°C for up to 5 days. A red color indicated urease activity.

Gelatinase test:

A heavy inoculum from standard culture was stabbed into the gelatin media (Oxoid) and incubated for 48 hr to 2 weeks at 37°C. After incubation in refrigerator for approximately 30 minutes, the gelatin was not liquefied.

Sugar fermentation test:

A heavy inoculum from standard culture was stabbed into peptone water media (Oxoid) containing 1% Andrade's indicator and inverted Durham's tube to which 1 % of one of the following sugars was added (glucose, galactose, lactose, sucrose, maltose, sorbitol and mannitol), and incubated at 37° c for 48 hr.

Results

Phenotypic characteristics:

All obtained isolates showed, low convex bright translucent, 0.5–1.5 mm in diameter, fine, circular and smooth-edged with grey-white colour. On blood agar colonies, were bright translucent exhibited -β- haemolysis and measured 0.5–1.5 mm in diameter, low convex, fine, round smooth-edged grey-white in color. On MacConkey agar, colonies were small (pin point-like), flat, and pink (lactose Glucose phosphate medium (Oxoid) was inoculated by tested isolates and incubated at 37°C for two days, then 0.6 ml of 5% alpha naphthol solution and 0.2 ml of 40% KOH aqueous solution were added. The tubes were shaken and then examined after 15 minutes and one hr. A bright red color indicates positive reaction.

Citrate utilization test:

A light suspension of the test organism was made in normal saline. Then the citrate medium (Oxoid) was inoculated with a straight wire, incubated at 37°C for up to 7 days and examined daily for turbidity.

Nitrate reduction:

A loopful of culture was introduced to peptone water (Oxoid) containing 0.1 % potassium nitrate and cultured for 2 days at 37°C. Nitrite was identified in nitrate broth culture by adding roughly 1.0 ml of sulfanilic acid and 1.0 ml of -naphthylamine reagent. The development of a definite red color quickly turned brown indicates a favorable result. In the absence of nitrate or nitrite reduction, a pinch of zinc dust was added to the tube to confirm the presence of nitrate or nitrite reduction; the development of a red color after adding zinc indicated that the bacteria were not reducing nitrate.

Urease test:

were negative for citrate utilization, indole, urease, gelatinase and maltose fermentation tests. Besides, the recovered isolates were negative for the motility test.

The prevalence of *G. anatis* in the examined samples:

Twenty-eight isolates were recovered from 80 bacteriologically examined tracheal swabs samples of chickens. The prevalence of *G. anatis* was 35% (28/80) in the examined birds.

fermenter). Gram staining revealed bacteria ranging from coccoid to pleomorphic rods.

Biochemical identification:

All suspected isolates were positive oxidase (formation of a deep purple-blue/blue colour indicates oxidase production within 5-10 seconds), catalase (produce copious bubbles) and nitrate reduction (Development of a cherry red colouration), as well as sucrose, sorbitol, and mannitol fermentation tests (produce yellow colour with gas production). However, suspected isolates

Table (1): *The biochemical characteristics of G. anatis:*

The test	Reaction result
Oxidase	+ Ve
Catalase	+ Ve
Nitrate reduction	+Ve
Indole	- Ve
MR	- Ve
VP	- Ve
Citrate utilization	- Ve
Urease	- Ve
Gelatinase	- Ve
Sucrose fermentation	+ Ve
Sorbitol fermentation	+ Ve
Mannitol fermentation	+ Ve
Maltose fermentation	- Ve

+ Ve: Positive - Ve: Negative

and eggs are seen as critical and basic ingredients of the global diet of the future ***(AVEC, 2011).*** Pasteurellaceae are important as primary or opportunistic

Discussion

Worldwide necessity for meat and animal products is increasing in lockstep with global population and wealth growth. Poultry meat

Additionally, the results of this study indicated that *G. anatis* isolates reacted positively to catalase, oxidase, nitrate reduction, mannitol, sucrose, and sorbitol, but negatively to indole, urease, gelatinase, and maltose. These findings corroborated those of ***Christensen et al. (2003)*** and ***Bojesen et al. (2007),*** who observed comparable reactions in *G. anatis* isolates, indicating that all typical *G. anatis* strains are catalase, oxidase, and nitrate reduction positive.

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infections in domestic and wild animals, primarily respiratory pathogens ***(Yaman and Sahan (2019).***

In our investigation, *G. anatis* was diagnosed in bids that suffered from respiratory signs and occasionally head swelling. The post mortem examination of sacrificed and/or freshly dead chickens revealed mild tracheitis, peritonitis. In recent years highly economic losses were detected in different poultry farms due to *G. anatis* bacteria infection ***(Yaman and Sahan 2019; Lozica et al., 2020).*** *G. anatis* was regularly isolated from ill but otherwise healthy layers and cockerels of unusual chicken breeds, and various other birds, including turkeys, geese, ducks, pheasants, partridges, and cattle egrets ***(Paudel et al., 2014; Sorour et al., 2015).***

The overall prevalence of *G. anatis* was 35% in the present study, based on 80 samples obtained from the trachea of sick chickens. ***Bojesen et al. (2003)*** collected tracheal and cloacal swabs from probable infected flocks and isolated and identified *G. anatis* at a high percentage. While ***Elbestawy et al. (2018)*** isolated six *G. anatis* isolates (19.6 %) from tracheal, ovarian, and oviduct swabs obtained from Egyptian egg-laying chickens.

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دراسة مبدئية لاستقصاء مدى انتشار ميكروب الجاليبيكتيريم اناتيس في الدجاج

الملخص العربي

في هذه الدراسة تم تجميع عدد (80) عينة بطريقة عشوائية (مسحة من القصبة الهوائية) من مزارع الدجاج المصاب بمحافظة الاسماعيلية في مصر وجمعت كل العينات في اكياس بلاستيك نظيفة وحفظت في المبرد مع قطع ثلجية لنقلها من مزارع الدواجن لقسم البكتريا بمعهد البحوث صحة الحيوان بمحافظة الشرقية في مصر. ثم اخضعت العينات المجمعة للفحص البكتيريولوجي لعزل وتصنيف ميكروب الجاليبيكتيريم اناتيس وتم تلخيص النتائج كالاتي: قد تم التعرف علي ميكروب الجاليبيكتيريم اناتيس علي أساس الشكل المجهري ,التفاعل لصبغة الغرام والاختبارت الكيميائية الحيوية وقد اثبت أنها عصيات أو متعددة الاشكال سالبة للغرام ، غير متحركة . موجبة لاختبارات الكاتلاز ، الاوكسيداز ، و اختزال النيترات.وسالبة لاختبارات اليورياز،الاندول ،الجيلتيناز وسترات يتوليزاشن. وتم عزل عدد(28) عترة من الجالي بكتيريوم أناتس من الدجاج المصاب بمعدل انتشار 35%.