PROTEIN PROFILE OF PASTEURELLA MICROORGANISMS ISOLATED FROM DIFFERENT AVIAN SPECIES

WAFAA M. M. HASSAN AND M.G. AGGOUR

Animal Health Research Institute, ARC, Ministry of Agriculture, Dokki , Giza

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Abstract

Bacteriological examination of 18 outbreaks of avian pasteurellosis including 10 chickens, 4 ducks, 3 turkeys and one ostrich was carried out. The examination included microscopic examination, cultural, biochemical, pathogenicity and drug susceptibility test. The obtained results were recorded and discussed revealing the characteristics of *Pasteurella multocida* Furthermore, the protein profiles of *Pasteurella multocida* strains isolated from chickens, ducks, turkeys and ostrichs were investigated using SDS –PAGE. The four isolats shared in 3 polypeptide bands ranging from 11 to 26 KDa. The major difference between isolates was in the position between 34 and 72 KDa region.

INTRODUCTION

Pasteurella multocida is the causative agent of fowl cholera, a septicemic disease that affects both domestic and wild avian species. High morbidity and mortality associated with fowl cholera result in significant economic losses to the poultry industry. Serotype A strains of *Pasteurella multocida* are the major cause of fowl cholera in poultry (Rhoades and Rimler, 1991). Highly virulent strains of *Pasteurella multocida* are usually encapsulated, whereas the non-capsulated strains showed less virulence (Rhoades and Rimler, 1991). Snipes and Hirsh (1986) reported that the encapsulated strains of *Pasteurella multocida* were able to multiply in the blood vascular system after intravenous inoculation of turkey to a greater extent than the non-capsulated strains. Anderson *et al.* (1984) reported that the capsular hyaluronic acid acts as an antiphagocytic factor. The isolated *Pasteurella multocida* strains have been identified as 5 capsular types designated as A, B, D, E and F (Rimler and Rhoaders, 1987) and 16 somatic types on the basis of antigenic differences in their somatic lipopolysaccharides (Heddleston *et al.,* 1972). A standardized system designating *Pasteurella multocida* serotypes has been recommended by Carter and Chengappa (1981) where the 3 important avian serotypes are A: 1, A: 3 and A: 4. Kumar *et al.* (2004) revealed the presence of various *Pasteurella multocida* serotypes (A: 1,A:3, A: 1, 3, A: 4 , B: 2, D:1 and E : 1) among different animals and avian species . Shivachandra *et al.* (2005) indicated that molecular methods for detection and typing are rapid in comparison with conventional tests which are helpful for differentiation of *Pasteurella multocida* strains in fowl cholera outbreaks.The main objective of the study reported here was to differentiate between four isolates of

Pasteurella multocida on basis of protein profiles extracted form whole cell lysates. The chicken, duck, turkey and ostrich isolates were analyzed using sodium dodecyle sulfate – polyacrylamide gel elctrophoresis (SDS- PAGE).

MATERIALS AND METHODS

Bacterial strains and culture conditions

During a period of two years (Jan 2004 to Dec 2005) , eighteen outbreaks of avian pasteurellosis were investigated in different species under Egyptian conditions. These field outbreaks included ten chickens, four ducks, 3 turkeys and, one ostrich in poultry farms.

Direct heart blood smears, pericardial fluid and impression smears of liver and spleen were stained with methylene blue and Gram's stain and microscopically examined . The organisms were isolated on blood agar, McConkey's agar and brain heart infusion (BHI) agar. These isolates were subjected to biochemical identification according to standard procedure of Namioka (1979). *Pasteurella multocida* was subjected to pathogenicity test in mice by inoculating 0.2 ml of 8 hours grown BHI broth culture intraperitoneally with control inoculated with 0.2 ml sterile BHI broth.

Sensitivity tests

The isolates of *Pasteurella multocida* were subjected to antibiotic sensitivity test using 12 commonly used antibiotic discs as per-established procedure (Bauer *et al.* , 1966).

Preparation of protein extract for electrophoresis (according to Ireland *et al.*, 1991)

The isolates were grown in 200 ml of nutrient broth for 18 h at 37°C and collected by centrifugation at 8000g for 20 min. at 4°C. The cells were washed twice in PBS and resuspended in 10 ml PBS. The suspension was sonicated in an ultrasonics (VerSonic 475) at 70% power for 3 min. and stored at - 20°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein extract prepared from 4 strains of *Pasteurella multocida* was subjected to discontinuous SDS - PAGE, using 10% separating gel according to the methods of Laemmli (1970). Electrophoresis was performed at a constant voltage 100 V using a Hoefer mini – gel system with PS 500 XT power supply. Prior to loading onto the gel, the protein extracts were heated at 100°C for 4 minutes in sample buffer containing 0.06 M Tris, 1.2 % SDS , 5% B- mercaptoethanol and 11.9% glycerol. Sample containing 20 µg of protein in 50 ml of sample buffer was loaded into each lane. The protein content of the sample was determined by the modified lowery procedure of Markwell *et al.* (1978). Staining was done with Coomassie blue R- 250, and molecular weight was determined using standard proteins of known molecular weight (Fermentas) containing a mixture of 10 proteins with the apparent molecular weights form 11 KDa to 170 KDa.

RESULTS

Bacteriological examination

Direct microscopic examination of the obtained samples revealed Gram negative pleomorphic bipolar organisms.On blood agar, significant growth of nonhaemolytic, tiny (0.5 – 1 mm), low convex, translucent, smooth glistening surface and entire edges were noticed. The organisms did not grow on McConkey's agar plate. Biochemical tests revealed that the organisms were positive for catalase, oxidase, indol, nitrite production, ornithine and arginine decarboxylase tests. The mice subjected to pathogenicity tests died after 24 hours of inculcation and the organisms were reisolated from the heart blood indicating the virulent and septicemic nature of the isolates. The clinical signs, necropsy lesions, cultural and biochemical tests confirmed the nature of the isolates. The incidence of *Pasteurella multocida* in chicken, duck, turkey and ostrich samples were presented in Table 1. Antibiotic sensitivity test of twenty *Pasteurella multocida* isolates from each avian species revealed that the organisms were highly sensitive to sulphamethoxazol, colistin sulphate, tetracycline, enrofloxacin and amoxicillin (Table 2).

Table 1. *Pasteurella multocida* isolation from the obtained field samples of avian pasteurellosis outbreaks.

Samples	Chicken (10 outbreaks)	Duck (4 outbreaks)	Turkey (3 outbreaks)	Ostrich (1 outbreak)
Heart blood	96/150 (64.0%)	93/60 (65.0%)	30/45 (66.7%)	5/7 (71.4%)
Pericardial fluid	101/150 (64.3%)	43/60 (71.7%)	31/45 (68.9%)	6/7 (85.7%)
Bone marrow	112/150 (74.7 %)	47/60 (78.3%)	33/45 (73.3%)	7/7 100.0 %)
Liver	93/150 (62.0%)	37/60 (61.7%)	28/45 (62.2%)	5/7 (71.4%)
Brain	71/150 (47.3%)	25/60 (41.7%)	21/45 (46.7%)	4/7 (57.1%)
Spleen	99/150 (66.0%)	39/60 (65%)	29/45 (64.4%)	5/7 (71.4%)
Lung	90/150 (60.0%)	34/60 (56.7%)	26/45 (57.8%)	4/7 (57.1%)
Joint capsule	59/150 (39.3%)	22/60 (36.7%)	10/45 (22.2%)	2/7 (28.6%)
Air sacs	83/150 (55.3%)	33/60 (55.0%)	28/45 (62.2%)	5/7 (71.4%)
Nasal sinus	87/150 (58.0 %)	35/60 (58.3%)	30 /45 (66.7%)	5/7 (71.4%)
Overall	795/1500 (53.0%)	354/600 (54.0%)	266/450 (59.1%)	48/70 (68.6%)

The values were expressed as positive / examined (percent)

Table 2. Antimicrobial susceptibility for *Pasteurella multocida* isolates.

Antimicrobial drugs	Susceptility of chicken isolates	S. of ducks isolates	S. of turkey isolates	S. of ostrich isolates
Amoxycillin	90%	85%	90%	90
Ampicillin	75%	70%	80%	75%
Chloramphenicol	65%	70%	80%	75%%
Colistin-sulpahte	95%	95%	100%	95%
Danofloxacin	65%	70%	60%	75%
Erythromycin	5.0%	5.0%	0.0%	10%
Enrofloxacin	95%	90%	90%	95%
Gentamycin	70%	75%	70%	75%
Flumequine	5.0%	10%	0.0%	5.0%
Penicillin	15%	15%	20%	20%
Sulphamethoxazol	100%	100%	100%	100%
Tetracycline	90%	95%	100%	90.0%

Protein of *Pasteurella multocdia* isolates

The protein of the 4 serotypes isolated of *Pasteurella multocida* from chicken, ducks, turkey and ostrich were shown in Fig. 1. The profiles showed some differences. The four isolates shared in 3 polypeptide bands ranging from 11 to 26 KDa. The major difference between isolates was in the position between 34 and 72 KDa region. In chicken isolates, 4 distinct polypeptide bands appeared with molecular weight ranging from 32 to 85 KDa. On the otherhand, duck isolate showed 3 distinct polypeptide bands ranging from 47 to 72 KDa. In turkey, the protein profile of the isolate revealed the presence of 10 polypeptide bands with molecular weight ranging from 11 to 85 KDa. In ostrich, the protein profile revealed the presence of 3 polypeptide bands ranging from 11 to 26 KDa.

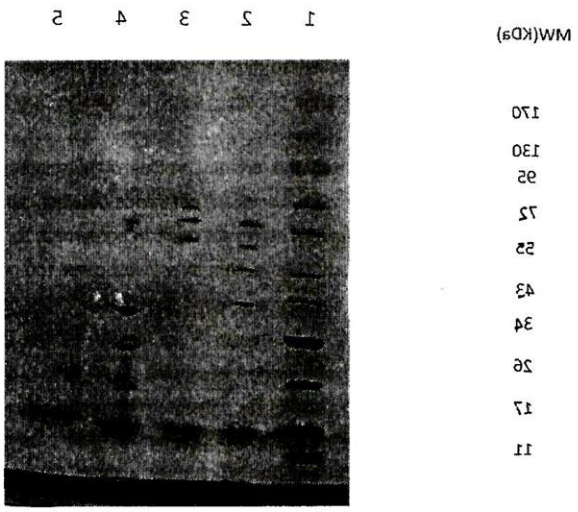

Fig. 1. SDS - PAGE of *Pasteurella multocida* isolates stained with coomassie blue. Lane 1, molecular weight markers (KDa). Lane 2, *Pasteurella multocida* isolate from chicken. Lane 3, *Pasteurella multocida* isolate from ducks. Lane 4, *Pasteurella multocida* isolate from turkey. Lane 5 *Pasteurella multocida* isolate from ostrich.

DISCUSSION

Microscopic, cultural growth and biochemical tests confirmed the isolated organisms as *Pasteurella multocida*. These observations were in accordance with those of Rhoades and Rimler (1991), Kumar *et al.* (2004) and Bhattacharya (2005). The isolation rate of *Pasteurella multocida* as shown in Table 1, was similar to that reported by Kumar *et al.* (2004). The isolation rate reported here was higher than that reported by Bhattacharya (2005) and lower than that reported by Gunawardana *et al.* (2000). The pathogenicity test on mice indicated the virulent and septicemic nature of *Pasteurella multocida* isolates. This observation was in harmony with that reported by Rhoades and Rimler (1991). Antibiotic sensitivity tests of all the *Pasteurella multocida* isolates as shown in Table 2 were in agreement with those of Waltman and Horne (1993). The superiority of enrofloxacin as reported here is in accordance with that reported by Knott and Lister (2003). The superiority of colistin sulphate and the superiority of amoxycillin was also reported by Olson *et al.* (2002). Tetracycline was previously recorded as an effective drug against *Pasteurella multocida* (Olson *et al.,* 2002, Knott and Lister, 2003 and Shivachandra *et al.,* 2005).

In general, pasteurellae were sensitive to most antibiotics. The degree of this sensitivity varied according to the localities, the prevalent serotype and the emergence of multi drug resistant strains.

In this study we have investigated the protein profiles of 4 isolates from chicken, duck, turkey and ostrich. There were significant difference between isolates, in the position between 34 to 72 KDa region. The results were in agreement to some extent with those reported by Ireland *et al.* (1991) where they investigated the protein of *Pasteurella multocida* serotype 1 from chicken, and found quantitative differences in the molecular weight of prtoteins present in the range 34 to 38 KDa. Similar results have been reported for *Pasteurella multocida* isolates of porcine origin by Lugtenberg *et al.* (1984) who named this major protein the heavy "H" protein. However, Lugtenberg *et al.* (1984) were unable to find any association between the presence of the "H" protein and the virulence of the isolates. So, further work is needed with different isolates to establish any correlation of virulence with specific protein molecules.

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الصورة البروتينية لميكروبات الباستريلا المعزولة من فصائل الطيور المختلفة

وفاء محمد محمد حسن ، محمد جلال عجور

معهد بحوث صحة الحيوان – مركز البحوث الزراعية – وزارة الزراعة – دقى – جيزة

شمل الفحص البكترويولوجي ثماني عشر وباء لميكروب الباستريلا في عدد ١٠ مزارع من الدجاج و ٤ مزارع من البط و٣ مزارع من الرومي ومزرعة واحدة فقط من النعام .

اشتمل الفحص الميكروبي علي الفحص ميكروسكوبياً وصفات الزرع والفحص بيوكيميائيـاً والقدرة الممرضه وتم عمل اختبار حساسية لمختلف العترات المعزولة وتم تدوين النتائج ومناقشـتها وتوضيح الصفات المميزة للباستريلا ملتوسيدا .

علاوة علي ذلك تم دراسة الصورة البروتينية لميكروبات الباستريلا ملتوسيدا المعزولة من دجاج وبط ورومي ونعام وذلك بأستخدام اختبار الـ أس دي أس باج (SDS - PAGE)

كانت الأربع معزولات متشاركة في ٣ روابط بولي ببتيد تتراوح من ١١ – ٢٦ كيلو دالتون . كان الاختلاف الكبير بين المعزولات في الموقع بين ٣٤ – ٧٢ كيلو دالتون .