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STUDY ON PROTEOLYTIC BACTERIA AFFECTING THE RESPIRATORY TRACT OF CHIKENS

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ABSTRACT:

Ninety five samples from trachea of both alive and freshly dead chickens (different ages) were collected from different farms of Assiut Governorate. These samples were cultured on different media. The proteolytic bacteria were identified by using caseinate agar. After biochemical tests, the proteolytic bacteria were classified into: *Staph. aureus-Staph. hyicus. Staph. epidermis-Flavobacterium sp.* and *Vibrio alginolyticus*. Experimental infection of 7-day-old chicks was done. Intranasal and oral infection of chicks with *Staph. hyicus* led to mortality rate between 20-40% within 6 days postinoculation, mucus secretion from the nose and respiratory signs. Intranasal infection of chicks with *Flavobacterium* gave neither death nor respiratory signs, but double dose of the bacterial suspenstion showed mortality rate of 10% with mild respiratory signs.

In vitro sensitivity test for *Staph. hyicus* showed that enrofloxacin, streptomycin and amikacin were the most effective drugs. But tetracycline, spectinomycin and gentamycin were the most effective drugs for *Flavobacterium sp*.

INTRODUCTION:

Easterday and Hinshaw, (1991) reported that bacteria in the respiratory flora of poultry secretes proteolytic enzymes and so are named proteolytic bacteria. These enzymes may increase the severity of avian influenza virus in poultry due to cleavage of avian influenza virus hemagglutinin molecules by proteolytic enzymes. The potential existence of this mechanism is especially appealing as an explanation for the frequent observation that many A1 viruses associated with severe clinical illness under field conditions are non pathogenic under laboratory conditions (Newman *et al*., 1981), and (king *et al*., 2009).

Byrum and Slemons (1995) showed that proteolytic bacteria were readily demonstrated among the upper respiratory tract flora of poultry included five species of Staphylococci (*Staph. aureus, Staph. epidermis, Staph. sciuri, Staph. xylosus and Staph. hyicus*) as well as two gram–negative bacterial species: *Flavobacterium sp*. and Vibrio alginolyticus, where the later 2 organisms usually reported to be associated with soil and surface water origins. The genus Vibrio found in fresh water, brackish or estuarine water and marine or salt water. The large number of proteolytic bacteria in the

marine environment, coupled with the multiplicity of enzyme production by each bacterium, (Merkel 1971). Mancini *et al*., (2008) reported that interaction of extracellular proteases with the hemagglutinin may activate viral infection in natural infection.

The present study was under taken to cover the following points:

- Isolation and identification of proteolytic bacteria which affect the respiratory tract.
- Experimental infection of chicks with isolated proteolytic bacteria.
- In vitro sensitivity test to show the most effective drugs against the isolated organisms.

MATERIAL AND METHODS:

Material:

Specimens:

Ninety five samples (trachea) from alive and freshly dead chickens (different ages) with respiratory signs were collected from different farms of Assiut Governorate.

Media, sugars used were:

Tryptose soy agar with 5% sheep blood, caseinate agar, MacConkey's agar sodium chloride, glucose, mannose, sucrose, maltose, mannitol, lactose, esculin, yeast extract, ferric citrate, urea agar and gelatin.

Reagents:

Kovac's, oxidase and Voges – Proskauer :

Stain : Gram's stain.

Chicks for experimental infection :

Fifty, 1-day-old chicks were kept under observation for one week before the experiment.

In vitro antibiotic sensitivity discs used were:

Neomycin (30 µg), gentamycin (10 µg), ampicillin (30 µg), amikacin (30 µg), tetracycline (30 µg), spectinomycin (25 µg), streptomycin (10 µg), amoxycillin (25 µg), penicillin (10 µg), enrofloxacin (5 µg), kanamycin (30 µg), cephalexin (30 µg), oxytetracycline (30 µg) and doxycycline (30 µg).

Methods:

Isolation:

Tracheal samples were inoculated on to caseinate agar and tryptose soy agar with 5% sheep blood. The bacterial culture plates were incubated aerobically at 37^{o}C for 24-48h. Susbected colonies were subjected to bacteriological examination to identify the organism by showing: (shape – size - colour) of the colonies, the typical morphology of the organisms by Gram's stain and studying the biochemical reactions according to Ellen *et al*., (1994) and Connie and George (1995).

Pathogenicity test:

Fifty, 1-day old balady chicks were used. They kept under observation for 7 days before the experiment, then they were divided as follow:

1st group: Ten, 1-week-old chicks were inoculated intranasally with 0.5 ml of a bacterial suspension of the isolated *Staph. hyicus* containing 10^6 C.FU of 24 h. culture of blood agar.

2nd group: Ten, 1-week-old chicks. were inoculated orally with 0.5 ml of a bacterial suspension of the isolated *Staph. hyicus* containing 10^6 CFU/ml of 24h. cultures of blood agar.

3rd group: Five, 1-week-old chicks were left as control .

4th group: Ten, 1-week-old chicks, were inoculated intranasaly with 0.5 ml of a bacterial suspension of the isolated *Flavobacterium sp*. containing 10^6 CFU/ml of 24 h. culture of blood agar.

5th group: Ten, 1-week-old chicks, were inoculated intranasally with 1ml of a bacterial suspension of the isolated *Flavobacterium sp*. containing 10^6 CFU/ml of 24 h. culture of blood agar .

6th group: Five -1week-old chicks were left as control.

In vitro antibiotic sensitivity test:

Sensitivity test were carried out on blood agar using sensitivity discs.

RESULTS:

Postmortem (PM) examination of naturally infected chickens reveald accumulation of mucus or bloody mucus in the anterior part of trachea, congestion of lungs and airsacculitis.

The results of bacteriological examinations are illustrated in table (1).

According to the morphology and culture characters and biochemical reactions, we could identify:

- ***Staph. aureus*** **3/95 isolates (2.8%).**
- ***Staph. hyicus*** **11/95 isolates (10.4%).**
- ***Staph. epidermis*** **16/95 isolates (15.2%).**
- ***Flavobacterium sp.*** **85/95 isolates (80.7%).**
- **Vibrio alginolyticus. 2/95 isolates (1.9%).**

On caseinate agar, highly proteolytic bacteria were classified as colonies surrounded by an inner clear zone and an outer zone of caseinate precipitates while proteolytic bacteria were identified as colonies surrounded only by caseinate precipitates. Colonies without surrounding caseinate precipitates were classified as nonproteolytic. Based on this classification, *Flavobacterium* and Vibrio alginolyticus are highly proteolytic gram-negative rods and *Staph. aureus, Staph. hyicus* and *Staph. epidermis* are highly proteolytic gram positive cocci.

Pathogeicity test:

Intranasal infection of 7-day–old chicks with the isolated *Staph. hyicus* resulted in 20-40% mortality within 6 days (p1). Before death, the birds were listless, depressed with eye affection (Fig. 1). Some birds had secretion from the eyes, mucous secretion from the nostrils and mouth breathing. PM examination showed bloody mucus in the anterior part of trachea (Fig. 2) with airsacculitis and congestion of lungs (Fig. 3). Oral infection in the 2nd group also gave the same results. Experimental infection of 7-day-old chicks intranasally with the isolated *Flavobacterium* gave neither signs nor lesions but double dose of the inoculated organism (5th group) gives mild respiratory signs with 10% moratlity. PM examination revealed bloody mucus in the trachea of some birds (Fig. 4) .

There were no signs and no lesions in the control birds. Reisolation of *Staph. hyicus* and *Flavobacterium* from trachea of the inoculated chicks was successful.

The effects of the different antibiotics on the isolated organisms are illustrated in tables (2 and 3).

Table (1): Illustrates the results of bacteriological examinations

Suspected colony	Colony on blood agar	Colony on nutrient agar with 6% NaCl	Colony on Macconkey's agar	Gram's stain
Flavobacterium sp	Lavender green discoloration of the colony	No growth	No growth	Gram-negative bacilli
Staph. sp	some give β haemolysis	No growth	No growth	Gram –positive cocci
Vibrio sp	Give β or α haemolytic colony	Small white colony	Non lactose fermenter colony	Gram-negative, slightly curved rod.

Table (2): Illustrates in vitro sensitivity test for *Staph. hyicus*

Antibiotic discs	Sensitivity of *Staph hyicus* isolates
Enrofloxacin	+++
Streptomycin	+++
Amikacin	+++
Ampicillin	++
Tetracycline	++
Amoxycillin	++
Gentamycin	+
Kanamycin	-
Neomycin	-
Penicillin	-

Table (3): Illustrates in vitro sensitivity test for Flavobacterium

Antibiotic discs	Sensitivity of *Flavobacterium* isolates
Tetracycline	+++
Gentamycin	+++
Spectinomycin	+++
Oxytetracycline	++
Doxycycline	++
Cephalexin	+
Ampicillin	+
Kanamycin	-

(+++) highly sensitive (++) moderate sensitive (+) weak sensitive (-) not sensitive

Fig. (1): Experimentally infected chicks with the isolated *Staph. hyicus* showing depression and eye affection

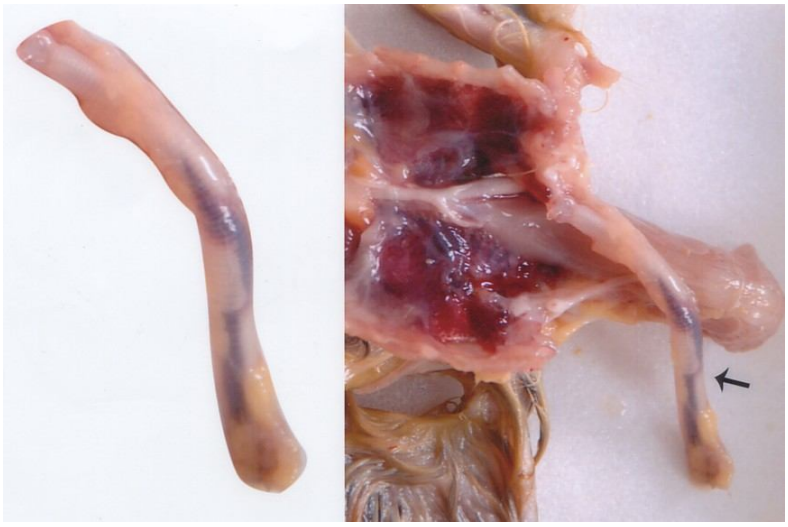

Fig. (2) Showing bloody mucus in the trachea of experimentally infected chick with *Staph. hyicus*

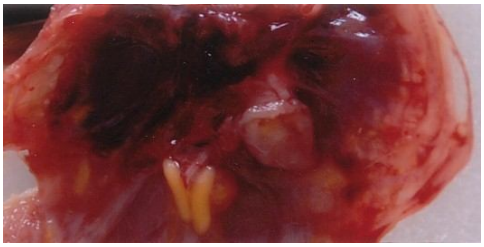

Fig. (3): Showing congestion of lungs

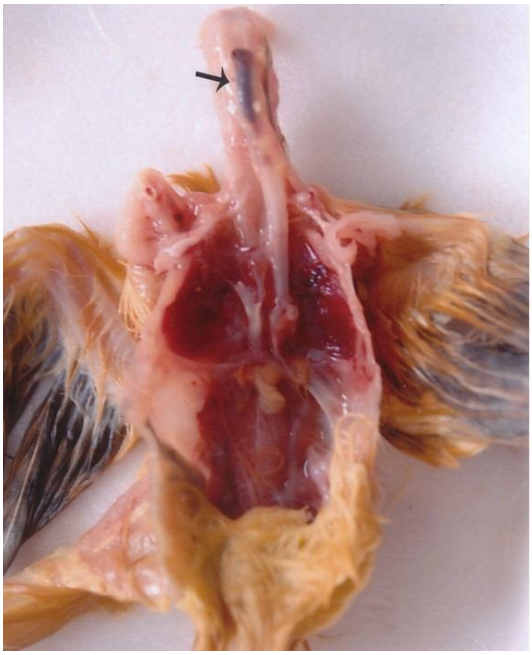

Fig. (4): Experimentally infected chick with high dose of *Flavobacterium*, showing bloody mucus in the trachea

DISCUSSION:

The proteolytic bacteria may play a role in the enhancement of Avian Influenza virus infectivity in field outbreaks where the protease enzyme which secreted by proteolytic bacteria is capable of enhancing the infectivity of influenza (Mancini *et al.*, 2008).

Our results indicated that highly proteolytic and proteolytic bacteria are commonly present in upper trachea of poultry in varying proportions. These bacteria produces proteases that digest detectable quantities of casein in caseinate agar plates.

In our study, bacteriological examination revealed isolation of proteolytic bacteria *Staph. aureus, Staph. hyicus, Staph. epidermis, Flavobacterium sp* and *Vibrio alginolyticus*, Byrum and Slemons (1995) isolated also *Staph. sciuri* and *Staph. xylosus* beside the previous organisms.

Experimental infection of 7-day-old chicks intranasally and orally with the isolated *Staph. hyicus* revealed respiratory signs while intranasal infection of chicks with the isolated *Flavobacterium* gave no signs or lesions, while the overdose resulted in mild respiratory signs. This result is inagrrement with that recorded by Byrum and Selemons (1995). They reported that the isolated highly proteolytic bacteria from poultry may be pathogenic and associated with disease or nonpathogenic and not associated with disease.

Our conclusion proved that, highly proteolytic bacteria present in the respiratory tract of poultry may be pathogenic causing respiratory signs or non pathogenic and so the proteolytic bacteria may play a role in the enhancement of avian influenza virus infectivity in field outbreaks due to the protease enzyme, that secreted by these bacteria. So hygienic measure must be done and proper cleaning and disinfection can reduce environmental contamination by such bacteria.

REFERENCES:

Byrum B.R. and Slemons R.D. (1995): Detection of proteolytic bacteria in the upper respiratory tract flora of poultry. Avian Diseases 39 : 622–626 .

Connie, M.R. and George, M. (1995): laboratory identification of significant isolates in: Text book of Diagnostic Microbiology. Part II p. 492–499. page 530 – 531 .

Easterday, B. C., and Hinshaw V. S. (1991): Influenza in : Diseases of poultry 9th ed . Calnek B.W., Barnes H.J., Berd, C.W, Reid, W.M. and Yoder H.W. Jr., eds. low state university press, Ames lowa. P.P. 531 – 549.

Ellen, J.B., lance, R.P. and Sydney, M.F. (1994): Methods for identification of etiological agents of infectious diseases in: Diagnostic Microbiology Edit by: Baily and Scott's.

King, M.D, Guentzel M.N., Arulanandam, B.P., lupiani, B. and chamebers J.P. (2009): Proteolytic bacteria in the lower digestive tract of poultry may affect avian influenza virus pathogenicity. Poult. Sci, 88 : 1388–1393 .

Mancini, D., Assunc, D. and portari, O. (2008): Influenza virus and proteolytic bacteria co-infection in respiratory tract from individuals presenting respiratory manifestions Rev. inst. Med. Trop. S. Paulo. Vol. 50, pp. 41-46 .

Merkel. J.R. (1971): Proteolytic enzyme production by Marine bacteria. Scientific Technical information Network. At the Defense Technical information Center (DTIC).

New man , J., Halvorson D., Karunakaran, D., Poss, P. and Johnson, J. (1981): Complications associated with avian influenza infection. proc. First international Avian Influenza symposium R.A. Bankowski, ed. Carter printing Co., Richmond, Va. PP.8-12 .

دراسة عن البكتريا المحللة للبروتين المؤثرة على الجهاز التنفسي للدجاج

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تم جمع 95عينة من القصبة الهوائية لدجاج حي ونافق حديثاً (أعمار مختلفة) من عدة مصادر بمحافظة أسيوط، وتم زرع هذه العينات على مستنبتات غذائية مختلفة، وأمكن التعرف على البكتريا المحللة للبروتين باستخدام الكازيين أجار، وبعد عمل الاختبارات البيوكيميائية أمكن تصنيف تلك البكتريا المحللة للبروتين إلى: المكور العنقودي الذهبي، المكور العنقودي هايكس، المكور العنقودي ابيدرمس، فلافوباكتريوم، البكتريا الواوية. وتم إجراء عدوى صناعية على كتاكيت سليمة عمر 7 أيام، وقد سببت العدوى الصناعية عن طريق الأنف والفم باستخدام ميكروب المكور العنقودي هايكس نسبة نفوق تراوحت من 20–40% خلال 6 أيام من بداية العدوى مع وجود إفرازات من العين وسائل مخاطي بالأنف وأعراض تنفسية لباقي الكتاكيت أما العدوى عن طريق الأنف باستخدام ميكروب فلافوباكتريوم لم تسبب أي نفوق أو أعراض ولكن باستخدام جرعة مضاعفة من الميكروب أمكن إحداث نفوق للكتاكيت بنسبة 10%، وظهور أعراض تنفسية. أظهر اختبار الحساسية لميكروب المكور العنقودي هايكس المعزول أن الأنروفلوكساسين والاستربتوميسين والإميكاسين هي الأدوية الأكثر تأثيراً عليه أما ميكروب الفلافوباكتريوم فوجد أن التتراسيكلين الاسبكتينوميسين والجنتاميسين هي الأدوية الأكثر فاعلية.