Molecular Studies Employed for Diagnosis of *Mycoplasma gallisepticum* in Chickens

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ABSTRACT

Four hundred and fifty tissue samples (trachea, lungs and air sacs) and 450 blood samples were obtained from chickens from different chicken laboratories at Zagazig city. They were of different sex, breed and age. Sixety were apparently healthy and 90 chickens were diseased suffering from respiratory manifestation. The samples were submitted to laboratory examination using the culture (isolation) method. Thirty three (18.3%) out of 180 isolates were identified as *M. gallisepticum* from apparently healthy chickens and *M. gallinarum* 1 (0.5%). While, 95 Mycoplasma isolates (35.18%) were recovered from 270 diseased chickens. They were identified as MG 91 (33.4%), *M. gallinarum* 3 (1.1%) and *M. iners* 1 (0.3%). The sera were examined by slide agglutination test (SPA). It was found that 25% and 60% were positive to MG in sera of apparently healthy and diseased chickens, respectively. Thirty tracheal swabs were examined, resulted in 10 (33.3%) MG positive samples by culture and 15 (53%) MG positive samples PCR method. An antibiogram was performed on the Mycoplasma isolates. It was concluded that enrofloxacin was the most effective antimicrobial agent against MG isolates in vitro followed byoxytetracyclin.

INTRODUCTION

Mycoplasma *gallisepticum* (MG) is an avian pathogen that cause CRD in chickens resulting in significant economic losses to poultry industry worldwide due to decreased egg production, reduced feed efficiency, downgrading of carcasses and high medication and vaccination cost. In addition, it causes infectious sinusitis in turkey and conjunctivitis in finches ***(1)***. So the importance of MG requires accurate and rapid diagnosis

. PCR was previously applied by various authors who used it for the diagnosis and epidemiological studies of mycoplasmosis ***(2)***. who used PCR was used for the detection of Mycoplasma from migratory birds ***(3,4)***.

MG has shown sensitivity *in vitro* and *in vivo* to several antimicrobial agents including macrolides, tetracyclines, fluoroquinolones and others ***(5-7)***.

The aim of the present study:

(1) Study the prevalence of *M. gallispeticum* chickens of different ages, sex and breed in different localities at Zagazig city. (2) Isolation and identification of *M. gallisepticum* by traditional cultural method. (3) Applying biochemical and serotyping of the isolate by growth inhibition test, growth pp and SPA. (4) Applying PCR for diagnosis of *M. gallisepticum*. (5) Applying antimicrobial sensitivity test for some antimicrobial agents.

MATERIAL AND METHODS

One hundred and fifty chicken (60 apparently healthy and 90 diseased were used in this study). Blood samples were collected from wing vein of each chicken and serum were obtained for serological examination. Chickens were slaughter and samples from trachea , lung and airsacs (450 samples) were obtained from each chicken.

Culture method

The media was PPLO and the culture procedures were according to ***OIE (8)***. Culture and antisera were supplied by Prof. Dr. Laila El-Shabiny, Mycoplasma Dept., Animal Health Research Institute Dokki, Giza which was kindly provided by Dr. Shin Diagnostic Lab. Cornell Univ., Ithaca NY.

Biochemical characterization was done, including glucose fermentation and arginine determination *(9)*.

Serologic examination

Growth inhibition *(10)* and growth precipitation *(11)* were applied SPA was performed *(12)* and genus determination (Digitonin test) was applied *(13)*.

Polymerase **Chain Reaction:** PCR was carried out briefly as follows *(8)*:

a) DNA extraction: DNA was extracted from 30 tracheal swabs (every 3 were pooled) suspended in 1 ml PCR-grad pbs in an ependorf tube. The suspension was centrifuged for 30 minutes at 14.000 rpm at 4°C. The supernatant was removed and the pellet suspended in 25 µl PCR grade water, boiled for 10 minutes in heat block (100°C) then placed in -20°C freeze for 10 minutes, centrifuged at 14.000 for 5 minutes. The DNA was in the supernatant.

b) The following MG primers were Chosen *(14)*.

F.5 CGCAATTTGGTCCTAATCCCCAACA-3
R 5 TAAACCCACCTCCAGCTTTATTTCC-3

c) PCR was performed in Biometra thermal cycler model according to especial program.

d)Electrophoresis: PCR products were detected by agarose gel electrophorsis in corporating appropriate size ladder (Ab gene, United Kingdom 100-1000bp).

The end result was examined under UV light. The sample was positive for MG when a fragment with 230 bp was amplified.

Antibiogram was done *(15)*

RESULTS

It is clear from the results recorded in Table (1) that from apparently healthy chickens 33 (18.3%) from 180 examined samples were positive for *Mycoplasma*. While, in diseased chickens 95 (35.18%) from 270 were positive for *Mycoplasma*.

It is also observed that the highest isolation rate was from air sacs followed by trachea then lungs.

It is clear from the results recorded in Table (2) that 33.7% of the isolates were MG and 1.1% *M. gallinarum* and 0.3% were *M. iners* in diseased chickens and in apparently healthy chicken 1.7% of the isolates were *M. gallispticum* and 0.5% *M. gallinarum*.

Incidence of Mycoplasma isolates from tracheal swabs from naturally infected chicken:

1-By standard cultural method: Using standard cultured method for detection of MG in 30 tracheal swabs. The results recorded in Table (3) showed that 10 samples (33.3%) were positive for *M. galliseticum*.

2-Results of PCR for detection of *M. gallisepticum* in tracheal swabs from naturally infected chickens: 30 tracheal swabs, 15 samples were positive to *M. gallisepticum* Fig. (1).

3. Results of antibiogram Table (4)

Table 1. Incidence of Mycoplasma isolates from apparently healthy and diseases chickens

Organs	Samples from apparently healthy chickens			Samples from diseases chickens		
	No. of exam. samples	No. of positive samples	%	No. of exam. samples	No. of positive samples	%
Air sacs	60	17	28.3	90	72	46.6
Trachea	60	10	16.6	90	30	30.3
Lungs	60	6	10	90	23	25.5
Total	180	33	18.3	270	95	35.18

Table 2. Serotyping of Mycoplasma isolates

Source	Healthy chickens								Diseased chickens							
	No. of exam. Samples	No. of isolates	*MG*	%	*M. gallinarum*	%	*M. ineris*	%	No. of exam. Samples	No. of isolates	*MG*	%	*M. gallinarum*	%	*M. ineris*	%
Air sacs	60	17	17	28.3	0	0	0	0	90	42	41	45.5	1	1.1	0	0
Trachea	60	10	10	16.6	0	0	0	0	90	30	27	30	2	2.2	1	1.1
Lung	60	6	5	7.3	1	1.6	0	0	90	23	23	25.5	0	0	0	0
Total	180	33	32	1.7	1	0.5	0	0	270	95	91	33.7	3	1.1	1	0.3

Table 3. Comparison between standard method and PCR for identification of Mycoplasma isolates

Type of exam. Sample	No. of exam. Sample	Standard cultural method		PCR	
		+ ve	%	+ ve	%
Tracheal swabs	30	10	33.3	15	53.3

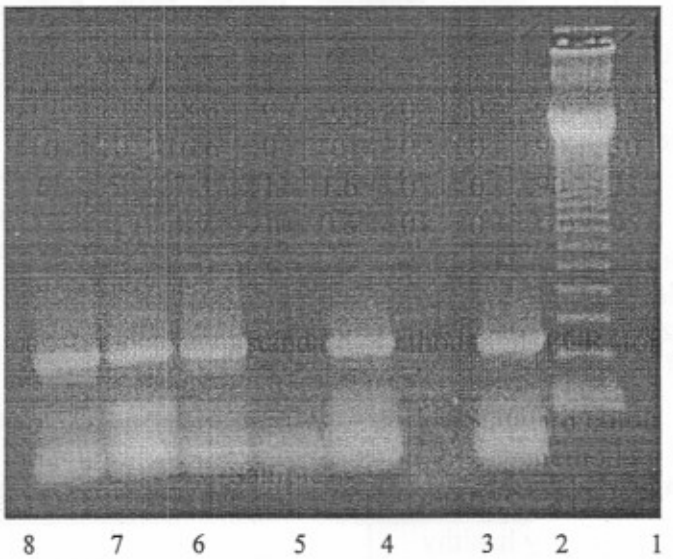

Fig. 1. Agarose gel electrophoresis of PCR products from *Mycoplasma gallisepticum* reference strains and some field isolates.

Lane (1) Ladder (abgen United Kingdom from 100 to 1000).

Lane (2) *M. gallispeticum* positive control (band at 230 bp).

Lane (3) *M. gallispeticum negative* control.

Lane (4, 6, 7, 8) *M. gallispeticum*, positive samples (band at 230 bp).

Lane (5) *M. gallisepticum*, negative sample.

Table 4. Results of in vitro sensitivity test of *M. gallisepticum* isolates (91) to 14 antimicrobial agent

Antibiotic	Disc potency mg	No. of sensitivity isolates	Activity %
Ciprofloxacin	20	36	55.5
Erythromycin	15	18	83.3
Oxytetracyclien	30	44	68.1
Tylosin	15	20	75
Lincospectin	20	39	51.2
Norfloxacin	20	30	66.6
Amoxicillin	10	46	21.7
Enrofloxacin	10	46	21.7
Gentamycin	10	35	28.5
Danofloxacin	30	52	57.6
Trospecpmycin	20	59	33.89
Tilmicosin	20	38	52.6
Tiamulin	30	58	51.7
Neomycien	30	88	34

$$\text{Calculation of \%} = \frac{\text{Disc potency } \textit{M. gallisepticum} \times 100}{\text{No. of sensitive isolates}}$$

DISCUSSION

MG is the most pathogenic avian mycoplasma which continued to be a problem in commercial layers. In addition, it causes CRD and leads to economic losses to poultry industry ***(16)***.

In the present study, using culture method for diagnosis, revealed that 18.3% of the samples from apparently healthy chickens were positive. MG (33 from 180) and (35.15%) of the samples from diseased chicken were positive (95 from 270 samples). These results coincided with previously cited work ***(17,18)***.

Biochemical and serotyping of the isolates revealed different types of *Mycoplasma* species involving, MG, *M. gallispeticum, M. gallinarum* and *M. iners*. Similar finding were recorded previously ***(19)***.

SPA was used as a rapid serologic test, it was concluded that 45 MG positive serum samples were detected from 180 apparently healthy chickens (25%) while 162 positive MG positive serum samples were found in 270 examined diseased chickens (60%). This result agree with ***El-Makarem (20)***.

SPA is the most commonly used serologic test especially for screening purposes as a flock test ***(21)***.

Antibiogram was made against various antimicrobial agents which clarified that enrofloxacin was the most effective against MG followed by oxytetracyclin. Similar sensitivity antibiogram was recorded in M.G, isolated from migrataory quil by ***El-Shabiny et al. (4)***.

PCR was previously used for the diagnosis of MG by many authors ***(22)*** who applied conventional PCR and also restriction fragment length polymerase chain assay. Artificially PCR primed PCR for the detection of MG ***(3, 23)*** and MS. and identified avian Mycoplasma including MG using nested PCR ***(24)***. To differentiate reference and field MG strains ***(25)***. It was used for the identification of MG from tissue of chicken ***(26)***. ***El-Shabiny et al. (27)*** who used PCR for the differentiation between virulent MB strain and

vaccinal strain and for identification of MG from migratory birds.

Duplex PCR was used to differentiate between MG and MS in a single reaction on the basis of conserved species specific sequences of their hemagglutination genes *(28)*). Real-time PCR was used for quantitative and qualitative detection of MG *(29).*

In the present study conventional PCR was applied and compared with culture method for the diagnosis of MG in tracheal swabs. It was found that PCR was more sensitive, specific and accurate as shown by high activity percentages 100%, 75% and 83.3%, respectively.

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الملخص العربى

دراسات جزيئية لتشخيص الميكوبلازما جاليسبتكم فى الدجاج

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تم جمع ٤٥٠ عينة أنسجة من القصبة الهوائية والرئة والأكياس الهوائية و٤٥٠ عينة دم من الدجاج المختلف الأعمار والأجناس من مدينة الزقازيق وتم فحص عينات الأنسجة بطريقة الزرع وقد أسفرت عن ٣٣ عترة ميكوبلازما (١٨,٣٪) من ١٨٠ عينة من الدجاج السليم ظاهريا صنفت ٣٢ (١,٧٪) ميكوبلازما جالسبتكم وعينة واحدة بنسبة (٠,٥٪) ميكوبلازما جالينيرم بينما تم عزل ٩٥ عزلة بنسبة (٣٥,٨٪) من ٢٧٠ عينة من الدجاج المصاب صنفت ٩١ (٣٣,٧٪) ميكوبلازما جالسبتكم و٣ (١,١٪) ميكوبلازما جالينيرم و١ (٠,٣٪) ميكوبلازما اينرز.

وبفحص عينات الدجاج السليم ظاهريا باختبار تلازن الدم وجد أن ٢٥٪ منها كانت ايجابية للميكوبلازما جالسبتكم بينما كان ٦٠٪ منها ايجابى من مصل الدجاج المصاب. وتم فحص ٣٠ عينة من القصبة الهوائية بالزرع وباختبار تفاعل البلمرة المتسلسل حيث وجد أن نسبة الميكوبلازما جالسبتكم كانت ١٠ (٣٣,٣٪) و١٥ (٥٣,٣٪) على التوالى وبإجراء اختبار الحساسية للمضادات الحيوية وجد أن الأنروفلوكساسين كان أكثر تأثيراً على الميكوبلازما جالسبتكم يليه الاوكسى تتراسيكلين.