Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Molecular Characterization of Avian Mycoplasma with Special Reference to Antibiotic Sensitivity in Egypt

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Abstract

AVIAN MYCOPLASMOSIS is one of the most significant financial threats affecting the global poultry business so, convenient approach for detecting etiological agents; *Mycoplasma gallisepticum (MG)* and *Mycoplasma synoviae (MS)*to characterize the circulating field strains and determine their antimicrobial susceptibility profiles. A study conducted between 2017 to 2022 in Kafrelshekh, Sharkia and Dakahlia, governorates in Egypt on different avian sectors, Twelve *MG* and four *MS i*solates were identified by culture and confirmed by PCR. Partial sequence of *mgc*2 gene of two *MG* isolates from turkey (*MG*B.T.) and (*MG*W.T.) was submitted on gene bank under accession no.OM632677.and OM632678 respectively and revealed that there are two different *MG* circulating strains in turkey, for determining the antimicrobial susceptibility profiles , ten *MG*, one *MS* isolates from different avian sector were tested against eight antimicrobials for determination of lowest MIC, three antibacterials, tiamulin (0.009µg/ml), tilmicosin (0.039µg/ml) and tylosin (0.019µg/ml), offered the lowest MICs of all efficient medications .*MS* isolate was sensitive for the eight antimicrobials, most *MG* isolates were sensitive to ciprofloxacin and streptomycin while Lincospectin has intermediate effect. Isolate *MG*11from baladi chicks was completely resistant to all antimicrobial. The two *MG* isolates from turkey were resistant to (erythromycin and tylosin), so, further investigation for macrolides resistant genes detection is required. The two *MG* isolates from turkey were resistant to (erythromycin and tylosin), so, further investigation for macrolides resistant genes detection is required. In conclusion, the present study confirms the presence of *MG* infection in turkey and chicken flocks of Sharkia and Dakahlia governorates.

Keywords: PCR, MIC, MG, MS, Turkey.

Introduction

Mycoplasmas are the smallest and simplest self replicating bacteria [1]. These bacteria need a peptidoglycan-based hard cell wall and are therefore resistant to antibiotics like penicillin and its equivalents that are successful against the majority of bacterial cell culture chemicals. All the species that belong to the class Mollicutes are collectively referred to by the meaningless term *Mycoplasma*. Due to their extremely small genomes (0.58-2.20 Mb compared to 4.64 Mb for *E-coli*), which are incapable of replication, *Mycoplasmas* have limited metabolic options for both living and reproduction[2].*Mycoplasma gallisepticum* and *Mycoplasma synoviae* are bacterial pathogens responsible for poultry illness[3].*M. gallisepticum* produces chronic respiratory disorder of farm chicken and turkey, particularly with managerial stressors and/or other respiratory infections. Lachrymation, conjunctivitis, sneezing, cough, sinusitis, and reduction of egg production in poultry define the disease [4,5]. Direct or indirect contact with susceptible birds, contact with infected

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(Received 31/01/2024, accepted 15/05/2024)
DOI: 10.21608/EJVS.2024.266946.1815

carriers, or contact with contaminated trash all contribute to the vertical or horizontal spread of the *M. gallisepticum* infection[6]. *MS* can result in respiratory disorder, synovitis, or silent infection. *Mycoplasma* cultivation methods are hard, time-consuming, and need sterile conditions; hence, molecular detection is the most common way for routinely diagnosing *MG* infection [7]. Methods for preventing and controlling avian *Mycoplasmosis* consists predominantly of biosecurity, therapy, and immunization. One of the effective ways to manage the illness and reduce economic loss caused by *MG* infection is the wide spread use of antibiotics. Numerous drug classes have been shown to be effective against *MG*, including fluoroquinolones, macrolides, tetracyclines, and pleuromutilin [8,9]. It was found that *MG* could develop antibacterial resistance as a result of prolonged antibiotic use [10,11]**.** The prevention and treatment of *Mycoplasmosis* frequently involves the use of antibiotics from the macrolide family, such as tylosin and tilmicosin [12]. Prior research has demonstrated the influence of point mutations in the 23S rRNA-encoding genes on the emergence of macrolide resistance in a number of *Mycoplasma* species, including *M. gallisepticum* and *M. synoviae.* According to [12,13].

Material and Methods

Sampling

One hundred and twenty tissues of trachea, lungs and air sacs were collected from twelve broiler flocks ,five layers flocks ,three broiler breeder flocks and seven turkey flocks from different localities Dakahlia and Sharkia governorates.These samples are taken from diseased or freshly died bird,under complete aseptic condition also, ten flocks of day old chicks were taken from ten various area with various breeds were investgated for isolation (trachea,lung) and serological examination (SPA)of *MG* and *MS*. Take serum samples from day old chicks under aseptic condition. These samples were taken from Kafrelsheikh governorate.

Serological identification of MG and MS

By using serum plate agglutination test (SPA) according to [14]**.** Hundred serum samples were examined using (Lilli dale diagnostic *MG* RSA antigen, batch no:122001. Lilli dale diagnostic *MS* RSA antigen, batch no:012101), according to the manufacturer's guidelines. Take serum samples from day old chicks. Mix one drop of serum with drop of antigen then wait two minutes till appearance of agglutination.

Isolation and Identification of MG and MS:

Mycoplasma was cultivated on pleuropneumonia like organism PPLO broth and agar base media described by [15]. We have taken pieces of organs (trachea, lungs and air sacs) in sterile conditions in *Mycoplasma* broth (about 5 grams of tissue sample per 25 ml broth), then kept at 37 ºC for 3 days, following that, 20 µl of the incubated broth culture was transferred and streaked on PPLO agar. The agar plates were kept at 37 ° C in a damp candle jar with decreased oxygen tension [16]. Using an inverted microscope, the cultures were then examined every day till 30 days of incubation in the existence of the same fried-egg colonies of *MG* and *MS.* Biochemical tests, such as those for digitonin sensitivity, glucose fermentation, arginine hydrolysis, film and spot production, were carried out as originally described by [17,18]**.**

Molecular Identification

By using Genomic DNA extraction kit (kit Gene Direx. Taiwan), following the manufacturer's instructions. All mycoplasma isolates were confirmed as *M. gallisepticum* and *M. synovae* by PCR using species-specific primers for 16S rRNA and mgc2 genes, following previously published methods [7,19].

Detection of 16s ribosomal RNA gene by PCR:

The *MG* 16s ribosomal RNA gene was detected using the PCR test on every sample. 1 µL of each primer and 25 µL of the 10X PCR mix were added to a 50µL reaction container to perform the PCR reaction. (20µM) MGF (5'GAGCTAATCTGTAAAGTTGGTC3') and MGR (5'GCTTCCTTGCGGTTAGCAAC3'), MS primer MSF ('5GAGAAGCAAAATAGTGATATCA3) MSR: ('5CAGTCGTCTCCGAAGTTAACAA3') 2 µL Taq DNA polymerase, 19 µL deionized distilled water and 2 µL template DNA. The following three steps were part of the thermal cycle: First, primary denaturation was carried out for three minutes at 94 ˚C. In the subsequent phase, there were forty cycles with three segments: denaturation at 94˚C for 30 seconds, annealing at 55˚C for 30 seconds, and extension at 72˚C for 60 seconds. The third and final extension was eventually carried out for five minutes at 72 ˚C [7]. The PCR products were visualized by staining with ethidium bromide after being electrophoresed on 1.5% agarose gel for 1 hour at 100 V. In all PCR assays, reference vaccine *Mycoplasma gallisepticum* strain Ts-11 from (IFT company) was used as the positive control and distilled water as the negative control.

Detection of mgc2 gene by PCR

The second specific MG primers, mgc2-2F (5'-CGC AAT TTG GTC CTA ATC CCC AAC A-3')

and mgc2-2R (5'-TAA ACC CAC CTC CAG CTT TAT TTC C-3') were used for amplifying mgc2 gene (300bp) [19]. For the mgc2-PCR, a mixture with a total volume of 50 µL was used. It contained 25 µL of Dream Taq Green master mix (10X PCR mix), 1 µL of each primer (50 pmol µL-1), 2 µL of Taq DNA polymerase, 19 µL of deionized distilled water, and 2 µL of extracted DNA as template. The reaction was run through 40 cycles, with denaturation at 95 ˚C for one minute, annealing at 60 ˚C for forty seconds, primary extension at 72 ˚C for ten seconds, and final extension at 72 ˚C for one minute. The three rounds of different temperature and time (°C/min) segments—94°C for 20 seconds, 51°C for 30 seconds, and 72°C for one minute—corresponded to denatured target DNA. Every amplification reaction was carried out in a thermal cycler. UV trans illumination equipment (Spector line, Model 312A, 312 nm Ultraviolet, USA) was used to visualize 1% agarose gel electrophoresis stained with ethidium bromide in 1X Tris-acetic acid-EDTA buffer.

Minimal Inhibitory Concentration (MIC) Method

The micro-broth method test was carried out twice precisely as[9]. The tested antimicrobials were made with concentrations of antimicrobial agents ranging from 0.016 to 16 ug/ml. The maximum concentration of antibiotics that inhibited the metabolic activity of the tested organisms was noted. The persistence of the initial color without changing served as the metric for determining the minimum inhibitory concentration (MIC). Eight antimicrobials were tested, including erythromycin 20% (Mefeco), lincospectin 100% (Pfizer), streptomycin 100% (Mefco), doxycycline 50% (Atco Pharma), and ciprofloxacin 20% (Arabco-med). There are three types of phenolic compounds: tilmicosin (25%), tylosin (100%), and tiamulin (45%).

Sequencing of mgc2 gene and Phylogenetic Analysis

QIA fast PCR Product extraction kit was used to purify PCR products. (Qiagen, Valencia). The sequence reaction was done with a big dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) and subsequently purified with a Centrisep spin column. A BLAST® (Basic Local Alignment Search Tool) [20], analysis (Applied Biosystems 3130 genetic analyzer, HITACHI, Japan) was primarily done to determine sequence identity to Gen Bank accessions. Meg Align was used to generate the phylogenetic tree, and neighbor-joining was used to conduct phylogenetic studies in MEGA X: Molecular Evolutionary Genetics Analysis on several computing systems [21].

Results

Prevalence of *M.gallisepticum* and *M.synoviae* infection in day old chick ,*MG* detection was 37% by serum plate agglutination(SPA), 3.3% and 10% by culture and PCR respectively, while *MS* isolates were 11% by SPA , 1.1% and 4.4% by culture and PCR respectively. Cobb breed is negative SPA,culture and negative PCR as in table(1). *Mycoplasma* isolates appear as tiny smooth colonies fried egg appearance on agar with acharacterstic feature (film and spot) in *MS* as in Table (1) and Figs (2,3).

Twelve MG isolates and four *MS* isolates were characterized by culture, biochemical tests and PCR. *MG* isolates were50% by PCR where *MS* isolates were 16.7%. Three *MG* isolate and two *MS* isolates was isolated and identified from twelve broiler flocks (from one hundred and twenty samples of trachea, lungs and air sacs) from dakhlia while one *MG* and one *MS* isolates was identified from five layer flock examened from kafrelsheikh . Also, one *MG* and one *MS* from three broiler breeder flocks from dakhlia and four *MG* isolates from seven turkey flocks(three from back yard black turkey from dakhlia and three *MG* from four white turkey flocks from sharkia as in Table (2).

The PCR results for MG and MS are shown in Fig. (5, 6) by using OIE primer giving 185bp and 211bp respectively . results for mgc2 gene are shown in Fig. (7). Based on the nucleotide phylogenetic tree of mgc2 gene, 7 positive samples giving 300bp.

MG isolates were examined for eight antibiotics. The three antibiotics with the lowest MICs were tiamulin (0.009g/ml), tilmicosin (0.039g/ml), and tylosin (0.019g/ml). Nasi6, *MG*64,T19 are susceptible to all antimicrobial while *MG11* is completely resistant to them while *MG*-B.T of black turkey and *MG*-W.T of white turkey were resistant to doxycycline and tylosin . Also, *MS* was sensitive to macrolides (erythromycin, tylosin, tilmicosin). but *MS* was resistant to (doxycycline, lincospectin) further studies are recommended for this point as in Table (3).

Sequence examination of MG mgc2 gene of black turkey

The sequence of mgc2 gene of *MG* was submitted to gene bank database under accession no. OM632677. *MG*mgc2 **(**OM632677) showed 100% nucleotide identity and amino acids identity with other isolates such KT943467.1 *M. gallisepticum*/ Eis-9-CK-15, FJ234839.1 *M. gallisepticum*/ RabE1-08, and JN113343.2 *M. gallisepticum*/ MG NMH-and97%with HQ591356 .1 *M.gallisepticum* /Eis4-C-10 as in Fig(8), table (4)

The sequence of *mgc*2 gene of *MG* was submitted to gene bank database under accession no. OM632678.1 M. gallisepticum/ Eidmg9.2-2-022. Although nucleotide identity between *MG*OM632678.1 and other isolates KX268626.1, KX268627.1, KX268628.1, KX268629.1, KX268630.1, KX268622.1, KP300761.1, KP279743.1, KP300757.1, KP300758.1 and KP300760.1 which were got from the gene bank were 76%, the amino acid identity was 100%as in Fig (9) and Table (5).

Discussion

Infections caused by avian *Mycoplasma* have been detected in Egypt for many years. Based on serological studies, bacteriological and recently, molecular diagnosis. The current study deals with the occurrence, diagnosis and treatment of Avian *Mycoplasmas* are causing CRD and infectious sinusitis in chicken and turkeys, respectively from the poultry flocks of Sharkia, Dakahlia and Kafrelsheikh governorates of Egypt. *MG* and *MS* are the two most common species of *Mycoplasma* that cause respiratory and joint diseases in chickens in the different Egyptian governorates (Giza, Fayoum, Benisuef, Menya, and Alexandria) [22] .In the current study prevalence of *M.gallisepticum* was 37% by serum plate agglutination (SPA), 3.3% by culture and 10% by PCR , while *M.synoviae* Prevalence in day old chick in Kafrelsheikh was 11% by SPA , 1.1% by culture and 4.4% by PCR so, *MG* infection is predominant in day old chicks than *MS*. results opposite to [23]In libia who carried sero-prevalence for *MS* and *MG* which found *Ms* more predominant than *MG* in local and imported flocks[24]. In their study, the seroprevalence was examined by age. Infection in breeders was indicated by the antibodies found in one- to three-day-old chicks, which ranged from 29 to 54%. Due to the degradation of maternal antibodies, the seropositive rate was lowest in 3- to 4-week-old hens. The higher sero-prevalence of 71 to 83% in older chickens older than 35 weeks may be the result of a natural infection. So, circulated *M.gallisepticum* strain vertically transmitted from breeder the main reservoir to day old chicks and still silent not make any disease in chickens until immuno suppression occurs due to viral vaccination, bacterial, viral infection or environmental stressors as bad hygiene or defect in biosafety measures .Twelve *MG* isolates and four *MS* isolates were characterized by culture, biochemical tests and PCR. *MG* isolates were50% by PCR where *MS* isolates were16.7% in Egypt 2020 [25]. For the purpose of this investigation, three identified *M. gallisepticum* strains, Zagazig/2014/1 (Mk310102), Zagazig/2014/2 (MK 310103), and Zagazig/2017 (MK310101), were sequenced and phylogenetically analysed. The results showed high similarity (96.3- 99.5%) with previous Egyptian published sequences of *MG* *mgc*2 (field isolates). In this work, the 16S rRNA gene in the avian samples was found using the PCR technique. Positive samples were sent for mgc2 gene sequencing. Two Positive samples from the black and white turkey were subjected to partial sequencing and analysis of mgc2 gene. The Egypt white turkey strain OM632678 isolated from Sharkia showed 100% similarity to KX268629 Thailand strain and Indian strain KP279743 as mentioned by [5]2021 and 47% to local Egyptian turkey strain HQ591357/2011 and 39% similarity to MG676447 local Egypt chicken2018 Although nucleotide identity between mgc2 gene of white turkey *MG*OM632678. and other isolates KX268626- KX268630 and KX268622. from Thailand and KP300761., KP279743., KP300757., KP300758. and KP300760 from India; which were got from the gene bank were 76%, but the amino acid identity was 100%. Additionally, isolates previously reported from Pakistan shared 98–99 percent similarity, as mentioned by [26].The type strain isolated from black turkey (OM632677) from Dakahlia governorate showed 100% nucleotide identity and amino acids identity with other Egyptian isolates such KT943467.1 *M. gallisepticum*/ Eis-9-CK-15, FJ234839.1 *M. gallisepticum*/ RabE1-08 which is similar to F strain, and JN113343.2 M. gallisepticum/ *MG* NMH-2and97%with HQ591356. 1M. gallisepticum /Eis4-C-10. Due to the potential virulence of the vaccine F-strain in broilers and turkeys, it is not advised for use in Italy[27] ,while in Egypt it is licensed and commonly used. The results of isolation and molecular characterization confirmed the presence of *MG* infection in chicken and turkey flocks of Dakhalia and Sharkia governorates. These results in accordance with [5] 2021who mentioned that, early diagnosis using PCR techniques would enable the adoption of biosecurity and control measures well in advance of any financial loss. There was just a slight difference between the isolates used in their investigation and the isolates that are common in Pakistan, India and USA [28, 29]. Others[30] in their study in USA concluded that, the *M. gallisepticum* ts-11vaccine and ts-11 isolates showed genomic changes compared to the *M. gallisepticum* R low genome in the form of insertions/deletions of sequences. These isolates were collected from the field and from ts-11 animal passage trials. In Iran 2017 [31] after the 16S r RNA gene PCR method the results of the sequencing of the mgc2 gene on positive samples indicated that *MG* was present in Iranian backyard and commercial turkey farms. The molecular study revealed significant sequence similarities between

several Iranian turkey isolates and *MG* isolates from Pakistan and India. Also, they identified two specific substitutions: a switch from proline to leucine at positions 62 and 90 in five isolates, and an exchange from polar methionine to nonpolar isoleucine. Some antigenic changes may result from substitutions of *MG* nucleic acids and corresponding amino acid sequences. The white turkey strain showed 47% to local Egyptian turkey strain HQ591357/2011 and 39% similarity to MG676447 local Egypt chicken2018 because they were containing nonpolar isoleucine while now the *Mg* strains that containing polar methionine are predominant. In Egypt, *Mycoplasma gallisepticum* has a severe impact on the chicken business [32]. One of the effective ways to control *Mycoplasmosis* is the widespread use of antibiotics .Numerous drug classes have been shown to be effective against *MG*, including fluoroquinolones, macrolides, tetracyclines, and pleuromutilin [8,9]but, *MG* could develop antibacterial resistance as a result of prolonged antibiotic use [10,11].The prevention and treatment of *mycoplasmosis* frequently involves the use of antibiotics from the macrolide family, such as tylosin and tilmicosin [12].Prior research has demonstrated the influence of point mutations in the 23S rRNA-encoding genes on the emergence of macrolide resistance in a number of *Mycoplasma* species, including *M. gallisepticum* and *M. synoviae.* According to [12,13]. Concerning sensitivity to different antibiotics mycoplasma isolates of this study were examined for eight antibiotics. The three antibiotics with the lowest MICs were tiamulin (0.009g/ml), tilmicosin (0.039g/ml), and tylosin (0.019g/ml). Nasi6, *MG*64, T19 are susceptible to all antimicrobial while *MG*11 is completely resistant to them while *MG*-B.T of black turkey and *MG*-W.T of white turkey were resistant to erythromycin, doxycycline and tylosin . Also, *MS* was sensitive to macrolides (erythromycin, tylosin, tilmicosin). but *MS* was resistant to doxycycline and lincospectin, further studies are recommended for this point. Our research in accordance with [33] who concluded that the circulated *MG* strains are mutant type of *MG* F vaccine strain. Using of PCR in diagnosis early enable to get rid of infection by *MG* this accordance with [34]who concluded that the PCR test the most effective due to its rapidity in *MG* diagnosis.

Conclusion

The present research verifies the existence of *MG* infection in backyard and commercial turkey farms as well as in flocks of chickens of sharkia and Dakahlia governorates in Egypt. Thus widespread vaccination and regular surveillance are required to stop the spread of illness. Early diagnosis using PCR techniques would enable the adoption of biosecurity and control measures well in advance of any economic loss.

Acknowledgment

The auther would like to thanks Department of Mycoplasma, Animal Health Research Institute Dokki, Giza, for financial support and necessary facilities to carry out this work.

Conflict of interest:

The authors have declared no conflict of interest.

Funding statement

Self-funding

Ethical approval

This study was conducted with the approval *(*KFS-IACUC/ 161/ 2023) from Animal Care and Use Experimental Committee, Faculty of Veterinary Medicine Kafrelsheikh University Egypt.

Authors' Contributions

All the authors are contributed equally in the search work.

TABLE 1. Serological, Culture and PCR *detection* of Mycoplasma gallisepticum and Mycoplasma synoviae in different day old chick Breeds flock in Kafrelsheikh

No.	Breed	*Mycoplasma gallisepticum (MG)*				*Mycoplasma Synoviae (MS)*			
		SPA		**Culture**	**PCR**	**SPA**		**Culture**	**PCR**
1	Baladi1	4/10	14/30= 49.3%	1/30=3.3%	1/30=3.3%	0/10	2/30=6.7%	0/30=0%	0/30=0%
2	Baladi2	5/10		(MG 11)		0/10			
3	Baladi3	5/10				2/10			
4	Avian (48) 1	2/10	7/20=35%	1/20=5%	3/20=15%	4/10	4/20=20%	0/20=0%	2/20=10%
5	Avian(48) 2	5|10		(MG10)		0/10			
6	Arbo plus1	3|10	6/20=30%	0/20=0%	4/20=20%	1/10	4/20=20%	1/20=5% (MS-D.O.)	2/20=10%
7	Arbo plus2	3|10				3/10			
`8	Saso	5|10	50%	1/10=10% (MG DO.1)	1/10=10%	0/10	0/10=0%	0/10=0%	0/10=0%
9	Fayomi	5|10	50%	0/10=0%	0/10=0%	0/10	0/10=0%	0/10=0%	0/10=0%
10	Cobb	0/10	0%	ND	ND	0/10	ND	ND	ND
Total		37/100	37%	3/90=3.3%	9/90= 10%	10/90	10/90=11%	1/90= 1.1%	4/90= 4.4%

●*MG* detection was 37% by serum plate agglutination(SPA), 3.3% and 10% by culture and PCR respectively, while *MS* isolates were 11% by SPA , 1.1% and 4.4% by culture and PCR respectively. Cobb breed is negative SPA,culture and negative PCR.

TABLE 2. Culture and PCR detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Chicken Breeds and Turkey in Kafrelsheikh, Dakahlia and Sharkia governorates:

Year of isolation	Governorate	Avian Breed	Mycoplasma isolated =24			Mycoplasma confirmed by PCR=20			Strain isolated in this study
			G+ F- D+	G+ F+ D+	G- A+ D+	Un-typed Mycoplasma G+ F- D+	MG	MS	
2017	Kafrelsheikh	Layers	3	-	2	2	1	1	Nas6,
2018	Dakhlia	Broiler breeder	2	-	1	1	1	1	MG64
2018	Dakhlia	Broiler	3	-	1	1	3	2	MG8, MS-
2019									ABO.T,
									BROI.19
2017	Dakhlia	Back yard turkey	5	-			4	-	T28,T30,T31,
2021									MG-B.T.
2022									T3
2018	Sharkia	Turkey joint	3	4			3	-	Tra.19,
2019		Turkeytrachea							T2
2019		Turkey sinus							MG-W.T.
2022		White turkey							
No.			16	4	4	4	12	4	

● G+ Glucose positive, G-Glucose Negative,A+ Arginine positive, F+ Film and spot positive,D+ Digitonin positive,No.Number

●12 *MG* isolates and 4 *MS* isolates were characterized by culture, biochemical tests and PCR. *MG* isolates were50% by PCR where *MS* isolates were16.7% .3 *MG* isolate and 2 *MS* isolates was isolated and identified from 12 broiler flocks from dakhlia while one *MG* and one *MS* isolates was identified from five layer flock examened from kafrelsheikh . Also ,one *MG* and one *MS* from three broiler breeder flocks from dakhlia and 4 *MG* isolates from seven turkey flocks(three from back yard black turkey from dakhlia and three *MG* from four white turkey flocks from sharkia.

Table 3. Sensitivity test: MIC levels of eight antimicrobial agents against *Mycoplasma gallisepticum* and *M. synoviae* isolates:

NO.	MG isolated Strains	Anti-microbial drugs															
		Ciprofluxacin		Doxycycline		Lincospectin		Streptomycin		Erythromycin		Tiamulin		Tilmicosin		Tylosin	
		MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
		1.25	5	0.039	5	0.156	2.5	0.313	5	0.039	10	0.009	5	0.039	20	0.009	5
1	MG10	2.5		0.156		0.313		0.313		0.313		0.78		0.313		0.156	
2	MG11	R		R		R		R		R		R		R		R	
3	BROI.19	2.5		0.78		1.25		0.625		0.78		0.313		5		0.039	
4	T19	2.5		0.78		1.25		0.156		0.039		0.156		0.313		0.78	
5	MG 8	1.25		0.039		0.313		0.625		10		0.009		0.039		0.019	
6	DO.1	2.5		0.156		0.625		5		0.78		0.156		2.5		0.313	
7	Nasi 6	5		0.313		1.25		0.625		0.156		0.156		1.25		0.313	
8	MG 64	2.5		0.625		2.5		1.25		0.625		0.625		0.313		0.313	
9	MG-W.T.	NA		5		2.5		NA		R		0.78		20		10	
10	MG-B.T.	NA		R		0.156		NA		R		5		0.039		R	
	MS	NA		20		10		NA		0.078		2.5		0.313		0.156	

●R: resistant , NA: not applied

●Three antibiotics with the lowest MICs were tiamulin (0.009g/ml), tilmicosin (0.039g/ml), and tylosin (0.019g/ml). Nasi6,*MG*64,T19 are susceptible to all antimicrobial while *MG11* is completely resistant to them while *MG*-B.T of black turkey and *MG*-W.T of white turkey were resistant to doxycycline and tylosin . Also, *MS* was sensitive to macrolides (erythromycin, tylosin, tilmicosin). but *MS* was resistant to(doxycycline, lincospectin)

Fig.1 . Infectious sinusitis of turkey

Isolation of Mycoplasma colonies on PPLO media give fried egg appearance under stereomicroscope (40x)

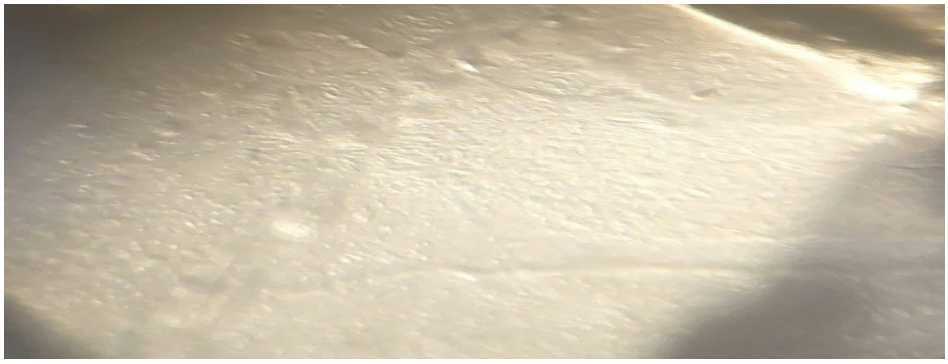

Fig. 2. *Mycoplasma synoviae(MS)* (Film and spot)

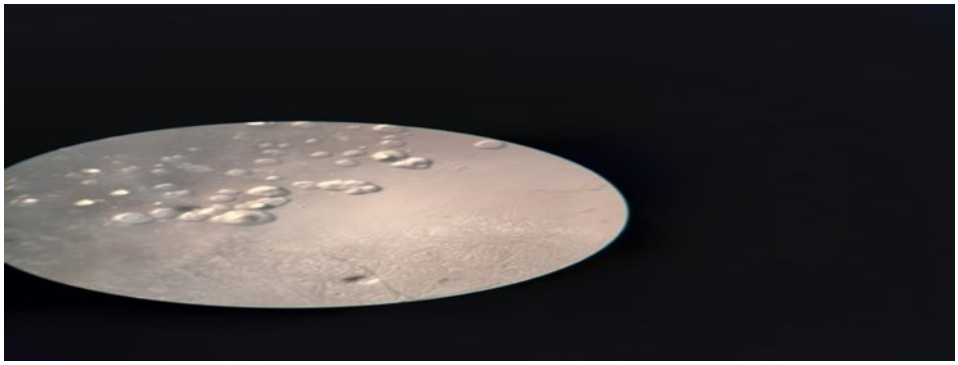

Fig. 3. Mycoplasma synoviae

Fig. 4.Mycoplasma gallisepticum (MG)

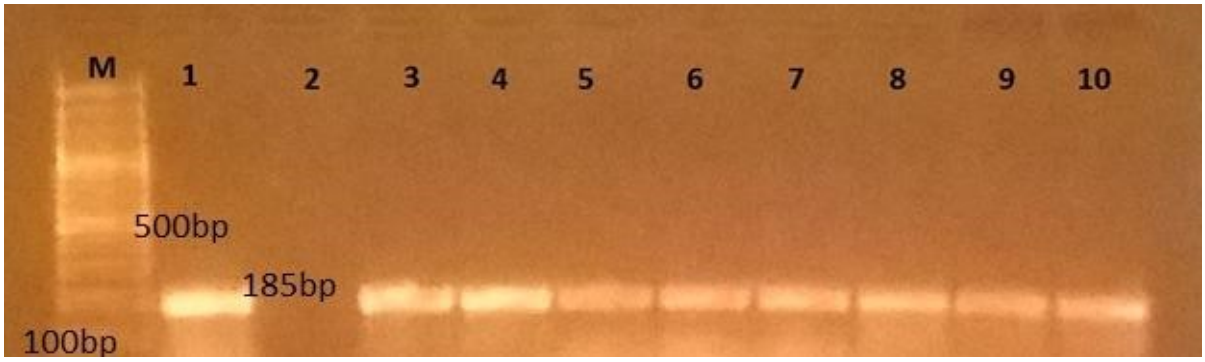

Fig. 5.Electrophoretic agarose gel of mycoplasma gallisepticum using Oie primers
M- 100bp-1000pb DNA ladder
1- Control positive MG 2. Control negative 3-10 Positive field isolates PCR results -16S rRNA gene giving characterstic band at 185bp.

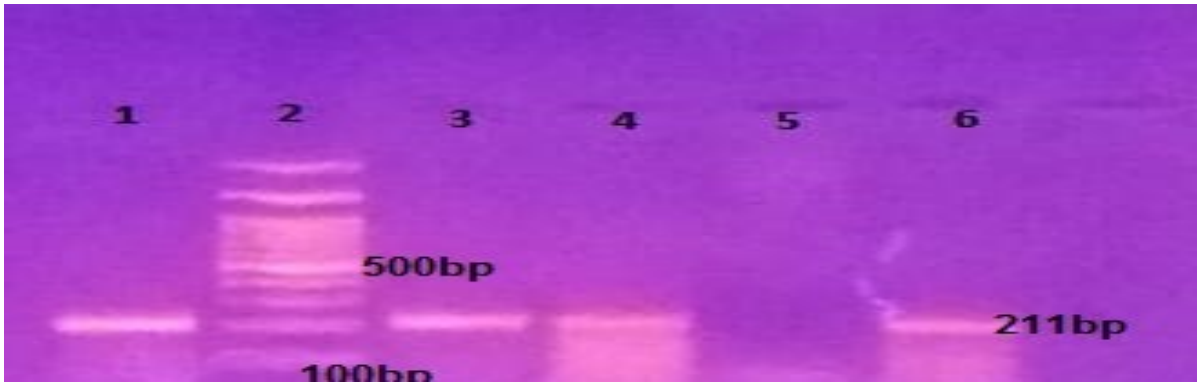

Fig. 6. Elecrophoretic gel of MS using specific primers
1- Control positive MS 2-100bp-500bp ladder 3- 3,4,6 field MS positive samples. Detection of 16S rRNA gene of *MS* three mycoplasma isolates were identified as specific band at 211 bp as in fig (6).

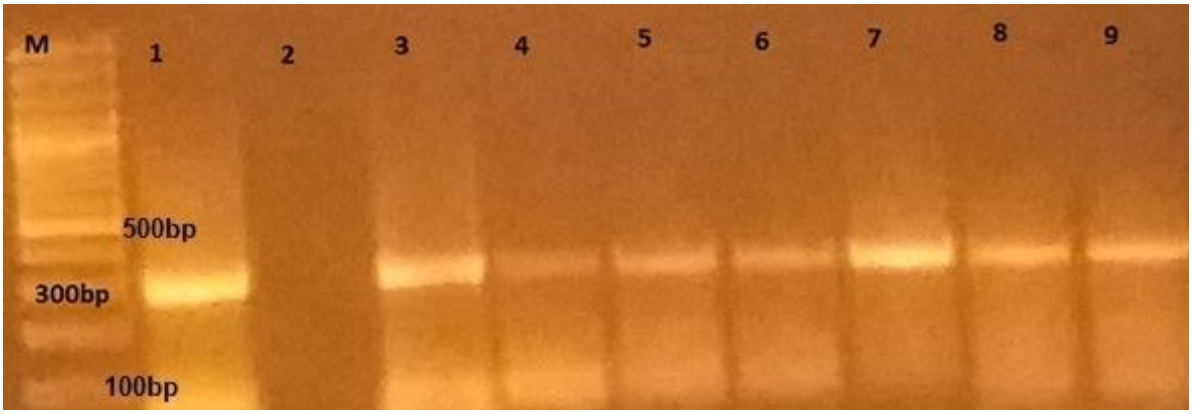

Fig. 7. Mycoplasma gallisepticum using Mgc2 primer
M :100bp-1000 ladder
lane1:Control positive *MG*
lane2: Control negative
lane3:3-9 positive *MG* field isolstes giving characteristic band at 300bp

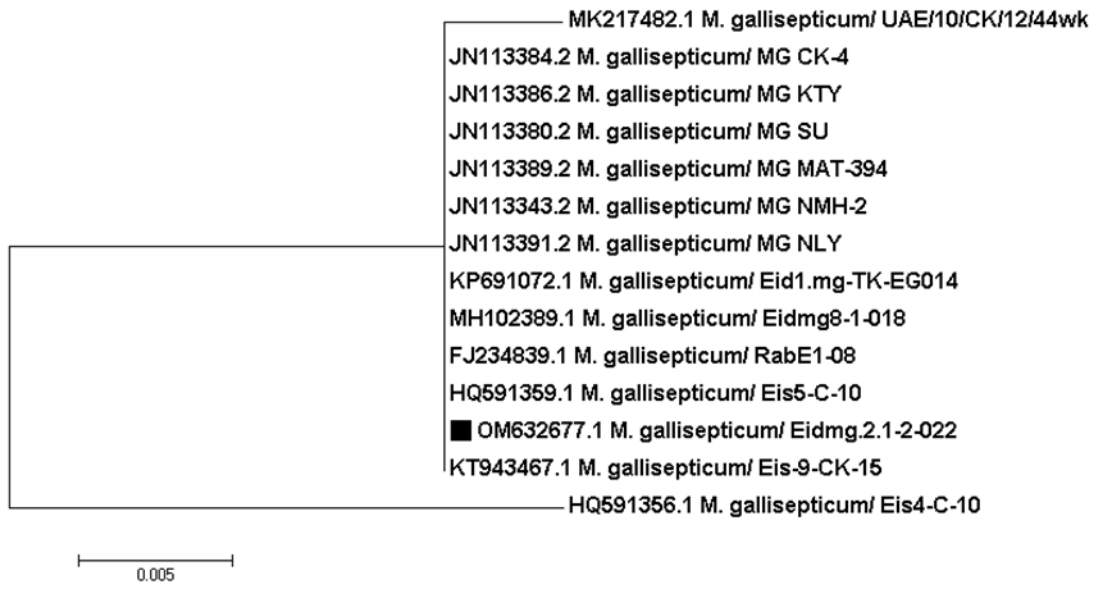

Fig. 8. Show the phylogenetic tree of *MG mgc2* black square: this study isolate.

Table 4. nucleotides (horizontal) and amino acids (vertical) identity between *MG mgc2*of black turkey and other gene bank isolates.

Seq->	>OM632677.1 M. gallisepticum/ Eidmg.2.1-2-022	>KT943467.1 M. gallisepticum/ Eis-9-CK-15	>HQ591359.1 M. gallisepticum/ Eis5-C-10	>HQ591356.1 M. gallisepticum/ Eis4-C-10	>FJ234839.1 M. gallisepticum/ RabE1-08	>MH102389.1 M. gallisepticum/ Eidmg8-1-018	>KP691072.1 M. gallisepticum/ Eid1.mg-TK-EG014	>MK217482.1 M. gallisepticum/ UAE/10/CK/12/44wk	>JN113391.2 M. gallisepticum/ MG NLY	>JN113343.2 M. gallisepticum/ MG NMH-2	>JN113389.2 M. gallisepticum/ MG MAT-394	>JN113380.2 M. gallisepticum/ MG SU	>JN113386.2 M. gallisepticum/ MG KTY	>JN113384.2 M. gallisepticum/ MG CK-4
>OM632677.1 M. gallisepticum/ Eidmg.2.1-2-022	ID	100%	100%	97%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>KT943467.1 M. gallisepticum/ Eis-9-CK-15	100%	ID	100%	97%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>HQ591359.1 M. gallisepticum/ Eis5-C-10	100%	100%	ID	97%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
>HQ591356.1 M. gallisepticum/ Eis4-C-10	95%	95%	95%	ID	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%
>FJ234839.1 M. gallisepticum/ RabE1-08	100%	100%	100%	95%	ID	100%	100%	100%	100%	100%	100%	100%	100%	100%
>MH102389.1 M. gallisepticum/ Eidmg8-1-018	100%	100%	100%	95%	100%	ID	100%	100%	100%	100%	100%	100%	100%	100%
>KP691072.1 M. gallisepticum/ Eid1.mg-TK-EG01	100%	100%	100%	95%	100%	100%	ID	100%	100%	100%	100%	100%	100%	100%
>MK217482.1 M. gallisepticum/ UAE/10/CK/12/4	100%	100%	100%	94%	99%	99%	99%	ID	100%	100%	100%	100%	100%	100%
>JN113391.2 M. gallisepticum/ MG NLY	100%	100%	100%	95%	100%	100%	100%	99%	ID	100%	100%	100%	100%	100%
>JN113343.2 M. gallisepticum/ MG NMH-2	100%	100%	100%	95%	100%	100%	100%	99%	100%	ID	100%	100%	100%	100%
>JN113389.2 M. gallisepticum/ MG MAT-394	100%	100%	100%	95%	100%	100%	100%	99%	100%	100%	ID	100%	100%	100%
>JN113380.2 M. gallisepticum/ MG SU	100%	100%	100%	95%	100%	100%	100%	99%	100%	100%	100%	ID	100%	100%
>JN113386.2 M. gallisepticum/ MG KTY	100%	100%	100%	95%	100%	100%	100%	99%	100%	100%	100%	100%	ID	100%
>JN113384.2 M. gallisepticum/ MG CK-4	100%	100%	100%	95%	100%	100%	100%	99%	100%	100%	100%	100%	100%	ID

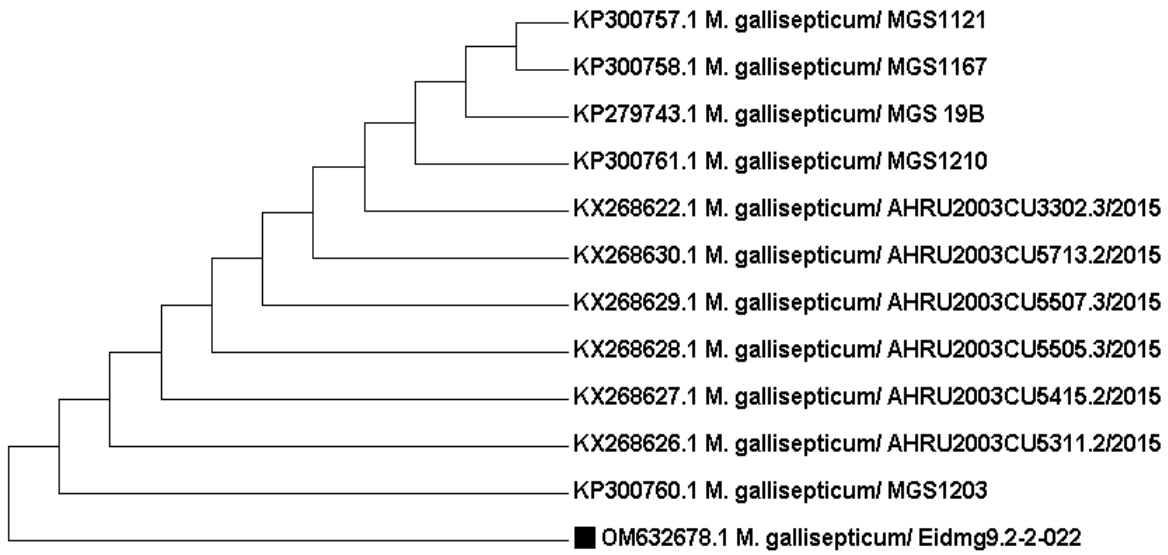

Fig. 9. show the phylogenetic tree of *MG mgc2* white turkey in this study isolate.

TABLE 5. Nucleotides (horizontal) and amino acids (vertical) identity between *MG mgc2* of black turkey and other gene bank isolates.

Seq->	>OM632678.1 M. gallisepticum/ Eidmg9.2-2-022	>HQ591357.1 M. gallisepticum/ Eis6-T-10	>MW679029.1 M. gallisepticum/ MG-EGY/ORABI/Raheel/2020	>MG676447.1 M. gallisepticum/ Heb.2/Mg/CK/EG017	>KX268626.1 M. gallisepticum/ AHRU2003CU5311.2/2015	>KX268627.1 M. gallisepticum/ AHRU2003CU5415.2/2015	>KX268628.1 M. gallisepticum/ AHRU2003CU5505.3/2015	>KX268629.1 M. gallisepticum/ AHRU2003CU5507.3/2015	>KX268630.1 M. gallisepticum/ AHRU2003CU5713.2/2015	>KX268622.1 M. gallisepticum/ AHRU2003CU3302.3/2015	>MG428415.1 M. gallisepticum/ clone HN1396.52	>KP300761.1 M. gallisepticum/ MGS1210	>KP279743.1 M. gallisepticum/ MGS 19B	>KP300757.1 M. gallisepticum/ MGS1121	>KP300758.1 M. gallisepticum/ MGS1167	>KP300760.1 M. gallisepticum/ MGS1203
>OM632678.1 M. gallisepticum/ Eidmg9.2-2-022	ID	36%	36%	30%	76%	76%	76%	76%	76%	76%	36%	76%	76%	76%	76%	76%
>HQ591357.1 M. gallisepticum/ Eis6-T-10	47%	ID	97%	83%	48%	48%	48%	48%	48%	48%	99%	48%	48%	48%	48%	48%
>MW679029.1 M. gallisepticum/ MG-EGY/ORABI/Raheel/2020	46%	97%	ID	85%	47%	47%	47%	47%	47%	47%	97%	47%	47%	47%	47%	47%
>MG676447.1 M. gallisepticum/ Heb.2/Mg/CK/EG017	39%	82%	84%	ID	40%	40%	40%	40%	40%	40%	83%	40%	40%	40%	40%	40%
>KX268626.1 M. gallisepticum/ AHRU2003CU5311.2/2015	100%	47%	46%	39%	ID	100%	100%	100%	100%	100%	48%	100%	100%	100%	100%	100%
>KX268627.1 M. gallisepticum/ AHRU2003CU5415.2/2015	100%	47%	46%	39%	100%	ID	100%	100%	100%	100%	48%	100%	100%	100%	100%	100%
>KX268628.1 M. gallisepticum/ AHRU2003CU5505.3/2015	100%	47%	46%	39%	100%	100%	ID	100%	100%	100%	48%	100%	100%	100%	100%	100%
>KX268629.1 M. gallisepticum/ AHRU2003CU5507.3/2015	100%	47%	46%	39%	100%	100%	100%	ID	100%	100%	48%	100%	100%	100%	100%	100%
>KX268630.1 M. gallisepticum/ AHRU2003CU5713.2/2015	100%	47%	46%	39%	100%	100%	100%	100%	ID	100%	48%	100%	100%	100%	100%	100%
>KX268622.1 M. gallisepticum/ AHRU2003CU3302.3/2015	100%	47%	46%	39%	100%	100%	100%	100%	100%	ID	48%	100%	100%	100%	100%	100%
>MG428415.1 M. gallisepticum/ clone HN1396.52	47%	97%	97%	82%	47%	47%	47%	47%	47%	47%	ID	48%	48%	48%	48%	48%
>KP300761.1 M. gallisepticum/ MGS1210	100%	47%	46%	39%	100%	100%	100%	100%	100%	100%	47%	ID	100%	100%	100%	100%
>KP279743.1 M. gallisepticum/ MGS 19B	100%	47%	46%	39%	100%	100%	100%	100%	100%	100%	47%	100%	ID	100%	100%	100%
>KP300757.1 M. gallisepticum/ MGS1121	100%	47%	46%	39%	100%	100%	100%	100%	100%	100%	47%	100%	100%	ID	100%	100%
>KP300758.1 M. gallisepticum/ MGS1167	100%	47%	46%	39%	100%	100%	100%	100%	100%	100%	47%	100%	100%	100%	ID	100%
>KP300760.1 M. gallisepticum/ MGS1203	100%	47%	46%	39%	100%	100%	100%	100%	100%	100%	47%	100%	100%	100%	100%	ID

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التوصيف الجزيئي لميكوبلازما الطيور مع إشارة خاصة إلى حساسية المضادات الحيوية في مصر

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المستخلص

يعد داء الميكوبلازما في الطيور أحد أهم التهديدات المالية التي تؤثر على صناعة الدواجن العالمية ، لذا ، فإن النهج المناسب للكشف عن العوامل المسببة الا وهى الميكوبلازما جاليسيبتيكم (MG) والميكوبلازما سينوفى (MS) لتوصيف السلالات الحقلية المنتشرة وتحديد ملامح حساسيتها لمضادات الميكروبات.اجريت دراسة من 2017 إلى 2022 في محافظات كفر الشيخ والشرقية والدقهلية في مصر على قطاعات الطيور المختلفة، تم تحديد اثنتي عشرة عزلة من نوع MG وأربع عزلات من MS المتعدد عن طريق الزراعة والتأكد من تفاعل انزيم البلمرة المتسلسل. تم تقديم التسلسل الجزئي لجين mgc2 لعزلتين من MG من الرومي (MGB.T.) و (MGW.T.) على بنك الجينات تحت رقم الانضمام OM632677. وOM632678 على التوالي وكشف عن وجود سلالتين مختلفتين من MG المتداولة في الرومى ، لتحديد ملامح الحساسية لمضادات الميكروبات ، تم اختبار عشرةعزلات من MG،واحدة MS من مختلف قطاعات الطيور مقابل ثمانية مضادات ميكروبات لتحديد أدنى MIC ، وثلاثة مضادات للبكتيريا ، تيامبولين (0.009 ميكروجرام / مل) ، تيلميكوزين (0.039 ميكروجرام / مل) وتايلوزين (0.019 ميكروجرام / مل)، قدمت أقل MICs من جميع الأدوية الفعالة .MS كان عزل حساسا لمضادات الميكروبات الثمانية ، وكانت معظم عزلات MG حساسة للسيبروفلوكساسين والستربتومايسين بينما كان للينكوسبكتين تأثير متوسط. عزل MG11 من فراخ بلدي كان مقاوما تماما لجميع مضادات الميكروبات. كانت عزلتا MG من الديك الرومي مقاومتين ل (الإريثروميسين والتيلوزين) ، لذلك يلزم إجراء مزيد من التحقيق للكشف عن الجينات المقاومة للماكروليدات . نستنتج من الدراسة أن الدراسة الحالية تؤكد وجود الإصابة بالمرض في قطعان الرومي والدجاج بمحافظتي الشرقية والدقهلية.

الكلمات الافتتاحية: انزيم البلمرة المتسلسل ، اختبار الحساسية للمضادات الحيوية ، الميكوبلازما جاليسبتكم MG ، الميكوبلازما سينوفى MS ، الرومى.