# CHARACTERIZATION OF PASTEURELLA MULTOCIDA CAPSULE CAUSING PNEUMONIA IN CHICKEN

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## Abstract

**In the present investigation, trails for isolation of *P. Multocida* from dead (40), apparently healthy (25) and diseased chickens (25), with a history of respiratory disorders, were done for the reorganization of the organism. Moreover, 100 sera samples were collected from apparently healthy and diseased chickens and examined serologically for detection of *P. Multocida* antibodies. It was noticed that *P. Multocida* was detected from the diseased (42.9%) and apparently healthy chickens (20%). In regard to dead chickens, the organism was recovered form lung and heart blood, but could not be isolated form the liver samples. LD50 of *P. Multocida* capsular antigen was detected in chickens 1/40 dilution of prepared capsular antigen achieved 50% lethality in the inoculated group. The haemolytic activity of P. Multocida on sheep blood agar plates enhanced and become clearer at refrigeration temperature. In the meantime, the serological examination indicated that *P. Multocida* agglutinin existed in both apparently healthy and diseased chickens.**

## INTRODUCTION

***Pasteurella multocida*** causes fowl cholera in chickens and many avian species and hemorrhtagic septicemia in cattle and buffalos and atrophic rhinitis in swine. Serotype A capsular material is most often associated with avian cholera, function as virulence factor, and it is composed largely of hyaluronic acid *(12)*. Organisms belonging to capsular serotypes B, D and F had been isolated from diseased birds, with low incidence as compared to serotype A *(14)*

***P. Multocida*** is an important cause pneumonia, septicaemia and soft tissue infections; which accounts for 25- 43% of these infections *(18)*.

Pneumonia caused by ***P. Multocida*** is quiet dangerous because it is extremely difficult to be controlled, the mortality rate may exceed 50% even in the treated cases. The presence ***P. Multocida*** capsular antigen (KCA) in the serum of patients infected with ***P. Multocida*** appeared to correlate with severity of infection, persistence of active foci, and a poorer prognosis than in those patients which had no detectable antigen. The lipoplysaccharides of ***P. Multocida*** may contribute to the pathogencity of the organism in a variety of ways, whereas the capsule, which possess a hydrophilic character *(9)*; plays a well known antiphagocytic role and may also aid in protecting the organisms from complement- mediated lyses in serum of infected animals *(7)*.

The main clinical signs of infected animals with ***P. Multocida*** rise in body temperature, acceleration of respiration, abdominal respiration, abnormal sounds on lung auscultation ***(17)***.

The present study aimed to isolate and identify ***P. Multocida*** in chickens suffered from respiratory manifestation, and to study the role of the isolates in induction of disease symptoms by determination of some virulence factors.

## MATERIAL AND METHODS

A total of 100 samples were collected from apparently healthy as well as diseased and dead chickens with a history of respiratory manifestation, 25 arid 35 nasal swabs were collected from apparently healthy and diseased chickens respectively; and 40 samples (20 of lung tissues, and 10 each of heart blood, and liver samples) from chicken. Another 100 blood samples were obtained from both apparently healthy (60) and diseased chicken (40).

Nasal swabs and organs tissues were cultivated on sheep blood agar plate and MacCon-key agar, incubated at 37°C for 48 hrs.

Smears from suspected colonies were stained with Gram's stain to recognize Gram negative coco-bacilli. In the meantime, smears were stained by Indian ink to reveal the presence of the capsule,

The suspected colonies were further characterized by biochemical and serological identifications according to *(10)* and *(13)*

**Studying of some virulence features of isolates:**

1- **Pathogenicity test:**

**a) preparation of capsular antigen**

A loopful from organism suspension was inoculated onto the surface of lactose agar plate (containing 0.5% lactose and 0.5% peptone) which is sufficient to give good but not excessive growth of capsules *(3)* The cultures were incubated for 24 hrs at 37°C and the, colonies were removed from the agar surface (harvesting) and suspended in phosphate buffer saline, then checked for the presence of capsules by moist Indian Ink stain. The bacterial suspension were incubated one hour in water bath at 37°C, followed by centrifugation at 17,000 xg for 15 minutes. The resulting extract supernatant (capsular antigen) were removed and filtered *(4)*.

**b) Experimental design:** "Mice Lethality Test"

66 white mice were divided into two groups, 56 infected mice and another 10 were kept as control. The tested mice were divided into seven groups of eight mice each, and inoculated intraperitoneally with 1ml of one of five serial 10 fold dilutions of the freshly prepared capsule of P. Multocida in sterile PBS. Dead mice was recorded every 24 hr. *(11)*. The mortality rate was recorded in both inoculated and controlled mice during a period of two week observation.

2- **Haemolytic activity:** *(1)*:

The ***P. Multocida*** isolates were cultivated onto 0.6% sheep blood agar plates. The haemolytic activity was recorded after 2 hours incubation at 37°C, then after 24 hours incubation at 4°C.

3- **Serum agglutination test** *(11)*

Blood was collected from both diseased and apparently healthy chickens for serum separation ***P. Multocida*** antigen was prepared from

48 hours broth culture onto nutrient agar, incubated at 37°C and harvested into physiological saline. The viable cell suspension was heated in water bath at 60°C for 60 min. Antigen was standardized with 0.5% saline to an absorbance of 0.39 at 550 nm. Two fold serial dilutions were prepared from each serum sample. The end titer was recorded for each sample.

## RESULTS

From the results shown in table (1) and fig. (1); it is clear that, out of 100 samples collected from dead, diseased and apparently healthy chickens, 27 cases harbored ***P. Multocida*** (27%).

The results recorded in table (2) revealed that LD5Q was recorded in mice group No. IV "inoculated with (1/40) dilution of capsular antigen", whereas 4 mice out of 8 were dead (50%), the mortality rate reached to (43%). No symptoms of death were observed in the control mice during the two weeks of observation.
The haemolytic activity of the isolates (27) was studied as shown in table (3). 4 isolates of ***P. multocida*** (14.8%) showed haemolysis of sheep RBCs after 24 hours of incubation at 37°C. While 9 isolates (33.3%) showed haemolysis at 4°C after 24 hours of incubation. The haemolysis intensity increased by incubation of plates at 4°C and became sharper and cleaner.

The collected serum samples (100) were titrated by using tube agglutination test. Among the apparently healthy chickens, 8 out of 60 serum samples were positive (13.3%), 3 serum samples had 4 antibody titers (5%), 2 had 8, 16 (3.3%) each, and 1 had 128 (1.7%). Among the diseased chickens 9 out of 40 samples showed positive results (22.5%). The mean antibody liter was 4 in 3 samples (7.5%), 2 had titer of 8 and 32 (5%) each. Finally 1 sample had tillers of 128, 512 (2.5%) each as shown in table (4) and fig. (2).

**Table (1):** Prevalence rate ***P. Multocida*** isolated from apparently healthy, diseased, and dead chickens suffered from respiratory manifestation.

| General Health Condition | Type of Samples | No. of Examined Samples | Positives Cases | | Collective Positive % |
|---|---|---|---|---|---|
| | | | No. | % | |
| Apparently healthy chicken | Nasal swabs | 25 | 5 | 20 | 20 |
| Diseased chicken | Nasal swabs | 35 | 15 | 42.9 | 42.9 |
| Dead chicken | Lung Heart<br>blood Liver | 20<br>10<br>10 | 5<br>2<br>- | 25<br>20<br>-- | 17.5 |
| Total | | 100 | 27 | 27 | |

**Table (2):** Mice lethality test: I/P inoculation of mice with 10 fold serial dilution of capsular antiges of ***P. Multocida*** "Detection of $LD_{50}$ of ***P. Multocida*** capsular antigen"

| INOCULUM | No. of Cases | No. of dead mice at different groups inoculated (I/P) by 10 fold serial dilution of *P. Multocida* capsular antigen (%) | | | | | | | Mortality rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 56 | Gr. I* | Gr. II 1:10 | Gr. III 1:20 | Gr. IV 1:40 | Gr. V 1:80 | Gr. VI 1:160 | Gr. VII 1:320 | |
| *P. Multocida* "Capsular antigen" (infected group) | 10 | 7/8 (88%) | 6/8 (75%) | 5/8 (63%) | 4/8** (50%) | 2/8 (25%) | 0/8 (0.0%) | 0/8 (0.0%) | 24/56 43% |
| Physiological saline "control group" | 10 | (0/10) (0.0%) | | | | | | | 0/10 0.0% |

*Original concentration of capsular antigen

** ($LD_{50}$) of ***P. Multocida*** capsular antigen was detected at mice group No IV inoculated with 1:40 dilution of prepared capsular antigen.

**Table (3):** Haemolytic activity of 27 ***P. Multocida*** isolates recovered form chickens suffered form respiratory manifestation.

| No. of isolates had haemolytic activity | Incubation period per degree (°C) | Haemolytic activity on sheep RBCs (%) |
|---|---|---|
| 4 | 2 h at 37 °C | 14.8 |
| 9 | 24 h at 4 °C | 33.3 |

Percentage was calculated according to n= 27 isolates of ***P. Multocida*** examined for haemolytic activity on sheep blood agar plates.

**Table (4):** Results of agglutination antibody titers of apparently healthy and diseased chicken samples

| Examined cases | No. of cases | End serum titer | | | | | | | | Total results | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | Positive reactor | Negative reactor |
| Apparently healthy chicken | 60 | 4 (5) | 2 (3.3) | 2 (3.3) | 0 (0.0) | 0 (0.0) | 1 (1.7) | 0 (0.0) | 0 (0.0) | 8 (13.3) | 52 (86.7) |
| Diseased chicken | 40 | 3 (7.5) | 2 (5.0) | 0 (0.0) | 2 (5.0) | 0 (0.0) | 1 (2.5) | 0 (0.0) | 1 (2.5) | 9 (22.5) | 31 (77.5) |

Percentage calculated according to the tested sera of apparently healthy or diseased calves.

## DISCUSSION

***P. Multocida*** is an important cause of nasocornial infection. Different factors are encountered in the pathogencity of ***P. Multocida*** such as their serum resistance ***(6)***, although *Klebsiellae* are commonly described as non-haemolytic microorganisms, detection and determination of haemolytic effect of certain isolates were reported ***(1)***.

***P. Multocida*** is considered as environmental pathogenic microorganisms and they are frequently encountered in both upper and lower respiratory tract especially in animals housed at bad hygienic condition ***(13)***.

Table (1) and fig (1) illustrated that the isolation rate of P. Multocida (27%) among the examined cases (100). Nearly similar findings were recorded by ***(16)*** who isolated ***P. Multocida*** from 13 calve (28%) that exhibited combined with nasal discharge. In this concern ***(8)*** isolated ***P. Multocida*** from respiratory tract of chicken and stated that 64% of the isolates were more prevalent in nasal and lung infection. Those finding was in agreement with our studying. It could be attributed to bad hygienic measures (over crowded and long house standing) and nutritional deficiencies in farms. A high incidence of isolation was recorded in diseased chickens (42.9%), while the apparently healthy chickens recorded (20%) positive .cases. Meanwhile, 7 out of 40 dead chickens(17.5%) were positive for ***P. Multocida*** and it was isolated from both lung and heart blood of dead chicken with a prevalence rate of (25%) and (20%) respectively.

The polysaccharides of ***P. Multocida*** may contribute to the pathogencity of the organism in a variety of ways, where the capsule possess a hydrophilic character, which plays a well known antiphagocytic role ***(9)***, and may also aid in protecting the organism from complement-mediated lysis in serum ***(7)***.

Table (2) showed that the mice which previously inoculated intraperitoneally with undiluted capsular antigen of ***P. Multocida*** as in group (I) revealed that 7 mice out of 8 (88%) were dead, and with (diluted capsular antigen), as in group II (1/10) and group III (1/20) recorded 6/8 (75%) and 5/8 (63%) mortality rate respectively, whereas $LD_{50}$ was

recorded in group IV 4/8 (50%) which was inoculated with 1/40 diluted capsular antigen. No. mortality was recorded in group VI, group VII, and control group.

These findings were supported by *(11)* who found that the inoculation of mice with two different strains of different capsules size of ***P. Multocida*** showed variations in $LD_{50}$; whereas the strain owed the larger capsule, was more virulent than that owed the smaller one

The production of haemolysin among gram negative bacteria is indicative of other virulence and entrotoxigenic factors ***(2).***

As shown in table (3), ***P. Multocida*** were found to be haemolytic isolates, the primary cultivation of the isolates onto sheep blood agar plates was weakly noticed, but it enhanced and became more clear at refrigeration temperature, these obtained patterns of haemolysis percentages were consistent with the findings of **(15).**

Eight serum samples out of 60 samples form apparently normal chickens revealed positive agglutinins (13.3%), such incidences was lower than the positive reactors of diseased calves (22.5%). Serological examination indicated that ***P. Multocida*** agglutinins exist in both apparently healthy and diseased chicken (Table 3 and Fig. 2).

One of the most attractive theories to explain the differences in pathogencity of P. Multocida proposes that the amount of soluble capsular polysaccharide (CPS),
produced and exuded into the medium by a given strain, correlates with virulence *(5)*.

In the meantime *(3)* stated that the release of capsular material may inhibit the activation of other host defenses, and may also accentuate the antiphagocytic character of the strain, especially when it is synthesized at a rapid rate.

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# خصائص الكبسول للباستريلا ملتوسيدا المسببة للالتهاب الرئوى فى الدواجن

د. نجوى ربيع , د. سحر ذو الفقار

تم عزل الباستريلا ملتوسيدا من 40 حالة دجاج نافق و 25 حالة دجاج سليم و 25 حالة مريضة ظهر عليها أعراض تنفسية و كذلك 100 عينة سيرم من الحالات السليمة و المريضة تم فحصها سيرولوجيا للأجسام المناعية للباستريلا ملتوسيدا. و كانت النتيجة إيجابية بنسبة 42.9% من الحالات المريضة و بنسبة 20% من الحالات السليمة. تم عزل الميكروب من الرئة و القلب و الدم من الحالات النافقة و بالكشف عن الكبسول و بتخفيفة عند 40:1 كانت نسبة السمية له 50%. ووجد أن التحلل الدموى للباستريلا ملتوسيدا على أطباق الدم تكون أكبر سرعة و أكثر وضوحا عند درجة الحرارة المنخفضة.